# 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: Executive Summary

## A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines

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# 2. High Blood Cholesterol and ASCVD

## 2.1. Measurements of LDL-C and Non–HDL-C

| Recommendations for Measurements of LDL-C and Non–HDL-C<br>Referenced studies that support recommendations are summarized in Online Data Supplement 1. | | |
|---|---|---|
| **COR** | **LOE** | **Recommendations** |
| **I** | **B-NR** | **1. In adults who are 20 years of age or older and not on lipid-lowering therapy, measurement of either a fasting or a nonfasting plasma lipid profile is effective in estimating ASCVD risk and documenting baseline LDL-C (S2.1-1–S2.1-6).** |
| **I** | **B-NR** | **2. In adults who are 20 years of age or older and in whom an initial nonfasting lipid profile reveals a triglycerides level of 400 mg/dL (≥4.5 mmol/L) or higher, a repeat lipid profile in the fasting state should be performed for assessment of fasting triglyceride levels and baseline LDL-C (S2.1-1–S2.1-4).** |
| **IIa** | **C-LD** | **3. For patients with an LDL-C level less than 70 mg/dL (<1.8 mmol/L), measurement of direct LDL-C or modified LDL-C estimate is reasonable to improve accuracy over the Friedewald formula (S2.1-7–S2.1-9).** |
| **IIa** | **C-LD** | **4. In adults who are 20 years of age or older and without a personal history of ASCVD but with a family history of premature ASCVD or genetic hyperlipidemia, measurement of a fasting plasma lipid profile is reasonable as part of an initial evaluation to aid in the understanding and identification of familial lipid disorders.** |

# 3. Therapeutic Modalities

## 3.1. Lipid-Lowering Drugs

Among lipid-lowering drugs, statins are the cornerstone of therapy, in addition to healthy lifestyle interventions. Other LDL-lowering drugs include ezetimibe, bile acid sequestrants, and PCSK9 inhibitors. Triglyceride-lowering drugs are fibrates and niacin; they have a mild LDL-lowering action, but RCTs do not support their use as add-on drugs to statin therapy (S3.1-1). Characteristics of LDL-lowering drugs are summarized in Table S3 in the Web Supplement.

### *3.1.1. Statin Therapy*

The intensity of statin therapy is divided into 3 categories: high-intensity, moderate-intensity, and low-intensity (S3.1.1-1). High-intensity statin therapy typically lowers LDL-C levels by ≥50%, moderate-intensity statin therapy by 30% to 49%, and low-intensity statin therapy by <30% (Table 3). Of course, the magnitude of LDL-C lowering will vary in clinical practice (S3.1.1-2). Certain Asian populations may have a greater response to certain statins (S3.1.1-3). Pharmacokinetic profiles among statins are heterogeneous (Table S4 in the Web Supplement). Statin safety has been extensively evaluated (S3.1.1-4). Statin-associated side effects are discussed in Section 5. Common medications that may potentially interact with statins are listed in Table S5 in the Web Supplement. More information on statin drug–drug interactions can be obtained from the ACC LDL-C Manager (http://tools.acc.org/ldl) (S3.1.1-5).

**Table 3. High-, Moderate-, and Low-Intensity Statin Therapy***

| | High Intensity | Moderate Intensity | Low Intensity |
|---|---|---|---|
| **LDL-C lowering†** | ≥50% | 30%–49% | <30% |
| **Statins** | **Atorvastatin (40 mg‡) 80 mg**<br>**Rosuvastatin 20 mg (*40 mg* | **Atorvastatin 10 mg** (20 mg)<br>**Rosuvastatin** (5 mg) **10 mg**<br>**Simvastatin 20–40 mg§** | Simvastatin 10 mg |
| | … | **Pravastatin 40 mg** (80 mg)<br>**Lovastatin 40 mg** (80 mg)<br>Fluvastatin XL 80 mg<br>**Fluvastatin 40 mg BID**<br>Pitavastatin 1–4 mg | **Pravastatin 10–20 mg**<br>**Lovastatin 20 mg**<br>Fluvastatin 20–40 mg |

*Percent reductions are estimates from data across large populations. Individual responses to statin therapy varied in the RCTs and should be expected to vary in clinical practice (S3.1.1-2).
†LDL-C lowering that should occur with the dosage listed below each intensity.
‡Evidence from 1 RCT only: down titration if unable to tolerate atorvastatin 80 mg in the IDEAL (Incremental Decrease through Aggressive Lipid Lowering) study (S3.1.1-18).
§Although simvastatin 80 mg was evaluated in RCTs, initiation of simvastatin 80 mg or titration to 80 mg is not recommended by the FDA because of the increased risk of myopathy, including rhabdomyolysis.
Percent LDL-C reductions with the primary statin medications used in clinical practice (atorvastatin, rosuvastatin, simvastatin) were estimated using the median reduction in LDL-C from the VOYAGER database (S3.1.1-2).
Reductions in LDL-C for other statin medications (fluvastatin, lovastatin, pitavastatin, pravastatin) were identified according to FDA-approved product labeling in adults with hyperlipidemia, primary hypercholesterolemia, and mixed dyslipidemia (S3.1.1-6).
**Boldface type** indicates specific statins and doses that were evaluated in RCTs (S3.1.1-7–S3.1.1-19), and the Cholesterol Treatment Trialists' 2010 meta-analysis (S3.1.1-20). All these RCTs demonstrated a reduction in major cardiovascular events.
BID indicates twice daily; FDA, U.S. Food and Drug Administration; LDL-C, low-density lipoprotein cholesterol; RCT, randomized controlled trial; VOYAGER, an indiVidual patient data meta-analysis Of statin therapY in At risk Groups: Effects of Rosuvastatin, atorvastatin and simvastatin; and XL, extended release.

# 4. Patient Management Groups

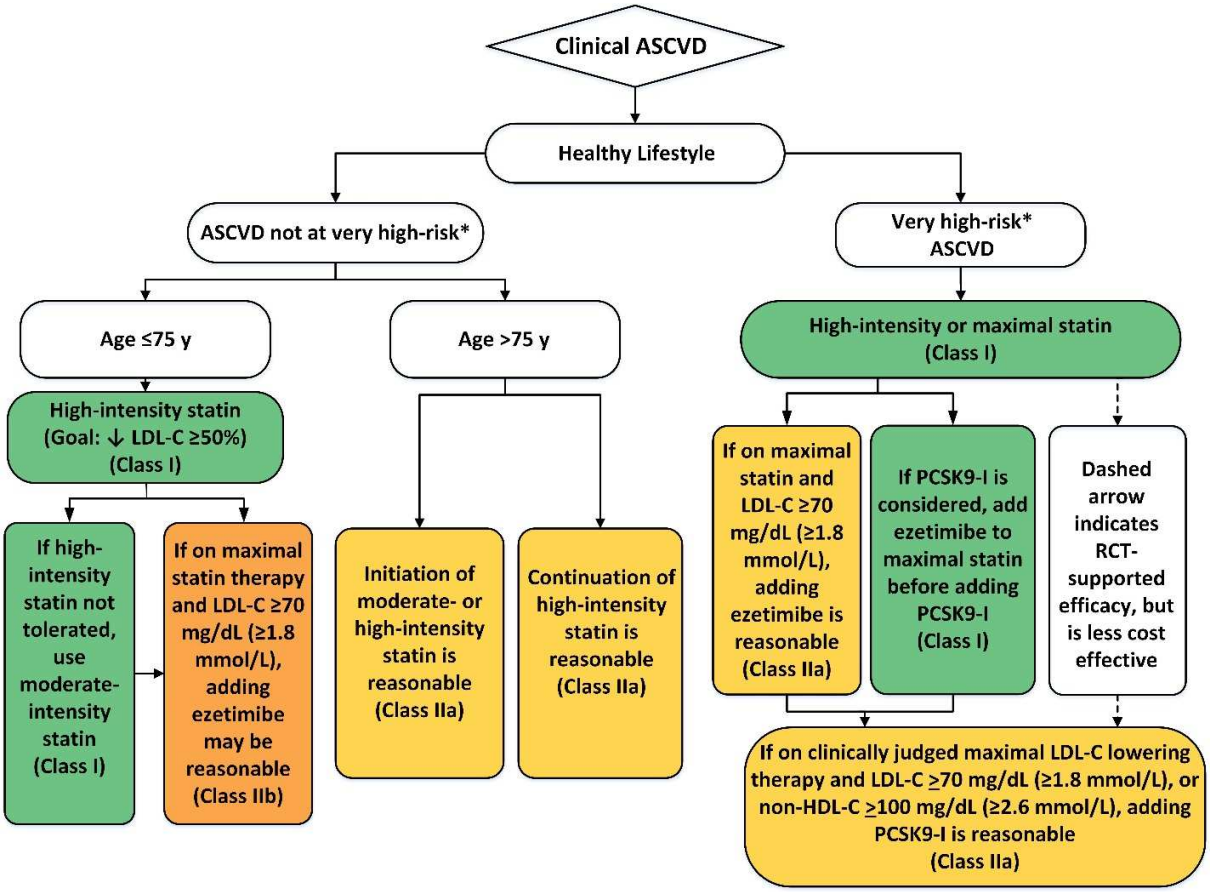
## 4.1. Secondary ASCVD Prevention

| **Recommendations for Statin Therapy Use in Patients With ASCVD**<br>Referenced studies that support recommendations are summarized in Online Data Supplements 6, 7, 8 and in the Systematic Review Report (Figure 1). | | |
|---|---|---|
| **COR** | **LOE** | **Recommendations** |
| **I** | **A** | **1. In patients who are 75 years of age or younger with clinical ASCVD,* high-intensity statin therapy should be initiated or continued with the aim of achieving a 50% or greater reduction in LDL-C levels (S4.1-1–S4.1-5).** |
| **I** | **A** | **2. In patients with clinical ASCVD in whom high-intensity statin therapy is contraindicated or who experience statin-associated side effects, moderate-intensity statin therapy should be initiated or continued with the aim of achieving a 30% to 49% reduction in LDL-C levels (S4.1-3, S4.1-6–S4.1-13).** |
| **I** | **B-NR** | **3. In patients with clinical ASCVD who are judged to be very high risk and *considered for PCSK9 inhibitor therapy,* maximally tolerated LDL-C lowering** |

| | | |
|---|---|---|
| | | **therapy should include maximally tolerated statin therapy and ezetimibe (S4.1-14, S4.1-15).** |
| **IIa** | **A[SR]** | **4. In patients with clinical ASCVD who are judged to be very high risk and who are on maximally tolerated LDL-C lowering therapy with LDL-C 70 mg/dL (≥1.8 mmol/L) or higher or a non–HDL-C level of 100 mg/dL (≥2.6 mmol/L) or higher, it is reasonable to add a PCSK9 inhibitor following a clinician–patient discussion about the net benefit, safety, and cost (S4.1-16–S4.1-20).** |
| **IIa** | **B-R** | **5. In patients with clinical ASCVD who are on maximally tolerated statin therapy and are judged to be at very high risk and have an LDL-C level of 70 mg/dL (≥1.8 mmol/L) or higher, it is reasonable to add ezetimibe therapy (S4.1-14, S4.1-15).** |
| **Value Statement: Low Value (LOE: B-NR)** | | **6. At mid-2018 list prices, PCSK9 inhibitors have a low cost value (>$150,000 per QALY) compared to good cost value (<$50,000 per QALY) (Section 7 provides a full discussion of the dynamic interaction of different prices and clinical benefit) (S4.1-21–S4.1-23).** |
| **IIa** | **B-R** | **7. In patients older than 75 years of age with clinical ASCVD, it is reasonable to initiate moderate- or high-intensity statin therapy after evaluation of the potential for ASCVD risk reduction, adverse effects, and drug–drug interactions, as well as patient frailty and patient preferences (S4.1-24–S4.1-32).** |
| **IIa** | **C-LD** | **8. In patients older than 75 years of age who are tolerating high-intensity statin therapy, it is reasonable to continue high-intensity statin therapy after evaluation of the potential for ASCVD risk reduction, adverse effects, and drug-drug interactions, as well as patient frailty and patient preferences (S4.1-3, S4.1-10, S4.1-24, S4.1-27, S4.1-32–S4.1-37).** |
| **IIb** | **B-R** | **9. In patients with clinical ASCVD who are receiving maximally tolerated statin therapy and whose LDL-C level remains 70 mg/dL (≥1.8 mmol/L) or higher, it may be reasonable to add ezetimibe (S4.1-15).** |
| **IIb** | **B-R** | **10. In patients with heart failure (HF) with reduced ejection fraction attributable to ischemic heart disease who have a reasonable life expectancy (3 to 5 years) and are not already on a statin because of ASCVD, clinicians may consider initiation of moderate-intensity statin therapy to reduce the occurrence of ASCVD events (S4.1-38).** |

*Clinical atherosclerotic cardiovascular disease (ASCVD) includes acute coronary syndrome (ACS), those with history of myocardial infarction (MI), stable or unstable angina or coronary or other arterial revascularization, stroke, transient ischemic attack (TIA), or peripheral artery disease (PAD) including aortic aneurysm, all of atherosclerotic origin.

## Figure 1. Secondary Prevention in Patients With Clinical ASCVD




Colors correspond to Class of Recommendation in Table 2.
Clinical ASCVD consists of acute coronary syndrome (ACS), those with history of myocardial infarction (MI), stable or unstable angina or coronary other arterial revascularization, stroke, transient ischemic attack (TIA), or peripheral artery disease (PAD) including aortic aneurysm, all of atherosclerotic origin.
Very high-risk includes a history of multiple major ASCVD events or 1 major ASCVD event and multiple high-risk conditions (Table 4).
ACS indicates acute coronary syndrome; ASCVD, atherosclerotic cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; MI, myocardial infarction; and PCSK9i, PCSK9 inhibitor.

**Table 4. Very High-Risk* of Future ASCVD Events**

| **Major ASCVD Events** |
|---|
| Recent ACS (within the past 12 mo) |
| History of MI (other than recent ACS event listed above) |
| History of ischemic stroke |
| Symptomatic peripheral arterial disease (history of claudication with ABI <0.85, or previous revascularization or amputation (S4.1-39)) |
| **High-Risk Conditions** |
| Age ≥65 y |
| Heterozygous familial hypercholesterolemia |
| History of prior coronary artery bypass surgery or percutaneous coronary intervention outside of the major ASCVD event(s) |
| Diabetes mellitus |
| Hypertension |
| CKD (eGFR 15-59 mL/min/1.73 $m^2$) (S4.1-15, S4.1-17) |
| Current smoking |
| Persistently elevated LDL-C (LDL-C ≥100 mg/dL [≥2.6 mmol/L]) despite maximally tolerated statin therapy and ezetimibe |
| History of congestive HF |

*Very high-risk includes a history of multiple major ASCVD events or one major ASCVD event and multiple high-risk conditions.
ABI indicates ankle-brachial index; ACS, acute coronary syndrome; ASCVD, atherosclerotic cardiovascular disease; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HF, heart failure; LDL, low-density lipoprotein cholesterol; and MI, myocardial infarction.

## 4.2. Severe Hypercholesterolemia (LDL-C ≥190 mg/dL [≥4.9 mmol/L])

| **Recommendations for Primary Severe Hypercholesterolemia (LDL-C ≥190 mg/dL [≥4.9 mmol/L])**<br>Referenced studies that support recommendations are summarized in Online Data Supplements 9 and 10. | | |
|---|---|---|
| **COR** | **LOE** | **Recommendations** |
| **I** | **B-R** | **1. In patients 20 to 75 years of age with an LDL-C level of 190 mg/dL (≥4.9 mmol/L) or higher, maximally tolerated statin therapy is recommended (S4.2-1–S4.2-7).** |
| **IIa** | **B-R** | **2. In patients 20 to 75 years of age with an LDL-C level of 190 mg/dL (≥4.9 mmol/L) or higher who achieve less than a 50% reduction in LDL-C while receiving maximally tolerated statin therapy and/or have an LDL-C level of 100 mg/dL (≥2.6 mmol/L) or higher, ezetimibe therapy is reasonable (S4.2-8–S4.2-10).** |
| **IIb** | **B-R** | **3. In patients 20 to 75 years of age with a baseline LDL-C level ≥190 mg/dL** |

| | | |
|---|---|---|
| | | (≥4.9 mmol/L), who achieve less than a 50% reduction in LDL-C levels and have fasting triglycerides ≤300 mg/dL (≤3.4 mmol/L). while taking maximally tolerated statin and ezetimibe therapy, the addition of a bile acid sequestrant may be considered (S4.2-11, S4.2-12). |
| IIb | B-R | 4. In patients 30 to 75 years of age with heterozygous FH and with an LDL-C level of 100 mg/dL (≥2.6 mmol/L) or higher while taking maximally tolerated statin and ezetimibe therapy, the addition of a PCSK9 inhibitor may be considered (S4.2-9, S4.2-13–S4.2-15). |
| IIb | C-LD | 5. In patients 40 to 75 years of age with a baseline LDL-C level of 220 mg/dL (≥5.7 mmol/L) or higher and who achieve an on-treatment LDL-C level of 130 mg/dL (≥3.4 mmol/L) or higher while receiving maximally tolerated statin and ezetimibe therapy, the addition of a PCSK9 inhibitor may be considered (S4.2-13–S4.2-17). |
| Value Statement: Uncertain Value (B-NR) | | 6. Among patients with FH without evidence of clinical ASCVD taking maximally tolerated statin and ezetimibe therapy, PCSK9 inhibitors provide uncertain value at mid-2018 U.S. list prices. |

## 4.3. Diabetes Mellitus in Adults

| Recommendations for Patients With Diabetes Mellitus<br>Referenced studies that support recommendations are summarized in Online Data Supplements 11 and 12. | | |
|---|---|---|
| **COR** | **LOE** | **Recommendations** |
| I | A | 1. In adults 40 to 75 years of age with diabetes mellitus, regardless of estimated 10-year ASCVD risk, moderate-intensity statin therapy is indicated (S4.3-1–S4.3-9). |
| IIa | B-NR | 2. In adults 40 to 75 years of age with diabetes mellitus and an LDL-C level of 70 to 189 mg/dL (1.7 to 4.8 mmol/L), it is reasonable to assess the 10-year risk of a first ASCVD event by using the race and sex-specific PCE to help stratify ASCVD risk (S4.3-10, S4.3-11). |
| IIa | B-R | 3. In adults with diabetes mellitus who have multiple ASCVD risk factors, it is reasonable to prescribe high-intensity statin therapy with the aim to reduce LDL-C levels by 50% or more (S4.3-12, S4.3-13). |
| IIa | B-NR | 4. In adults older than 75 years of age with diabetes mellitus and who are already on statin therapy, it is reasonable to continue statin therapy (S4.3-5, S4.3-8, S4.3-13). |
| IIb | C-LD | 5. In adults with diabetes mellitus and 10-year ASCVD risk of 20% or higher, it may be reasonable to add ezetimibe to maximally tolerated statin therapy to reduce LDL-C levels by 50% or more (S4.3-14, S4.3-15). |
| IIb | C-LD | 6. In adults older than 75 years with diabetes mellitus, it may be reasonable to initiate statin therapy after a clinician–patient discussion of potential benefits and risks (S4.3-5, S4.3-8, S4.3-13). |

| | | |
|---|---|---|
| **IIb** | **C-LD** | **7. In adults 20 to 39 years of age with diabetes mellitus that is either of long duration (≥10 years of type 2 diabetes mellitus, ≥20 years of type 1 diabetes mellitus), albuminuria (≥30 mcg of albumin/mg creatinine), estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73 $m^2$, retinopathy, neuropathy, or ABI (<0.9), it may be reasonable to initiate statin therapy (S4.3-5, S4.3-6, S4.3-8, S4.3-16–S4.3-25).** |

**Synopsis**

Adults 20 to 39 years of age are mostly at low 10-year risk, although moderate-intensity statin therapy in those with long-standing diabetes mellitus or a concomitant higher-risk condition may be reasonable (Table 5) (S4.3-17, S4.3-20, S4.3-21). It may be reasonable to have a discussion about initiating moderate-intensity statin therapy with patients who have had type 2 diabetes mellitus for at least 10 years or type 1 diabetes mellitus for at least 20 years and with patients with ≥1 major CVD risk factors or complications, such as diabetic retinopathy (S4.3-19), neuropathy (S4.3-16), nephropathy (eGFR <60 mL/min/1.73 $m^2$ or albuminuria ≥30 mcg albumin/mg creatinine) (S4.3-25), or an ABI of <0.9 (S4.3-22, S4.3-24) (Table 5).

**Table 5. Diabetes-Specific Risk Enhancers That Are Independent of Other Risk Factors in Diabetes Mellitus**

| Risk Enhancers |
|---|
| • Long duration (≥10 years for type 2 diabetes mellitus (S4.3-20) or ≥20 years for type 1 diabetes mellitus (S4.3-6) |
| • Albuminuria ≥30 mcg of albumin/mg creatinine (S4.3-25) |
| • eGFR <60 mL/min/1.73 $m^2$ (S4.3-25) |
| • Retinopathy (S4.3-19) |
| • Neuropathy (S4.3-16) |
| • ABI <0.9 (S4.3-22, S4.3-24) |

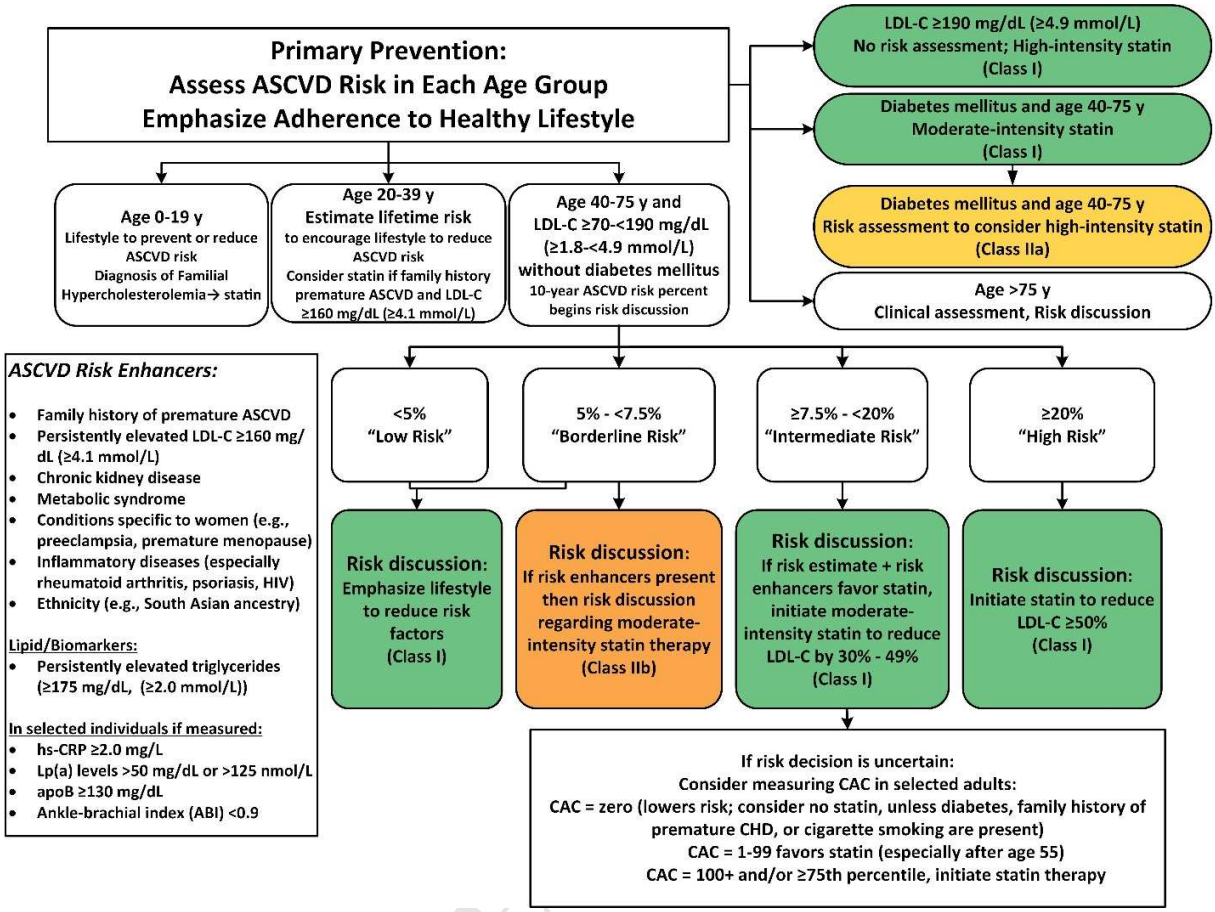
ABI indicates ankle-brachial index; and eGFR, estimated glomerular filtration rate.

## 4.4. Primary Prevention

Primary prevention of ASCVD over the life span requires attention to prevention or management of ASCVD risk factors beginning early in life (Figure 2). One major ASCVD risk factor is elevated serum cholesterol, usually identified clinically as measured LDL-C. Screening can be performed with fasting or nonfasting measurement of lipids. In children, adolescents (10 to 19 years of age), and young adults (20 to 39 years of age), priority should be given to estimation of lifetime risk and promotion of lifestyle risk reduction. Drug therapy is needed only in selected patients with moderately high LDL-C levels (≥160 mg/dL [≥4.1 mmol/L]) or patients with very high LDL-C levels (190 mg/dL [4.9 mmol/L]). Three major higher-risk categories are patients with severe hypercholesterolemia (LDL-C levels ≥190 mg/dL [≥4.9 mmol/L]), adults with diabetes, and adults 40 to 75 years of age. Patients with severe hypercholesterolemia and adults 40 to 75 years of age with diabetes mellitus are candidates for immediate statin therapy without further risk assessment. Adults with diabetes mellitus should start with a moderate-intensity statin, and as they accrue multiple risk factors, a high-intensity statin may be indicated. In other adults 40 to 75 years of age, 10-year ASCVD risk should guide therapeutic considerations. The higher the estimated ASCVD risk, the more likely the patient is to benefit from

evidence-based statin treatment. The risk discussion should also consider several “risk enhancers” that can be used to favor initiation or intensification of statin therapy. When risk is uncertain or if statin therapy is problematic, it can be helpful to measure CAC to refine risk assessment. A CAC score predicts ASCVD events in a graded fashion and is independent of other risk factors, such as age, sex, and ethnicity (S4.4-1). A CAC score equal to zero is useful for reclassifying patients to a lower-risk group, often allowing statin therapy to be withheld or postponed unless higher risk conditions are present. For patients >75 years of age, RCT evidence for statin therapy is not strong, so clinical assessment of risk status in a clinician–patient risk discussion is needed for deciding whether to continue or initiate statin treatment (S4.4-2–S4.4-21).

**Figure 2. Primary Prevention**




Colors correspond to Class of Recommendation in Table 2.
apoB indicates apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; CAC, coronary artery calcium; human immunodeficiency virus; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; and Lp(a), lipoprotein (a).

## 4.4.1. Evaluation and Risk Assessment

### *4.4.1.1. Risk-Enhancing Factors*

Moderate intensity generic statins allow for efficacious and cost-effective primary prevention in patients with a 10-year risk of ASCVD ≥7.5% (S4.4.1.1-1). Since 2013 ACC/AHA guidelines (S4.4.1.1-2), the HOPE-3 RCT (S4.4.1.1-3) provided additional support for this finding. The pooled cohort equation (PCE) is the single most robust tool for estimating 10-year risk in U.S. adults 40 to 75 years of age. Its strength can be explained by inclusion of major, independent risk factors. One limitation on the PCE when applied to individuals is that age counts as a risk factor and dominates risk scoring with advancing age. Age is a powerful population risk factor but does not necessarily reflect individual risk. Another factor influencing risk are baseline characteristics of populations (baseline risk). These characteristics include both genetic and acquired risk factors other than established major risk factors. Variation in baseline risk accounts for difference in risk in different ethnic groups. Absolute risk predictions depend on the baseline risk of a population (e.g., the U.S. population). These considerations in patients at intermediate

risk leave room in the clinician-patient risk discussion to withhold or delay initiation of statin therapy, depending on age, pattern of risk factors, and patient preferences and values.

In sum, the PCE is a powerful tool to predict population risk, but it has limitations when applied to individuals. One purpose of the clinician patient risk discussion is to individualize risk status based on PCE as well as other factors that may inform risk prediction. Among these other factors are the risk-enhancing factors discussed in this guideline. These risk-enhancing factors are listed in Table 6, and evidence base and strength of association with ASCVD are shown in Table S6 in the Web Supplement. In the general population, they may or may not predict risk independently of PCE. But in the clinician–patient risk discussion they can be useful for identifying specific factors that influence risk. Their presence helps to confirm a higher risk state and thereby supports a decision to initiate or intensify statin therapy. They are useful for clarifying which atherogenic factors are present in a particular patient. And in some patients, certain risk-enhancing factors carry greater lifetime risk than denoted by 10-year risk prediction in the PCE. Finally, several risk-enhancing factors may be specific targets therapy beyond those of the PCE.

A few comments may illustrate the potential usefulness of risk-enhancing factors in the patient discussion. LDL-C ≥160 mg/dL (≥4.1 mmol/L), apoB ≥130 mg/dL (particularly when accompanied by persistently elevated triglycerides), and elevated Lp(a) denote high lifetime risk for ASCVD and favor initiation of statin therapy. The presence of family history of ASCVD, premature menopause, and patients of South Asian race appear to convey a higher baseline risk and are stronger candidates for statin therapy. Conditions associated with systemic inflammation (chronic inflammatory disorders, metabolic syndrome, chronic renal disease, and elevated hsCRP) appear to predispose to atherothrombotic events, which reasonably justifies statin therapy in intermediate-risk patients.

**Table 6. Risk-Enhancing Factors for Clinician–Patient Risk Discussion**

| Risk-Enhancing Factors |
|---|
| • **Family history of premature ASCVD** (males, age <55 y; females, age <65 y)<br>• **Primary hypercholesterolemia** (LDL-C, 160–189 mg/dL [4.1–4.8 mmol/L); non–HDL-C 190–219 mg/dL [4.9–5.6 mmol/L])*<br>• **Metabolic syndrome** (increased waist circumference, elevated triglycerides [>175 mg/dL], elevated blood pressure, elevated glucose, and low HDL-C [<40 mg/dL in men; <50 in women mg/dL] are factors; tally of 3 makes the diagnosis)<br>• **Chronic kidney disease** (eGFR 15–59 mL/min/1.73 $m^2$ with or without albuminuria; not treated with dialysis or kidney transplantation)<br>• **Chronic inflammatory conditions** such as psoriasis, RA, or HIV/AIDS<br>• **History of premature menopause (before age 40 y) and history of pregnancy-associated conditions that increase later ASCVD risk such as preeclampsia**<br>• **High-risk race/ethnicities** (e.g., South Asian ancestry)<br>• **Lipid/biomarkers**: Associated with increased ASCVD risk<br>  o Persistently* elevated, primary hypertriglyceridemia (≥175 mg/dL);<br>  o If measured:<br>  ▪ **Elevated high-sensitivity C-reactive protein** (≥2.0 mg/L)<br>  ▪ **Elevated Lp(a):** A relative indication for its measurement is family history of premature ASCVD. An Lp(a) ≥50 mg/dL or ≥125 nmol/L constitutes a risk-enhancing factor especially at higher levels of Lp(a). |

- **Elevated apoB** ≥130 mg/dL: A relative indication for its measurement would be triglyceride ≥200 mg/dL. A level ≥130 mg/dL corresponds to an LDL-C >160 mg/dL and constitutes a risk-enhancing factor
- **ABI** <0.9

*Optimally, 3 determinations.
AIDS indicates acquired immunodeficiency syndrome; ABI, ankle-brachial index; apoB, apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); and RA, rheumatoid arthritis.

## *4.4.2. Primary Prevention Adults 40 to 75 Years of Age With LDL-C Levels 70 to 189 mg/dL (1.7 to 4.8 mmol/L)*

**Primary Prevention Recommendations for Adults 40 to 75 Years of Age With LDL Levels 70 to 189 mg/dL (1.7 to 4.8 mmol/L)**
Referenced studies that support recommendations are summarized in Online Data Supplement 16 (Table 8).

| COR | LOE | Recommendations |
|---|---|---|
| I | A | **1. In adults at intermediate-risk, statin therapy reduces risk of ASCVD, and in the context of a risk discussion, if a decision is made for statin therapy, a moderate-intensity statin should be recommended (S4.4.2-1–S4.4.2-8).** |
| I | A | **2. In intermediate-risk patients, LDL-C levels should be reduced by 30% or more, and for optimal ASCVD risk reduction, especially in high-risk patients, levels should be reduced by 50% or more (S4.4.2-1, S4.4.2-4–S4.4.2-9).** |
| I | B-NR | **3. For the primary prevention of clinical ASCVD* in adults 40 to 75 years of age without diabetes mellitus and with an LDL-C level of 70 to 189 mg/dL (1.7 to 4.8 mmol/L), the 10-year ASCVD risk of a first "hard" ASCVD event (fatal and nonfatal MI or stroke) should be estimated by using the race- and sex-specific PCE, and adults should be categorized as being at low risk (<5%), borderline risk (5% to <7.5%), intermediate-risk (≥7.5% to <20%), and high-risk (≥20%) (S4.4.2-10, S4.4.2-11).** |
| I | B-NR | **4. Clinicians and patients should engage in a risk discussion that considers risk factors, adherence to healthy lifestyle, the potential for ASCVD risk-reduction benefits, and the potential for adverse effects and drug–drug interactions, as well as patient preferences, for an individualized treatment decision (S4.4.2-12–S4.4.2-14).** |
| IIa | B-R | **5. In intermediate-risk adults, risk-enhancing factors favor initiation or intensification of statin therapy (S4.4.2-6, S4.4.2-15–S4.4.2-22).** |

| | | |
|---|---|---|
| **IIa** | **B-NR** | **6. In intermediate-risk or selected borderline-risk adults, if the decision about statin use remains uncertain, it is reasonable to use a CAC score in the decision to withhold, postpone or initiate statin therapy (S4.4.2-15, S4.4.2-17, S4.4.2-23).** |
| **IIa** | **B-NR** | **7. In intermediate-risk adults or selected borderline-risk adults in whom a CAC score is measured for the purpose of making a treatment decision, AND**<br>• **If the coronary calcium score is zero, it is reasonable to withhold statin therapy and reassess in 5 to 10 years, as long as higher risk conditions are absent (diabetes mellitus, family history of premature CHD, cigarette smoking);**<br>• **If CAC score is 1 to 99, it is reasonable to initiate statin therapy for patients ≥ 55 years of age;**<br>• **If CAC score is 100 or higher or in the 75th percentile or higher, it is reasonable to initiate statin therapy (S4.4.2-17, S4.4.2-23).** |
| **IIb** | **B-R** | **8. In intermediate-risk adults who would benefit from more aggressive LDL-C lowering and in whom high-intensity statins are advisable but not acceptable or tolerated, it may be reasonable to add a nonstatin drug (ezetimibe or bile acid sequestrant) to a moderate-intensity statin (S4.4.2-9).** |
| **IIb** | **B-R** | **9. In patients at borderline risk, in risk discussion, the presence of risk-enhancing factors may justify initiation of moderate-intensity statin therapy (S4.4.2-17, S4.4.2-24).** |

*Definition of clinical ASCVD includes acute coronary syndrome (ACS), those with history of myocardial infarction (MI), stable or unstable angina or coronary or other arterial revascularization, stroke, transient ischemic attack (TIA), or peripheral artery disease (PAD) including aortic aneurysm, all of atherosclerotic origin.

**Table 7. Checklist for Clinician–Patient Shared Decision-Making for Initiating Therapy**

| Checklist Item | Recommendation |
|---|---|
| ASCVD risk assessment | • Assign to statin treatment group; use ASCVD Risk Estimator Plus.*<br>o In lower-risk primary-prevention adults 40-75 y of age with LDL-C ≥70 mg/dL (≥1.8 mmol/L).<br>o Not needed in secondary prevention, in those with LDL-C ≥190 mg/dL (≥4.9 mmol/L), or in those 40-75 y of age with diabetes mellitus.<br>• Assess other patient characteristics that influence risk. See Risk-Enhancing Factors (Section 4.4.1.3. and Table 6)<br>• Assess CAC (Section 4.4.1.4.) if risk decision is uncertain and additional information is needed to clarify ASCVD risk.<br>o Use decision tools to explain risk (e.g., ASCVD Risk Estimator Plus,* Mayo Clinic Statin Choice Decision Aid). |
| Lifestyle modifications | • Review lifestyle habits (e.g., diet, physical activity, weight or body mass index, and tobacco use).<br>• Endorse a healthy lifestyle and provide relevant advice, materials, or referrals. (e.g., CardioSmart, AHA Life's Simple 7, NLA Patient Tear Sheets, PCNA Clinicians' Lifestyle Modification Toolbox, cardiac rehabilitation, dietitian, smoking cessation program). |
| Potential net clinical benefit of pharmacotherapy | • Recommend statins as first-line therapy.<br>• Consider the combination of statin and nonstatin therapy in selected patients.<br>• Discuss potential risk reduction from lipid-lowering therapy.<br>• Discuss the potential for adverse effects or drug–drug interactions. |
| Cost considerations | • Discuss potential out-of-pocket cost of therapy to the patient (e.g., insurance plan coverage, tier level, copayment). |
| Shared decision-making | • Encourage the patient to verbalize what was heard (e.g., patient's personal ASCVD risk, available options, and risks/benefits).<br>• Invite the patient to ask questions, express values and preferences, and state ability to adhere to lifestyle changes and medications.<br>• Refer patients to trustworthy materials to aid in their understanding of issues regarding risk decisions.<br>• Collaborate with the patient to determine therapy and follow-up plan. |

*ASCVD Risk Predictor Plus is available at: http://tools.acc.org/ASCVD-Risk-Estimator-Plus/#!/calculate/estimate/. Accessed September 1, 2018.
AHA indicates American Heart Association; ASCVD, atherosclerotic cardiovascular disease; CAC, coronary artery calcium; CKD, chronic kidney disease; HIV, human immunodeficiency virus; LDL-C, low-density lipoprotein cholesterol; PCNA, Preventive Cardiology Nurses Association and NLA, National Lipid Association.

**Table 8. Selected Examples of Candidates for CAC Measurement Who Might Benefit From Knowing Their CAC Score Is Zero**

| CAC Measurement Candidates Who Might Benefit from Knowing Their CAC Score is Zero |
|---|
| • Patients reluctant to initiate statin therapy who wish to understand their risk and potential for benefit more precisely<br>• Patients concerned about need to reinstitute statin therapy after discontinuation for statin-associated symptoms<br>• Older patients (men, 55-80 y of age; women, 60-80 y of age) with low burden of risk factors (S4.4.2-25) who question whether they would benefit from statin therapy<br>• Middle-aged adults (40-55 y of age) with PCE-calculated 10-year risk of ASCVD 5% to <7.5% with factors that increase their ASCVD risk, although they are in a borderline risk group |

Caveats: If patient is intermediate risk and if a risk decision is uncertain and a CAC score is performed, it is reasonable to withhold statin therapy unless higher risk conditions such as cigarette smoking, family history of premature ASCVD, or diabetes mellitus are present, and to reassess CAC score in 5-10 years. Moreover, if CAC is recommended, it should be performed in facilities that have current technology that delivers the lowest radiation possible.

ASCVD indicates atherosclerotic cardiovascular disease; CAC, coronary artery calcium; LDL-C, low-density lipoprotein cholesterol; and PCE, pooled cohort equations.

## *4.4.3. Monitoring in Response to LDL-C–Lowering Therapy*

| **Recommendation for Monitoring**<br>Referenced studies that support the recommendation are summarized in Online Data Supplement 17. | | |
|---|---|---|
| **COR** | **LOE** | **Recommendation** |
| **I** | **A** | **1. Adherence to changes in lifestyle and effects of LDL-C–lowering medication should be assessed by measurement of fasting lipids and appropriate safety indicators 4 to 12 weeks after statin initiation or dose adjustment and every 3 to 12 months thereafter based on need to assess adherence or safety (S4.4.3-1–S4.4.3-3).** |

## *4.4.4. Primary Prevention in Other Age Groups*

### *4.4.4.1. Older Adults*

Additional recommendations for adults >75 years of age are included in Section 4.1. (Secondary ASCVD Prevention) and Section 4.3. (Diabetes Mellitus in Adults).

| **Recommendations for Older Adults**<br>Referenced studies that support recommendations are summarized in Online Data Supplements 18 and 19. | | |
|---|---|---|
| **COR** | **LOE** | **Recommendations** |
| **IIb** | **B-R** | **1. In adults 75 years of age or older with an LDL-C level of 70 to 189 mg/dL (1.7 to 4.8 mmol/L), initiating a moderate-intensity statin may be reasonable (S4.4.4.1-1–S4.4.4.1-8)** |

| | | |
|---|---|---|
| IIb | B-R | 2. In adults 75 years of age or older, it may be reasonable to stop statin therapy when functional decline (physical or cognitive), multimorbidity, frailty, or reduced life-expectancy limits the potential benefits of statin therapy (S4.4.4.1-9). |
| IIb | B-R | 3. In adults 76 to 80 years of age with an LDL-C level of 70 to 189 mg/dL (1.7 to 4.8 mmol/L), it may be reasonable to measure CAC to reclassify those with a CAC score of zero to avoid statin therapy (S4.4.4.1-10, S4.4.4.1-11). |

### *4.4.4.2. Children and Adolescents*

| Recommendations for Children and Adolescents<br>Referenced studies that support recommendations are summarized in Online Data Supplements 18, 19, 20, and 21. | | |
|---|---|---|
| **COR** | **LOE** | **Recommendations** |
| I | A | 1. In children and adolescents with lipid disorders related to obesity, it is recommended to intensify lifestyle therapy, including moderate caloric restriction and regular aerobic physical activity (S4.4.4.2-1–S4.4.4.2-4). |
| I | B-NR | 2. In children and adolescents with lipid abnormalities, lifestyle counseling is beneficial for lowering LDL-C (S4.4.4.2-1-3, S4.4.4.2-5–S4.4.4.2-12). |
| IIa | B-R | 3. In children and adolescents 10 years of age or older with an LDL-C level persistently 190 mg/dL (≥4.9 mmol/L) or higher or 160 mg/dL (4.1 mmol/L) or higher with a clinical presentation consistent with FH (see Section 4.2) and who do not respond adequately with 3 to 6 months of lifestyle therapy, it is reasonable to initiate statin therapy (S4.4.4.2-13–S4.4.4.2-16). |
| IIa | B-NR | 4. In children and adolescents with a family history of either early CVD* or significant hypercholesterolemia,† it is reasonable to measure a fasting or nonfasting lipoprotein profile as early as age 2 years to detect FH or rare forms of hypercholesterolemia (S4.4.4.2-17–S4.4.4.2-21). |
| IIa | B-NR | 5. In children and adolescents found to have moderate or severe hypercholesterolemia, it is reasonable to carry out reverse-cascade screening of family members, which includes cholesterol testing for first-, second-, and when possible, third-degree biological relatives, for detection of familial forms of hypercholesterolemia (S4.4.4.2-22–S4.4.4.2-24). |
| IIa | C-LD | 6. In children and adolescents with obesity or other metabolic risk factors, it is reasonable to measure a fasting lipid profile to detect lipid disorders as components of the metabolic syndrome (S4.4.4.2-25–S4.4.4.2-27). |

| | | |
|---|---|---|
| **IIb** | **B-NR** | **7. In children and adolescents without cardiovascular risk factors or family history of early CVD, it may be reasonable to measure a fasting lipid profile or nonfasting non HDL-C once between the ages of 9 and 11 years, and again between the ages of 17 and 21 years, to detect moderate to severe lipid abnormalities (S4.4.4.2-19, S4.4.4.2-21, S4.4.4.2-27–S4.4.4.2-29).** |

*Family history of early CVD is defined here as MI, documented angina, or atherosclerosis by angiography in parents, siblings, grandparents, aunts, or uncles (<55 years of age for men, <65 years of age for women).
†TC ≥240 mg/dL (≥6.2 mmol/L), LDL-C ≥190 mg/dL (≥4.9 mmol/L), non–HDL-C ≥220 mg/dL (≥5.7 mmol/L), or known primary hypercholesterolemia.
CVD indicates cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; and TC, total cholesterol.

### Synopsis

Selective screening for lipid disorders on the basis of family history (Recommendation 1) or lifestyle-related factors (Recommendation 2) identifies only a portion of childhood lipid abnormalities (S4.4.4.2-19, S4.4.4.2-21, S4.4.4.2-26) (Table 9).

**Table 9. Normal and Abnormal Lipid Values in Childhood*†**

| | Acceptable, mg/dL | Borderline, mg/dL | Abnormal, mg/dL |
|---|---|---|---|
| **TC** | <170 (<4.3 mmol) | 170–199 (4.3–5.1 mmol) | ≥200 (≥5.1 mmol) |
| **Triglycerides (0-9 y)** | <75 (<0.8 mmol) | 75–99 (0.8–1.1 mmol) | ≥100 (≥1.1 mmol) |
| **Triglycerides (10-19 y)** | <90 (<1.0 mmol) | 90–129 (1.0–1.5 mmol) | ≥130 (≥1.4 mmol) |
| **HDL-C** | >45 (>1.2 mmol) | 40–45 (1.0–1.2 mmol) | <40 (<1.0 mmol) |
| **LDL-C** | <110 (<2.8 mmol) | 110–129 (2.8–3.3 mmol) | ≥130 (≥3.4 mmol) |
| **Non–HDL-C** | <120 (<3.1 mmol) | 120–144 (3.1–3.7 mmol) | ≥145 (≥3.7 mmol) |

*Values for plasma lipid and lipoprotein levels are from the NCEP Expert Panel on Cholesterol Levels in Children. Non–HDL-C values from the Bogalusa Heart Study are equivalent to the NCEP Pediatric Panel cutpoints for LDL-C.
†The cutpoints for high and borderline high represent approximately the 95th and 75th percentiles, respectively. Low cutpoints for HDL-C represent approximately the 10th percentile.
Values given are in mg/dL. To convert to SI units, divide the results for TC, LDL-C, HDL-C, and non–HDL-C by 38.6; for triglycerides, divide by 88.6.
HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NCEP, National Cholesterol Education Program; SI, *Système international d'unités* (International System of Units); and TC, total cholesterol.

ORIGINAL ARTICLE

# Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia

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## ABSTRACT

### BACKGROUND

Patients with elevated triglyceride levels are at increased risk for ischemic events. Icosapent ethyl, a highly purified eicosapentaenoic acid ethyl ester, lowers triglyceride levels, but data are needed to determine its effects on ischemic events.

### METHODS

We performed a multicenter, randomized, double-blind, placebo-controlled trial involving patients with established cardiovascular disease or with diabetes and other risk factors, who had been receiving statin therapy and who had a fasting triglyceride level of 135 to 499 mg per deciliter (1.52 to 5.63 mmol per liter) and a low-density lipoprotein cholesterol level of 41 to 100 mg per deciliter (1.06 to 2.59 mmol per liter). The patients were randomly assigned to receive 2 g of icosapent ethyl twice daily (total daily dose, 4 g) or placebo. The primary end point was a composite of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina. The key secondary end point was a composite of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke.

### RESULTS

A total of 8179 patients were enrolled (70.7% for secondary prevention of cardiovascular events) and were followed for a median of 4.9 years. A primary end-point event occurred in 17.2% of the patients in the icosapent ethyl group, as compared with 22.0% of the patients in the placebo group (hazard ratio, 0.75; 95% confidence interval [CI], 0.68 to 0.83; P<0.001); the corresponding rates of the key secondary end point were 11.2% and 14.8% (hazard ratio, 0.74; 95% CI, 0.65 to 0.83; P<0.001). The rates of additional ischemic end points, as assessed according to a prespecified hierarchical schema, were significantly lower in the icosapent ethyl group than in the placebo group, including the rate of cardiovascular death (4.3% vs. 5.2%; hazard ratio, 0.80; 95% CI, 0.66 to 0.98; P=0.03). A larger percentage of patients in the icosapent ethyl group than in the placebo group were hospitalized for atrial fibrillation or flutter (3.1% vs. 2.1%, P=0.004). Serious bleeding events occurred in 2.7% of the patients in the icosapent ethyl group and in 2.1% in the placebo group (P=0.06).

### CONCLUSIONS

Among patients with elevated triglyceride levels despite the use of statins, the risk of ischemic events, including cardiovascular death, was significantly lower among those who received 2 g of icosapent ethyl twice daily than among those who received placebo. (Funded by Amarin Pharma; REDUCE-IT ClinicalTrials.gov number, NCT01492361.)

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*A complete list of the REDUCE-IT trial investigators is provided in the Supplementary Appendix, available at NEJM.org.

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Among patients with cardiovascular risk factors who are receiving treatment for secondary or primary prevention, the rates of cardiovascular events remain high.[1-3] Even in patients receiving appropriate treatment with statins, a substantial residual cardiovascular risk remains.[4] In such patients, an elevated triglyceride level serves as an independent marker for an increased risk of ischemic events, as shown in epidemiologic and mendelian randomization studies.[5-9] In randomized trials, medications that reduce triglyceride levels, such as extended-release niacin and fibrates, have not reduced the rates of cardiovascular events when administered in addition to appropriate medical therapy, including statins.[10] Contemporary trials and recent meta-analyses of n–3 fatty acid products have not shown a benefit in patients receiving statin therapy.[11-13]

In the Japan EPA Lipid Intervention Study (JELIS), 18,645 Japanese patients with hypercholesterolemia were randomly assigned to receive either low-intensity statin therapy plus 1.8 g of eicosapentaenoic acid (EPA) daily or statin therapy alone (there was no placebo group). The risk of major coronary events was significantly lower, by 19%, in the group that received EPA plus statin therapy than in the group that received statin therapy alone.[14]

These considerations led to the design of the Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial (REDUCE-IT).[15] Icosapent ethyl is a highly purified and stable EPA ethyl ester that has been shown to lower triglyceride levels and is used as an adjunct to diet in adult patients who have triglyceride levels of at least 500 mg per deciliter (5.64 mmol per liter).[16,17] In addition, icosapent ethyl may have antiinflammatory, antioxidative, plaque-stabilizing, and membrane-stabilizing properties.[18-21] We hypothesized that the risk of cardiovascular events would be lower with icosapent ethyl therapy than with placebo among patients in whom elevated triglyceride levels served as a marker of residual risk despite statin therapy.

## Methods

### Trial Design

The design of REDUCE-IT has been published previously.[15] In brief, REDUCE-IT was a phase 3b randomized, double-blind, placebo-controlled trial comparing icosapent ethyl (2 g twice daily with food [total daily dose, 4 g]) with a placebo that contains mineral oil to mimic the color and consistency of icosapent ethyl. Randomization was stratified according to cardiovascular risk stratum (secondary-prevention cohort or primary-prevention cohort, with primary prevention capped at 30% of enrolled patients), use or no use of ezetimibe, and geographic region. Further details of the study design are provided in Figure S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org. Patients were enrolled and followed at 473 participating sites in 11 countries. The first patient underwent randomization on November 28, 2011, and the last on August 4, 2016.

The trial was sponsored by Amarin Pharma. The steering committee, which consisted of academic physicians (see the Supplementary Appendix), and representatives of the sponsor developed the protocol, available at NEJM.org, and were responsible for the conduct and oversight of the study, as well as the interpretation of the data. The sponsor was responsible for the collection and management of the data. The protocol was approved by the relevant health authorities, institutional review boards, and ethics committees. All the data analyses were performed by the sponsor, and the primary, secondary, and tertiary adjudicated end-point analyses were validated by an independent statistician from the data and safety monitoring committee. The first author vouches for the completeness and accuracy of the data and analyses and for the fidelity of the trial to the protocol.

### Eligibility

Patients could be enrolled if they were 45 years of age or older and had established cardiovascular disease or were 50 years of age or older and had diabetes mellitus and at least one additional risk factor. Eligible patients had a fasting triglyceride level of 150 to 499 mg per deciliter (1.69 to 5.63 mmol per liter) and a low-density lipoprotein (LDL) cholesterol level of 41 to 100 mg per deciliter (1.06 to 2.59 mmol per liter) and had been receiving a stable dose of a statin for at least 4 weeks; because of the intraindividual variability of triglyceride levels, the initial protocol allowed for a 10% lower triglyceride level from the target lower limit, which permitted patients to be enrolled if they had a triglyceride level of at least 135 mg per deciliter (1.52 mmol per liter). The

first protocol amendment in May 2013 changed the lower limit of the acceptable triglyceride level from 150 mg per deciliter to 200 mg per deciliter (2.26 mmol per liter), with no allowance for variability. Patients were excluded if they had severe heart failure, active severe liver disease, a glycated hemoglobin level greater than 10.0%, a planned coronary intervention or surgery, a history of acute or chronic pancreatitis, or known hypersensitivity to fish, shellfish, or ingredients of icosapent ethyl or placebo. Further details regarding inclusion and exclusion criteria are provided in Tables S1 and S2 in the Supplementary Appendix. Written informed consent was obtained from all patients.

### END POINTS

The primary efficacy end point was a composite of cardiovascular death, nonfatal myocardial infarction (including silent myocardial infarction), nonfatal stroke, coronary revascularization, or unstable angina in a time-to-event analysis. While the steering committee and the sponsor remained unaware of the trial-group assignments, a second protocol amendment in July 2016 designated the key secondary end point as a composite of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke in a time-to-event analysis. After the primary efficacy end-point analysis was performed, the prespecified secondary efficacy end points were examined in a hierarchical fashion in the following order: the key secondary efficacy end point; a composite of cardiovascular death or nonfatal myocardial infarction; fatal or nonfatal myocardial infarction; emergency or urgent revascularization; cardiovascular death; hospitalization for unstable angina; fatal or nonfatal stroke; a composite of death from any cause, nonfatal myocardial infarction, or nonfatal stroke; and death from any cause. Prespecified tertiary end points are listed in the Supplementary Appendix. Adjudication of all the above events was performed by an independent clinical end-point committee whose members were unaware of the trial-group assignments and lipid levels.

### STATISTICAL ANALYSIS

In this event-driven trial, it was estimated that approximately 1612 adjudicated primary end-point events would be necessary to provide the trial with 90% power to detect a 15% lower risk of the primary composite end point in the icosapent ethyl group than in the placebo group. We estimated that a sample size of approximately 7990 patients would be required to reach that number of primary end-point events. The primary efficacy analysis was based on the time from randomization to the first occurrence of any component of the primary composite end point. If the risk of the primary composite end point was significantly lower with icosapent ethyl than with placebo at a final two-sided alpha level of 0.0437 (as determined with the use of O'Brien–Fleming boundaries generated with the Lan–DeMets alpha-spending function approach after accounting for two prespecified interim efficacy analyses), the key secondary end point and other prespecified secondary end points were to be tested in a hierarchical fashion at the same final alpha level of 0.0437. All analyses were performed according to the intention-to-treat principle. Hazard ratios and 95% confidence intervals were generated with the use of a Cox proportional-hazards model that included trial-group assignment as a covariate, stratified according to cardiovascular risk category, geographic region, and use of ezetimibe. Log-rank P values from a Kaplan–Meier analysis that was stratified according to the three randomization factors are reported to evaluate the timing of events in the two trial groups. With respect to the tertiary and subgroup efficacy analyses, 95% confidence intervals (which were not adjusted for multiple comparisons) are reported. An independent data and safety monitoring committee oversaw the study and performed two prespecified interim efficacy reviews.

## RESULTS

### PATIENTS

A total of 19,212 patients were screened, of whom 8179 (43%) underwent randomization. At the time of database lock, vital status was available for 99.8% of the patients; 152 patients (1.9%) did not complete the final study visits, and 578 patients (7.1%) withdrew consent. Details regarding the disposition of the patients are provided in Figure S2 in the Supplementary Appendix.

The baseline characteristics of the patients are shown in Table 1. Among the patients who underwent randomization, 70.7% were enrolled on the basis of secondary prevention (i.e., patients had established cardiovascular disease) and 29.3% on the basis of primary prevention (i.e., patients

**Table 1. Characteristics of the Patients at Baseline.***

| Characteristic | Icosapent Ethyl (N = 4089) | Placebo (N = 4090) |
|---|---|---|
| Age | | |
| Median (IQR) — yr | 64.0 (57.0–69.0) | 64.0 (57.0–69.0) |
| ≥65 yr — no. (%) | 1857 (45.4) | 1906 (46.6) |
| Male sex — no. (%) | 2927 (71.6) | 2895 (70.8) |
| White race — no. (%)† | 3691 (90.3) | 3688 (90.2) |
| Body-mass index‡ | | |
| Median (IQR) | 30.8 (27.8–34.5) | 30.8 (27.9–34.7) |
| ≥30 — no. (%) | 2331 (57.0) | 2362 (57.8) |
| Geographic region — no. (%)§ | | |
| United States, Canada, the Netherlands, Australia, New Zealand, and South Africa | 2906 (71.1) | 2905 (71.0) |
| Eastern European | 1053 (25.8) | 1053 (25.7) |
| Asia–Pacific | 130 (3.2) | 132 (3.2) |
| Cardiovascular risk stratum — no. (%) | | |
| Secondary-prevention cohort | 2892 (70.7) | 2893 (70.7) |
| Primary-prevention cohort | 1197 (29.3) | 1197 (29.3) |
| Ezetimibe use — no. (%) | 262 (6.4) | 262 (6.4) |
| Statin intensity — no. (%) | | |
| Low | 254 (6.2) | 267 (6.5) |
| Moderate | 2533 (61.9) | 2575 (63.0) |
| High | 1290 (31.5) | 1226 (30.0) |
| Data missing | 12 (0.3) | 22 (0.5) |
| Diabetes — no. (%) | | |
| Type 1 | 27 (0.7) | 30 (0.7) |
| Type 2 | 2367 (57.9) | 2363 (57.8) |
| No diabetes at baseline | 1695 (41.5) | 1694 (41.4) |
| Data missing | 0 | 3 (0.1) |
| Median high-sensitivity CRP level (IQR) — mg/liter | 2.2 (1.1–4.5) | 2.1 (1.1–4.5) |
| Median triglyceride level (IQR) — mg/dl | 216.5 (176.5–272.0) | 216.0 (175.5–274.0) |
| Median HDL cholesterol level (IQR) — mg/dl | 40.0 (34.5–46.0) | 40.0 (35.0–46.0) |
| Median LDL cholesterol level (IQR) — mg/dl | 74.0 (61.5–88.0) | 76.0 (63.0–89.0) |
| Distribution of triglyceride levels — no./total no. (%) | | |
| <150 mg/dl | 412/4086 (10.1) | 429/4089 (10.5) |
| ≥150 to <200 mg/dl | 1193/4086 (29.2) | 1191/4089 (29.1) |
| ≥200 mg/dl | 2481/4086 (60.7) | 2469/4089 (60.4) |
| Triglyceride level ≥200 mg/dl and HDL cholesterol level ≤35 mg/dl — no. (%) | 823 (20.1) | 794 (19.4) |
| Median eicosapentaenoic acid level (IQR) — μg/ml | 26.1 (17.1–40.1) | 26.1 (17.1–39.9) |

* Median low-density lipoprotein (LDL) cholesterol level at baseline differed significantly between the trial groups (P=0.03); there were no other significant between-group differences in baseline characteristics. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. In general, the baseline value was defined as the last nonmissing measurement obtained before randomization. The baseline LDL cholesterol value as measured by means of preparative ultracentrifugation was used in our analyses; however, if the preparative ultracentrifugation value was missing, the LDL cholesterol value measured by another method was used in the following order of priority: the value obtained by means of direct measurement of LDL cholesterol, the value derived with the use of the Friedewald equation (only for patients with a triglyceride level <400 mg per deciliter), and the value derived with the use of the calculation published by Johns Hopkins University investigators.[22] At the first and second screening visits, the LDL cholesterol value obtained by direct measurement was used if at the same visit the triglyceride level was higher than 400 mg per deciliter. At all remaining visits, the LDL cholesterol value was obtained by means of direct measurement or preparative ultracentrifugation if at the same visit the triglyceride level was higher than 400 mg per deciliter. For all other measures of lipid and lipoprotein markers, whenever possible, the baseline value was derived as the arithmetic mean of the value obtained at visit 2 (day 0) and the value obtained at the preceding screening visit. If only one of these values was available, that single value was used as the baseline value. CRP denotes C-reactive protein, HDL high-density lipoprotein, and IQR interquartile range. Percentages may not total 100 because of rounding.

† Race was reported by the investigators.

‡ Body-mass index is the weight in kilograms divided by the square of the height in meters.

§ Eastern European region includes Poland, Romania, Russia, and Ukraine, and Asia–Pacific region includes India.

had diabetes mellitus and at least one additional risk factor). The median age of the patients was 64 years; 28.8% were female, and 38.5% were from the United States. At baseline, the median LDL cholesterol level was 75.0 mg per deciliter (1.94 mmol per liter), the median high-density lipoprotein cholesterol level was 40.0 mg per deciliter (1.03 mmol per liter), and the median triglyceride level was 216.0 mg per deciliter (2.44 mmol per liter).[22]

### FOLLOW-UP AND EFFECTS ON LIPIDS

The median duration of follow-up was 4.9 years (maximum, 6.2 years). The median change in triglyceride level from baseline to 1 year was a decrease of 18.3% (–39.0 mg per deciliter [–0.44 mmol per liter]) in the icosapent ethyl group and an increase of 2.2% (4.5 mg per deciliter [0.05 mmol per liter]) in the placebo group; the median reduction from baseline (as estimated with the use of the Hodges–Lehmann approach) was 19.7% greater in the icosapent ethyl group than in the placebo group (a 44.5 mg per deciliter [0.50 mmol per liter] greater reduction; P<0.001). The median change in LDL cholesterol level from baseline was an increase of 3.1% (2.0 mg per deciliter [0.05 mmol per liter]) in the icosapent ethyl group and an increase of 10.2% (7.0 mg per deciliter [0.18 mmol per liter]) in the placebo group — a 6.6% (5.0 mg per deciliter [0.13 mmol per liter]) lower increase with icosapent ethyl than with placebo (P<0.001). The results with respect to levels of EPA and lipid, lipoprotein, and inflammatory biomarkers are provided in Table S4 in the Supplementary Appendix.

### CLINICAL END POINTS

A total of 1606 adjudicated primary end-point events occurred. A primary end-point event occurred in 17.2% of the patients in the icosapent ethyl group, as compared with 22.0% of the patients in the placebo group (hazard ratio, 0.75; 95% confidence interval [CI], 0.68 to 0.83; P<0.001), an absolute between-group difference of 4.8 percentage points (95% CI, 3.1 to 6.5); the number needed to treat to avoid one primary end-point event was 21 (95% CI, 15 to 33) over a median follow-up of 4.9 years.[23,24] The event curves based on a Kaplan–Meier analysis of the primary efficacy end point are provided in Figure 1A. The results of time-to-event analyses of each component of the primary end point are provided in Figure S3 in the Supplementary Appendix. A key secondary efficacy end-point event (Fig. 1B) occurred in 11.2% of the patients in the icosapent ethyl group, as compared with 14.8% of the patients in the placebo group (hazard ratio, 0.74;

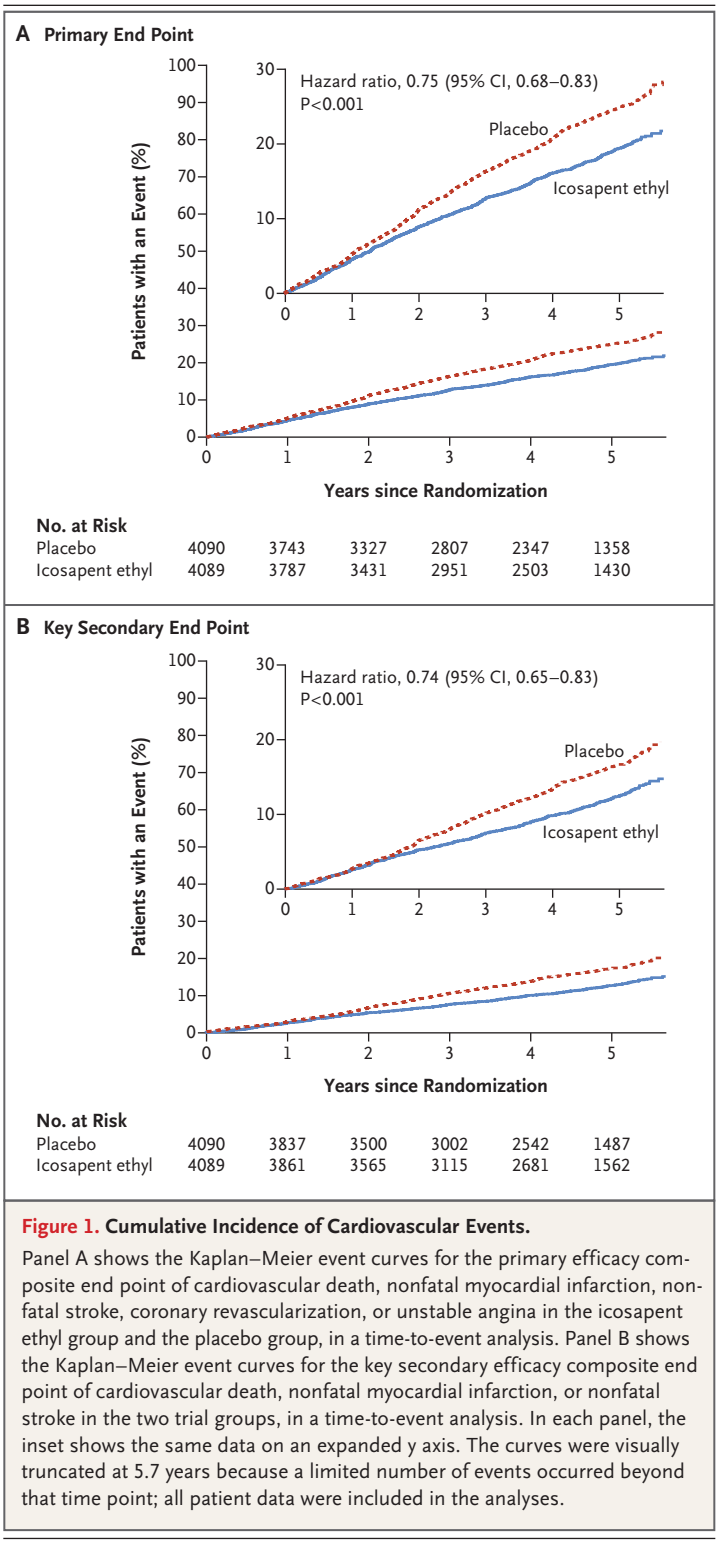


**Figure 1. Cumulative Incidence of Cardiovascular Events.**

Panel A shows the Kaplan–Meier event curves for the primary efficacy composite end point of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina in the icosapent ethyl group and the placebo group, in a time-to-event analysis. Panel B shows the Kaplan–Meier event curves for the key secondary efficacy composite end point of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke in the two trial groups, in a time-to-event analysis. In each panel, the inset shows the same data on an expanded y axis. The curves were visually truncated at 5.7 years because a limited number of events occurred beyond that time point; all patient data were included in the analyses.

| Subgroup | Icosapent Ethyl | Placebo | Hazard Ratio (95% CI) | P Value for Interaction |
|---|---|---|---|---|
| | *no. of patients with event/total no. of patients (%)* | | | |
| All patients | 705/4089 (17.2) | 901/4090 (22.0) | 0.75 (0.68–0.83) | |
| Risk stratum | | | | 0.14 |
| Secondary-prevention cohort | 559/2892 (19.3) | 738/2893 (25.5) | 0.73 (0.65–0.81) | |
| Primary-prevention cohort | 146/1197 (12.2) | 163/1197 (13.6) | 0.88 (0.70–1.10) | |
| Region | | | | 0.30 |
| United States, Canada, the Netherlands, Australia, New Zealand, and South Africa | 551/2906 (19.0) | 713/2905 (24.5) | 0.74 (0.66–0.83) | |
| Eastern Europe | 143/1053 (13.6) | 167/1053 (15.9) | 0.84 (0.67–1.05) | |
| Asia–Pacific | 11/130 (8.5) | 21/132 (15.9) | 0.49 (0.24–1.02) | |
| Ezetimibe use | | | | 0.64 |
| No | 649/3827 (17.0) | 834/3828 (21.8) | 0.75 (0.67–0.83) | |
| Yes | 56/262 (21.4) | 67/262 (25.6) | 0.82 (0.57–1.16) | |
| Sex | | | | 0.33 |
| Male | 551/2927 (18.8) | 715/2895 (24.7) | 0.73 (0.65–0.82) | |
| Female | 154/1162 (13.3) | 186/1195 (15.6) | 0.82 (0.66–1.01) | |
| Race | | | | 0.18 |
| White | 646/3691 (17.5) | 812/3688 (22.0) | 0.77 (0.69–0.85) | |
| Other | 59/398 (14.8) | 89/401 (22.2) | 0.60 (0.43–0.83) | |
| Age | | | | 0.004 |
| <65 yr | 322/2232 (14.4) | 460/2184 (21.1) | 0.65 (0.56–0.75) | |
| ≥65 yr | 383/1857 (20.6) | 441/1906 (23.1) | 0.87 (0.76–1.00) | |
| From the United States | | | | 0.14 |
| Yes | 281/1548 (18.2) | 394/1598 (24.7) | 0.69 (0.59–0.80) | |
| No | 424/2541 (16.7) | 507/2492 (20.3) | 0.80 (0.71–0.91) | |
| Diabetes at baseline | | | | 0.56 |
| Yes | 433/2394 (18.1) | 536/2393 (22.4) | 0.77 (0.68–0.87) | |
| No | 272/1695 (16.0) | 365/1694 (21.5) | 0.73 (0.62–0.85) | |
| Baseline estimated GFR | | | | 0.41 |
| <60 ml/min/1.73 $m^2$ | 197/905 (21.8) | 263/911 (28.9) | 0.71 (0.59–0.85) | |
| ≥60 to <90 ml/min/1.73 $m^2$ | 380/2217 (17.1) | 468/2238 (20.9) | 0.80 (0.70–0.92) | |
| ≥90 ml/min/1.73 $m^2$ | 128/963 (13.3) | 170/939 (18.1) | 0.70 (0.56–0.89) | |
| Baseline triglycerides | | | | 0.45 |
| ≥200 mg/dl | 430/2481 (17.3) | 559/2469 (22.6) | 0.73 (0.64–0.83) | |
| <200 mg/dl | 275/1605 (17.1) | 342/1620 (21.1) | 0.79 (0.67–0.93) | |
| Baseline triglycerides | | | | 0.83 |
| ≥150 mg/dl | 640/3674 (17.4) | 811/3660 (22.2) | 0.75 (0.68–0.83) | |
| <150 mg/dl | 65/412 (15.8) | 90/429 (21.0) | 0.79 (0.57–1.09) | |
| Baseline triglycerides ≥200 mg/dl and HDL cholesterol ≤35 mg/dl | | | | 0.04 |
| Yes | 149/823 (18.1) | 214/794 (27.0) | 0.62 (0.51–0.77) | |
| No | 554/3258 (17.0) | 687/3293 (20.9) | 0.79 (0.71–0.88) | |
| Baseline statin intensity | | | | 0.12 |
| High | 232/1290 (18.0) | 310/1226 (25.3) | 0.69 (0.58–0.82) | |
| Moderate | 424/2533 (16.7) | 543/2575 (21.1) | 0.76 (0.67–0.86) | |
| Low | 48/254 (18.9) | 45/267 (16.9) | 1.12 (0.74–1.69) | |
| Baseline LDL cholesterol (derived) in thirds | | | | 0.62 |
| ≤67 mg/dl | 244/1481 (16.5) | 302/1386 (21.8) | 0.72 (0.61–0.85) | |
| >67 to ≤84 mg/dl | 248/1347 (18.4) | 307/1364 (22.5) | 0.81 (0.68–0.96) | |
| >84 mg/dl | 213/1258 (16.9) | 292/1339 (21.8) | 0.74 (0.62–0.89) | |
| Baseline high-sensitivity CRP | | | | 0.07 |
| ≤2 mg/liter | 288/1919 (15.0) | 407/1942 (21.0) | 0.68 (0.58–0.79) | |
| >2 mg/liter | 417/2167 (19.2) | 494/2147 (23.0) | 0.81 (0.71–0.93) | |

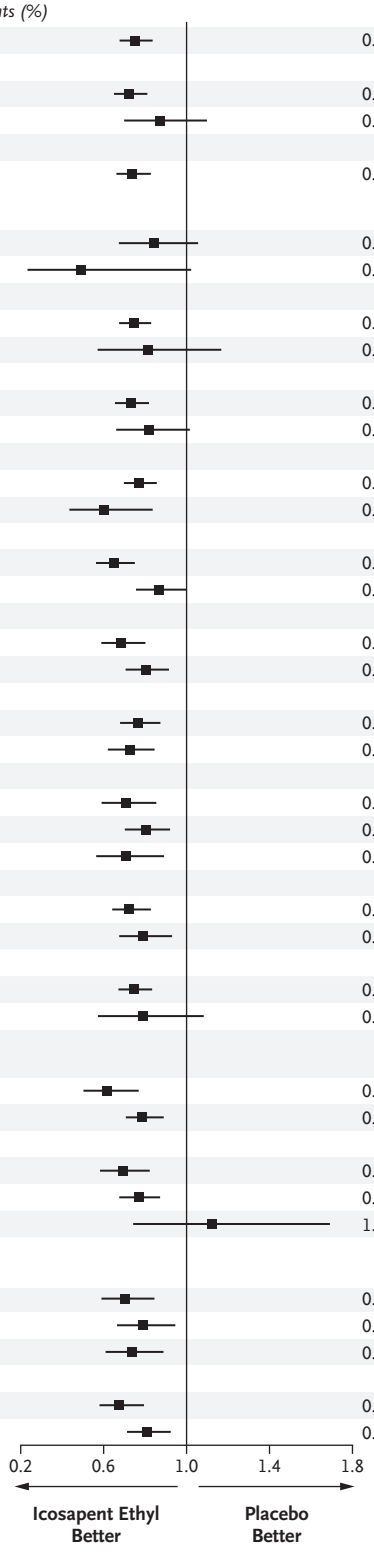

**Figure 2 (facing page). Primary Efficacy Composite End Point in Selected Prespecified Subgroups.**

Shown are the hazard ratios and 95% confidence intervals for the primary efficacy composite end point of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina, as assessed in a time-to-event analysis, in selected prespecified subgroups of the intention-to-treat population (all patients who underwent randomization). The confidence intervals shown for the subgroup analyses have not been adjusted for multiple testing, and inferences drawn from the intervals may not be reproducible. Race was reported by the investigators. Eastern European region includes Poland, Romania, Russia, and Ukraine, and Asia–Pacific region includes India. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. CRP denotes C-reactive protein, GFR glomerular filtration rate, HDL high-density lipoprotein, and LDL low-density lipoprotein. The LDL cholesterol value obtained by means of preparative ultracentrifugation was used. If the preparative ultracentrifugation value was missing, the LDL cholesterol value measured by another method was used in the following order of priority: the nonmissing value obtained by means of direct measurements of LDL cholesterol, the value derived with the use of the Friedewald equation, and the value derived with the use of the calculation published by Johns Hopkins University investigators.[22]

95% CI, 0.65 to 0.83; P<0.001), corresponding to an absolute between-group difference of 3.6 percentage points (95% CI, 2.1 to 5.0); the number needed to treat to avoid one key secondary endpoint event was 28 (95% CI, 20 to 47) over a median follow-up 4.9 years.[23,24]

The rates of the primary and key secondary efficacy end points in selected prespecified subgroups are provided in Figures 2 and 3; the findings show a consistent benefit with icosapent ethyl. Baseline triglyceride levels (≥150 vs. <150 mg per deciliter or ≥200 or <200 mg per deciliter) had no influence on the primary or key secondary efficacy end points (Figs. 2 and 3). The attainment of triglyceride levels of 150 mg per deciliter or higher or below 150 mg per deciliter at 1 year after randomization also had no influence on the efficacy of icosapent ethyl as compared with placebo with respect to the primary or key secondary efficacy end point (Fig. S4 in the Supplementary Appendix). In a post hoc analysis, we found no substantial difference in the benefit of icosapent ethyl as compared with placebo with respect to the primary end point according to whether the patients who received placebo had an increase in LDL cholesterol levels at 1 year or had no change or a decrease in LDL cholesterol levels.

In the prespecified hierarchical testing of end points (Fig. 4), the rates of all individual and composite ischemic end points (except for death from any cause — the last secondary end point in the hierarchy) were significantly lower in the icosapent ethyl group than in the placebo group, including the rate of cardiovascular death (4.3% vs. 5.2%; hazard ratio, 0.80; 95% CI, 0.66 to 0.98; P=0.03). The rate of death from any cause was 6.7% in the icosapent ethyl group and 7.6% in the placebo group (hazard ratio, 0.87; 95% CI, 0.74 to 1.02). The results for selected prespecified tertiary end points, which were not adjusted for multiple comparisons, are provided in Table S3 in the Supplementary Appendix. Among these results, the rates of adjudicated sudden cardiac death were 1.5% in the icosapent ethyl group and 2.1% in the placebo group (hazard ratio, 0.69; 95% CI, 0.50 to 0.96), and the rates of cardiac arrest were 0.5% and 1.0%, respectively (hazard ratio, 0.52; 95% CI, 0.31 to 0.86).

### SAFETY AND ADVERSE EVENTS

The overall rates of adverse events that occurred while the patients were in the trial and the rates of serious adverse events leading to discontinuation of the trial drug or placebo did not differ significantly between the trial groups (Table S5 in the Supplementary Appendix). The only serious adverse event that occurred at a frequency of at least 2% was pneumonia (2.6% in the icosapent ethyl group and 2.9% in the placebo group, P=0.42). Adverse events that occurred in at least 5% of patients are reported in Table S6 in the Supplementary Appendix. The rate of atrial fibrillation was significantly higher in the icosapent ethyl group than in the placebo group (5.3% vs. 3.9%), as was the rate of peripheral edema (6.5% vs. 5.0%), but the rate of anemia was significantly lower in the icosapent ethyl group than in the placebo group (4.7% vs. 5.8%), as were the rates of diarrhea (9.0% vs. 11.1%) and gastrointestinal adverse events (33.0% vs. 35.1%) (Table S7 in the Supplementary Appendix). The rate of the prespecified adjudicated tertiary end point of heart failure did not differ significantly between the icosapent ethyl group and the placebo group (4.1% and 4.3%, respectively). The rate of the

| Subgroup | Icosapent Ethyl | Placebo | Hazard Ratio (95% CI) | P Value for Interaction |
|---|---|---|---|---|
| | *no. of patients with event/total no. of patients (%)* | | | |
| All patients | 459/4089 (11.2) | 606/4090 (14.8) | 0.74 (0.65–0.83) | |
| Risk stratum | | | | 0.41 |
| Secondary-prevention cohort | 361/2892 (12.5) | 489/2893 (16.9) | 0.72 (0.63–0.82) | |
| Primary-prevention cohort | 98/1197 (8.2) | 117/1197 (9.8) | 0.81 (0.62–1.06) | |
| Region | | | | 0.54 |
| United States, Canada, the Netherlands, Australia, New Zealand, and South Africa | 358/2906 (12.3) | 473/2905 (16.3) | 0.73 (0.64–0.84) | |
| Eastern European | 93/1053 (8.8) | 117/1053 (11.1) | 0.78 (0.59–1.02) | |
| Asia–Pacific | 8/130 (6.2) | 16/132 (12.1) | 0.47 (0.20–1.10) | |
| Ezetimibe use | | | | 0.46 |
| No | 426/3827 (11.1) | 569/3828 (14.9) | 0.73 (0.64–0.82) | |
| Yes | 33/262 (12.6) | 37/262 (14.1) | 0.87 (0.54–1.39) | |
| Sex | | | | 0.44 |
| Male | 353/2927 (12.1) | 474/2895 (16.4) | 0.72 (0.62–0.82) | |
| Female | 106/1162 (9.1) | 132/1195 (11.0) | 0.80 (0.62–1.03) | |
| Race | | | | 0.13 |
| White | 418/3691 (11.3) | 538/3688 (14.6) | 0.76 (0.67–0.86) | |
| Other | 41/398 (10.3) | 68/401 (17.0) | 0.55 (0.38–0.82) | |
| Age | | | | 0.06 |
| <65 yr | 200/2232 (9.0) | 290/2184 (13.3) | 0.65 (0.54–0.78) | |
| ≥65 yr | 259/1857 (13.9) | 316/1906 (16.6) | 0.82 (0.70–0.97) | |
| From the United States | | | | 0.38 |
| Yes | 187/1548 (12.1) | 266/1598 (16.6) | 0.69 (0.57–0.83) | |
| No | 272/2541 (10.7) | 340/2492 (13.6) | 0.77 (0.66–0.91) | |
| Baseline diabetes | | | | 0.29 |
| Yes | 286/2394 (11.9) | 391/2393 (16.3) | 0.70 (0.60–0.81) | |
| No | 173/1695 (10.2) | 215/1694 (12.7) | 0.80 (0.65–0.98) | |
| Baseline estimated GFR | | | | 0.77 |
| <60 ml/min/1.73 $m^2$ | 152/905 (16.8) | 205/911 (22.5) | 0.71 (0.57–0.88) | |
| ≥60 to <90 ml/min/1.73 $m^2$ | 229/2217 (10.3) | 296/2238 (13.2) | 0.77 (0.64–0.91) | |
| ≥90 ml/min/1.73 $m^2$ | 78/963 (8.1) | 105/939 (11.2) | 0.70 (0.52–0.94) | |
| Baseline triglycerides | | | | 0.62 |
| ≥200 mg/dl | 290/2481 (11.7) | 371/2469 (15.0) | 0.75 (0.65–0.88) | |
| <200 mg/dl | 169/1605 (10.5) | 235/1620 (14.5) | 0.71 (0.58–0.86) | |
| Baseline triglycerides | | | | 0.68 |
| ≥150 mg/dl | 421/3674 (11.5) | 546/3660 (14.9) | 0.74 (0.65–0.84) | |
| <150 mg/dl | 38/412 (9.2) | 60/429 (14.0) | 0.66 (0.44–0.99) | |
| Baseline triglycerides ≥200 mg/dl and HDL cholesterol ≤35 mg/dl | | | | 0.50 |
| Yes | 101/823 (12.3) | 136/794 (17.1) | 0.68 (0.53–0.88) | |
| No | 356/3258 (10.9) | 470/3293 (14.3) | 0.75 (0.65–0.86) | |
| Baseline statin intensity | | | | 0.10 |
| High | 151/1290 (11.7) | 210/1226 (17.1) | 0.66 (0.54–0.82) | |
| Moderate | 270/2533 (10.7) | 361/2575 (14.0) | 0.74 (0.63–0.87) | |
| Low | 37/254 (14.6) | 32/267 (12.0) | 1.20 (0.74–1.93) | |
| Baseline LDL cholesterol (derived) in thirds | | | | 0.97 |
| ≤67 mg/dl | 157/1481 (10.6) | 196/1386 (14.1) | 0.73 (0.59–0.90) | |
| >67 to ≤84 mg/dl | 157/1347 (11.7) | 208/1364 (15.2) | 0.75 (0.61–0.93) | |
| >84 mg/dl | 145/1258 (11.5) | 202/1339 (15.1) | 0.74 (0.60–0.91) | |
| Baseline hsCRP | | | | 0.97 |
| ≤2 mg/liter | 183/1919 (9.5) | 245/1942 (12.6) | 0.73 (0.61–0.89) | |
| >2 mg/liter | 276/2167 (12.7) | 361/2147 (16.8) | 0.73 (0.63–0.86) | |

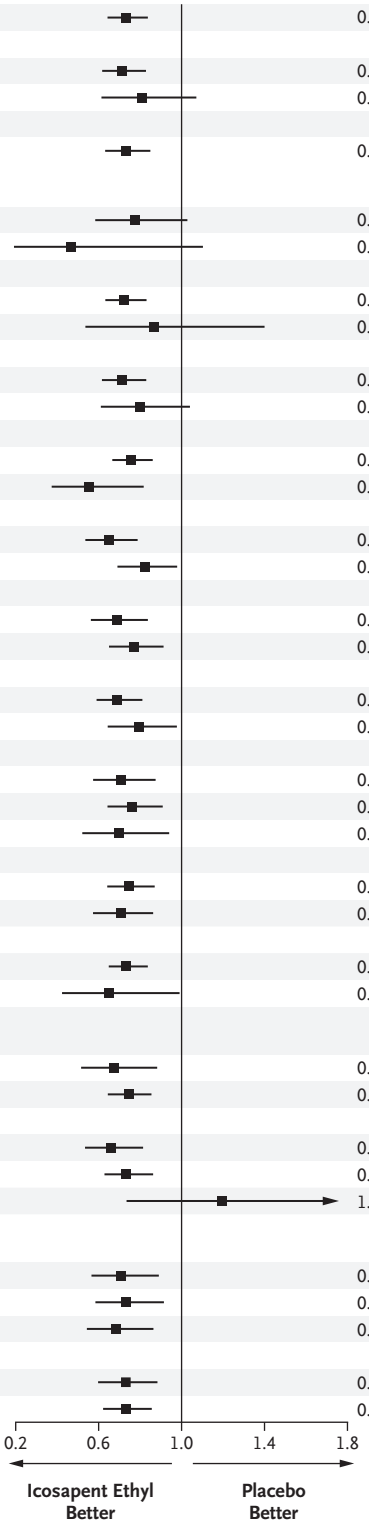

**Figure 3 (facing page). Key Secondary Efficacy Composite End Point in Selected Prespecified Subgroups.**

Shown are the hazard ratios and 95% confidence intervals for the key secondary efficacy composite end point of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke, as assessed in a time-to-event analysis, in selected prespecified subgroups of the intention-to-treat population. The confidence intervals shown for the subgroup analyses have not been adjusted for multiple testing, and inferences drawn from the intervals may not be reproducible.

prespecified adjudicated tertiary end point of hospitalization for atrial fibrillation or flutter was significantly higher in the icosapent ethyl group than in the placebo group (3.1% vs. 2.1%, P=0.004). The overall rates of serious adverse bleeding events that occurred while the patients were in the trial were 2.7% in the icosapent ethyl group and 2.1% in the placebo group (P=0.06), although there were no fatal bleeding events in either group; there were no significant differences between the icosapent ethyl group and the placebo group in the rates of adjudicated hemorrhagic stroke (0.3% vs. 0.2%, P=0.55), serious central nervous system bleeding (0.3% vs. 0.2%, P=0.42), or gastrointestinal bleeding (1.5% vs. 1.1%, P=0.15) (Table S8 in the Supplementary Appendix).

## DISCUSSION

In REDUCE-IT, the risk of the primary composite end point of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina, assessed in a time-to-event analysis, was significantly lower, by 25%, among the patients who received 2 g of icosapent ethyl twice daily than among those who received placebo, corresponding to an absolute between-group difference of 4.8 percentage points in the rate of the end point and a number needed to treat of 21. The risk of the key secondary composite end point of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke in a time-to-event analysis was also significantly lower, by 26%, in the icosapent ethyl group than in the placebo group, corresponding to an absolute between-group difference of 3.6 percentage points in the rate of the end point and a number needed to treat of 28. Prespecified hierarchical testing of other secondary end points revealed that the risks of a variety of fatal and nonfatal ischemic events were lower in the icosapent ethyl group than in the placebo group, including a 20% lower risk of cardiovascular death. The benefits were observed against a background of appropriate statin use among patients who had a median LDL cholesterol level of 75.0 mg per deciliter at baseline.

The overall rates of adverse events were similar in the trial groups. Serious adverse events related to bleeding occurred in more patients in the icosapent ethyl group than in the placebo group, although the overall rates were low; there were no fatal bleeding events in either group, and the rates of adjudicated hemorrhagic stroke, serious central nervous system bleeding, and serious gastrointestinal bleeding were not significantly higher in the icosapent ethyl group than in the placebo group. The rate of hospitalization for atrial fibrillation or flutter was significantly higher in the icosapent ethyl group than in the placebo group, although the rates were low. The rates of adverse events and serious adverse events leading to discontinuation of trial drug were similar in the two groups.

The results of REDUCE-IT stand apart from the negative findings of several contemporary trials of other agents that also lower triglyceride levels, including other n–3 fatty acids, extended-release niacin, fenofibrate, and cholesteryl ester transfer protein inhibitors.[10-13] It is not known whether the lack of benefit from n–3 fatty acids in previous trials may be attributable to the low dose or to the low ratio of EPA to docosahexaenoic acid (DHA).[12,13] Both the formulation (a highly purified and stable EPA ethyl ester) and dose (total daily dose of 4 g) used in REDUCE-IT were different from those in previous outcome trials of n–3 fatty acids. JELIS, which compared a combination of statin therapy and pure EPA with statin therapy alone, showed that the risk of ischemic events was significantly lower in the group that received the combination treatment than in the group that received statin therapy alone.[14] Although the dose of EPA administered in JELIS (1.8 g daily) was lower than the EPA-equivalent dose used in REDUCE-IT (4 g daily), it resulted in a plasma EPA level (170 μg per milliliter in a Japanese population) similar to that attained in a previous 12-week lipid study in which a total daily dose of 4 g of icosapent ethyl was used in a Western population (183 μg per milliliter)[25,26]

| End Point | Icosapent Ethyl (N=4089) | Placebo (N=4090) | Hazard Ratio (95% CI) | P Value for Interaction |
|---|---|---|---|---|
| | *no. of patients with event (%)* | | | |
| Primary composite | 705 (17.2) | 901 (22.0) | 0.75 (0.68–0.83) | <0.001 |
| Key secondary composite | 459 (11.2) | 606 (14.8) | 0.74 (0.65–0.83) | <0.001 |
| Cardiovascular death or nonfatal myocardial infarction | 392 (9.6) | 507 (12.4) | 0.75 (0.66–0.86) | <0.001 |
| Fatal or nonfatal myocardial infarction | 250 (6.1) | 355 (8.7) | 0.69 (0.58–0.81) | <0.001 |
| Urgent or emergency revascularization | 216 (5.3) | 321 (7.8) | 0.65 (0.55–0.78) | <0.001 |
| Cardiovascular death | 174 (4.3) | 213 (5.2) | 0.80 (0.66–0.98) | 0.03 |
| Hospitalization for unstable angina | 108 (2.6) | 157 (3.8) | 0.68 (0.53–0.87) | 0.002 |
| Fatal or nonfatal stroke | 98 (2.4) | 134 (3.3) | 0.72 (0.55–0.93) | 0.01 |
| Death from any cause, nonfatal myocardial infarction, or nonfatal stroke | 549 (13.4) | 690 (16.9) | 0.77 (0.69–0.86) | <0.001 |
| Death from any cause | 274 (6.7) | 310 (7.6) | 0.87 (0.74–1.02) | — |

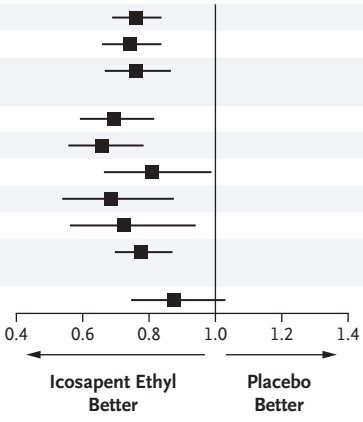


**Figure 4. Hierarchical Testing of End Points.**

Shown is the prespecified plan for hierarchical testing of end points. The rates of all end points up to death from any cause were significantly lower in the icosapent ethyl group than in the placebo group.

and similar to that attained in the current trial. However, unlike the current trial, JELIS included an open-label design without a placebo group, used a low-intensity statin, and was conducted in a single country; patients also had higher levels of LDL cholesterol at baseline (182 mg per deciliter [4.71 mmol per liter] before initiation of statin therapy) and lower baseline triglyceride values (151 mg per deciliter [1.70 mmol per liter]) than the patients in REDUCE-IT.

Metabolic data provide evidence that icosapent ethyl–based formulations do not raise LDL cholesterol levels, whereas DHA-based formulations do.[27] The results of the current trial should not be generalized to other n–3 fatty acid preparations — in particular, dietary-supplement preparations of n–3 fatty acid mixtures, which are variable and unregulated and which have not been shown to have clinical benefit.

A triglyceride level of 150 mg per deciliter or higher was an initial inclusion criterion in REDUCE-IT (although the required level was subsequently changed to ≥200 mg per deciliter); however, owing to allowance for variability in these levels, 10.3% of enrolled patients had triglyceride levels lower than 150 mg per deciliter at baseline. The observed cardiovascular benefits were similar across baseline levels of triglycerides (<150, ≥150 to <200, and ≥200 mg per deciliter). In addition, the significantly lower risk of major adverse cardiovascular events with icosapent ethyl than with placebo appeared to occur irrespective of the attained triglyceride level at 1 year (≥150 or <150 mg per deciliter), which suggests that the cardiovascular risk reduction was not associated with attainment of a more normal triglyceride level. These observations suggest that at least some of the effect of icosapent ethyl that resulted in a lower risk of ischemic events than that with placebo may be explained by metabolic effects other than a reduction of triglyceride levels.[28]

Mechanisms responsible for the benefit of icosapent ethyl observed in REDUCE-IT are currently not known. The timing of the divergence of the Kaplan–Meier event curves suggests a delayed onset of benefit, which may reflect the time that is needed for a benefit from a reduction in triglyceride levels to be realized or may indicate that other mechanisms are involved. The modestly higher rate of bleeding events with icosapent ethyl suggests that there may be an antithrombotic mechanism of action. However, it is unlikely that an antithrombotic effect would reduce the rate of elective revascularization. Also, if the full explanation involved an antiplatelet or anticoagulant effect, one might expect a large increase in the rate of major bleeding events, which was not observed.[29] It is possible that membrane-stabilizing effects could explain part of the ben-

efit.[20,21,30] Stabilization or regression of coronary plaque (or both) may also play a part.[19,31] Our observation of lower rates of cardiac arrest and sudden cardiac death with icosapent ethyl than with placebo in the current trial might support that mechanism, although these findings should be viewed as exploratory. It is also possible that the difference in high-sensitivity C-reactive protein level observed in REDUCE-IT may contribute to the benefit; the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) showed a significant reduction in the risk of ischemic events with treatment targeted at inflammation.[32-35] Blood samples obtained during REDUCE-IT have been banked for biomarker and genetic analyses that may provide more information regarding mechanisms of action.

Ongoing trials of moderate-to-high doses of pure EPA ethyl ester will provide further information on the effects of these agents.[10,36] These trials include the Randomized Trial for Evaluation in Secondary Prevention Efficacy of Combination Therapy–Statin and EPA (RESPECT-EPA; UMIN Clinical Trials Registry number, UMIN000012069), a secondary prevention outcomes trial involving statin-treated patients in Japan, and the Effect of Vascepa on Improving Coronary Atherosclerosis in People with High Triglycerides Taking Statin Therapy (EVAPORATE; ClinicalTrials.gov number, NCT02926027), which is examining changes in coronary plaque over 9 to 18 months.

Our trial has certain limitations. First, at the time the trial was designed, there was relatively little use of ezetimibe or data supporting its use.[37] However, subgroup analyses do not suggest a differential benefit for patients taking ezetimibe. Similarly, proprotein convertase subtilisin–kexin type 9 (PCSK9) inhibitors were not available for the majority of the patients in the trial.[38] Second, if mineral oil in the placebo affected statin absorption in some patients, this might have contributed to differences in outcomes between the groups. However, the relatively small differences in LDL cholesterol levels between the groups would not be likely to explain the 25% lower risk observed with icosapent ethyl, and a post hoc analysis suggested a similar lower risk regardless of whether there was an increase in LDL cholesterol level among the patients in the placebo group. Although JELIS was designed as an open-label study that did not use a mineral oil placebo, it showed a 19% lower risk of ischemic events with statin therapy plus EPA than with statin therapy alone.

In conclusion, among patients with elevated triglyceride levels who were receiving statin therapy, the risk of major ischemic events, including cardiovascular death, was significantly lower with 2 g of icosapent ethyl twice daily (total daily dose, 4 g) than with placebo.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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# Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome

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## ABSTRACT

### BACKGROUND

Patients who have had an acute coronary syndrome are at high risk for recurrent ischemic cardiovascular events. We sought to determine whether alirocumab, a human monoclonal antibody to proprotein convertase subtilisin–kexin type 9 (PCSK9), would improve cardiovascular outcomes after an acute coronary syndrome in patients receiving high-intensity statin therapy.

### METHODS

We conducted a multicenter, randomized, double-blind, placebo-controlled trial involving 18,924 patients who had an acute coronary syndrome 1 to 12 months earlier, had a low-density lipoprotein (LDL) cholesterol level of at least 70 mg per deciliter (1.8 mmol per liter), a non–high-density lipoprotein cholesterol level of at least 100 mg per deciliter (2.6 mmol per liter), or an apolipoprotein B level of at least 80 mg per deciliter, and were receiving statin therapy at a high-intensity dose or at the maximum tolerated dose. Patients were randomly assigned to receive alirocumab subcutaneously at a dose of 75 mg (9462 patients) or matching placebo (9462 patients) every 2 weeks. The dose of alirocumab was adjusted under blinded conditions to target an LDL cholesterol level of 25 to 50 mg per deciliter (0.6 to 1.3 mmol per liter). The primary end point was a composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization.

### RESULTS

The median duration of follow-up was 2.8 years. A composite primary end-point event occurred in 903 patients (9.5%) in the alirocumab group and in 1052 patients (11.1%) in the placebo group (hazard ratio, 0.85; 95% confidence interval [CI], 0.78 to 0.93; P<0.001). A total of 334 patients (3.5%) in the alirocumab group and 392 patients (4.1%) in the placebo group died (hazard ratio, 0.85; 95% CI, 0.73 to 0.98). The absolute benefit of alirocumab with respect to the composite primary end point was greater among patients who had a baseline LDL cholesterol level of 100 mg or more per deciliter than among patients who had a lower baseline level. The incidence of adverse events was similar in the two groups, with the exception of local injection-site reactions (3.8% in the alirocumab group vs. 2.1% in the placebo group).

### CONCLUSIONS

Among patients who had a previous acute coronary syndrome and who were receiving high-intensity statin therapy, the risk of recurrent ischemic cardiovascular events was lower among those who received alirocumab than among those who received placebo. (Funded by Sanofi and Regeneron Pharmaceuticals; ODYSSEY OUTCOMES ClinicalTrials.gov number, NCT01663402.)

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Drs. Schwartz and Steg contributed equally to this article.

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Despite the availability of current evidence-based treatments, patients who have had an acute coronary syndrome remain at high risk for recurrent ischemic cardiovascular events.[1,2] This residual risk is attributable in part to elevated levels of low-density lipoprotein (LDL) cholesterol and other atherogenic lipoproteins. Previous clinical trials have shown that the risk is lower among patients who receive statin therapy to lower the LDL cholesterol level than among those who receive placebo,[3] among patients who receive high-intensity statins than among those who receive moderate-intensity statins,[4] and among patients who receive ezetimibe added to statin therapy than among those who receive a statin alone.[5]

Proprotein convertase subtilisin–kexin type 9 (PCSK9) promotes degradation of LDL receptors, thereby diminishing the clearance of LDL from the circulation.[6] Studies have shown that mutations conveying gain or loss of function of PCSK9 result in a higher or lower level of LDL cholesterol, respectively, which in turn is associated with a corresponding higher[7] or lower[6] risk of incident coronary heart disease. These findings have led to the development of monoclonal antibodies to PCSK9 that produce substantial reductions in LDL cholesterol when administered alone or with a statin.[8-12] Two of these agents were reported to reduce the risk of ischemic cardiovascular events in patients who had stable atherosclerotic disease or high cardiovascular risk and an elevated level of atherogenic lipoproteins despite statin treatment,[11,13] with one agent showing benefit only among patients who had a baseline LDL cholesterol level of at least 100 mg per deciliter (2.6 mmol per liter).[12]

To date, the potential for a PCSK9 antibody to reduce cardiovascular risk after an acute coronary syndrome remains undetermined. In the ODYSSEY OUTCOMES trial, we tested the hypothesis that treatment with alirocumab, a fully human monoclonal antibody to PCSK9,[13-15] would result in a lower risk of recurrent ischemic cardiovascular events than placebo among patients who had an acute coronary syndrome within the preceding 1 to 12 months and who have levels of atherogenic lipoproteins that exceed specified thresholds despite statin therapy at a high-intensity dose or at the maximum tolerated dose.

## Methods

### Trial Organization and Oversight

Details of the trial design have been reported previously.[14] ODYSSEY OUTCOMES was a multicenter, randomized, double-blind, placebo-controlled trial that was sponsored by Sanofi and Regeneron Pharmaceuticals. The protocol and statistical analysis plan (available with the full text of this article at NEJM.org) were conceived by the first three authors, developed in conjunction with the other members of the executive steering committee and sponsors, and approved by the responsible regulatory authorities and ethics committees. The sponsors participated in the selection of the trial sites, the monitoring of the trial, and the supervision of data collection. Duke Clinical Research Institute led the blinded adjudication of the end points. An independent data and safety monitoring committee monitored the safety and efficacy data. Analyses were performed independently by the academic statistician (the third author) in parallel with the sponsors. The manuscript was prepared by the first author with input from all the authors. The members of the executive steering committee made the decision to submit the manuscript for publication and vouch for the completeness and accuracy of the data and for the fidelity of the trial to the protocol.

### Trial Population

Patients were eligible for enrollment in the trial if they were 40 years of age or older, had been hospitalized with an acute coronary syndrome (myocardial infarction or unstable angina) 1 to 12 months before randomization, and had an LDL cholesterol level of at least 70 mg per deciliter (1.8 mmol per liter), a non–high-density lipoprotein (HDL) cholesterol level of at least 100 mg per deciliter, or an apolipoprotein B level of at least 80 mg per deciliter. All qualifying lipid levels were measured after a minimum of 2 weeks of stable treatment with atorvastatin at a dose of 40 to 80 mg once daily, rosuvastatin at a dose of 20 to 40 mg once daily, or the maximum tolerated dose of one of these statins (including no statin in the case of documented unacceptable side effects). Full trial enrollment criteria are provided in the Supplementary Appendix, available

at NEJM.org. All the patients provided written informed consent.

### TRIAL PROCEDURES

During a prerandomization run-in phase (described in the Supplementary Appendix), patients received instruction in injecting themselves (with placebo), and lipid levels were verified for patient eligibility. Patients who met trial entry criteria were randomly assigned, in a 1:1 ratio, to receive alirocumab at a dose of 75 mg or matching placebo; randomization was stratified according to country (Table S1 in the Supplementary Appendix). All doses of alirocumab or placebo were administered by subcutaneous injection every 2 weeks.

The trial-group assignments and lipid levels during the trial were concealed from the patients and investigators. LDL cholesterol levels were calculated with the use of the Friedewald formula unless the triglyceride level exceeded 400 mg per deciliter (4.52 mmol per liter) or the calculated LDL cholesterol level was found to be less than 15 mg per deciliter (0.39 mmol per liter), in which case values were determined by beta quantification. Among patients assigned to the alirocumab group, protocol-specified dose-adjustment algorithms[14] were used to target an LDL cholesterol level of 25 to 50 mg per deciliter (0.6 to 1.3 mmol per liter) and to avoid sustained levels below 15 mg per deciliter (details can be found in the Additional Information on the Methods and Results section and in Figs. S1 and S2 in the Supplementary Appendix). Dose adjustments were performed under blinded conditions, without either the patient or the investigator being aware of the adjustment, including substitution of placebo for alirocumab in the case of sustained levels of LDL cholesterol below 15 mg per deciliter.

### TRIAL END POINTS

The primary end point was a composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization. Prespecified main secondary end points were any coronary heart disease event (death from coronary heart disease, nonfatal myocardial infarction, unstable angina requiring hospitalization, or an ischemia-driven coronary revascularization procedure); major coronary heart disease event (death from coronary heart disease or nonfatal myocardial infarction); any cardiovascular event (death from cardiovascular causes, nonfatal ischemic stroke, nonfatal myocardial infarction, unstable angina requiring hospitalization, or an ischemia-driven coronary revascularization procedure); a composite of death from any cause, nonfatal myocardial infarction, or nonfatal ischemic stroke; death from coronary heart disease; death from cardiovascular causes; and death from any cause. Individual components of the primary end point, an ischemia-driven coronary revascularization procedure, and hospitalization for congestive heart failure were additional secondary end points. All primary and secondary end points were adjudicated by physicians who were unaware of the trial-group assignments.

### STATISTICAL ANALYSIS

Efficacy was determined by the time to the first occurrence of any component of the composite primary end point; analyses were performed according to the intention-to-treat principle and included data from all patients and for all events that occurred from the time of randomization to the common trial end date. Design assumptions included an incidence of the composite primary end point of 11.4% at 4 years in the placebo group and a median baseline LDL cholesterol level of 90 mg per deciliter (2.3 mmol per liter), with an anticipated 50% lower LDL cholesterol level in the alirocumab group than in the placebo group, which would result in an expected 15% lower risk of the primary end point with alirocumab than with placebo. It was estimated that 1613 composite primary end-point events occurring in 18,000 patients over a median follow-up of approximately 3 years would provide the trial with 90% power to detect the expected difference in risk at a significance level of 0.05. In China, 614 patients underwent randomization after random assignment of the main trial cohort had been completed (as described in the Supplementary Appendix). The protocol specified that the trial was to continue until at least 1613 primary end-point events had occurred and all patients who could be evaluated were followed for at least 2 years (except the patients from

China), which would ensure a sufficient observation time in which to assess safety and efficacy. Patients from China were not followed for 2 years because a lengthy regulatory approval process delayed their random assignment to a trial group until after completion of the randomization process for the rest of the trial cohort.

LDL cholesterol was evaluated in an intention-to-treat analysis that included levels measured after premature discontinuation of the trial regimen, levels measured after dose adjustments were made under blinded conditions, and levels measured after blinded substitution of placebo for alirocumab. LDL cholesterol was also evaluated in the alirocumab group in an on-treatment analysis that excluded levels measured after premature discontinuation of alirocumab and levels measured after blinded substitution of placebo for alirocumab but included levels measured after dose adjustments of alirocumab between the 75-mg dose and the 150-mg dose were made under blinded conditions.

Hazard ratios and 95% confidence intervals were estimated with the use of a Cox proportional-hazards model, stratified according to geographic region; P values were determined with the use of stratified log-rank tests. To adjust for multiplicity, the results of the main secondary end points were to be tested in hierarchical fashion in the sequence listed above if the risk of the composite primary end point was found to be significantly lower in the alirocumab group than in the placebo group. Two prespecified interim analyses were performed when approximately 50% and 75% of the planned primary end-point events for the final analysis had occurred; neither led to early termination of the trial. To account for the two interim analyses, a two-sided P value of less than 0.0498 was required to declare statistical significance for the primary end point at the final analysis. Absolute treatment effects in prespecified subgroups were compared with the use of the Gail–Simon test.[15] The statistical analysis plan and the Supplementary Appendix provide details of the descriptive safety analyses and analytical methods.

## RESULTS

### PATIENTS, TRIAL REGIMEN, AND FOLLOW-UP

A total of 18,924 patients underwent randomization at 1315 sites in 57 countries; 9462 were assigned to the alirocumab group and 9462 to the placebo group (Fig. S3 in the Supplementary Appendix). Except in China, patients underwent randomization from November 2012 through November 2015. In China, 613 patients underwent randomization from May 2016 through February 2017. At the time of randomization, the characteristics of the two trial groups were well balanced (Table 1, and Table S2 in the Supplementary Appendix). The qualifying acute coronary syndrome was myocardial infarction in 83.0% of the patients and unstable angina in 16.8%. Most of the patients (92.1%) qualified with an LDL cholesterol level of 70 mg or more per deciliter; a majority of the remaining patients (7.2%) met only the non–HDL cholesterol criterion. The median time from the qualifying acute coronary syndrome to randomization was 2.6 months (interquartile range, 1.7 to 4.3).

Most of the patients received guideline-recommended medications and had undergone coronary revascularization for the index event. At the time of randomization, 88.8% of the patients were receiving atorvastatin at a dose of 40 mg to 80 mg daily or were receiving rosuvastatin at a dose of 20 mg to 40 mg daily. After 1 year of follow-up, 84.7% of the patients in the alirocumab group and 86.2% in the placebo group were receiving such treatment; after 3 years of follow-up, the percentages were 82.8% in the alirocumab group and 86.6% in the placebo group. Information on the adjustment of alirocumab doses under blinded conditions can be found in the Supplementary Appendix.

Patients were followed for a median of 2.8 years (interquartile range, 2.3 to 3.4); the common trial end date was November 11, 2017. Premature discontinuation of the assigned alirocumab or placebo for reasons other than death occurred in 1343 patients (14.2%) in the alirocumab group and in 1496 patients (15.8%) in the placebo group (Fig. S3 in the Supplementary Appendix). Exposure to the intended trial regimen as a percentage of the total follow-up time

**Table 1. Demographic and Baseline Characteristics of the Patients.***

| Characteristic | Alirocumab (N = 9462) | Placebo (N = 9462) |
|---|---|---|
| Age — yr | 58.5±9.3 | 58.6±9.4 |
| Female sex — no. (%) | 2390 (25.3) | 2372 (25.1) |
| Race — no. (%)† | | |
| White | 7500 (79.3) | 7524 (79.5) |
| Asian | 1251 (13.2) | 1247 (13.2) |
| Black | 235 (2.5) | 238 (2.5) |
| Other | 475 (5.0) | 451 (4.8) |
| Region of enrollment — no. (%) | | |
| Central and Eastern Europe | 2719 (28.7) | 2718 (28.7) |
| Western Europe | 2084 (22.0) | 2091 (22.1) |
| Canada or United States | 1435 (15.2) | 1436 (15.2) |
| Latin America | 1293 (13.7) | 1295 (13.7) |
| Asia | 1150 (12.2) | 1143 (12.1) |
| Rest of world | 781 (8.3) | 779 (8.2) |
| Medical history before index acute coronary syndrome — no. (%) | | |
| Hypertension | 6205 (65.6) | 6044 (63.9) |
| Diabetes mellitus | 2693 (28.5) | 2751 (29.1) |
| Current tobacco smoker | 2282 (24.1) | 2278 (24.1) |
| Family history of premature coronary heart disease | 3408 (36.0) | 3365 (35.6) |
| Myocardial infarction | 1790 (18.9) | 1843 (19.5) |
| Percutaneous coronary intervention | 1626 (17.2) | 1615 (17.1) |
| Coronary-artery bypass grafting | 521 (5.5) | 526 (5.6) |
| Stroke | 306 (3.2) | 305 (3.2) |
| Peripheral artery disease | 373 (3.9) | 386 (4.1) |
| Congestive heart failure | 1365 (14.4) | 1449 (15.3) |
| Index acute coronary syndrome — no. (%) | | |
| ST-segment elevation myocardial infarction | 3301 (34.9) | 3235 (34.2) |
| Non–ST-segment elevation myocardial infarction | 4574 (48.3) | 4601 (48.6) |
| Unstable angina | 1568 (16.6) | 1614 (17.1) |
| Missing data | 19 (<0.1) | 12 (<0.1) |
| Percutaneous coronary intervention or coronary-artery bypass grafting for index acute coronary syndrome — no. (%) | 6798 (71.8) | 6878 (72.7) |
| Median time from index acute coronary syndrome to randomization (IQR) — mo | 2.6 (1.7–4.4) | 2.6 (1.7–4.3) |
| Body-mass index‡ | 28.5±4.9 | 28.5±4.8 |

* Plus–minus values are means ±SD. There were no significant differences between the two groups in demographic or baseline characteristics. Additional baseline characteristics are listed in Table S2 in the Supplementary Appendix. Percentages may not sum to 100 because of rounding. IQR denotes interquartile range.
† Race was reported by the patient.
‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

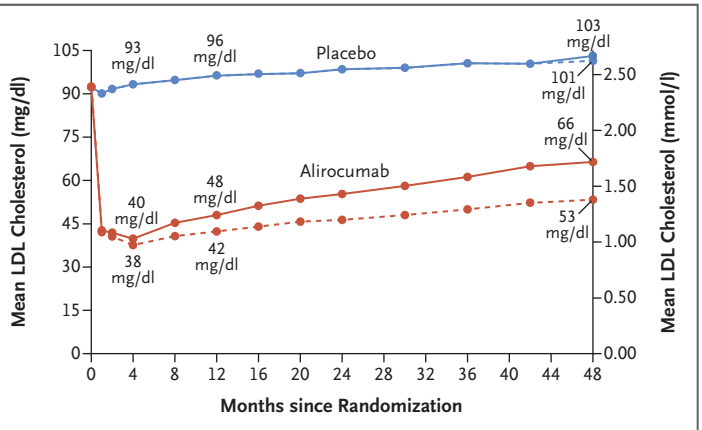


**Figure 1. LDL Cholesterol Levels during the Trial.**

The intention-to-treat analysis (results shown with solid lines) included all low-density lipoprotein (LDL) cholesterol values, including levels measured after premature discontinuation of the trial regimen, levels measured after dose adjustments were made under blinded conditions, and levels measured after blinded substitution of placebo for alirocumab. The on-treatment analysis (results shown with dashed lines) excluded LDL cholesterol levels measured after premature discontinuation of the trial regimen and levels measured after blinded substitution of placebo for alirocumab (but included LDL cholesterol levels measured after dose adjustments of alirocumab were made under blinded conditions between the 75-mg dose and the 150-mg dose). To convert the values for LDL cholesterol to millimoles per liter, multiply by 0.02586.

was 90.7% in the alirocumab group (including time after blinded substitution of placebo for alirocumab) and 90.0% in the placebo group. Ascertainment of the composite primary end point was complete for 99.1% of potential patient-years of follow-up, and ascertainment of death was complete for 99.8% of potential patient-years of follow-up.

### EFFECT OF TRIAL REGIMEN ON LIPID LEVELS

At baseline, the mean (±SD) LDL cholesterol level was 92±31 mg per deciliter (2.38±0.80 mmol per liter). In the alirocumab group, the mean LDL cholesterol level (intention-to-treat analysis) at 4 months, 12 months, and 48 months after randomization was 40 mg per deciliter (1.0 mmol per liter), 48 mg per deciliter (1.2 mmol per liter), and 66 mg per deciliter (1.7 mmol per liter), respectively; in the placebo group, the mean LDL cholesterol level at 4 months, 12 months, and 48 months after randomization was 93 mg per deciliter (2.4 mmol per liter), 96 mg per deciliter (2.5 mmol per liter), and 103 mg per deciliter (2.7 mmol per liter), respectively (Fig. 1). In the on-treatment analysis in the alirocumab group (which excluded values measured after discontinuation of alirocumab and after blinded substitution of placebo for alirocumab), the mean LDL cholesterol level at 4 months, 12 months, and 48 months was 38 mg per deciliter (0.98 mmol per liter), 42 mg per deciliter (1.1 mmol per liter), and 53 mg per deciliter (1.4 mmol per liter), respectively; these levels were an average of 62.7%, 61.0%, and 54.7% lower than the respective levels in the placebo group. Other lipid measurements are provided in Figure S4 in the Supplementary Appendix.

### END POINTS

A composite primary end-point event occurred in 903 patients (9.5%) in the alirocumab group and in 1052 patients (11.1%) in the placebo group (Table 2); The Kaplan–Meier probability estimate at 4 years was 12.5% in the alirocumab group and 14.5% in the placebo group (hazard ratio, 0.85; 95% confidence interval [CI], 0.78 to 0.93; P<0.001) (Fig. 2). To prevent the occurrence of one primary end-point event, 49 patients (95% CI, 28 to 164) would need to be treated for 4 years. The effect of alirocumab on the relative risk of the composite primary end point did not differ significantly according to any of the prespecified subgroup variables (Fig. S5 in the Supplementary Appendix).

As would be expected, the incidence of the composite primary end point in the placebo group differed across three categories of baseline LDL cholesterol levels (<80, 80 to <100, and ≥100 mg per deciliter), with the greatest incidence among patients in the highest category. Correspondingly, in a nonprespecified analysis, the greatest absolute reduction in risk of the composite primary end point with alirocumab was also shown among the patients who had a baseline LDL cholesterol level of 100 mg or more per deciliter (P<0.001 for the interaction between treatment and baseline LDL cholesterol level) (Table S3 and Fig. S6 in the Supplementary Appendix). To prevent the occurrence of one primary end-point event among patients with a baseline LDL cholesterol level of 100 mg or more per deciliter, 16 patients (95% CI, 11 to 34) would need to be treated for 4 years. Additional analyses related to categories of baseline LDL cholesterol are

**Table 2. Composite Primary End Point and Secondary End Points (Intention-to-Treat Population).**

| End Point | Alirocumab (N=9462) | Placebo (N=9462) | Hazard Ratio (95% CI) | P Value |
|---|---|---|---|---|
| | *number of patients (percent)* | | | |
| Primary end point: composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization | 903 (9.5) | 1052 (11.1) | 0.85 (0.78–0.93) | <0.001 |
| Major secondary end points, in order of hierarchical testing | | | | |
| Any coronary heart disease event* | 1199 (12.7) | 1349 (14.3) | 0.88 (0.81–0.95) | 0.001 |
| Major coronary heart disease event† | 793 (8.4) | 899 (9.5) | 0.88 (0.80–0.96) | 0.006 |
| Any cardiovascular event‡ | 1301 (13.7) | 1474 (15.6) | 0.87 (0.81–0.94) | <0.001 |
| Composite of death from any cause, nonfatal myocardial infarction, or nonfatal ischemic stroke§ | 973 (10.3) | 1126 (11.9) | 0.86 (0.79–0.93) | <0.001 |
| Death from coronary heart disease | 205 (2.2) | 222 (2.3) | 0.92 (0.76–1.11) | 0.38¶ |
| Death from cardiovascular causes | 240 (2.5) | 271 (2.9) | 0.88 (0.74–1.05) | |
| Death from any cause | 334 (3.5) | 392 (4.1) | 0.85 (0.73–0.98) | |
| Other end points‖ | | | | |
| Nonfatal myocardial infarction | 626 (6.6) | 722 (7.6) | 0.86 (0.77–0.96) | |
| Fatal or nonfatal ischemic stroke | 111 (1.2) | 152 (1.6) | 0.73 (0.57–0.93) | |
| Unstable angina requiring hospitalization | 37 (0.4) | 60 (0.6) | 0.61 (0.41–0.92) | |
| Ischemia-driven coronary revascularization procedure | 731 (7.7) | 828 (8.8) | 0.88 (0.79–0.97) | |
| Hospitalization for congestive heart failure | 176 (1.9) | 179 (1.9) | 0.98 (0.79–1.20) | |

* This end point includes death from coronary heart disease, nonfatal myocardial infarction, unstable angina requiring hospitalization, and an ischemia-driven coronary revascularization procedure (definitions can be found in the Supplementary Appendix).
† This end point includes death from coronary heart disease and nonfatal myocardial infarction.
‡ This end point includes any death from cardiovascular causes, nonfatal myocardial infarction, unstable angina requiring hospitalization, an ischemia-driven coronary revascularization procedure, or nonfatal ischemic stroke.
§ The widths of the confidence intervals for the secondary end points were not adjusted for multiplicity, so the intervals for the outcomes listed below this outcome should not be used to infer definitive treatment effects.
¶ The hierarchical analysis was stopped after the first nonsignificant P value was observed, in accordance with the hierarchical testing plan.
‖ The analysis for other end points was not adjusted for multiplicity; therefore, no P values are reported.

provided in Table S3 in the Supplementary Appendix.

Among the main secondary end points, the risks of any coronary heart disease event, major coronary heart disease events, any cardiovascular event, and a composite of death from any cause, nonfatal myocardial infarction, or nonfatal ischemic stroke were lower among patients treated with alirocumab than among those who received placebo (Table 2, and Fig. S7 in the Supplementary Appendix). A total of 334 patients (3.5%) in the alirocumab group and 392 patients (4.1%) in the placebo group died (hazard ratio, 0.85; 95% CI, 0.73 to 0.98).

### SAFETY

The incidence of adverse events and of laboratory abnormalities was similar in the alirocumab group and the placebo group (Table 3), with the exception of local injection-site reaction (3.8% in the alirocumab group vs. 2.1% in the placebo group, P<0.001). Injection-site reactions (itching, redness, or swelling) were usually mild and self-limited and led to discontinuation of the trial regimen in 26 patients in the alirocumab group, at a median of 8.3 months after randomization, and in 3 patients in the placebo group. Neurocognitive events were reported in 1.5% of the patients in the alirocumab group and in 1.8% of

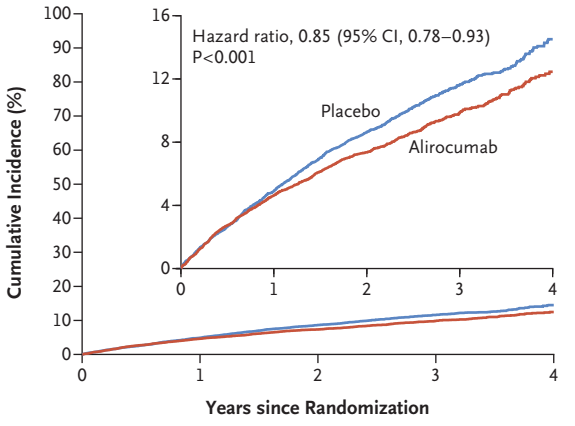


**Figure 2. Cumulative Incidence of the Composite Primary End Point.**

Shown is the cumulative incidence of the primary efficacy end point (a composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization). The Kaplan–Meier rates for the primary end point at 4 years were 12.5% (95% CI, 11.5 to 13.5) in the alirocumab group and 14.5% (95% CI, 13.5 to 15.6) in the placebo group. The inset shows the same data on an enlarged y axis. The P value was calculated with the use of log-rank tests, stratified according to geographic region.

the patients in the placebo group, new-onset diabetes (as defined in the Supplementary Appendix) in 9.6% and 10.1%, respectively, and hemorrhagic stroke (confirmed by adjudication) in less than 0.1% and 0.2%. Neutralizing antidrug antibodies were detected in 0.5% of the patients in the alirocumab group and in less than 0.1% in the placebo group.

## DISCUSSION

Among patients who had a previous acute coronary syndrome and in whom lipid levels exceeded specified thresholds despite atorvastatin or rosuvastatin therapy at a high-intensity dose or at the maximum tolerated dose, the risk of a composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization was lower among those who were treated with alirocumab than among those who received placebo. These benefits were observed in the context of background care that included extensive use of evidence-based treatments[16-19] as well as the use of a dose-adjustment strategy for alirocumab that targeted an LDL cholesterol level of 25 to 50 mg per deciliter and allowed a level of 15 to 25 mg per deciliter, but that avoided sustained levels below 15 mg per deciliter.

The absolute benefit of alirocumab with respect to the composite primary end point was more pronounced among patients who had a baseline LDL cholesterol level of 100 mg or more per deciliter than among patients with a lower baseline LDL cholesterol level. Similarly, a recent meta-analysis showed that intensive lowering of LDL cholesterol (primarily with the use of statins) resulted in a mortality benefit that was observed only among patients with a baseline LDL cholesterol level of 100 mg or more per deciliter.[20]

Over a median follow-up period of 2.8 years, with more than 8000 patients who were eligible to be followed for 3 to 5 years and 6444 patients who received the assigned alirocumab or placebo for at least 3 years, the incidence of adverse events did not differ significantly between the two groups, with the exception of local injection-site reactions. Whether the safety and efficacy of alirocumab were influenced by the blinded dose-adjustment strategy, which was designed to mitigate the occurrence of very low levels of LDL cholesterol, is unknown. Serious safety concerns were also not observed with evolocumab in the FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) trial,[11] which had no lower limit for allowable LDL cholesterol levels; however, that trial had a shorter median follow-up, and very few patients were followed for 3 or more years. Neither trial can fully predict longer-term safety of treatment with a PCSK9 monoclonal antibody.

Lowering of LDL cholesterol levels with alirocumab was sustained but to a lesser extent than that reported in previous trials that had a shorter duration.[9] The increase in LDL cholesterol over time in the intention-to-treat analysis reflects premature discontinuation of treatment, dose reduction or substitution of placebo for alirocumab under blinded conditions, and attenuation of the intensity of statin treatment. The last factor probably also contributed to the rise in LDL cholesterol observed in the placebo group, in the

**Table 3. Adverse Events and Laboratory Abnormalities.**

| Variable | Alirocumab (N=9451) | Placebo (N=9443) |
|---|---|---|
| Adverse events — no. (%) | | |
| Any adverse event | 7165 (75.8) | 7282 (77.1) |
| Serious adverse event | 2202 (23.3) | 2350 (24.9) |
| Adverse event that led to death | 181 (1.9) | 222 (2.4) |
| Adverse event that led to discontinuation of the trial regimen | 343 (3.6) | 324 (3.4) |
| Local injection-site reaction | 360 (3.8) | 203 (2.1) |
| General allergic reaction | 748 (7.9) | 736 (7.8) |
| Diabetes worsening or diabetic complication among patients with diabetes at baseline — no./total no. (%) | 506/2688 (18.8) | 583/2747 (21.2) |
| New-onset diabetes among patients without diabetes at baseline — no./total no. (%)* | 648/6763 (9.6) | 676/6696 (10.1) |
| Neurocognitive disorder | 143 (1.5) | 167 (1.8) |
| Hepatic disorder | 500 (5.3) | 534 (5.7) |
| Cataracts | 120 (1.3) | 134 (1.4) |
| Hemorrhagic stroke, adjudicated | 9 (<0.1) | 16 (0.2) |
| Laboratory abnormalities at any time — no./total no. (%) | | |
| Alanine aminotransferase >3 times upper limit of normal range | 212/9369 (2.3) | 228/9341 (2.4) |
| Aspartate aminotransferase >3 times upper limit of normal range | 160/9367 (1.7) | 166/9338 (1.8) |
| Total bilirubin >2 times upper limit of normal range | 61/9368 (0.7) | 78/9341 (0.8) |
| Creatine kinase >10 times upper limit of normal range | 46/9369 (0.5) | 48/9338 (0.5) |
| Antidrug antibodies† | 67/9091 (0.7) | 32/9097 (0.4) |
| Neutralizing antidrug antibodies | 43/9091 (0.5) | 6/9097 (<0.1) |

* New-onset diabetes was defined according to the presence of one or more of the following, with confirmation of the diagnosis by blinded external review by experts in the field of diabetes: an adverse-event report, a new prescription for diabetes medication, a glycated hemoglobin level of at least 6.5% on two occasions (and a baseline level of <6.5%), or a fasting serum glucose level of at least 126 mg per deciliter (7.0 mmol per liter) on two occasions (and a baseline level of <126 mg per deciliter).

† Antidrug antibodies were defined by the presence of positive responses detected after the start of administration of the trial regimen in at least two consecutive postbaseline serum samples, separated by at least a 16-week period.

on-treatment analysis in the alirocumab group, and in previous trials involving patients who had an acute coronary syndrome.[5,21] Antidrug antibodies were detected in few patients and have been shown not to influence the lipid-lowering efficacy of alirocumab.[22]

There are noteworthy similarities and differences between our trial and the previous FOURIER and SPIRE (Studies of PCSK9 Inhibition and the Reduction of Vascular Events) trials, which evaluated the PCSK9 antibodies evolocumab and bococizumab, respectively.[11,13] The current trial and the FOURIER trial showed similar improvements in composite cardiovascular outcomes with PCSK9 inhibition among patients who had a baseline LDL cholesterol level of 70 mg or more per deciliter and whose average baseline LDL cholesterol level was approximately 90 mg per deciliter.[10] Both our trial and the SPIRE trial showed a more prominent absolute reduction in the risk of cardiovascular outcomes with PCSK9 inhibition among patients who had a baseline LDL cholesterol level of 100 mg or more per deciliter.[12] The current trial showed the efficacy of PCSK9 inhibition in high-risk patients who had a previous acute coronary syndrome, 89% of whom received high-intensity statin therapy, and used a blinded dose-adjustment strategy to achieve a target range of LDL cholesterol with PCSK9 inhibition. The longer duration of follow-up in the

current trial than in previous trials, owing to the mandatory minimum 2-year follow-up, facilitated the assessment of efficacy and safety. A limitation of all three trials is the infrequent use of ezetimibe, for which cardiovascular efficacy was established[5] after most of the patients had already been enrolled and the trials were well under way.

In conclusion, among patients who had a previous acute coronary syndrome and whose levels of atherogenic lipoproteins remained elevated despite statin therapy at a high-intensity dose or at the maximum tolerated dose, the risk of major adverse cardiovascular events was lower among those who were treated with alirocumab than among those who received placebo.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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## APPENDIX

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Special Report

# Quantifying Atherogenic Lipoproteins: Current and Future Challenges in the Era of Personalized Medicine and VLDL Cholesterol. A Consensus Statement from EAS and EFLM

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**BACKGROUND:** The European Atherosclerosis Society–European Federation of Clinical Chemistry and Laboratory Medicine Consensus Panel aims to provide recommendations to optimize atherogenic lipoprotein quantification for cardiovascular risk management.

**CONTENT:** We critically examined LDL cholesterol, non-HDL cholesterol, apolipoprotein B (apoB), and LDL particle number assays based on key criteria for medical application of biomarkers. (*a*) Analytical performance: Discordant LDL cholesterol quantification occurs when LDL cholesterol is measured or calculated with different assays, especially in patients with hypertriglyceridemia >175 mg/dL (2 mmol/L) and low LDL cholesterol concentrations <70 mg/dL (1.8 mmol/L). Increased lipoprotein(a) should be excluded in patients not achieving LDL cholesterol goals with treatment. Non-HDL cholesterol includes the atherogenic risk component of remnant cholesterol and can be calculated in a standard nonfasting lipid panel without additional expense. ApoB more accurately reflects LDL particle number. (*b*) Clinical performance: LDL cholesterol, non-HDL cholesterol, and apoB are comparable predictors of cardiovascular events in prospective population studies and clinical trials; however, discordance analysis of the markers improves risk prediction by adding remnant cholesterol (included in non-HDL cholesterol) and LDL particle number (with apoB) risk components to LDL cholesterol testing. (*c*) Clinical and cost-effectiveness: There is no consistent evidence yet that non-HDL cholesterol-, apoB-, or LDL particle-targeted treatment reduces the number of cardiovascular events and healthcare-related costs than treatment targeted to LDL cholesterol.

**SUMMARY:** Follow-up of pre- and on-treatment (measured or calculated) LDL cholesterol concentration in a patient should ideally be performed with the same documented test method. Non-HDL cholesterol (or apoB) should be the secondary treatment target in patients with mild to moderate hypertriglyceridemia, in whom LDL cholesterol measurement or calculation is less accurate and often less predictive of cardiovascular risk. Laboratories should report non-HDL cholesterol in all standard lipid panels.



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**Table 1. Current challenges for LDLC quantification.**

| Cause | Problem | Recommendation[a] |
|---|---|---|
| Analytical | | |
| Novel therapies: very low LDLC concentrations | Magnification of measurement and calculation errors (e.g., Friedewald) | CBR2, CBR3, CBR4 |
| Nonfasting lipid testing | Postprandial variation of TG in LDLC calculation | CBR4, CBR5 |
| Increasing prevalence of obesity, diabetes, and moderate or major increases in TG | Nonspecificity bias in hypertriglyceridemic (>175 mg/dL; >2 mmol/L) and dyslipidemic samples | CBR2, CBR3, CBR4, CBR9, FR1, FR2 |
| High Lp(a) | Overestimation of LDLC | CBR10 |
| Clinical | | |
| Increasing prevalence of obesity and diabetes | LDLC is a less predictive marker | CBR1, CBR5, CBR6, CBR7, FR3 |
| Residual (on-treatment) CVD risk | Residual risk unexplained by LDLC | CBR8, FR3, FR4 |
| Personalized medicine | LDLC has low or no diagnostic and predictive performance in certain patients | CBR1, CBR8, FR4, FR5 |

[a] CBR and future research recommendation (FR) to address the problem, listed in Table 2 (CBR) and Table 8 (FR).

Measurement of LDL cholesterol (LDLC)[20] is a key component in the assessment of risk of cardiovascular disease (CVD) and the management of dyslipidemia *(1–4)*. Indeed, the causality of LDL particles in the pathophysiology of atherosclerotic CVD is indisputable *(5)*. Furthermore, there is a direct graded relationship between LDLC concentration and the incidence of CVD observed in randomized controlled trials and metaanalyses *(5, 6)*. All guidelines concur that lowering LDLC to concentrations below a target of 70 mg/dL (1.8 mmol/L) (or by ≥50% if this target cannot be attained) is of critical importance in subjects at high or very high risk of CVD *(1–4)*.

Despite the overwhelming evidence that LDLC-targeted strategies effectively reduce CVD, there is substantial between-subject variability in the response to lipid-lowering therapies and the reduction of CVD risk *(7)*. Furthermore, accumulating evidence indicates that a focus solely on the assessment and management of LDLC is not an optimal strategy for all patients, in part as emerging evidence has established that VLDL, their remnants, and lipoprotein(a) [Lp(a)] likewise are causally related to CVD *(8–11)*. Major concerns equally relate to the potential for substantial errors in risk estimation given recognized LDLC measurement or calculation errors in patients with moderate or marked hypertriglyceridemia or low LDLC concentrations (Table 1). Clearly, additional biomarkers beyond LDLC are needed to identify and treat more persons at high CVD risk, especially in this era of novel therapies, such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition, which efficaciously target and reduce concentrations of atherogenic lipoprotein particles to unprecedented low levels *(11, 12)*.

For many years, the inaccuracy in measured or calculated LDLC could be tolerated because of limited clinical impact at the average to high LDLC range. However, this issue must be readdressed in the contemporary treatment era, in which much lower LDLC concentrations are seen *(12)* and moderate hypertriglyceridemia is potentially a greater problem because of the increasing prevalence of obesity, metabolic syndrome, and diabetes mellitus *(13, 14)*. Furthermore, limitations of direct LDLC assays depend on the type of assay. Despite the widespread belief that direct LDLC measurements are standardized and unequivocal, data indicate that results can vary significantly between different assays from different manufacturers *(15)*. Difficulties encountered with HDL cholesterol (HDLC) assays also raise concerns about the reliability of calculated LDLC and non-HDLC, as HDLC is used in both calculations *(15)*.

[20] Nonstandard abbreviations: LDLC, low-density lipoprotein cholesterol; CVD, cardiovascular disease; Lp(a), lipoprotein(a); PCSK9, proprotein convertase subtilisin/kexin type 9; HDLC, high-density lipoprotein cholesterol; LDLP, LDL particle number; apoB, apolipoprotein B; CBR, consensus-based recommendation; dLDLC, direct LDL cholesterol measurement; cLDLC, calculated LDL cholesterol; TC, total cholesterol; TG, triglycerides; LPL, lipoprotein lipase; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; IDL, intermediate-density lipoprotein; Remnant-C, remnant lipoprotein cholesterol; NMR, nuclear magnetic resonance; KIV-2, kringle IV type 2; CRMLN, Cholesterol Reference Method Laboratory Network; dHDLC, direct HDL cholesterol measurement; NCEP, National Cholesterol Education Program; EQA, external quality assessment; VLDLC, very low-density lipoprotein cholesterol; HR, hazard ratio; RR, relative risk; MESA, Multi-Ethnic Study of Atherosclerosis; VLDLP, VLDL particle number; OR, odds ratio.

**Table 2. Key CBR to improve the clinical use of atherogenic lipoprotein assays.**

| | |
|---|---|
| CBR1 | Comprehensive assay(s) of atherogenic lipoproteins should assess the risk conferred by LDL particles, remnant particles, and Lp(a). |
| CBR2 | Laboratories and clinical trial centers should report lipid profiles with declaration of the assay method/manufacturer used. |
| CBR3 | Follow-up of lipid profiles of a patient, from baseline at diagnosis to on-treatment measurements, should be ideally performed with the same assay method (and preferably the same laboratory and instrument). |
| CBR4 | Values near the treatment decision cutpoints should be confirmed by ≥2 repeated measurements by the same method and then averaged. |
| CBR5 | Laboratories should automatically calculate and report non-HDLC on all lipid profiles. |
| CBR6 | Non-HDLC adds Remnant-C to LDLC and can be calculated in the fasting and nonfasting state, independent of TG variability. |
| CBR7 | ApoB assay can estimate LDLP (~95% of apoB) plus Remnant-P and Lp(a) particle numbers in the fasting and nonfasting state. |
| CBR8 | LDLC is the primary target of lipid-lowering therapy. When LDLC goal is achieved, then non-HDLC or apoB should be preferred as secondary treatment targets in patients with TG >175 mg/dL (>2 mmol/L), obesity, metabolic syndrome, or type 2 diabetes. |
| CBR9 | When LDLC is unavailable because of an invalid Friedewald equation (TG >400 mg/dL; 4.5 mmol/L), follow-up calculation of non-HDLC should be used at higher TG concentrations rather than additional direct LDLC measurement. |
| CBR10 | Lp(a)-corrected LDLC should be assessed at least once in patients with suspected or known high Lp(a), or if the patient shows a poor response to LDL-lowering therapy. |

In addition to analytical limitations, there is clinical concern regarding the failure to prevent a large proportion of CVD events that occur despite aggressive LDLC-targeted statin therapy. Many individuals experience CVD-related events or progression of atherosclerosis despite having optimal LDLC even at concentrations <70 mg/dL *(16)*. This residual or "hidden" risk—not identifiable by LDLC—contributes substantially to CVD-related morbidity and mortality and underscores the need for a personalized medicine approach using additional markers to better understand and manage interindividual heterogeneity *(17, 18)*. These markers include LDL subclasses and particle concentration (LDLP), apolipoprotein B (apoB) and mass spectrometry-based proteomics, non-HDLC, remnant cholesterol and particle concentration, Lp(a), renal function and inflammation biomarkers, among others *(17, 18)*.

The analytical validity of these markers and their incremental value beyond LDLC is strongly debated among laboratory professionals and clinicians. Other expert panels have undertaken efforts to investigate emerging biomarkers that are not related to lipid metabolism *(17, 18)*. The current multidisciplinary consensus panel has been established by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and the European Atherosclerosis Society (EAS) with the aim of critically addressing the key issues of the lipoprotein and apolipoprotein markers identified above, as well as reaching a consensus on contemporary laboratory testing for CVD risk assessment and management of lipid therapies. This article embodies the consensus-based recommendations (CBRs) of this expert panel, summarized in Table 2.

## Which Atherogenic Lipoproteins Should Be Measured?

Are we using the appropriate biomarker? Standard LDLC assays measure the cholesterol content of LDL particles, expressed as milligrams per deciliter (or mmol/L) of cholesterol. LDLC concentration can be measured directly (dLDLC) with several different assays, but is still often calculated (cLDLC) from a standard lipid profile that includes measurements of total cholesterol (TC), HDLC, and triglycerides (TG). Fasting blood samples have been the standard for determining lipids because measuring them in the fasting state reduces variability of TG concentrations, thus allowing for a more accurate cLDLC estimation with the Friedewald equation. When the initial clinical decision cutpoints were developed, they were mainly derived from population studies using fasting samples. Thus, recommendations about use of fasting samples in patient care aimed at reproducing the conditions used in these studies to ensure cutpoints are applied properly and results are comparable with those used in these studies. However, fasting is no longer routinely required for the determination of a lipid profile *(19, 20)*. Nonfasting lipid profiles are now endorsed by several

societies' guidelines, including those in Europe, Canada, and the US *(1, 3, 4)*.

Findings from population studies show that despite minor postprandial increases in TG and remnant cholesterol, quantitative changes in other lipids, lipoproteins, and apolipoproteins appear to be negligible in response to the habitual meal intake for most individuals *(19–21)*. Nonfasting lipid profiles are, as we have earlier recommended *(19)*, likely to be more relevant to the estimation of an individual's CVD risk than fasting lipids *(22)*, including TG *(23)*, because in real life we spend most of our time in a postprandial state *(20)*. However, even when measured in the nonfasting state, LDLC alone does not account for all the risk conferred by atherogenic lipoproteins described hereafter. Please note that although we use the term *atherogenic lipoproteins* when we refer jointly to LDL, remnant lipoproteins, and Lp(a), this does not imply that the mechanism by which these lipoproteins cause CVD is identical.

### REMNANT PARTICLES

Postprandial accumulation of TG-rich remnant particles in blood is an important factor in atherogenesis *(8, 9, 24)*. These lipoproteins contain a higher load of cholesterol that is not considered in typical fasting TG- or LDLC-related risk estimations. TG-rich chylomicrons secreted from the intestine, as well as VLDL secreted primarily from the liver, are remodeled in the circulation primarily through the actions of lipoprotein lipase (LPL), hepatic lipase, and cholesteryl ester transfer protein (CETP). The hydrolysis of TG by LPL and acquisition of cholesteryl esters from HDL by CETP generate smaller remnant particles that are depleted of part of their TG content. Consequently, TG-rich lipoproteins also encompass cholesterol-enriched remnants—with the rare exception in individuals with familial chylomicronemia owing to complete deficiency of LPL or 1 of its key cofactors such as apoC-II and apoA-V (estimated incidence, 1/million) *(13)*. There is persuasive experimental evidence that these remnant particles may enter the arterial intima and contribute to atherosclerosis, whereas nascent chylomicrons and very large VLDL particles are too large initially to cross the endothelial layer *(8, 24)*. Unlike LDL particles, which need to be modified (e.g., by oxidation) to generate ligands of the macrophage scavenger receptor, TG-rich remnants can be taken up directly (without modification) by monocyte-derived macrophages, leading to the formation of foam cells, a key step in development of atherosclerotic plaques. Another mechanism by which these particles may predispose an individual to CVD involves the concept that LPL activity catalyzes the release of free fatty acids from TG-rich remnant particles, resulting in local endothelial injury and arterial inflammation *(23)*. Mendelian randomization studies suggest that life-long high plasma concentrations of TG-rich lipoproteins or their remnants are causally associated with increased risk of coronary heart disease (CHD) *(25–28)* and all-cause mortality *(29)*.

Accurate isolation and quantification of remnants has previously been problematic, as remnants are difficult to differentiate from their larger and more TG-rich precursors; furthermore, their plasma concentration is typically much lower compared with other lipoproteins. There are several early assays that claim to specifically measure cholesterol in remnants, but they show poor agreement *(30)*. Some have been validated in cohort studies and have revealed significant associations of remnant cholesterol with cardiovascular events *(31, 32)*.

Larger data sets have been obtained by the calculation of "remnant cholesterol" (Remnant-C = TC − HDLC − LDLC) because Remnant-C corresponds to all cholesterol not found in LDL and HDL, i.e., in all TG-rich lipoproteins (Fig. 1). In the fasting state, this constitutes cholesterol in VLDL and intermediate-density lipoproteins (IDLs), whereas in the nonfasting state, a relatively small amount of cholesterol can also be found in chylomicron remnants. Because both newly secreted chylomicrons and VLDL are acted on rapidly by LPL, any circulating chylomicrons and VLDL have undergone some partial lipolysis and hence can be considered remnants *(24)*. If Friedewald-cLDLC is used in the calculation, then Remnant-C simply equals TG/2.2 (in mmol/L) or TG/5 (in mg/dL) and does not provide any clinical information beyond TG concentrations; however, this is not the case if dLDLC is used in the calculation. Importantly, a homogeneous direct assay to measure Remnant-C in all TG-rich lipoproteins combined has become available to be used with standard hospital autoanalyzers, and direct Remnant-C is highly correlated with calculated Remnant-C, although not identical *(33)*.

Remnant-C also contributes to non-HDLC, which is calculated as the difference between TC and HDLC *(19)*. This term is independent of the Friedewald term and, therefore, not confounded as much with TG concentrations as calculated Remnant-C; thus, it represents an additional clinically valuable marker *(19)*. Remnant-C, measured or calculated, differs from non-HDLC in that non-HDLC contains Remnant-C plus LDLC (Fig. 1). Some individuals with high Remnant-C have low LDLC, and if interpreting non-HDLC instead of Remnant-C, then high Remnant-C will be masked in these individuals.

### LDL PARTICLES

All LDL particles are highly atherogenic, but their concentration is not always reflected by LDLC measurements, particularly in hypertriglyceridemic patients with diabetes or related conditions such as visceral obesity and insulin resistance, which are critical components of the metabolic syndrome *(13)*. LDLC concentration does not automatically equal LDLP because the cholesterol/TG

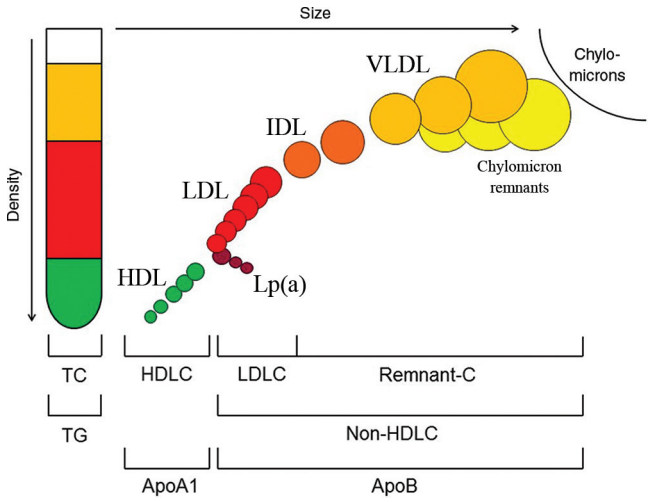


**Fig. 1. Lipoproteins separated according to density and size and their representative laboratory markers measured in a blood sample.**

Standard lipid profiles consist of measurements of TC, TG, HDLC, and LDLC; however, a standard lipid profile could also report calculated Remnant-C and calculated non-HDLC. Remnant-C, calculated as TC − HDLC − LDLC, is all cholesterol not found in LDL and HDL, i.e., in all TG-rich lipoproteins: VLDL, IDL, and, in the nonfasting state, chylomicron remnants. Non-HDLC, calculated as TC − HDLC, represents a comprehensive measure of all cholesterol found in atherogenic lipoproteins: LDLC, Remnant-C, and Lp(a) cholesterol. ApoB and apoA1 can be used as alternatives to non-HDLC and HDLC. The cholesterol content of Lp(a), corresponding to approximately 30% of Lp(a) total mass, is included in TC, non-HDLC, and LDLC measurements and its apoB content in the apoB measurement.

ratio in the particles can vary widely between individuals, reflecting differences in LDL subfraction profile *(34)*. Small LDL particles contain less (LDL) cholesterol than large ones. Although LDLC is typically not increased in patients with type 2 diabetes, such patients tend to have smaller average LDL particle size and will have concomitantly more LDL particles than a patient with the same LDLC concentration who has larger average LDL size *(34)*. These small LDL particles, typically predominant in those with mild to moderate hypertriglyceridemia, are the products of intravascular remodeling of larger TG-rich VLDL particles by 2 processes: first, the progressive hydrolysis of TG-rich VLDL by LPL; second, the increased exchange and transfer of TG and cholesteryl ester mediated by CETP to produce TG-enriched LDL particles *(13)*. TGs are then hydrolyzed by hepatic lipase, resulting in smaller and denser LDL particles with less cholesteryl ester per particle. These compact, lipid-depleted LDL particles are less efficiently cleared via hepatic LDL receptors *(35)*, leading to higher LDLP in patients with increased TG than would be predicted based on LDLC measurement. Concomitantly, TG-enriched HDL resulting from the action of CETP is also modified by hepatic lipase, producing smaller HDL and contributing to lower concentrations of HDLC, as typically manifested in the atherogenic dyslipidemic triad involving hypertriglyceridemia, increased small dense LDLP, and low HDLC *(35)*.

Measurement of apoB, the major protein component of LDL, can also be used to assess the number of LDL particles *(36)*; however, an apoB measurement also includes Lp(a), IDL, VLDL, and chylomicron remnants (Fig. 1). ApoB measurement is not, however, usually part of the standard lipid profile. Monogenic disorders that impair the removal of LDL particles from the circulation, such as familial hypercholesterolemia, can be easily recognized from the standard lipid profile without apoB. In contrast, polygenic hypertriglyceridemia or combined hyperlipidemia can be more adequately characterized based on TG and apoB *(37)*. Although not widely available, LDLP measured by nuclear magnetic resonance (NMR) spectroscopy provides an alternative measure of the number of LDL particles and has been shown to be at least equivalent to apoB and non-HDLC in predicting cardiovascular risk *(38)*.

### Lp(a) PARTICLES

Lp(a) is an LDL-like particle with 1 molecule of apoB to which an additional apolipoprotein, apo(a), is covalently attached. This apolipoprotein shows considerable size polymorphism originating from a variable number of kringle IV type 2 (KIV-2) repeats of apo(a), as encoded

**Table 3. CBRs for the clinical indication for atherogenic lipid and lipoprotein quantification.**

| | CVD risk estimation | Dyslipidemia characterization | Treatment choice | Treatment target | Desirable value |
|---|---|---|---|---|---|
| TC | Yes[a] | Optional[b] | Optional[b] | Optional[b] | <190 mg/dL (5.0 mmol/L) |
| LDLC | Yes | Yes | Yes | Yes | Low to moderate risk <115 mg/dL (3.0 mmol/L) |
| | | | | | High risk <100 mg/dL (2.5 mmol/L) |
| | | | | | Very high risk <70 mg/dL (1.8 mmol/L) |
| TG | Yes | Yes | Yes | No | Fasting <150 mg/dL (1.7 mmol/L) |
| | | | | | Nonfasting <175 mg/dL (2.0 mmol/L) |
| Non-HDLC | Yes | No | No | Yes[c] | Moderate risk <145 mg/dL (3.8 mmol/L) |
| | | | | | High risk <130 mg/dL (3.3 mmol/L) |
| | | | | | Very high risk <100 mg/dL (2.5 mmol/L) |
| ApoB[d] | Optional[c] | Yes[c] | No | Optional[c] | High risk <100 mg/dL (1.0 g/L) |
| | | | | | Very high risk <80 mg/dL (0.8 g/L) |

[a] In combination with HDLC.
[b] To be considered when LDLC is not available.
[c] In patients with mild to moderate hypertriglyceridemia (2–10 mmol/L; 175–880 mg/dL).
[d] Or LDLP if available.
To convert mmol/L to mg/dL, multiply by 38.6 for cholesterol and 88.5 for TG.

by tandem repeats of a genomic sequence in the *LPA*[21] gene *(10, 39)*. This size polymorphism is the most important determinant of the hepatic production rate of Lp(a), and results in marked interindividual variation of plasma Lp(a) concentrations by >1000-fold. Individuals expressing a low number of KIV-2 repeats [small apo(a) phenotypes] show, on average, markedly higher Lp(a) concentrations than those with a high number of KIV-2 repeats [large apo(a) phenotypes], who, on average, have low Lp(a) concentrations. An increased Lp(a) concentration is a strong genetic risk factor for CVD and calcific aortic valve stenosis independent of LDLC in the general population *(10, 39, 40)*. One of the major differences between these 2 particles is that LDLs are effectively lowered by statins, whereas Lp(a) is typically resistant to this treatment. Although PCSK9 inhibitors and other novel agents reduce both LDLC and Lp(a) *(12)*, it is unknown whether Lp(a) lowering per se contributes to the clinical benefit of these novel agents.

Lp(a) should be measured in all patients screened for high risk of CVD or aortic stenosis, in cases of premature CVD, and in those with a positive family history of CVD or high Lp(a) *(10)*. It is often a likely reason of otherwise unexplained CVD cases. However, Lp(a) measurement should not be included in repeated lipid profile measurements within the same patient [as Lp(a) concentrations exhibit little variation over a lifetime], unless treatment is known to influence Lp(a) concentrations. Importantly, the cholesterol content of Lp(a) is included in calculated and measured LDLC and, consequently, also TC and non-HDLC values *(19)*.

*Consensus-based recommendation*. Comprehensive testing of atherogenic lipoproteins should use a biomarker, or a panel of multiple markers, that can be measured in either the fasting or nonfasting state and assesses the risk associated not only with LDL particles but also remnant particles and Lp(a) (CBR1). The use of atherogenic lipoprotein testing in different clinical settings such as CVD risk estimation, dyslipidemia diagnosis, risk management, and treatment has been emphasized in other guidelines *(1)* (Table 3).

## Are LDLC Measurements or Calculations Reliable?

### OPERATIONAL DEFINITION OF LDL

Most manufacturers of lipid assay kits certify and standardize their assays by comparison with a Cholesterol Reference Method Laboratory Network (CRMLN) laboratory. The CRMLN standardization process ensures that the calibrators and reagents sold by manufacturers produce test results that are traceable to the CDC reference methods, i.e., $\beta$-quantification for LDLC and ultracentrifugation/heparin-$Mn^{2+}$ precipitation for HDLC *(41)*.

$\beta$-Quantification requires ultracentrifugation of serum or plasma at a density of 1.006 g/mL to separate the supernatant, which contains VLDL and chylomicrons, from the infranatant, which contains LDL, HDL, and

[21] Human Gene: *LPA,* lipoprotein(a).

Lp(a). Cholesterol is measured in the infranatant to provide the sum of LDL, HDL, and Lp(a) cholesterol, and then LDL particles, including Lp(a), are precipitated from the infranatant, and HDLC is measured in the remaining supernatant. LDLC is then calculated as infranatant cholesterol minus supernatant (HDL−) cholesterol, both measured with the Abell–Kendall cholesterol reference method *(41)*. However, it is not widely recognized that this LDLC also contains the cholesterol from Lp(a), which can be substantial in the case of high Lp(a) concentrations >50 mg/dL *(10)*.

An important prerequisite for reference standardization of the LDLC and HDLC assays is an unambiguous definition of the lipoproteins intended to be measured. With β-quantification, the lipoprotein fraction in the density range of 1.006 to 1.063 g/mL is defined as LDL, and the fraction in the density range of 1.063 to 1.21 g/mL is defined as HDL *(41)*. Yet, these operational definitions allow variable degrees of cross-reactivity of cholesterol from IDL with a density of 1.006 to 1.019 g/mL and Lp(a) with a density of 1.04 to 1.13 g/mL in the LDLC fraction using β-quantification. Direct assays that attempt to specifically measure cholesterol in LDL may, therefore, show discordant results compared with the reference method *(15)*.

#### DIRECT LDLC AND HDLC ASSAYS

In the previous century, the earliest measurements of lipoproteins involved ultracentrifugation and (most popular) precipitation techniques for isolation of LDL and HDL *(42)*. In the late 1990s, "homogeneous" or "direct" LDLC and direct HDLC (dHDLC) methods were introduced in clinical laboratories and have since largely replaced the older assays, particularly for HDLC *(15, 42)*. The homogeneous methods are commercially available as ready-to-use reagents, enabling fully automated dLDLC and dHDLC measurements from the primary blood sample tube, without any need for ultracentrifugation or precipitation to separate HDL and LDL particles. There are various dLDLC and dHDLC assays available based on different principles from different manufacturers to selectively isolate and measure cholesterol in these lipoproteins. Despite the ease of use and cost savings, mostly because of automation, substantial nonselectivity errors have been reported for many of the direct assays *(15, 42)*.

According to National Cholesterol Education Program (NCEP) criteria, the total error of LDLC and HDLC measurements should be within 12% and 13%, respectively, of the true value *(41, 43)*. The *total error* term combines 2 analytical components: imprecision and bias. Imprecision refers to the reproducibility of a method, the "random error" often reported as a CV. Bias, or inaccuracy, refers to a systematic difference in results between a method and the "true" or reference value: It should be ≤4% for LDLC and ≤5% for HDLC to allow the methods to meet the total error goals *(41, 43)*. The discordance between measurements and "true" values, assigned by the reference method, is caused by both bias (which in theory can be eliminated) and imprecision (which can only be minimized but not avoided) in the tests. The direct methods are well standardized using normal patient samples and demonstrate improved precision because of the elimination of sample pretreatment steps, but their bias is a major point of concern in case of aberrant sample matrices, e.g., owing to dyslipidemia. Unsolvable errors—regardless of the method used (ultracentrifugation, precipitation, or homogeneous assays)—relate to the ambiguity in the operational definition of "LDL" and "HDL" fractions and the heterogeneity of LDL and HDL particles. Both LDL and HDL fractions comprise different subclasses of particles that vary in size, density, shape, and lipid and apolipoprotein composition, without any definitive chemical structure, making development of specific assays difficult *(15, 42)*. Direct assays based on different principles may select different subclasses of LDL or HDL that may or may not be equally quantified, depending on the assay procedure and reagents. The reaction specificities of dLDLC assays vary regarding reactivity to small dense LDL subfractions and nonspecific reactivity to VLDL subfractions *(15)*. Consequently, nonspecificity bias is caused by the inaccuracy of the tests in selectively quantifying what is intended to be measured; this bias is inevitable and varies per sample *(15, 42, 44)*.

Most discrepancies—with marked deviations from the CDC reference methods—are observed in samples from patients with hypertriglyceridemia >2 mmol/L (>175 mg/dL), mixed dyslipidemia, or other conditions involving altered lipoprotein composition and remodeling, such as diabetes and chronic kidney disease *(44–46)*. In a comprehensive study of direct methods using fresh samples from individuals with and without CVD and/or various dyslipidemias, Miller et al. showed that only 5 of 8 dLDLC methods and only 6 of 8 dHDLC methods met the NCEP total error goal with samples from nondyslipidemic individuals, and all methods failed to meet NCEP performance criteria with those from dyslipidemic individuals *(44)*. The total error of dHDLC and dLDLC measurements ranged approximately ±13% in normolipidemic samples but from −20% to +36% for dHDLC and −26% to +32% for dLDLC in dyslipidemic samples *(44)*. Most discordant results were observed at lower concentration ranges of HDLC (<40 mg/dL) and LDLC (<70 mg/dL) *(44)*, which are now more clinically relevant given the highly efficacious LDL-lowering therapies presently available.

Test results differ substantially between the various direct methods from different manufacturers, particularly in hypertriglyceridemic (>2 mmol/L; >175 mg/dL) samples. For all dHDLC and dLDLC methods, a

high proportion (≥10%; for some methods, 30%–45%) of test results fall outside the NCEP total error goals in dyslipidemic samples *(44)*. We cannot recommend a specific manufacturer method, as no consistent pattern has been documented for the frequency of analytical errors with each method in normolipidemic or dyslipidemic samples. This is also evident from large-scale accuracy-based quality surveys organized across different laboratories *(47, 48)*. In a survey of 190 US laboratories, all dLDLC methods failed the bias criterion of ≤4% on a fresh frozen pooled serum with a TG concentration of 2.2 mmol/L (193 mg/dL) *(47)*. An external quality assessment (EQA) study of 200 clinical laboratories in the Netherlands, representing common dLDLC and dHDLC methods used in most countries, revealed unacceptable bias (up to 20%) with most manufacturers' methods in fresh and frozen serum pools, with TG concentrations of 7 mmol/L (620 mg/dL) *(48)*. Reported dLDLC data showed values for mean bias of +16% with Abbott Diagnostics, +14% with Beckman Coulter, and −7% with Roche Diagnostics methods as compared with the CRMLN reference laboratory measurement *(48)*. Mean dHDLC biases were also method-dependent [−3% (Abbott), −7% (Beckman Coulter), −19% (Roche), and −22% (Siemens)] and contributed to between-laboratory variability of cLDLC and non-HDLC calculations *(48)*. These errors resulted in misclassifications with respect to CVD risk assessment, depending on the laboratory where LDLC or HDLC was measured *(48)*.

The biases noted in dLDLC and dHDLC assays when analyzing dyslipidemic samples with atypical lipoproteins suggest that nonspecific cross-reaction takes place, with high variability between the different chemical procedures used to isolate the lipoproteins intended to be measured. This shortcoming is not revealed in current CDC certification programs.

**Table 4. Analytical and biological sources of error in variables included in the Friedewald-cLDLC equation.**

| |
|---|
| TC |
| (Pre-)analytical and intraindividual (biological) variations of TC |
| Lp(a) cholesterol in patients with increased Lp(a) is not subtracted from TC |
| HDLC |
| (Pre-)analytical and intraindividual (biological) variations of HDLC |
| Inaccurate dHDLC measurement (nonspecificity bias) in dyslipidemic samples |
| VLDLC |
| TG/cholesterol ratio increases with increasing hypertriglyceridemia |
| Invalid use of fixed TG/cholesterol ratio in type III dyslipoproteinemia and chylomicronemia |
| Intraindividual (biological) variations of TG |
| Postprandial variations of TG, when using nonfasting samples |
| Increased free glycerol concentration in nonglycerol blanked TG assays[a] |

[a] The free or endogenous glycerol concentration in a sample can usually be ignored [1 mg/dL, equivalent to approximately 10 mg/dL (0.11 mmol/L) of TG]. Increased baseline glycerol concentrations are found in patients with diabetes and chronic kidney disease, and will add to underestimation of cLDLC using nonglycerol blanked TG assay.

### cLDLC

In most laboratories, LDLC is calculated by the Friedewald formula, cLDLC = TC − HDLC − VLDL cholesterol (VLDLC), wherein VLDLC is estimated as TG/2.2 in mmol/L or TG/5 in mg/dL *(49)*.

Like dLDLC measurements, cLDLC calculation is not without flaws. The Friedewald formula also includes cholesterol in IDL and Lp(a) and assumes a constant VLDL TG/cholesterol ratio, lack of chylomicrons, and lack of excessive remnant lipoproteins. Nonfasting samples do not necessarily meet these assumptions, as chylomicrons may be present and are more TG-rich than VLDL particles *(42)*. Because the TG/cholesterol ratio in TG-rich VLDL and chylomicrons progressively increases as hypertriglyceridemia and (postprandial) chylomicronemia become more severe, the equation overestimates VLDLC and, therefore, underestimates LDLC at high TG concentrations *(42)*. In a study of type 2 diabetes patients, nonfasting cLDLC showed a negative bias of −12% with respect to β-quantification, and fasting cLDLC had a mean bias of −0.21 mmol/L (−8 mg/dL) vs β-quantification at 5 h postprandially *(50)*. Hence, cLDLC exaggerates the (usually minor) postprandial decrease in LDLC, which is clinically insignificant in most individuals *(19)*. The equation is increasingly inaccurate at TG concentrations from 200 to 400 mg/dL (2.3–4.5 mmol/L) and is regarded as invalid when TGs are >400 mg/dL (4.5 mmol/L) or in rare cases of type III dyslipoproteinemia in which cholesterol-rich β-VLDL remnants are present and VLDLC will be underestimated (therefore, LDLC will be overestimated) *(13, 42)*. Several alternative cLDLC equations and adjustable factors for the TG/VLDLC ratio have been proposed *(51–54)*, but it remains to be determined as to whether improvements over the Friedewald equation improve risk prediction or are substantive enough to justify their implementation in clinical practice *(55)*. The fact that cLDLC depends on 3 laboratory measures, i.e., TG, TC, and dHDLC, means that 3 CVs are involved, which increases the potential for measurement error (Table 4).

Both imprecision and bias of cLDLC increase at lower LDLC concentrations, an aspect that is more relevant because highly effective LDL-lowering therapies, including

combination therapies (e.g., statins with ezetimibe or statins with PCSK9 inhibitors), are now available *(11, 12)*. The original Friedewald equation in 1972 was not designed to be used in patients receiving such treatments. In a correlation analysis of pooled data from 14 trials of alirocumab-treated patients in the ODYSSEY program, only minor differences were observed between LDLC values derived by Friedewald calculation and β-quantification *(56)*. In patients with LDLC in the range of 15 to <25 mg/dL (0.4 to <0.6 mmol/L) as measured by β-quantification, there was a median difference of 3.5 mg/dL (0.1 mmol/L) compared with cLDLC; in those with measured LDLC <15 mg/dL (0.4 mmol/L), there was a median 3-mg/dL (0.1-mmol/L) difference *(56)*. These small differences are likely to have little clinical impact. Another report showed that underestimation of LDLC by the Friedewald equation compared with ultracentrifugation (β-quantification), especially an LDLC <70 mg/dL (1.8 mmol/L) with a median difference of −4 mg/dL (0.1 mmol/L), resulted in treatment group misclassification of 29% of patients with respect to the guideline-recommended cutpoint of 70 mg/dL (1.8 mmol/L) *(55)*. These studies reported pooled data from predominantly (>75%) normotriglyceridemic study populations *(55, 56)*. In a subanalysis of 33 106 hypertriglyceridemic patients with TGs of 200 to 399 mg/dL (2.3–4.5 mmol/L) from a large study sample (n = 191 333) with cLDLC ranging from 2 to <70 mg/dL (0.05 to <1.8 mmol/L), median cLDLC bias was −18 mg/dL (−0.5 mmol/L) (5th to 95th percentile, −7 to −36 mg/dL; −0.2 to −0.9 mmol/L) compared with LDLC measured by vertical spin density gradient ultracentrifugation *(57)*. Median biases were larger at the lowest concentration ranges: −26 mg/dL (−0.7 mmol/L) at cLDLC of 15 to <25 mg/dL (0.4 to <0.6 mmol/L), and −33 mg/dL (−0.9 mmol/L) at cLDLC of <15 mg/dL (0.4 mmol/L) *(58)*. The same investigators found that cLDLC was frequently classified as <70 mg/dL (1.8 mmol/L) despite true LDLC concentrations ≥70 mg/dL; indeed, 39% of patients were misclassified when TG levels were 150 to 199 mg/dL (1.7–2.3 mmol/L), and 59% were done so when levels ranged from 200 to 399 mg/dL (2.3–4.5 mmol/L) *(57)*. These patients may be excluded from treatment because of underestimated LDLC *(57, 58)*. It should be noted that the target (i.e., 70 mg/dL; 1.8 mmol/L) is based on population studies using cLDLC and not ultracentrifugation; thus, it could be argued that ultracentrifugation (β-quantification) is the method that misclassifies risk.

These observations reflect the inaccuracy of the Friedewald equation over the full range of LDLC values seen with novel therapies. In persons with very low LDLC and concurrently high TG, VLDLC estimation (TG/5 in mg/dL or TG/2.2 in mmol/L) constitutes a relatively larger portion of the equation. In this situation, the error of overestimated VLDLC with increasing TG-rich VLDL has a significant impact on the total error of estimated cLDLC.

#### EFFECT OF Lp(a) CHOLESTEROL ON LDLC

Friedewald-estimated cLDLC and most dLDLC methods include the cholesterol that is present in Lp(a) particles *(15, 59)*. Considering that an Lp(a) particle is composed of about 30% to 45% cholesterol by weight, a substantial overestimation of TC, non-HDLC, and LDLC concentration occurs in individuals with high and very high Lp(a) concentrations *(59)*; for example, if a person has an Lp(a) concentration of 100 mg/dL, cLDLC and dLDLC will be overestimated by 30 to 45 mg/dL (0.8–1.2 mmol/L). These circumstances might explain some cases of nonresponse or low response to statin treatment. Statins are known to have a pronounced effect on LDLC but do not lower Lp(a) concentrations *(60)*.

If a patient receives a statin with the aim to reduce LDLC to a target of <70 mg/dL, and if that patient already has a cLDLC value of 100 mg/dL and an Lp(a) concentration of 100 mg/dL, the Lp(a)-corrected LDLC is only approximately 55 to 70 mg/dL [i.e., 100 mg/dL cLDLC minus 30%–45% of measured Lp(a)]. In the new era of potent LDLC-lowering therapies, the achieved true LDLC concentrations [after correction for Lp(a) cholesterol] can be as low as 10 mg/dL (0.3 mmol/L) *(61)*. This scenario is particularly likely in African-Americans, who often have 2- to 3-fold higher Lp(a) concentrations than whites, or in patients with nephrotic syndrome or in those undergoing peritoneal dialysis in whom Lp(a) concentrations can reach up to >300 mg/dL, which corresponds to an Lp(a)-corrected LDLC that is 135 mg/dL (3.5 mmol/L) lower than the uncorrected LDLC value *(62)*. For example, patients with nephrotic syndrome had, on average, 27-mg/dL higher LDLC concentrations if not corrected for cholesterol derived from Lp(a) (compared with only 9 mg/dL in controls) *(62)*.

Lp(a)-corrected LDLC can be estimated with the Dahlen modification of the Friedewald formula, which assumes that 30% of Lp(a) weight consists of cholesterol: cLDLC = TC − HDLC − TG/5 − [Lp(a) × 0.30] in mg/dL *(63)*. We recommend that Lp(a)-corrected LDLC be applied at least once in patients with suspected high Lp(a), or in a patient who does not respond sufficiently to statin therapy, to identify or exclude potential interference by high or very high Lp(a) in making treatment decisions (CBR10). If a high Lp(a) concentration is the cause for an apparently disappointing LDLC-lowering response with a statin, then it might not be useful to increase the dosage of statin under such conditions *(60)*.

#### DISCORDANT cLDLC VS dLDLC

Measured and calculated LDLC correlate well using both fasting and nonfasting lipid profiles in general population statistics *(19)*, but often cLDLC may not agree with dLDLC in an individual subject *(15)*. Friedewald-cLDLC with a constant TG/cholesterol ratio cannot adjust for the postprandial increase in TG *(42)*. β-Quantification removes TG-rich lipoproteins by ultracentrifugation before measurement, and most dLDLC assays attempt to selectively measure cholesterol in LDL particles by either blocking or solubilizing non-LDL particles *(15)*. Thus, technically, β-quantification and dLDLC are less sensitive to TG-rich lipoproteins and should not be influenced by a nonfasting state, but the direct assays have varying specificity limitations when abnormal lipoproteins and increased TG are present *(44–46)*.

Among 1508 men (including 173 CHD events during follow-up) and 1680 women (including 74 incident CHD events), the Framingham Study found good agreement of cLDLC with dLDLC (Kyowa Medex assay) *(64)*. Discrepancies of >10% were assessed in 7.7% of LDLC determinations, but at higher TG concentrations and in patients with diabetes, CHD, or on cholesterol-lowering medications, there was a greater bias between the 2 methods *(64)*. This study, as in the Women's Health Study below, did not compare either the cLDLC or the dLDLC method with a gold standard such as ultracentrifugation. The Women's Health Study (n = 27 331) also observed good correlations between cLDLC and dLDLC (Roche Diagnostics assay) in fasting and nonfasting samples *(65)*. However, the average dLDLC concentration was 5 to 10 mg/dL (0.1–0.3 mmol/L) lower than cLDLC. Associations of fasting cLDLC and dLDLC concentrations with CHD events showed similar hazard ratios (HRs) of 1.22 (95% CI, 1.14–1.30) and 1.23 (95% CI, 1.15–1.32) per 1-SD increase (35 and 34 mg/dL; 0.9 and 0.9 mmol/L), respectively. However, the lower dLDLC resulted in classification of about 20% of individuals with discrepant risk as compared with cLDLC *(65)*.

Even in normotriglyceridemic samples, dLDLC methods do not offer advantage over cLDLC in classifying patients into NCEP risk categories when compared with the reference method *(66)*. In a study of 145 fasting individuals with TG <200 mg/dL (2.3 mmol/L), 7 of 8 commercially available dLDLC methods failed to show improved CVD risk score classification over the corresponding cLDLC estimated using the dHDLC method from each manufacturer in the calculation *(66)*. The overall misclassification rate for the CVD risk score ranged from 5% to 17% for cLDLC methods and from 8% to 26% for dLDLC methods when compared with β-quantification, and most normotriglyceridemic individuals were classified into a lower risk category by the dLDLC methods (3%–26%) *(66)*.

These observations suggest no substantial advantage for using dLDLC compared with cLDLC in normotriglyceridemic and hypertriglyceridemic blood samples up to TG concentrations of 400 mg/dL (4.5 mmol/L). dLDLC measurement is clearly more expensive than the "free of charge" cLDLC. Furthermore, most clinical trials demonstrating the evidence base for clinical benefit of LDLC lowering with statin therapy have used the Friedewald equation.

#### LDLC TEST ERRORS: ARE THEY CLINICALLY RELEVANT?

Clinical or epidemiological studies can validate whether analytical errors in LDLC testing are relevant in clinical decision-making and whether they influence outcomes. The ranges of uncertainty across different LDLC methods are not negligible (Table 5). Difficulties in treatment options may arise when LDLC test results are close to guideline-driven critical values that determine the decision of therapeutic intervention (Fig. 2). Misclassification—and, thus, inappropriate treatment—may occur if a true LDLC concentration is above the optimal target value but the reported LDLC value is in a desirable range, or if a true LDLC concentration is in a desirable range but the reported LDLC value is above target, e.g., 70 mg/dL (1.8 mmol/L), in a patient with a very high-risk score *(1)*. The former underdiagnosis bears the risk of insufficient treatment and adverse clinical outcome; the latter overdiagnosis will increase costs by leading to unnecessary prescriptions of statins in general and specifically combination treatments.

Depending on the methods used, different treatment decisions may be taken, or confusion may arise if the patient's samples for monitoring are sent to different laboratories using different methods or when a laboratory changes the method. Not uncommonly, changes in a patient's LDLC test result over time are within the range of uncertainty of laboratory method variation and may not be because of therapeutic intervention. Between-method and between-laboratory differences in on-treatment LDLC may even mimic (or mask) a nutraceutical or pharmaceutical effect, e.g. ezetimibe, when values differ >15% over time when measured by different methods during follow-up *(67)*. Based on the Cholesterol Treatment Trialists' metaanalysis *(6)*, measurement biases of up to −0.5 mmol/L (−20 mg/dL) can be falsely interpreted as a 10% CVD risk reduction (Fig. 3). For example, in the REVEAL study, the mean LDLC reduction in the anacetrapib-treated group compared with the placebo group was −26 mg/dL (−0.68 mmol/L; −41%) as measured with a direct assay (Beckman Coulter) but only −11 mg/dL (−0.28 mmol/L; −17%) as measured on β-quantification in the same samples *(68)*. These issues are, however, less relevant for the monitoring of the patient by the same laboratory and method over time. In this situation, the bias remains constant over time and

**Table 5. Examples of uncertainty when plasma lipid parameters are determined by different methods.**

| Assay | Assumed total error | Defined concentration in model patient | Range of uncertainty |
|---|---|---|---|
| TC | 9%[a] | 200 mg/dL (5.2 mmol/L) | 182–218 mg/dL (4.7–5.7 mmol/L) |
| TG | 15%[a] | 250 mg/dL (2.8 mmol/L) | 212–288 mg/dL (2.4–3.3 mmol/L) |
| HDLC | −20% to +36%[b] | 40 mg/dL (1.0 mmol/L) | 32–54 mg/dL (0.8–1.4 mmol/L) |
| Non-HDLC | Derived from TC and dHDLC | 160 mg/dL (4.1 mmol/L) | 128–186 mg/dL (3.3–4.8 mmol/L) |
| Measured LDLC | −26% to +32%[b] | 110 mg/dL (2.8 mmol/L) | 82–145 mg/dL (2.1–3.8 mmol/L) |
| Estimated LDLC (Friedewald) | Derived from TC, TG, and dHDLC | 110 mg/dL (2.8 mmol/L) | 70–144 mg/dL (1.8–3.7 mmol/L) |
| ApoB | 12%[c] | 110 mg/dL (1.1 g/L) | 97–123 mg/dL (0.9–1.2 g/L) |

[a] Based on NCEP analytical performance criteria *(41)*.
[b] Total error ranges observed by Miller et al. *(44)* across different dLDLC and dHDLC methods in dyslipidemic samples. The total error combines systematic bias and random imprecision. The tables are not relevant for the monitoring of a patient by the same laboratory/method over time. In this situation, the bias remains constant and only the (inevitable) imprecision is relevant. It will be considerably lower than the total error, at least for dHDLC and dLDLC (<10%), but not for TC, TG, and the derived measures cLDLC or non-HDLC.
[c] Based on AACC Lipoprotein and Vascular Diseases Division–Working Group on Best Practices assessment *(38)*.

only the imprecision (random error) is relevant, which may not be important given that clinicians are not aiming to achieve the LDLC targets exactly but often concentrations below it. In the present era, the extent of LDLC reduction is more important than achieving specific targets, and recent guidelines suggest that achievement of a >50% reduction in high- and very high-risk patients is paramount, irrespective of baseline LDLC concentration *(2)*.

The risk that errors in LDLC measurement affect the clinical decision is further attenuated by the NCEP recommendation that decisions to initiate a treatment, or adjusting or shifting to another treatment, should not be taken on 1 LDLC measurement but rather after multiple repeated measurements (at least 2 times) to allow for intraindividual (biological) variation *(43)*.

*Consensus-based recommendation*. Analytical performances of dLDLC and cLDLC are acceptable in blood samples with normal TG. That said, variable nonspecificity errors may confound measurements in samples with hypertriglyceridemia >175 mg/dL (2 mmol/L), which is seen in approximately 25% of individuals in the general population *(24)*, or in samples with low LDLC <70 mg/dL (1.8 mmol/L); however, these errors may still be less than the between-method, between-laboratory, biological, and, thus, total error. We recommend that laboratories and clinical trial centers report lipid levels together with the test method used, and in a similar manner to recommendations made to report other laboratory tests used for monitoring, e.g., tumor markers, to make clinicians aware of changes in methods as a potential cause of implausible laboratory test results (CBR2). Follow-up of lipid profiles in a patient, from baseline to on-treatment measurements, should ideally be performed with the same method (and preferably in the same laboratory and instrument) to minimize CVD-risk misclassifications (CBR3). Values near the therapeutic decision cutpoints should ideally be confirmed by repeated measurement(s) (≥2) by the same method and then averaged (CBR4).

## Are Alternative Atherogenic Lipoprotein Measures Reliable?

### CALCULATED NON-HDLC

Calculated by simply subtracting HDLC from TC, non-HDLC represents the cholesterol in all atherogenic particles, i.e., LDL, VLDL, IDL, chylomicron remnants, and Lp(a). In contrast to LDLC, non-HDLC considers the atherogenic potential of remnant lipoproteins *(19)*. Therefore, non-HDLC provides a more comprehensive risk estimation than does LDLC in patients with hypertriglyceridemia by adding VLDLC (= Remnant-C) to LDLC *(13)*.

Like LDLC, non-HDLC is managed with existing lipid-lowering agents, and there is a direct consistent relationship between the magnitude of non-HDLC lowering and CHD-risk reduction *(69)*. In a metaanalysis of 14 statin (n = 100 827), 7 fibrate (n = 21 647), and 6 niacin (n = 4445) trials, a 1% decrease in non-HDLC was associated with a 1% decrease of relative risk (RR) for CHD *(69)*. The estimated 1:1 relationship translated into an RR of 0.78 (95% CI, 0.64–0.94) for a 25% decrease in non-HDLC *(69)*. In another metaanalysis of 49 statin and nonstatin trials (n = 312 175), the RR of CVD per 1-mmol/L (39 mg/dL) reduction in non-HDLC was 0.80 (0.77–0.82), which was similar to the RR of 0.77 (0.75–0.79) with LDLC reduction *(70)*. All drugs including fibrates (except for CETP inhibitors) fitted the regression line to predict RR reduction when

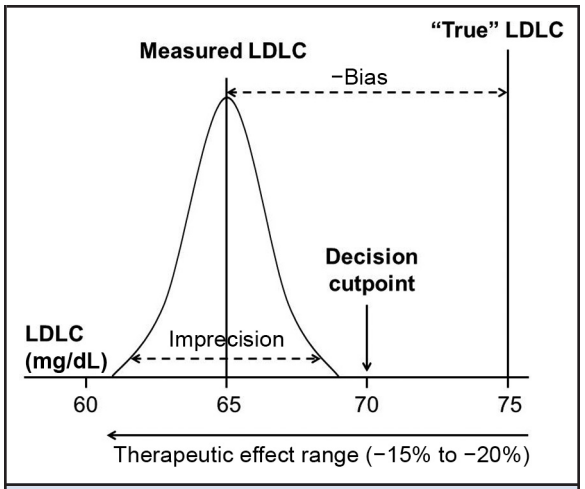


**Fig. 2. Example of potential confounding of negative measurement bias and between-laboratory imprecision in the clinical interpretation of LDLC concentration.**

In this example, LDLC is measured with a certain assay method in different laboratories and with the reference method β-quantification ("true" LDLC) in a patient with very high risk (desirable LDLC concentration, <70 mg/dL; 1.8 mmol/L). With LDLC measured in laboratories using this type of assay, it will be falsely concluded that the patient is at goal and can be excluded from LDLC-lowering therapy. The negative bias of this assay method compared with β-quantification is in the same range as can be observed with a low-potency therapeutic effect, e.g., ezetimibe, or nutraceutical effect. To convert LDLC to mmol/L, divide value in mg/dL by 38.6.

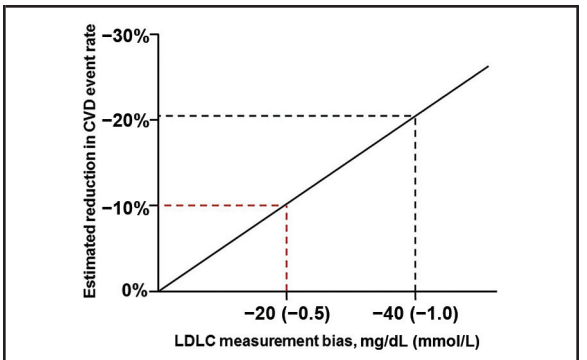


**Fig. 3. Potential confounding of biased LDLC measurement in the estimation of CVD risk reduction.**

Estimated CVD risk reduction in study populations is based on the Cholesterol Treatment Trialists' metaanalysis of LDLC-lowering trials. Measurement bias up to −0.5 mmol/L (−20 mg/dL) can be falsely interpreted as a 10% CVD risk reduction when discordant LDLC assay methods are used from baseline to on-treatment measurements.

non-HDLC was used, but not when LDLC was used *(70)*. In the REVEAL study, the CETP inhibitor anacetrapib fitted the regression line when non-HDLC reduction was applied *(68)*.

These findings support the use of non-HDLC as a target of therapy as recommended by guidelines *(1–4)*. The recommended targets for non-HDLC typically are arbitrarily set 30 mg/dL (0.8 mmol/L) higher than LDLC targets; this value assumes that the "normal" VLDLC level associated with the fasting TG cutpoint of 150 mg/dL (1.7 mmol/L) is 30 mg/dL (0.8 mmol/L), as estimated by the Friedewald formula (TG/5 in mg/dL or TG/2.2 in mmol/L). Thus, a non-HDLC goal of <100 mg/dL (2.5 mmol/L) is equivalent to an LDLC goal of <70 mg/dL (1.8 mmol/L) in very high-risk subjects *(19)* (Table 3). For very low or very high ranges of non-HDLC, simple addition of a fixed term of 30 mg/dL (0.8 mmol/L) is not appropriate. It may be more appropriate to use a multiplier of 1.3, so that an LDLC of 2 mmol/L is equivalent to a non-HDLC of 2.6 mmol/L (100 mg/dL), and a low LDLC of 1 mmol/L is equivalent to a non-HDLC closer to 1.3 mmol/L (50 mg/dL) rather than 1.8 mmol/L (70 mg/dL). This is in line with the previously proposed adjustable factor for VLDLC estimation based on TG and non-HDLC concentrations *(53, 54)*.

Non-HDLC can be obtained in the nonfasting state and does not require TG to be <400 mg/dL (4.5 mmol/L) *(19)*. Therefore, it is a useful alternative to cLDLC when the Friedewald equation is invalid, essentially making the need for a dLDLC assay obsolete. Although non-HDLC is not dependent on TG variability, dHDLC measurement errors in hypertriglyceridemic samples still affect the calculation of non-HDLC. Accuracy-based EQA surveys using the different dHDLC assays in the calculation revealed median bias of <10% for all methods, as compared with the expected non-HDLC value based on TC and HDLC reference measurements, although the range of individual laboratory biases reported was broad in moderate to severe hypertriglyceridemic serum samples *(47, 48)*. Therefore, not only calculated LDLC but also non-HDLC values must be interpreted with caution when making treatment decisions for individuals with dyslipidemia (Table 5). However, in samples from those with moderate hypertriglyceridemia in the 200 to 399 mg/dL (2.3–4.5 mmol/L) range, non-HDLC calculated with different dHDLC assays showed much better concordance with CVD risk classification according to NCEP non-HDLC cutpoints (overall misclassification range, 0%–14%) than either dLDLC or cLDLC test results according to LDLC cutpoints (overall misclassification range, 7%–37%) *(66)*. Consequently, under conditions of high TG when cLDLC is likely to be inaccurate, non-HDLC can be calculated. The

benefit of non-HDLC over dLDLC may arguably be cost, as it can be calculated at no additional expense above conventional lipid testing.

#### LDL PARTICLE NUMBER

LDL can be subfractionated by NMR spectroscopy, density-gradient ultracentrifugation, electrophoresis, ion-mobility analysis, or chromatography *(71, 72)*. NMR spectroscopy is by far the least labor-intensive method *(72)*. NMR spectroscopy provides rapid quantification of size and concentration (LDLP) of LDL particles and various lipoprotein subclasses, including VLDL, IDL, LDL, and HDL; Lp(a) is typically included in the measured LDL fraction with NMR. Lipoproteins are analyzed by NMR according to the spectral signals produced by the terminal methyl groups contained within the lipid particles. The number of methyl groups present in TG, cholesterol, and phospholipids is consistent for particles of a given size, allowing for translation into particle concentration *(72)*. Nonetheless, this technology has not been exhaustively standardized to account for the wide compositional variability occurring in neutral core lipids in different dyslipidemic conditions; therefore, caution is warranted in data interpretation under these conditions. NMR lipoprotein analyses have been evaluated against other existing technologies to determine numbers, size, and subclasses of lipoprotein particles, but the results agree poorly with each other, in part because the various techniques measure different physical properties *(71, 73)*. However, even different NMR methods yield discrepant results, as the algorithms to decipher the NMR signals are proprietary developments of each provider *(74)*. Therefore, standardization of NMR spectral analysis is urgently needed, as well as harmonization to a unique reference material to allow comparability of NMR data with other subfractionation techniques *(72)*.

Regardless, multiple large prospective cohort studies that have monitored clinical outcomes, including EPIC-Norfolk, Framingham Offspring, Multi-Ethnic Study of Atherosclerosis (MESA), and Women's Health Studies have demonstrated that LDLP is superior to LDLC in predicting CVD, consistent with the fact that many individuals with atherogenic dyslipidemia have increased numbers of LDLP without having high LDLC *(75–79)*; in this situation, patients often have higher concentrations of TG-rich lipoproteins, which may explain the higher CVD risk rather than high LDLP numbers per se. LDLP also appears to be a better indicator of subclinical CVD because it associates more strongly with coronary artery calcium and carotid intima–media thickness than LDLC or non-HDLC *(80)*. Whereas earlier studies emphasized the atherogenicity of small LDL particles, we now know that all LDL particles are atherogenic *(81)*, as evidenced by patients with familial hypercholesterolemia who display a predominance of large, buoyant LDL particles and early atherosclerosis *(82)*. Thus, the primary focus of patient treatment should remain the reduction of the number (concentration) of LDL particles, without efforts to distinguish between large and/or small LDL particles. Indeed, a review of 24 studies that reported relationships between LDL subfractions and cardiovascular outcomes concluded that higher LDLP but not LDL size was consistently associated with increased risk of CVD events *(83)*.

A major impediment to LDLP testing is its limited availability outside the US, although it is offered by some larger reference laboratories and a small automated NMR analyzer (Vantera) has been developed by Liposcience (now LabCorp) for high-throughput clinical laboratory testing *(84)*. Another potential barrier is its cost, which is about twice that of a standard lipid panel, although it does provide additional information on other lipoprotein fractions. NMR quantification of VLDL particle concentration (VLDLP) revealed that the smallest remnant subclass concentration was associated with a 68% per SD (HR, 1.68; 95% CI, 1.28–2.22) increase in residual risk among statin-treated patients with low LDLC *(85)*. The use of LDLP and VLDLP subclass monitoring as an additional treatment target beyond LDLC needs more widely accessible and standardized assays in clinical laboratories before it can be recommended for routine clinical practice.

#### APOLIPOPROTEIN B

ApoB is the structural protein for all non-HDL lipoproteins and modulates the centrifugal and centripetal transportation of lipoproteins with reference to liver, intestine, and peripheral tissues *(36)*. There are 2 isoforms of apoB: the full-length form of apoB, apoB100 with a molecular mass of 550 kDa, the major isoform synthesized in hepatocytes and found in VLDL, IDL, LDL, and Lp(a); and a truncated form, apoB48, with a molecular mass of 265 kDa (48% of the molecular weight of apoB100), that is synthesized in intestinal enterocytes and is the structural protein of chylomicrons and chylomicron remnants *(36)*. ApoB100, but not apoB48, contains the ligand that binds to the LDL receptor. Because each atherogenic particle contains 1 molecule of apoB, concentrations of apoB are considered to be a direct measure of the total number of atherogenic lipoproteins in the circulation *(36)*.

Because of its relatively high abundance, apoB100 can be readily measured by immunoassays. Immunoturbidimetric and immunonephelometric assays are commercially available on commonly used automated systems from several manufacturers. Most immunoassays detect both apoB100 and apoB48, depending on the specificity of the antibodies used, which are typically generated against apoB100. In fasting samples, >90% of apoB in plasma is apoB100. Because of the longer half-

life of LDL (3 or 4 days) compared with VLDL (3 or 4 h), almost all of it is associated with LDL when TG concentrations are <200 mg/dL (2.3 mmol/L) *(36)*. Therefore, at low TG concentrations, the measurement of apoB is essentially an estimate of LDL–apoB concentration or LDLP. Simple apoB testing obviates the need for more expensive technologies such as NMR spectroscopy to measure LDLP, although apoB cannot substitute for NMR-based particle size measurements *(38)*. LDLP can be directly compared with apoB100 measurements by adding VLDLP to LDLP and converting nanomoles per liter (nmol/L) to mass units (mg/dL) and by multiplication by the factor 0.055 based on the molecular weight of apoB100 (550 kDa) *(38)*. This conversion recognizes that both apoB100 and LDLP measurements include IDL and Lp(a), in addition to LDL *(38)*.

From an analytical viewpoint, apoB100 is a clearly defined protein. Its measurement can be standardized because of the availability of serum-based IFCC/WHO SP3–08 secondary reference material, value-assigned using immunonephelometry and the primary reference material—a stable LDL fraction prepared by ultracentrifugation (density, 1.030–1.050 g/mL) that excludes IDL and Lp(a) *(86)*. ApoB100 measurements are easily automated and yield more accuracy compared with direct assays for LDLC or HDLC, although concerns about the accuracy of apoB immunoassays still exist by comparison with apoB concentrations derived from NMR and vertical autoprofile ultracentrifugation in subjects with hypertriglyceridemia *(87)*. Uniform calibration has reduced between-laboratory variability (CV) of apoB immunoassays from >19% to approximately 7% to 9% *(88)*. In the EQA program in the Netherlands, 28% of participating laboratories exceeded the NCEP bias recommendation for apoB (≤6%), whereas 68% exceeded the recommendation for LDLC (≤4%) *(88)*. Notwithstanding the availability of internationally recognized reference systems, more efforts from in vitro-diagnostic industry and laboratory professionals have the potential to further improve apoB standardization and precision to approximately 2% to 4% *(88)*. Far higher than the maximum allowed TG concentration of 4.5 mmol/L (400 mg/dL) for cLDLC, most turbidimetric and nephelometric apoB100 immunoassays allow TG up to at least 10 mmol/L (880 mg/dL) without interference; a TG concentration above this limit in nonfasting blood samples is seen in only approximately 0.1% of individuals in the general population *(24)*.

More recently, LC-MS/MS-based quantification of apolipoproteins has been introduced *(89, 90)*. Automated mass spectrometry enables precise measurements of apoB100 (total CV, 2.5%–4%) that are interchangeable with immunoturbidimetric assays in both normotriglyceridemic and hypertriglyceridemic sera with TG ≤20 mmol/L (1770 mg/dL) *(90)*. However, the clinical applicability and throughput of these LC-MS/MS assays are limited by the complexity and processing time of the sample preparation that is typically performed before protein measurement. With improvements in the work flow using automation and shorter chromatographic run times, LC-MS/MS protein assays may be translated to large clinical studies and eventually to regular clinical laboratory operations. Another advantage of LC-MS/MS is that it enables the simultaneous (multiplexed) measurement of multiple apolipoproteins in a single run of the assay, thus making it possible to achieve a complete apolipoprotein profile in the patient. In the case of VLDL-associated apolipoproteins apoC-I, apoC-II, apoC-III, and apoE, standardized clinical immunoassays are generally not available, which impedes discovery of pathophysiological clues (e.g., apoC-II deficiencies or apoC-III increases) for adequate diagnosis and management of dyslipidemia. Mass spectrometry-based proteomics revealed strong associations of VLDL-associated apolipoproteins with incident CVD in the prospective Bruneck Study population, which supports the concept of targeting TG-rich lipoproteins to reduce risk of CVD *(91)*.

The use of traceable serum-based calibrators will improve interlaboratory reproducibility of LC-MS/MS methods and may contribute to a more rapid transition of biomarker discovery to clinical utility to identify personalized treatment opportunities for dyslipidemia in CVD patients. For this purpose, the IFCC Scientific Division established a working group in 2017 to achieve standardization of a panel of the clinically relevant serum apolipoproteins A-I, B, C-I, C-II, C-III, E, and apo(a), and to develop an LC-MS/MS-based reference measurement system for the aforementioned analytes that are unaffected by genetic variants, posttranslational modifications, and other factors.

#### PREANALYTICAL ADVANTAGES OF apoB

In addition to analytical variability, biological within-subject (intraindividual) variability should be considered. The average biological variability (intraindividual CV) of apoB is 6% to 7% (range, 3%–12%), making repeated measurements of apoB in the single patient more reliable than calculation of non-HDLC because the latter includes the biological variations of both TC (6% CV, range 2%–12%) and HDLC (7% CV, range 2%–14%) *(92)*. The intraindividual CV of TG (average, 28%) is a major contributor to the overall variability of cLDLC and ranges widely to as high as 75% *(91)*. This presents a major advantage when testing apoB or non-HDLC, which are not affected by biological variation in TG.

Similar to non-HDLC, fasting is never required for apoB measurement. Although each chylomicron and chylomicron remnant particle contains 1 molecule of apoB48, this does not present a problem in the apoB

immunoassay. Except for type III hyperlipoproteinemia (dysbetalipoproteinemia), there are so few chylomicron and chylomicron remnant particles, even compared with VLDL particles, that they do not appreciably influence total apoB concentrations *(13, 36)*. Even at peak postprandial concentrations, the number of chylomicron–apoB48 particles in healthy individuals is usually <1% and the number of VLDL–apoB100 particles is <10% of the number of LDL–apoB100 particles *(36)*. Thus, even if the number of chylomicrons increases 5- to 10-fold, no substantial changes will occur in total apoB concentration. In contrast, the contribution of Lp(a)–apoB100 to apoB concentration can be substantial (>10%) among individuals with very high Lp(a) >100 mg/dL *(93)*. Considering that apoB100 contributes approximately 16% of Lp(a) mass, Lp(a)-corrected apoB concentration can be estimated as apoB (mg/dL) − [Lp(a) (mg/dL) × 0.16] *(93)*.

*Consensus-based recommendation*. Non-HDLC and apoB tests are more accurate than dLDLC and cLDLC, especially for measurements in samples that are hypertriglyceridemic, nonfasting, or at low LDLC concentrations. Non-HDLC offers the advantage of adding Remnant-C to LDLC, independent of TG variability (CBR6), but is still compromised by nonspecificity bias of dHDLC used in the calculation. ApoB has the potential to meet analytical performance criteria in terms of accuracy, standardization, availability on common laboratory instruments, and relatively low cost, and can be used to estimate numbers (concentrations) of LDLP plus remnant-P (VLDLP) and Lp(a) particle numbers, but not size (CBR7) *(38)*. Multiplex LC-MS/MS apolipoprotein profiles or NMR-based LDLP and VLDLP subclass numbers and size are promising approaches for use in personalized (precision) medicine for cardiovascular risk management but still need standardization if laboratory data from several epidemiological studies and clinical trials are pooled for evaluation of clinical performance of the tests. Furthermore, wide accessibility is critical to enable clinical use.

## Should Non-HDLC or apoB Replace LDLC Tests?

### OBSERVATIONAL STUDIES IN GENERAL POPULATIONS

Studies have reported inconsistent findings as to whether non-HDLC or apoB identifies CVD risk more effectively than traditional LDLC testing. A metaanalysis of 12 epidemiological studies including 233 455 individuals concluded that apoB, with an RR of 1.43 (95% CI, 1.35–1.51) per 1-SD increment, was superior to non-HDLC (1.34; 1.24–1.44) and LDLC (1.25; 1.18–1.33) in the association with future fatal or nonfatal CVD events ($P < 0.001$) *(94)*. In 10% of women and 20% of men in the Copenhagen City Heart Study (n = 9231) included in this metaanalysis, apoB predicted a higher risk than cLDLC-related risk for developing CHD ($P < 0.01$); these individuals had higher TG and an optimal cLDLC concentration *(95)*. However, in 4679 MESA study participants, associations of lipoprotein particle measures (i.e., apoB and LDLP) with CHD were attenuated after adjustment for standard lipid variables, indicating that their measurement does not detect risk that is unaccounted for by the standard lipid panel *(96)*.

A large-scale metaanalysis performed by the Emerging Risk Factors Collaboration group on data of 302 430 patients (involving 12 785 CHD cases) from 68 prospective studies showed that adjusted HRs for CHD risk for 1-SD higher values were 1.50 (95% CI, 1.39–1.61) for non-HDLC and 0.99 (0.94–1.05) for TG after adjustment for non-HDLC and HDLC *(97)*. For a subset of 44 234 participants from 8 studies with measured dLDLC, HRs were similar for non-HDLC and dLDLC, 1.42 (1.06–1.91) and 1.38 (1.09–1.73), respectively. HRs were at least as strong in nonfasting participants than in those who did fast *(97)*. The same group performed another metaanalysis of 26 observational studies (139 581 participants, 12 234 events) that also had information on apolipoproteins and found similar prognostic values: HRs per 1-SD increase for apoB and non-HDLC were 1.24 (1.19–1.29) and 1.27 (1.22–1.33), respectively *(98)*. Replacement of information on TC with various lipid parameters, including non-HDLC and apoB, did not improve CVD prediction *(98)*.

A systematic review of 9 relevant studies by the Laboratory Medicine Best Practices method indicated that addition of apoB to standard risk factors (RR = 1.31; 95% CI, 1.22–1.40) resulted in significant improvement in long-term CVD risk assessment *(99)*; however, because there were an insufficient number of studies, no conclusion was made for the effectiveness of non-HDLC in predicting CVD events *(99)*.

### INTERVENTIONAL STUDIES

On-treatment apoB adds prognostic information to LDLC and even to non-HDLC in some but not all primary prevention and secondary prevention trials. A metaanalysis of on-treatment data from 38 153 participants in 8 randomized controlled statin trials revealed adjusted HRs per 1-SD higher of 1.13 (95% CI, 1.10–1.17) for LDLC, 1.16 (1.12–1.19) for non-HDLC, and 1.14 (1.11–1.18) for apoB *(100)*. All 3 were generally similar in magnitude of CVD residual risk, although only the small difference between LDLC and non-HDLC was statistically significant ($P = 0.002$), whereas the difference between LDLC and apoB was not significant *(100)*. Changes in non-HDLC also explained a larger proportion (64%) of the statin-induced atheroprotective effect than did LDLC (50%) or apoB (54%) *(100)*. It should

be noted that most of the studies included in the metaanalysis used heparin-$Mn^{2+}$ precipitation for HDLC measurement, which differs from direct assays mostly used nowadays; therefore, these results based on non-HDLC may not translate to current clinical laboratory practice.

A larger metaanalysis of 25 trials conducted in 131 134 patients including 12 on statin, 4 on fibrate, 5 on niacin, and 2 on simvastatin-ezetimibe, came to similar conclusions using Bayesian random-effect analysis: Non-HDLC was slightly superior to apoB for prediction of CHD (Bayes factor, 1.45) and CVD (Bayes factor, 2.07) *(101)*. Only the combination of apoB with non-HDLC or LDLC slightly improved CVD risk prediction (Bayes factor, 1.13). Combining the 25 trials, each 10-mg/dL decrease in apoB was associated with a 9% decrease in CHD, no decrease in stroke, and a 6% decrease in major CVD risk *(101)*. In the JUPITER trial in adults without diabetes or CVD, with baseline cLDLC <130 mg/dL (3.4 mmol/L) and C-reactive protein concentrations ≥2 mg/L, standardized HRs did not differ for on-statin cLDLC, non-HDLC, and apoB: 1.31 (95% CI, 1.09–1.56), 1.25 (1.04–1.50), and 1.27 (1.06–1.57), respectively *(102)*. Interestingly, in the JUPITER trial, baseline apoB, LDLP, VLDLP, Remnant-C, and non-HDLC were all associated with increased CVD risk, whereas cLDLC was not *(85)*.

In another metaanalysis, the mean CHD risk reduction per SD decrease was higher for apoB (24%; 95% CI, 19%–29%) compared with 20% (16%–24%) for LDLC and 20% (15%–25%) for non-HDLC across 7 major statin trials *(103)*. Within each trial, risk reduction per change in apoB averaged 22% (12%–31%) greater than changes in LDLC and 24% (22%–26%) greater than changes in non-HDLC ($P < 0.001$). In the same metaanalysis, 1-SD decreases to equivalent target concentrations of LDLC (−42%), non-HDLC (−41%), and apoB (−42%) would yield expected risk reductions of 30%, 32%, and 39%, respectively, suggesting that benefit would be greater if therapy were targeted to apoB rather than to LDLC or non-HDLC *(103)*.

*Consensus-based recommendation*. Metaanalyses among study populations suggest that the clinical performance of non-HDLC and apoB, although superior to LDLC in some studies, is, on average, comparable with LDLC to predict risk. However, in a subset of individuals in whom apoB or non-HDLC is high despite a normal or low LDLC, CVD risk tracks with apoB or non-HDLC (CBR8).

## Should Non-HDLC or apoB Be Used as "Add-on" Tests to LDLC?

Although there is high overall correlation and equivalent predictive power in large population-based studies and trials, data from concordance/discordance analyses reveal that the addition of non-HDLC or apoB to LDLC has the potential to improve risk prediction by identifying more high-risk individuals in a personalized approach. Discordances (1 normal, the other high) exist between LDLC and non-HDLC, LDLC and apoB, and non-HDLC and apoB, and may be present in up to 25% of the general population (Fig. 4).

### LDLC VS NON-HDLC

Despite the correlation between non-HDLC and either cLDLC or dLDLC measurements in study populations, it is evident that non-HDLC and LDLC do not consistently provide the same clinical information in individual patients. Among individuals with the same non-HDLC value, there can be a 40- to 60-mg/dL (1.0–1.6 mmol/L) difference in cLDLC, depending on the range of TG concentrations between 100 and 300 mg/dL (1.1–3.4 mmol/L) *(104)*. The correlation between non-HDLC and dLDLC significantly decreases with increasing TG concentrations: In a study of 1590 patients, the percentage of patients with non-HDLC concentration above the guideline-based cutpoint of 130 mg/dL (3.6 mmol/L), despite having dLDLC concentration on target at ≤100 mg/dL (2.6 mmol/L), increased significantly with increasing TG *(105)*. Replacing dLDLC with non-HDLC as a therapeutic target in hypertriglyceridemic patients almost doubled the number of patients requiring treatment *(105)*.

In a large database of lipid profiles of 1 310 440 patients, non-HDLC reclassified a significant proportion of patients to a higher guideline-based treatment category compared with cLDLC, especially at low LDLC in the treatment range and at TG ≥150 mg/dL (1.7 mmol/L) *(106)*. Of patients with LDLC <70 mg/dL (1.8 mmol/L), 15% had non-HDLC ≥100 mg/dL (2.6 mmol/L) and 22% had this value if TG concentrations were 150 to 199 mg/dL (1.7–2.3 mmol/L) concurrently *(106)*. Similarly, of patients with LDLC concentrations between 70 and 99 mg/dL (1.8 and 2.6 mmol/L), 12% had non-HDLC ≥130 mg/dL (3.4 mmol/L) and 17% had this value if TG concentrations were concurrently 150 to 199 mg/dL (1.7–2.3 mmol/L), and discordance between LDLC and non-HDLC percentiles increased at lower LDLC and higher TG concentrations *(106)*.

The potential clinical implications of discordant LDLC and non-HDLC are most evident at normal or low LDLC concentrations. Among apparently healthy individuals with cLDLC <100 mg/dL (2.6 mmol/L) in the EPIC-Norfolk prospective population study (n = 21 448), those with non-HDLC >130 mg/dL (3.4 mmol/L) had an HR for future CHD of 1.84 (95% CI, 1.12–3.04) compared with those with non-HDLC <130 mg/dL (3.4 mmol/L) *(107)*. The risk associated with a 1-SD increase of non-HDLC (HR = 1.54; 95%

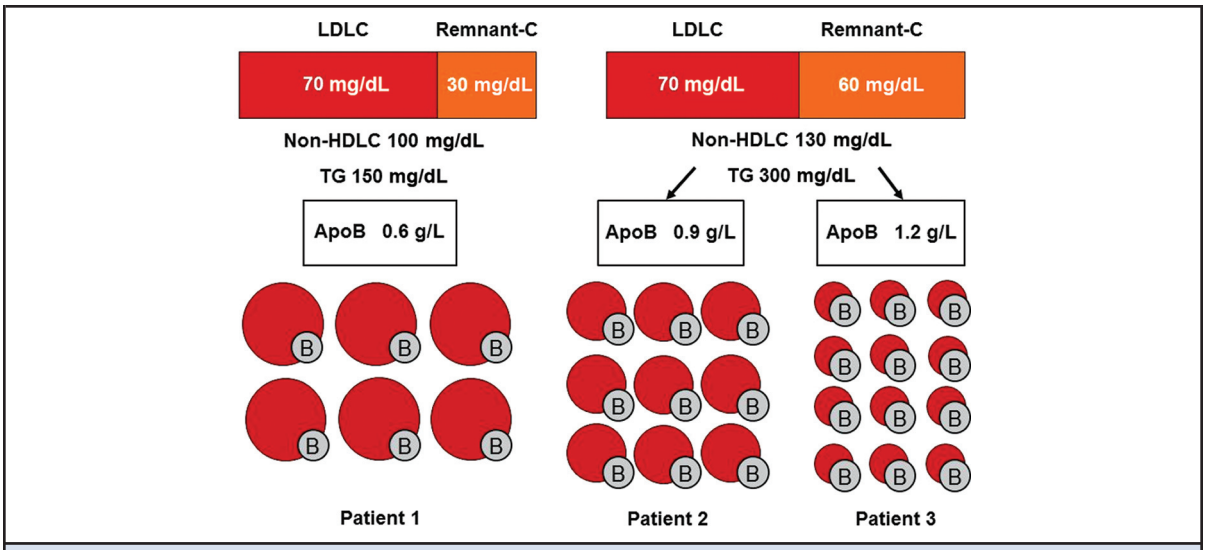


**Fig. 4. Relative LDL particle size and number and Remnant-C in 3 patients with identical low LDLC (70 mg/dL; 1.8 mmol/L) but with discordant non-HDLC and apoB with regard to desirable treatment targets for very high-risk patients.**

Patient 1 has all 3 targets at goal and normal numbers of (predominantly larger sized) LDL particles. Patient 2 with moderate hypertriglyceridemia has discordant non-HDLC above target (100 mg/dL; 2.6 mmol/L) because of increased Remnant-C. Patient 3 also has moderate hypertriglyceridemia and increased Remnant-C but concurrently higher apoB concentration than patient 2 because of a high number of small dense LDL particles not detected by standard LDLC measurement.

CI, 1.35–1.74) was higher than the risk associated with a 1-SD increase of cLDLC (HR = 1.22; 1.17–1.27) or TG (HR = 1.14; 1.09–1.19). The higher risk associated with increased non-HDLC was present at any category of LDLC concentrations, and particularly at low LDLC concentrations, whereas LDLC did not provide any additional risk to non-HDLC for CHD *(107)*. This notion implies that there is no reason to use non-HDLC as an "add-on" test: If concordant, it performs equally as well as LDLC. If discordant, it performs better. This implies that the net result is an overall better performance of non-HDLC compared with LDLC.

The same reflection is applicable to the metaanalysis by Boekholdt et al. of 8 statin trials *(100)*. In this analysis, HRs for major CVD risk were calculated for 4 categories of 38 153 treated patients based on whether they reached the LDLC target of 100 mg/dL (2.6 mmol/L) and the non-HDLC target of 130 mg/dL (3.4 mmol/L) *(100)*. Statin-treated patients reaching the non-HDLC target but not the LDLC target had an HR of 1.01 (95% CI, 0.92–1.12) compared with those reaching both targets. Patients reaching the LDLC target but not the non-HDLC target had an HR of 1.32 (1.17–1.50) *(100)*.

These data suggest that calculation of non-HDLC is at least equally good at predicting CHD as compared with measurement or calculation of LDLC in the overall population, and may be superior to LDLC if discordant in individuals with high TG because it includes VLDLC (= Remnant-C). The only concern is the selection of the cutoff value: It may be the more sensitive risk cutpoint for non-HDLC as compared with LDLC rather than the biomarker that makes the difference. Guideline-based non-HDLC cutpoints have been arbitrarily defined by consensus of expert groups and societies, based on the assumption that a normal VLDLC concentration exists when TG are <150 mg/dL (1.7 mmol/L), which is <30 mg/dL (0.8 mmol/L) as estimated by the Friedewald formula. Lowering non-HDLC cutpoints leads to upward reclassification of patients (if the goal is to reduce undertreatment), and higher cutpoints lead to downward reclassification (if the goal is to reduce overtreatment). Risk cutoff values need to be validated for diagnostic performance. For present purposes, the combination of non-HDLC with cLDLC data seems an appropriate strategy to guide therapy. This may compensate for the underestimation or overestimation of LDLC in terms of clinical decision-making, given the uncertainty of the measurement or calculation when LDLC approaches 70 mg/dL (1.8 mmol/L). Utilizing non-HDLC, at no additional expense, appears to be preferable to dLDLC when cLDLC is not available because of an invalid Friedewald equation (CBR9). However, the compromised accuracy of dHDLC assays in samples with hypertriglyceridemia reduces the benefit in reporting non-HDLC in some individuals *(48)*.

### LDLC VS apoB

The implication of discordant apoB vs LDLC is most evident in patients with atherogenic dyslipidemias, who present with "normal" concentrations of TC and LDLC—a profile that is especially prevalent among individuals with the metabolic syndrome or insulin resistance and in those taking medications, such as statins, that reduce LDLC more than apoB *(108)*. In 1522 individuals in the Insulin Resistance Atherosclerosis Study, increased apoB with normal cLDLC in each NCEP Adult Treatment Panel III risk category was more strongly associated with risk factors including abdominal obesity, dyslipidemia, hyperinsulinemia, and thrombosis than increased cLDLC with normal apoB *(109)*, consistent with the notion that cardiovascular risk is more directly related to the number of apoB-containing particles (reflected by apoB measurement) than to the cholesterol content of lipoproteins *(81)*. Therefore, risk may be underestimated on the basis of TC and LDLC alone in subjects with predominant small, cholesterol-depleted LDL particles, whereas increased apoB concentration helps identify these high-risk individuals who would have otherwise been overlooked because of their "optimal" LDLC, a situation estimated to apply to approximately 30% of the population *(108)*.

For example, when evaluating a patient with diabetes and an LDLC of 90 mg/dL (2.3 mmol/L), it is often concluded that they are at goal because their LDLC is <100 mg/dL (2.6 mmol/L) *(1)*. However, this level represents higher numbers of cholesterol-poor LDL particles and puts the patient at higher risk compared with a person with an LDLC of 90 mg/dL (2.3 mmol/L) but without atherogenic dyslipidemia and associated insulin resistance. Another problem is that these patients tend to have increased levels of TG-rich lipoproteins, and by the Friedewald equation, their LDLC will be underestimated *(57, 58)*.

The discordance analysis of the Women's Health Study *(110)* represents a comprehensive comparison of atherogenic lipoprotein markers. Despite significant LDLC correlations with non-HDLC, apoB, and LDLP observed in the 27 533 study participants, prevalence of LDLC discordance as defined by median cutpoints was 12%, 19%, and 24% for non-HDLC, apoB, and LDLP, respectively. Among women with dLDLC less than the median (121 mg/dL; 3.1 mmol/L), CHD risk was underestimated 3-fold for those with discordant (the median or more) non-HDLC (HR = 2.92; 95% CI, 2.33–3.67), apoB (HR = 2.48; 2.01–3.07), or LDLP (HR = 2.32; 1.88–2.85) compared with women with concordant concentrations; these individuals had increased TG, low HDLC, and smaller LDL particles that were cholesterol-depleted *(110)*. Conversely, among women with dLDLC equal to the median or more, risk was overestimated 3-fold for those with discordant (less than the median) non-HDLC (HR = 0.40; 0.29–0.57), apoB (HR = 0.34; 0.26–0.46), or LDL-P (HR = 0.42; 0.33–0.53); these individuals had increased LDLC because their LDL particles were larger and more cholesterol-enriched, despite having fewer overall numbers of LDLP or apoB *(110)*.

Additional analysis with cLDLC instead of dLDLC revealed that, among women with cLDLC below median, HRs for discordant apoB or LDLP were increased *(110)*. In a recent discordance analysis among 2794 young adults, high apoB and low cLDLC or non-HDLC discordance was associated with higher odds of developing coronary artery calcium in midlife: adjusted odds ratios [OR (95% CI)] were 1.55 (1.10–2.18) and 1.45 (1.01–2.09), respectively *(111)*. Discordance analyses of cLDLC vs LDLP in the Framingham and MESA studies both favored LDLP over cLDLC among discordant individuals *(75, 79)*.

These data suggest that for most individuals with concordant values of LDLC and an alternative measure (non-HDLC, apoB, or LDLP), the clinical performance of these measures is similar. However, among the subgroup of individuals (up to 25% of the Women's Health Study population) with discordance of LDLC with alternative measure, risk may be overestimated or underestimated when one relies on LDLC alone *(110)*. If apoB performs either equally well (if concordant) or better (if discordant) than LDLC, the 2-step procedure is redundant, and apoB could be the single test rather than an add-on test.

### NON-HDLC VS apoB

Non-HDLC is not a clinically accurate surrogate for apoB because the 2 markers represent different measures. Non-HDLC and apoB are highly correlated in large population-based studies but only moderately concordant between individuals *(108)*. At any non-HDLC value, there is considerable variation in apoB concentrations ranging from far above to far below the desirable values *(112)*. Such discordance between non-HDLC and apoB is even more pronounced in patients with hypertriglyceridemia, particularly familial combined hyperlipidemia, which is characterized by hyper-apoB *(37)*, and dysbetalipoproteinemia owing to the apoE2/2 genotype, which typically presents with low apoB concentration (and positively discordant non-HDLC with regard to apoB) because the $\beta$-VLDL remnants are not processed to LDL particles *(37)*. None of those dyslipidemias can be characterized with non-HDLC *(112)*.

Beyond LDLC, non-HDLC estimates the impact of 2 closely related metabolic biomarkers of atherogenic dyslipidemia, namely, (VLDL) remnant cholesterol and LDL particle number, but it does not accurately measure the latter. If LDL particles are cholesterol-depleted, as observed in atherogenic dyslipidemia, LDLC and non-

HDLC will underestimate the risk because of high LDL particle numbers (and consequently, discordant high apoB concentration) *(112)*. Also, the mass of cholesterol within VLDL particles is highly variable. VLDL– and IDL–apoB100 make up a relatively small fraction (<10%) of total plasma apoB, whereas VLDLC can easily range from 10% to 25% or more of non-HDLC, with the result that there is much greater variance in VLDLC as a percentage of non-HDLC than there is of VLDL–apoB100 as a percentage of total apoB *(108)*.

Discordance analysis of apoB vs non-HDLC in the INTERHEART case–control study demonstrated that apoB is not equivalent to non-HDLC in predicting CVD risk *(113)*. The OR for cases to controls with a "non-HDLC>apoB phenotype" (cholesterol-enriched apoB particles) was 0.72 (95% CI, 0.67–0.77), indicating risk was less than the reference concordant group, whereas the OR for the "non-HDLC<apoB phenotype" (cholesterol-depleted apoB particles) was 1.58 (1.38–1.58), indicating risk was significantly greater than the concordant group *(113)*.

In the Insulin Resistance Atherosclerosis Study (n = 1522), despite lower LDLC, the hyper-apoB group with normal non-HDLC had increased risk indicated by greater waist circumference, hyperinsulinemia, and lower insulin sensitivity *(114)*. ApoB was more closely associated with central adiposity, insulin resistance, and inflammation than non-HDLC, suggesting that apoB is a better risk parameter than non-HDLC for identifying a subgroup of individuals with or without metabolic syndrome with increased cardiovascular risk *(114)*. Once apoB concentrations are increased, then CHD risk can be considered high and non-HDLC yields negligible additional risk prediction.

Among 18 225 men in the Health Professionals Follow-up Study, non-HDLC and apoB were both strong predictors of CHD, more so than LDLC *(115)*. In this male cohort, the RR of CHD (top vs bottom quintile) was 3.01 (95% CI, 1.81–5.00) for apoB, 2.76 (1.66–4.58) for non-HDLC, and 1.81 (1.12–2.93) for dLDLC. When non-HDLC and dLDLC were mutually adjusted, only non-HDLC was predictive of CHD. When non-HDLC and apoB were mutually adjusted, only apoB was predictive. Within each tertile of non-HDLC, the risk of CHD increased with increasing tertiles of apoB, whereas within each tertile of apoB, the risk did not increase by tertiles of non-HDLC. Values for TG concentrations added significant information to non-HDLC but not to apoB for CHD risk prediction *(115)*.

In the Women's Health Study (n = 27 533), discordance among apoB, LDLP, and non-HDLC occurred in up to 20% of women *(116)*. Women with discordant high particle concentration measured by apoB and LDLP were more likely to have metabolic syndrome and diabetes. Women with high particle concentration relative to non-HDLC had increased CHD risk: Age-adjusted HR was 1.77 (95% CI, 1.56–2.00) for apoB and 1.70 (1.50–1.92) for LDLP. After adjustment for clinical risk factors including metabolic syndrome, these risks attenuated to 1.22 (1.07–1.39) for apoB and 1.13 (0.99–1.29) for LDLP. Importantly, most of these individuals with discordant high apoB or LDLP would be missed (deemed very low risk) by global risk algorithms *(2)*. Discordant low apoB or LDLP relative to non-HDLC was not associated with lower risk *(116)*.

The relative merits of apoB vs non-HDLC have been a point of ongoing debate and controversy. Both offer the practical benefits of assessment independent of the prandial state. Although apoB measurement comes at an extra cost, the findings from discordance analyses support our consensus that in certain patients the number of atherogenic lipoprotein particles measured by apoB (or LDLP) is more predictive of development of CHD than the cholesterol carried by these particles, measured by non-HDLC. It should be noted, however, that apoB and LDLP measurements may not always be equivalent in their ability to predict CVD risk. A metaanalysis in 2013 including 25 clinical studies revealed 21% discordance of apoB immunoassays and NMR-based LDLP measurements in their associations between diverse clinical outcomes, and the strength of association (as indicated by OR, RR, and HR) was more often higher for LDLP than it was for apoB *(38)*.

#### ON-TREATMENT DISCORDANCES

The reduction of LDLC, non-HDLC, and apoB concentrations achieved with statin therapy displays large intra-individual variation, even at very low LDLC <70 mg/dL (1.8 mmol/L), as observed in a metaanalysis of 8 statin trials *(117)*. Statins lower LDLC and non-HDLC significantly more than they lower apoB or LDLP *(118)*. This is because statins lower larger cholesterol-rich LDL particles proportionately more than they do for smaller cholesterol-poor LDL particles *(119)*. A metaanalysis of 17 000 patients from 11 statin trials showed that LDLC was reduced by 43%, non-HDLC by 39%, and apoB by 33% *(118)*. This necessarily results in an on-treatment LDLP concentration that, on average, is higher than would be anticipated from the concurrent LDLC or non-HDLC. However, high Lp(a) concentrations in some patients will also help explain why apoB and LDLP are reduced less than LDLC or non-HDLC. The average on-treatment LDLC is reduced to the 20th percentile and non-HDLC to the 29th percentile, but apoB and LDLP decrease only to the 55th and 51st percentile of the population, respectively *(120)*. This discordance points to substantial residual risk in many statin-treated patients and the opportunity for further benefit of LDL-lowering therapy that is lost if apoB or LDLP is not measured. In the AFCAPS/TexCAPS trial, on-treatment apoB main-

tained its strong risk relationship with major coronary events after 1 year of lovastatin therapy, whereas LDLC lost statistical significance *(121)*. Residual risk is also associated with on-treatment non-HDLC and more prominently with concentrations of smaller VLDLP, which may represent remnants of TG-rich lipoproteins that are insufficiently reduced by LDL-targeted statin dosages *(85, 119, 122)*.

A large proportion of treated patients achieving their LDLC and even non-HDLC goals fail to meet their apoB target without more aggressive therapy *(123–125)*. In the US National Health and Nutrition Examination Survey 2009–2010, 64% of statin-treated adults were at goal for LDLC and 63% were at goal for non-HDLC, but only 52% were at goal for apoB *(126)*. Among those at goal for non-HDLC, 50% of those with CHD and 33% of other high-risk adults (including patients with diabetes and chronic kidney disease) were not at apoB goal *(126)*. The presence of atherogenic dyslipidemia is associated with failure to meet all 3 targets of LDLC <70 mg/dL (1.8 mmol/L), non-HDLC <100 mg/dL (2.6 mmol/L), and apoB <0.8 g/L, such a nonachievement being found in a large proportion (one-third) of very high risk type 2 diabetes patients with on-statin LDLC <70 mg/dL (1.8 mmol/L) *(127)*.

Discordances persist with increasing intensity of statin treatment. Combined analysis of 2 randomized secondary prevention trials with statins, TNT and IDEAL, showed that non-HDLC was reduced to the 30th percentile and apoB was reduced only to the 60th percentile of the low-intensity statin group *(128)*. In the high-intensity statin group, non-HDLC was reduced to the 5th percentile, whereas apoB was reduced to only the 30th percentile *(128)*. In the JUPITER trial of high-intensity statin, 46% of treated patients showed an LDLC reduction ≥50%, 28% showed non-HDLC reduction ≥50%, and only 18% showed apoB reduction ≥50% *(7)*. It remains to be demonstrated whether intensifying treatment strategies to further reduce undesirable apoB concentrations would yield an improvement in outcomes. A smaller change in apoB concentrations may not necessarily need to translate into smaller risk reduction. If 30% apoB decrease confers the same risk reduction as 50% LDLC decrease, it will not be a superior target. To test this, one would need to compare 2 interventions that lower apoB and LDLC disproportionately and assess the population attributable risks associated with the changes, i.e., the number (or proportion) of CVD cases that would not occur in a population if the risk factor were further lowered. In the JUPITER trial, similar relationships between percent reduction and treatment efficacy were observed in clinical outcome analyses focusing on LDLC, non-HDLC, or apoB *(7)*.

Discordances in significant reductions in LDLC, non-HDLC, and apoB concentrations are also observed in anti-PCSK9 trials. Typically, PCSK9 inhibition causes average reductions of 60% to 65% in LDLC, 50% to 55% reductions in non-HDLC, and 45% to 50% reductions in apoB from baseline depending on PCSK9 monotherapy or addition to moderate- or high-intensity statin (±ezetimibe) therapies in patients with hypercholesterolemia *(129–132)*.

The reductions of LDLC, non-HDLC, and apoB obtained with anti-PCSK9 therapy are similar in patients with and without mixed hyperlipidemia, reflecting higher circulating concentrations of TG and remnant-like lipoproteins. Efficacy analysis of 3146 participants in 4 studies of evolocumab showed that the mean percentage change from baseline for patients with or without increased fasting TG >150 mg/dL (1.7 mmol/L) followed a similar pattern for LDLC (−67% and −65% vs placebo), non-HDLC (−53% and −54%), and apoB (−49% and −50%) *(133)*. A similarly high proportion of evolocumab-treated NCEP III high-risk patients with and without increased TG achieved the LDLC target of <70 mg/dL (1.8 mmol/L) (82% vs 81%, respectively) and <100 mg/dL (2.6 mmol/L) (92% vs 92%, respectively). However, significantly more patients without increased TG achieved the apoB target of <0.8 g/L than those with increased TG (93% vs 85%). Additionally, significantly more patients without increased TG achieved the non-HDLC target of <100 mg/dL (2.6 mmol/L) than those with increased TG (85% vs 77%), suggesting that PCSK9 inhibition less efficiently reduces Remnant-C than LDLC in patients with hypertriglyceridemia *(133)*.

*Consensus-based recommendation*. Discordance analysis of LDLC, non-HDLC, and apoB goals suggests opportunities to identify "hidden" CVD risk and to judge the adequacy of therapy. To reach the current proposed non-HDLC and apoB goals, novel therapies in addition to high-dose statins would inevitably be needed to further lower non-HDLC or apoB at very low concentrations of LDLC; however, the evidence base for this approach is still incomplete. The cutpoints for non-HDLC and apoB are arbitrarily defined by consensus cutoffs and need to be validated for diagnostic performance before they can be used as "add-on" tests to identify and treat additional persons at high risk. One would need to compare the reduction in CVD events achieved at each level of LDLC, non-HDLC, or apoB changes in the study data sets; otherwise, it is not clear whether the biomarker or the cutoff makes the difference.

For now, we recommend that every lipid profile report should add non-HDLC (CBR5). For those who determine TC, TG, and HDLC (and calculate cLDLC), this comes at no extra cost, as does calculated Remnant-C, which likewise can be reported free of charge. To improve patient comfort and compliance, there are advantages of this approach using

nonfasting blood samples *(19)*. When LDLC is unavailable owing to an invalid Friedewald equation (TG >400 mg/dL; 4.5 mmol/L), additional dLDLC measurement is not necessary, and non-HDLC calculation can be used instead of cLDLC to evaluate therapeutic response (CBR9).

## Which Is the Ideal Atherogenic Lipoprotein Test?

The traditional lipid profile of TC, TG, HDLC, and LDLC remains the primary approach for dyslipidemia diagnosis and CVD risk categorization. The position of LDLC is, however, challenged under treatment. The substantial residual risk that persists in LDLC-targeted therapies even at low LDLC <70 mg/dL (1.8 mmol/L) has fueled the debate about the cost-effectiveness of the clinical use of non-HDLC or apoB as an index of treatment efficacy *(134)*. Thus, although the lipid profile will remain essential for initial diagnosis and risk categorization, one might suggest that follow-up of lipid-lowering therapy could be simplified and expenses reduced by calculation of non-HDLC (by only 2 measurements of TC and HDLC) or single measurement of apoB, without the need to fast and without regard to TG *(134)*. Use of either non-HDLC or apoB adds an element of simplicity to guidelines by combining all "atherogenic lipoproteins" (apoB) or "atherogenic cholesterol" (non-HDLC) into a single marker. There are, however, significant barriers to replace LDLC by non-HDLC or apoB as a primary biomarker for management of dyslipidemia-related risk *(135)* (Table 6).

What makes a good marker of atherogenic lipoproteins? It should be easily measured in a nonfasting sample with a low cost and accurate procedure, and should not increase healthcare expenses to identify patients in whom CVD prevention strategies or therapies will be cost-effective. For monitoring purpose, the test should not only be able to predict clinically significant events but also respond to changes in the condition or treatment. None of the present tests in question has been completely validated according to key criteria to become a medically useful test, as defined by the EFLM Working Group for Test Evaluation *(136)*. Although the analytical performance of apoB measurements is superior to measurements or calculations of LDLC and even non-HDLC, and the clinical performance of apoB testing may be of added benefit over standard lipid assessment in selected patients, more definitive evidence in rigorously performed outcome studies is needed to confirm that apoB testing will effectively lead to reduced CVD events (clinical effectiveness) and health-economic costs (cost-effectiveness) compared with LDLC-targeted strategies (Table 7). These key test components should be validated in the setting of novel therapies aiming at very low LDLC targets <50 mg/dL (1.3 mmol/L).

A well-defined unmet clinical need should act as the architect for biomarker test development *(137)*. Desirable clinical performance specifications should be predefined (for which rate of misclassification and/or mistreatment is acceptable) for each test indication because they determine the analytical performance specifications *(136)*. Clinical studies can then be designed to evaluate clinical and cost-effectiveness of the test to improve health and economic outcomes *(138)*. In randomized controlled trials of diagnostic investigations, patients are randomized to receive the new test or the standard test, and the impact on clinical decision for detecting or treating more patients at high risk (i.e., not detected or treated when using the standard test) is assessed *(138)*. Biomarker tests per se do not bear directly on health outcomes. Investigations guide the decisions and actions of clinicians and patients, and it is these that directly impact on health outcomes. New tests can improve outcomes by optimizing the selection of treatment, through more accurate risk classification or prediction of CVD or CVD outcomes (i.e., mortality, morbidity, and complication rates), or by offering other patient benefits (i.e., more appropriate treatment options, the impact of medical care on patient well-being, quality of life) *(138)*. Indeed, the emerging approach of precision medicine (or personalized medicine) requires novel biomarkers for targeted therapies, tailored to the individual patient's condition. Operational outcomes can include time to test results, time to treatment, and other operational advantages such as nonfasting blood sampling *(19)*. Economic outcomes include the cost of the test and all downstream consequences on healthcare, such as the financial benefits of morbidity avoided, quality-adjusted life-years gained, or reductions in length of hospital stay *(136, 138)*.

The use of non-HDLC, apoB, or LDLP would be cost-effective if these tests provide information to guide therapy to reduce the risk (and cost) of CVD events more than standard therapy guided by LDLC alone. In 1 meta-analysis, it was estimated that a non-HDLC targeting strategy would prevent approximately 300 000 more CVD events than an LDLC strategy, whereas an apoB strategy would prevent approximately 500 000 more events than a non-HDLC strategy in the US adult population over a 10-year statin treatment period *(94)*. Recent studies on the cost-effectiveness of LDLP-guided statin therapy, either alone or in combination with LDLC, showed potential cost-saving because of reductions in medical expenses from fewer CVD events, and this approach was estimated to increase quality-adjusted life-years compared with standard LDLC-alone treatment *(139–141)*. LDLP-guided therapy was expected to result in LDLC reductions that exceed those guided by LDLC alone because of the use of higher doses of statins to achieve LDLP goals *(139–141)*. With the availability of inexpensive generic statins, the cost-effectiveness of

**Table 6. Strengths, weaknesses, opportunities, and threats (SWOT analysis) of LDLC, non-HDLC, apoB, and LDLP.**

| LDLC | Non-HDLC | apoB | LDLP |
|---|---|---|---|
| Strengths | | | |
| Widely available laboratory assays for dLDLC measurement or cLDLC calculation<br>Clinical performance: strong evidence-based, causal risk factor<br>Clinical effectiveness: LDLC-targeted treatment reduces risk | Not dependent on TG variability<br>Can always be calculated in the nonfasting state<br>Includes remnant cholesterol | Unequivocally defined protein<br>International standard available<br>Analytical performance: fasting and nonfasting test accuracy<br>Fully automated test; can be easily integrated in widely available laboratory autoanalyzer platforms<br>Diagnostic performance for characterization of complex, mixed dyslipidemias | Measures LDL particle number and size<br>Often provides simultaneous quantification of VLDL, IDL, LDL, HDL particle numbers and size, and in some cases other metabolic or inflammatory biomarkers, in 1 single run of the assay |
| Weaknesses | | | |
| dLDLC measurement errors in dyslipidemic samples and samples from diseased patients<br>cLDLC influenced by HDLC measurement errors<br>cLDLC influenced by postprandial TG variability; invalid at TG >400 mg/dL (4.5 mmol/L)<br>cLDLC and dLDLC influenced by increased Lp(a)<br>Manufacturer-dependent nonspecificity bias compared with reference method | HDLC measurement errors in dyslipidemic samples<br>Different assays for HDLC affect between-laboratory measurement variability<br>Arbitrary risk cutpoints and treatment targets, not validated for clinical performance | Controversial clinical performance vs LDLC and non-HDLC for risk estimation in general populations due to high correlations with TC and LDLC<br>Arbitrary risk cutpoints and treatment targets<br>No consistent evidence of significant population health-economic benefit of intensified or novel therapies aiming to reduce apoB | No standardization<br>Inconsistent agreement between different methods<br>Expensive and/or not widely available technology<br>Not easily integrated in high-throughput laboratory operations |
| Opportunities | | | |
| Novel therapies appear to safely reduce LDLC to very low concentrations (long-term follow-up is limited)<br>Novel therapies confirm the LDL hypothesis "the lower the better"<br>Increasing awareness and demand to screen for familial hypercholesterolemia<br>Improved cLDLC equations published | Healthcare budget: no additional cost<br>Increasing use of nonfasting blood samples<br>Additional risk reclassification by discordance analysis vs LDLC<br>Clinical utility particularly when LDLC is low or TGs are increased (needs to be validated) | Increasing prevalence of obesity, diabetes, and atherogenic dyslipidemias with increased apoB<br>Additional risk reclassification by discordance analysis vs LDLC (and in some studies vs non-HDLC)<br>Clinical utility particularly when LDLC is low or TGs are increased (needs to be validated)<br>Emerging mass spectrometry applications in the clinical laboratories, enabling more precise and multiplex apolipoprotein tests | Increasing prevalence of atherogenic dyslipidemias with increased LDLP and VLDLP<br>Additional risk reclassification by discordance analysis vs LDLC<br>Emerging evidence of cost-effectiveness by metaanalyses<br>Automated NMR assays are being developed |
| Threats | | | |
| Residual on-treatment CVD risk, not explained by LDLC<br>Increasing prevalence of obesity, diabetes, and atherogenic dyslipidemia, in which LDLC is less predictive<br>Uncertain analytical performance at very low concentrations obtained with novel therapies | No conceptual understanding by most practicing physicians and patients | Potential increases in estimated healthcare costs<br>Poor goal attainment rates on statin therapies, including high-dose statins<br>No conceptual understanding by most practicing physicians and patients | Assays not widely available in clinical laboratories<br>No conceptual understanding by most practicing physicians and patients |

intensifying pharmacological intervention aiming to reduce non-HDLC, apoB, or LDLP will be enhanced.

*Consensus-based recommendation*. The ideal biomarker(s) of atherogenic lipoproteins should be validated for analytical performance, clinical performance, clinical effectiveness, and cost-effectiveness to justify its/their use in clinical practice in different well-defined settings: screening, dyslipidemia diagnosis, treatment choice, and follow-up. For now and until these issues are clarified, non-HDLC is the best choice, as it incurs no additional expense to the patient or health system. A cost-efficient approach is to measure 3 markers (TC, TG, HDLC) and to calculate cLDLC and non-HDLC. Clinical laboratories should proactively calculate and report non-HDLC together with cLDLC on all lipid profiles (CBR5) *(142)*.

## Conclusion

The principal aim of laboratory medicine is to provide a high-quality service that delivers unequivocal test information and improves patient outcomes across the continuum of care. Laboratory medicine is a key component of models of care for all types of lipid disorders *(142)*. We

**Table 7. Contemporary evidence for the medical use of LDLC, non-HDLC, apoB, and LDLP as biomarkers based on essential criteria.**

| Test characteristics | LDLC | Non-HDLC | ApoB | LDLP |
|---|---|---|---|---|
| Analytical performance[a] | | | | |
| Precise assays | Yes | Yes | Yes | Yes |
| Accurate assays (method independency) | No | No | Yes | No |
| Nonfasting measurement possible | With TG <4.5 mmol/L | Yes | Yes | Yes |
| Widely accessible assays | Yes | Yes | Yes | No |
| High throughput and rapid turnaround | Yes | Yes | Yes | Yes |
| Reasonable operational costs | Yes | No extra cost | Yes | Not yet |
| Clinical performance[b] | | | | |
| Robust associations with incident CVD? | Yes | Yes | Yes | Yes |
| Novel information beyond existing markers? | (Reference) | Yes | Yes | Yes |
| Validated decision limits? | No | No | No | No |
| Clinical effectiveness[c] | | | | |
| Superiority to existing tests? | (Reference) | Probably | Probably | Probably |
| Modifiable risk association (treatment target)? | Yes | Yes | Yes | Yes |
| Biomarker-guided treatment reduces CVD risk? | Yes | Probably | Probably | Unknown |
| Cost-effectiveness[d] | | | | |
| Biomarker-guided treatment saves healthcare costs? | Yes | Unknown | Unknown | Unknown |

[a] Analytical validity: ability of the test to conform to predefined quality specifications to measure the marker of interest.
[b] Diagnostic or prognostic accuracy: ability of the test to consistently detect patients with a high risk for developing CVD.
[c] Clinical utility: ability of the test to improve health outcomes of the patient under standard clinical care.
[d] Health-economic advantage of introducing the test in medical practice (value for money).

have provided CBRs for improving the use of the lipid profile to assess CVD risk conferred by atherogenic lipoproteins (Table 1). EQA programs, which structurally evaluate laboratory test performance, demonstrate that improvements are necessary *(47, 48)*. New therapies demand accuracy of dyslipidemia testing at very low LDLC concentration ranges. Incorrect diagnosis and mismanagement of treatment, which are based on laboratory measures, are both costly to society and harmful to the patient. Even modest improvements in laboratory testing to predict risk of a disease as common as CVD translate into thousands of people who may be treated more appropriately and could benefit. Therefore, it is essential that diagnostic test results produced in laboratories worldwide are comparable across different medical facilities, enabling unequivocal diagnosis, treatment, and monitoring of patients. This prerequisite is increasingly recognized by CE marking and Food and Drug Administration regulatory bodies in their patient-focused risk assessment during premarket approval of in vitro diagnostic medical devices *(136)*. To that end, global standardization and harmonization of lipid tests should be the key for sustainable patient care with universal application of desirable values and decision cutpoints, as well as preparing for future exchange and interoperability of electronic health records *(142, 143)*.

Some questions remain unanswered and need further investigation (Table 8). In hypertriglyceridemic patients, several analytical and clinical performance limitations make LDLC an unreliable marker of atherogenic dyslipidemia; hence, there is a possibility of undertreatment. ApoB has the potential to be standardized across laboratories, which is not possible for LDLC and non-HDLC with the current CDC standardization program. Non-HDLC and apoB have the potential to address clinical needs unmet with LDLC testing, and they can always be used in nonfasting samples. To combine the strengths and to compensate for the weaknesses of the different markers, currently it seems the best strategy to consider is the use of LDLC, non-HDLC, and apoB as complementary rather than competitive markers of CVD risk and therapeutic response. Nonfasting non-HDLC can be used to assess the incremental risk of remnant lipoproteins, and apoB can detect increased LDLP often missed with LDLC alone. This suggestion is consistent with several guidelines and consensus documents that propose to use non-HDLC or apoB as a secondary treatment target in the treatment of high-risk or very high-risk patients

**Table 8 Unanswered questions and recommendations for future research.**

| | |
|---|---|
| | Unanswered questions (Q) |
| Q1 | Which (measured or calculated) LDLC method provides the best proxy for the gold standard measurement (ultracentrifugation) to ensure adequate translation of LDLC test results to the atherogenic risk of "true" LDL particles? |
| Q2 | Are guideline-recommended LDLC and non-HDLC goals used in clinical practice (based on Friedewald-cLDLC and non-HDLC calculations in study populations using the older HDLC precipitation methods) still applicable to LDLC and non-HDLC values as currently reported (i.e., as measured or calculated using contemporary assays)? |
| Q3 | Can follow-up of lipid-lowering therapies be simplified using a single-marker, nonfasting apoB assay to replace the traditional lipid profile to obtain at least equal, or even better, assessment of dyslipidemia-related risk? |
| Q4 | Can multimarker test panels of apolipoproteins and/or lipoprotein particles and subclasses provide precision medicine-individualized assessment of dyslipidemia-related risk in the patient? |
| | Future research (FR) action points to address the above questions |
| FR1 | Assay kit manufacturers and standardization programs should be closely linked to optimize the assays, or select only 1 best possible chemical procedure and type of assay, to ensure accurate and unequivocal measurements of LDLC and HDLC, including low concentrations, in the fasting and nonfasting state in both normotriglyceridemic and hypertriglyceridemic blood samples (Q1) |
| FR2 | New generation assays should be validated for clinical performance to discriminate between high- and low-risk patients, e.g., by reporting risk reclassification in observational studies using the net reclassification index (Q2) |
| FR3 | Clinical and cost-effectiveness studies should be designed to determine the health outcomes and economic implications of apoB-based treatment of high-risk patients (or subgroups of patients), relative to the standard care of LDLC management (Q3) |
| FR4 | NMR-based lipoprotein particle/subclass numbers and size measurement procedures should be standardized to ensure direct comparability of NMR data for the development of universally applicable guidelines and decision cutpoints (Q4) |
| FR5 | Multiplex LC-MS/MS apolipoprotein profiles (including apoA-I, B, C-I, C-II, C-III, and E) rather than single apoB tests should be further developed, standardized, and evaluated for the creation of a personalized clinical pathway: clarification of unmet clinical need(s) and diagnostic accuracy to identify the dyslipidemias of interest (Q4) |

with mild to moderate hypertriglyceridemia, defined as 2 to 10 mmol/L (175–880 mg/dL) (CBR8) *(14)*. If the primary target LDLC is at goal but non-HDLC or apoB is still high, attainment of all 3 targets will require intensified lipid-lowering therapy, lifestyle (re)inforcement, and/or additional TG-lowering drugs (e.g., fibrate or omega-3 fatty acids) *(1)*.

Which of the biomarkers to choose as secondary target: non-HDLC or apoB? To help train clinicians and patients in gaining an understanding of the concept and advantage of apoB (or LDLP) testing beyond LDLC, we need to gently transition into this process. To that end, as a first step we should move to include non-HDLC into the report of every lipid profile. Diabetes and abdominal obesity, the disorders that underlie the clinical expression of polygenic hypertriglyceridemia, are attaining epidemic proportions *(14)*; hence, apoB and LDLP tests will likely become more and more useful in the future. This underscores the need to standardize and harmonize innovative biomarkers such as NMR-based lipoprotein particles or LC-MS/MS proteomics, which have the potential to become innovative medical tests. To be prepared for the future of personalized medicine, obviously the most important challenge of apoB is to validate its use as part of a multiplex MS/MS-based apolipoprotein profile rather than a single marker test. These technologies provide complementary clinical information regarding the complex molecular basis of polygenic hypertriglyceridemias and, as such, will contribute by identifying better and individualized treatment options for dyslipidemic patients.

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# RNA-targeted therapeutics for lipid disorders

*Sotirios Tsimikas*

**Purpose of review**
To summarize recent developments in the field of RNA-directed therapeutics targeting lipid disorders that are not effectively managed.

**Recent findings**
Despite a number of approved therapies for lipid disorders, significant unmet needs are present in treating persistently elevated LDL-cholesterol, remnant-cholesterol, triglycerides and lipoprotein(a) [Lp(a)]. Small molecules and antibodies are effective modalities, but they are unable to adequately treat many patients with abnormal lipid parameters. Targeting mRNA with oligonucleotides to prevent protein translation is a relatively novel method to reduce circulating atherogenic lipoproteins. Small inhibiting RNA (siRNA) molecules targeting proprotein convertase subtilisin kexin type 9 to reduce LDL-C, and antisense oligonucleotides (ASO) targeting apolipoprotein C-III (*apoC-III*) to reduce triglycerides, angiopoietin-like 3 (*ANGPTL3*) to reduce LDL-C and triglycerides and apolipoprotein(a) (*LPA*) to reduce Lp(a) are currently in or just completed phase 1–3 trials. Fundamental differences exist in chemistry, delivery and mechanism of action of siRNA and ASOs.

**Summary**
Novel RNA therapeutics are poised to provide highly potent, specific and effective therapies to reduce atherogenic lipoproteins. As these compounds are approved, clinicians will be able to choose from a broad armamentarium to treat nearly all patients to acceptable goals in order to reduce risk of cardiovascular disease and events.

**Keywords**
angiopoietin-like 3, antisense oligonucleotides, apolipoprotein C-III, lipoprotein(a), small inhibiting RNA

## INTRODUCTION

The treatment of lipid disorders has improved significantly since the 1980s and effective therapies are approved to lower LDL-cholesterol (LDL-C). Treatments for elevated LDL-C include low-fat, low-cholesterol diet, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, bile-acid-binding resins, ezetimibe, niacin, and proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors [1]. Elevated LDL-C can now be reduced to an optimal level for all patients who can tolerate therapy, except for those with severe heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH). However, despite these advances, significant residual cardiovascular risk remains even with achievement of LDL-C less than 50 mg/dl [2,3].

Genetically validated targets for lipid disorders include LDL-C and apolipoprotein B-100, proteins involved in the metabolism of triglycerides such as apolipoprotein C-III (*apoC-III*), of both LDL-C and triglycerides such as angiopoietin-like 3 (ANGPTL3) and lipoprotein(a) [Lp(a)] (Fig. 1). These lipoproteins have been shown in multiple genome-wide association and Mendelian randomization studies to segregate patients to higher or lower risk categories by unique differences in genetic variability, such as in single nucleotide polymorphisms, which are associated with altered plasma levels of the respective lipoprotein, which in turn are associated with differences in risk of cardiovascular disease (CVD) [4,5,6▪,7,8,9]. From a therapeutic perspective, potential genetic causality provides a strong rationale to optimally understand their pathophysiology and their relative contribution to CVD [10▪] and to develop therapies to affect the lipid profile in a favorable manner. This review will, thus, focus on these lipoproteins as targets of novel therapeutic

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## KEY POINTS

- Significant unmet needs are present in treating persistently elevated LDL-cholesterol, remnant-cholesterol, triglycerides and lipoprotein(a).
- Targeting messenger RNA with oligonucleotides to prevent protein translation is a relatively novel method to reduce circulating atherogenic lipoproteins.
- Small inhibiting RNA molecules targeting proprotein convertase subtilisin kexin type 9 to reduce LDL-C, and antisense oligonucleotides targeting apolipoprotein C-III to reduce triglycerides, angiopoietin-like 3 to reduce LDL-C and triglycerides and apolipoprotein(a) to reduce Lp(a) are currently in or just completed phase 1–3 trials.
- Novel RNA therapeutics are poised to provide highly potent, specific and effective therapies to reduce atherogenic lipoproteins.

approaches using ribonucleic acid (RNA)-targeted drugs.

## RNA-TARGETED THERAPEUTICS

RNA therapeutics represent a novel platform through which treatment of lipid disorders may be affected [11▪▪]. Importantly, this approach allows potential therapies for what were previously thought to be undruggable targets. Traditional therapies in lipid metabolism include small molecules, such as HMG-CoA reductase inhibitors that inhibit enzyme activity intracellularly, or antibodies that inactivate circulating proteins involved in lipid metabolism, such as antibodies targeting PCSK9 in the circulation. Small molecules that inhibit enzyme function or receptor activity are not useful therapeutically for proteins which have no enzyme activity, such apoC-III, ANGPTL3 or Lp(a). Targeting such proteins with humanized monoclonal antibodies may be useful in some cases where the circulating concentration of the respective protein is low enough for the antibodies to completely inactivate the protein. They also have to be well tolerated and be effective at relatively low doses to be cost effective. Proteins at higher concentration, such as Lp(a) and possibly apoC-III, will require a large mass of antibodies that will generate large amounts of immune complexes, which will need to be metabolized and safely excreted, thus raising both safety and cost concerns.

RNA-targeted therapeutics represent an elegant and novel approach to lower circulating levels of lipoproteins involved by inhibiting their production within their sites of generation, which is predominantly the hepatocyte (Fig. 2). RNA-targeted therapies represent a platform for drug discovery involving chemically modified oligonucleotides, which are currently mainly used therapeutically as single stranded antisense oligonucleotides (ASO) or double-stranded small inhibiting RNA (siRNA) [11▪▪].

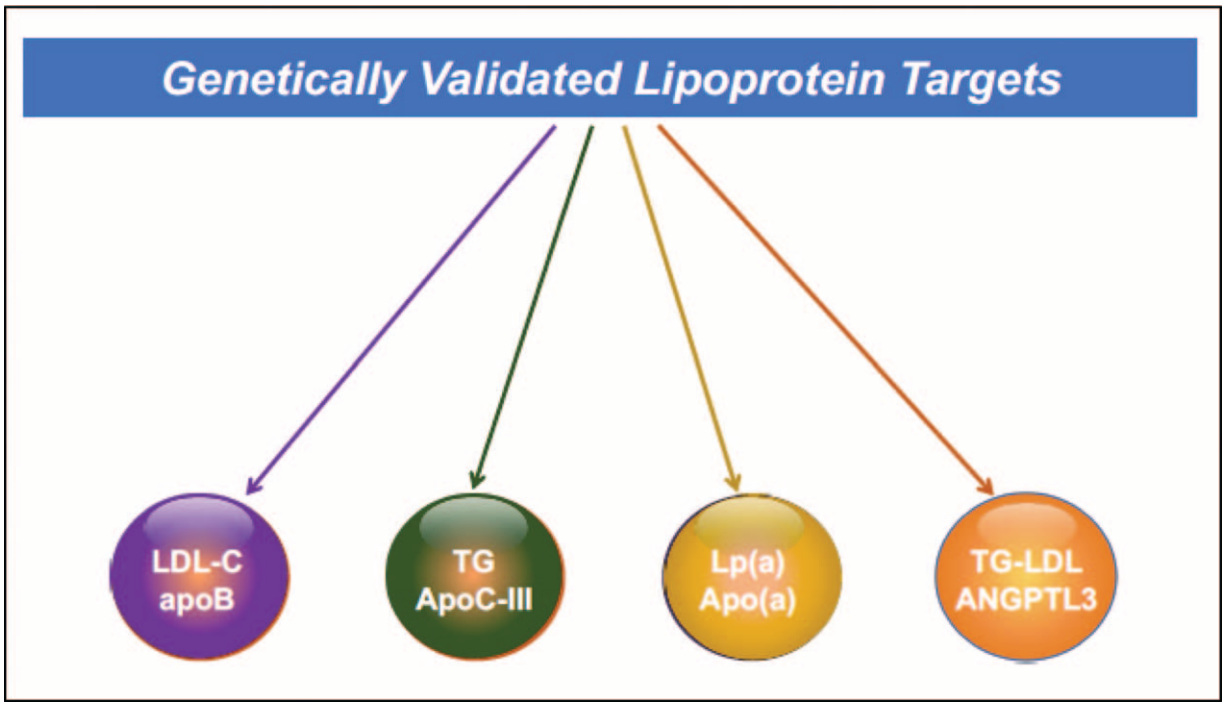


**FIGURE 1.** Genetically validated targets to reduce circulating atherogenic lipoproteins.

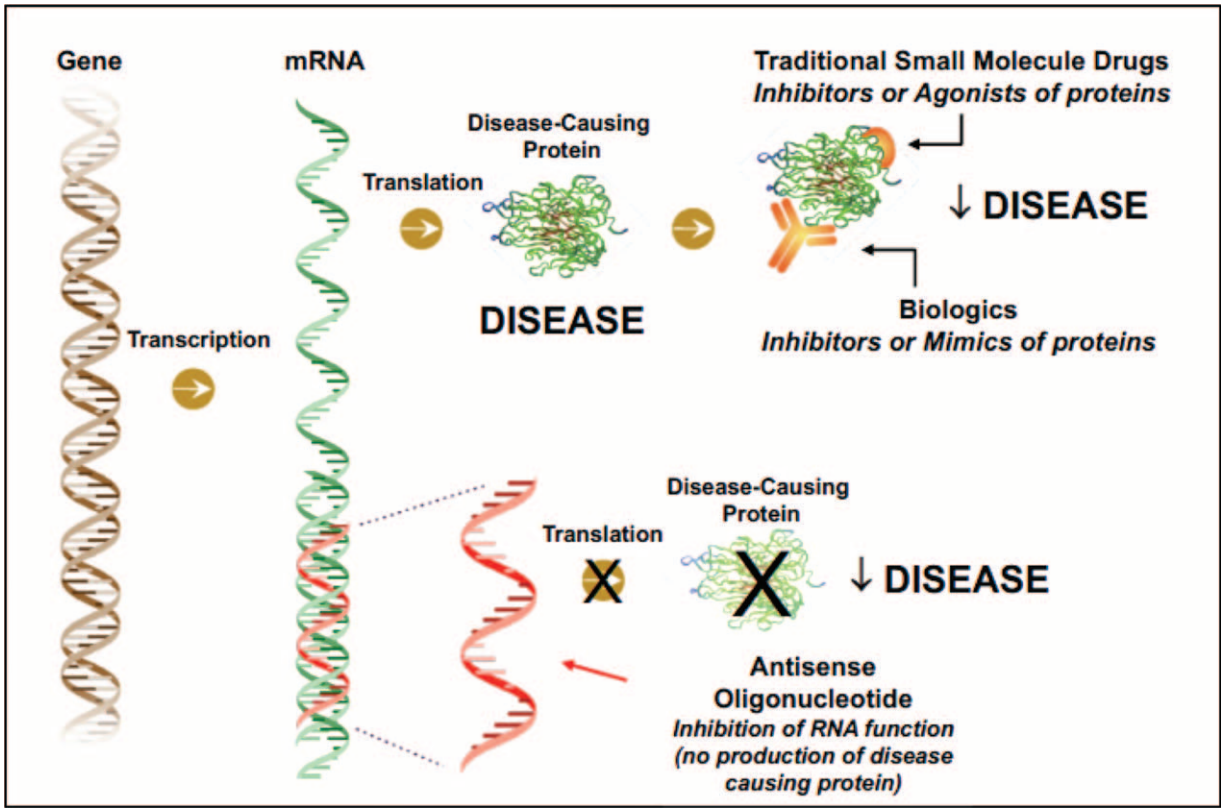


**FIGURE 2.** Conceptual rendition of differences in small molecules, antibodies and RNA therapeutics. Small molecule drugs target enzymes or receptors to mediate effects. Biologics target proteins and inactivate their biological activities. RNA therapeutics, among other mechanisms, function is to inhibit the cognate mRNA and prevent protein translation. The fundamental difference between RNA therapeutics and small molecules and antibodies is that there is decreased production of disease-causing proteins and no immune complexes generated in plasma with RNA targeted drugs.

The differences and similarities between ASOs and siRNAs are shown in Table 1. ASOs are single stranded molecules composed of modified DNA (so-called 'gapmers'). The middle 10 bases are composed of DNA whereas the five bases on the wings are modified DNA, generally with 2′ methoyethyl (2′ MOE). The phosphodiester backbone is often replaced by variable number of phosphorothiote moieties, whereas the nucleic acid bases are not modified. These chemical modifications are crucial for allowing enhanced stability in plasma and high-affinity and binding to messenger RNA (mRNA). They represent a fundamental reason why ASOs can be used as drugs, compared with unmodified, native oligonucleotides that are rapidly degraded in the circulation. When injected subcutaneously, ASOs bind proteins in serum, travel to the liver, and enter cell and nuclear membranes to mediate therapeutic effects. They bind their cognate mRNA through Watson–Crick interactions and can bind to mRNA in the cytoplasm or nucleus. As they are amphipathic, they dissolve is physiological saline and can be delivered subcutaneously without a carrier. ASOs have rapid plasma uptake and a short distribution half-life (<1 h) but have an intracellular half-life of 2–4 weeks, and therefore, a sustained duration of action. They are eliminated as short fragments by exonucleases and endonucleases [11▪▪].

siRNA are double-stranded RNA molecules that are configured not only with an antisense strand to mediate therapeutic effects, but also with a sense strand to maintain a double-stranded complex. siRNAs are hydrophilic and rapidly excreted unless injected in liposomes or targeted to the liver. They mediate protein knockdown by binding to RNA-induced silencing complex (RISC) complex in the cytoplasm. The RISC complex is a multiprotein complex, which allows the single strand to act as a template in order for RISC to recognize the target mRNA and use Argonaute-2 enzyme to cleave the mRNA.

Both ASO and siRNA can be targeted specifically to the highly prevalent asialoglycoprotein receptors present on hepatocytes to mediate cellular uptake. Using triantennary *N*-acetylgalactosamine (GalNAc$_3$)-conjugated oligonucleotides allows for similar or greater potency of unconjugated ASOs but with 20–30-fold lower dosing, thus minimizing systemic exposure [12▪▪,13▪▪,14,15].

**Table 1.** Main characteristics of antisense oligonucleotide and small inhibiting RNA drugs

| | ASO | siRNA |
|---|---|---|
| Chemical structure | Single stranded modified DNA. A central gap of DNA is flanked by 2′MOE or other modifications (2′OMe, cET, LNA) in the wings. Contains variable number of PS moieties. | Double stranded modified RNA. Contains both an antisense and sense strand with 2′OMe or 2′F modifications. Contains variable number of PS moieties. |
| Length of sequence | ~13–20 nucleic acids | ~40–44 nucleic acids, including carrier strand |
| Plasma/tissue distribution | Binds proteins in serum, cell membrane, and intracellularly | Rapidly excreted unless injected in liposomes or if contains targeting moiety to liver |
| Target specificity | High | High, but sense strand may have off-target effects |
| Mechanism binding to mRNA | Watson–Crick interactions | Watson–Crick interactions |
| Mechanism of action | RNAse H1/alteration of splicing, others | RISC complex-Ago2 |
| Location of activity | Nucleus and cytoplasm | Cytoplasm |
| Mode of delivery | Saline | Liposomes (requires adjunctive steroids), saline |
| Targeting moiety to liver | GalNac | GalNac |
| Uptake/half-life/elimination | Rapid uptake, distribution half-life less than 1 h, hepatocyte half-life 2–4 weeks, elimination by exonucleases and endonucleases | Rapid uptake, distribution half-life less than 1 h, hepatocyte half-life 2–4 weeks, elimination by exonucleases and endonucleases |

Ago2, argonaute 2; cET, constrained ethyl; GalNac, triantennary; LNA, locked nucleic acid;2′MOE, 2′ methoxyethyl; N, acetylgalactosamine; 2′OMe, 2′ o-methyl; RNAase H1, ribonuclease H1.

Currently 6 RNA therapeutics have been approved for clinical use, including fomivirsen for cytomegalovirus retinitis, mipomersen for HoFH, nusinersen for spinal muscular atrophy and eteplirsen for Duchenne muscular dystrophy, inotersen and patisiran for familial transthyretin amyloidosis, [11▪▪]. Fomivrsen, mipomersen and inotersen use the RNAse H1-mediated cleavage mechanism of the sense strand to prevent protein production, and nusinersen and eteplirsen affect splicing mechanisms to upregulate protein synthesis [16,17].

## RNA THERAPEUTICS FOR LIPID DISORDERS

### LDL-cholesterol

Mipomersen (Kynamro) is a second-generation ASO that binds to apolipoprotein B-100 mRNA to prevent synthesis of protein, and thus reduces circulating levels of apoB-containing lipoproteins. Mipomersen was the first, systemically dosed RNA therapeutic approved in the United States in January 2013. Mipomersen was approved for HoFH after being shown to significantly reduce LDL-C by 25% compared with placebo and on top of standard-of-care therapies [18]. Mipomersen has also been shown to reduce LDL-C in patients with HeFH [19], statin intolerant patients [20] and also significantly lowers Lp(a) [21]. In a post hoc analysis of prospectively collected data of three randomized trials and an open-label extension phase that included patients that were exposed to more than 12 months of mipomersen, MACE rates after initiation of mipomersen were shown to be significantly lower [22]. A phase 3 trial in patients with HeFH, FOCUS-FH, was completed and reported ~30% reduction in LDL-C levels [23]. Due to the emergence of PCSK9 inhibitors and side effect profile of mipomersen and the ultra-rare population it treats, the clinical use of mipomersen has been low. Mipomersen side effect profile includes injection site reactions and flu-like symptoms in some patients that has limited compliance. In addition, elevation of liver tests is noted, but this is felt to be primarily because of its mechanism of action of inhibiting apoB output by the liver, as this is also seen with microsomal triglyceride transfer inhibitors such as lomitapide [24]. An incremental increase in the median liver fat is also noted during the initial 6–12 months of therapy that appeared to diminish with continued mipomersen exposure beyond 1 year and returned towards baseline 24 weeks after last drug dose suggestive of adaptation [25].

Inclisiran is a GalNac-modified siRNA currently in clinical development for patients with elevated LDL-C. It binds to PCSK9 mRNA to inhibit hepatocyte production of PCSK9 protein and thereby reduce LDL-C levels. It is fundamentally different to the mechanism of inhibition of PCSK9 antibodies, whereby instead of binding PCSK9 in the circulation and generating immune complexes, it decreases hepatocyte production of PCSK9. To date, one phase 1 and one phase 2 trial have been published that

**Table 2.** Published clinical trials with RNA therapeutics currently in clinical development

| Drug | First author | Date published | Phase | Indication | Main dose | Number of patients | Main findings |
|---|---|---|---|---|---|---|---|
| Inclisiran | Fitzgerald *et al.* [26] | 2014 | 1 | Healthy volunteers with LDL-C greater than 3 mmol/l | 0.015–0.400 mg/kg i.v. | 32 | Up to mean 40% reduction in LDL-C |
| | Ray *et al.* [27] | 2017 | 2 | High-risk CVD/high LDL-C | 100–300 mg | 501 | Up to mean 53% reduction in LDL-C |
| Volanesorsen | Graham *et al.* [29] | 2013 | 1 | Healthy volunteers | 50–400 mg | 12 | Up to mean 44% reduction in TG |
| | Gaudet *et al.* [31] | 2014 | 2 | Patients with FCS with TG greater than 1000 mg/dl | 300 mg | 3 | 56–86% reduction in TG |
| | Gaudet *et al.* [30] | 2015 | 2 | Patients with elevated TG greater than 250–2000 mg/dl | 100–300 mg | 57 | Up to mean 71% reduction in TG |
| IONIS-APO(a)$_{Rx}$ | Tsimikas *et al.* [42] | 2015 | 1 | Healthy volunteers with elevated Lp(a) | 100–300 mg | 47 | Up to mean 78% reduction in Lp(a) |
| | Viney *et al.* [13▪▪] | 2016 | 2 | Patients with Lp(a) 50–175 mg/dl or greater than 175 mg/dl | 100–300 mg | 64 | Up to mean 72% reduction in Lp(a) |
| IONIS-APO(a)-L$_{Rx}$ | Viney *et al.* [13▪▪] | 2016 | 1/2a | Healthy volunteers with elevated Lp(a) greater than 30 mg/dl | 10–40 mg | 58 | Up to mean 92% reduction in Lp(a) |
| IONIS-ANGPTL3-L$_{Rx}$ | Graham *et al.* [12▪▪] | 2017 | 1 | Healthy volunteers with elevated TG greater than 150 mg/dl | 10–60 mg | 44 | Up to mean reductions 63% in TG, 60% in VLDL-C, 33% in LDL-C, 26% in apoB, 59% in apoC-III |

CVD, cardiovascular disease; FCS, familial chylomicronemia syndrome; Lp(a), lipoprotein(a); TG, triglycerides.

report the safety and efficacy of Inclisiran (Table 2) [26▪▪,27,28]. A total of 501 patients received a single dose of placebo or 200, 300, or 500 mg subcutaneously of inclisiran or two doses (at days 1 and 90) of placebo or 100, 200, or 300 mg of inclisiran. At day 180, the mean reductions in LDL-C levels were 27.9–41.9% after a single dose of inclisiran and 35.5–52.6% after two doses. Several ongoing trials are underway to develop additional evidence for regulatory approval of inclisiran (inclisiran for patients with ACSVD or ACSVD-risk equivalents and elevated LDL-C (ORION-11), NCT03400800).

## Apolipoprotein C-III

To date, three key studies have been published with volanesorsen, an ASO directed to apoC-III [29,30,31]. ApoC-III is present on all lipoproteins, including LDL, HDL and Lp(a) [32], and can be found on these lipoproteins in variable amounts. ApoC-III acts as an inhibitor of lipoprotein lipase but it also inhibits clearance of all triglyceride-rich lipoproteins through a combination of the LDL and LRP1 receptors, leading to chylomicronemia and/or elevated VLDL-C and elevated triglyceride levels in plasma [33]. A phase 1 trial in healthy volunteers initially demonstrated that volanesorsen decreased apoC-III and triglyceride levels by 78% and 44%, respectively [29]. A subsequent phase 2 trial was conducted in untreated patients with fasting triglyceride levels between 350 and 2000 mg/dl with 100–300 mg weekly volanesorsen as well as in patients receiving fibrates who had fasting triglyceride levels between 225–2000 mg/dl with 200–300 mg weekly volanesorsen. Treatment with volanesorsen at doses of 100–300 mg for 13 weeks resulted in decreases in plasma apoC-III levels of 45, 68.8, and 84.6% and 36.3, 63.6, and 75.9% in triglycerides in the 100, 200, and 300-mg groups, respectively, when the drug was administered as a single agent. When it was administered as an add-on to fibrates decreases in plasma apoC-III levels of 60.2 and 70.9% and 52.3 and 64.9% in triglycerides in the 200 and 300-mg groups were noted.

Familial chylomicronemia syndrome (FCS) is characterized by genetic abnormalities in lipoprotein lipase or proteins facilitating lipoprotein lipase activity and is associated with markedly elevated triglyceride levels that are mainly derived from dietary fat intake, lipemic plasma, lipemia retinalis and

eruptive xanthomas [34]. Such patients are predisposed to developing multiple episodes of acute pancreatitis, as well as long term complications including diabetes and cardiovascular disease. There are no approved or effective pharmacologic agents for FCS. A very low-fat diet (<20 grams per day) is prescribed in all patients but is difficult to follow due to its stringency and the effectiveness is variable.

A phase 2 trial, a 3-patient phase 2 trial was performed with volanesorsen in patients with FCS with triglyceride levels ranging from 1406 to 2083 mg/dl (15.9–23.5 mmol/l). After 13 weeks of study-drug administration, plasma apoC-III levels were reduced by 71–90% and triglyceride levels by 56–86% [31]. The decline in triglyceride levels in these patients lacking LPL activity established the role of apoC-III as a key regulator of LPL-independent pathways of triglyceride metabolism. It also provided the rationale for a phase 3 trial in FCS (APPROACH, NCT02211209), which was completed and reached its primary endpoint of significant reduction in triglyceride levels [35]. Patients with FCS can have thrombocytopenia as part of their natural history, but volanesorsen has also been associated with a higher incidence of thrombocytopenia in this particular patient cohort. With regular measurement of platelet counts and appropriate monitoring, the platelet count normalizes with withholding the drug. The full results of this trial including the side effect profile will be reported in the near future.

A GalNac-conjugated apoC-III ASO has recently finished a phase 1 study, showing mean reductions of −65, −84, and −83% in apoC-III and −61, −71, and −65% in triglycerides In multiple-dose cohorts of 15 and 30 mg weekly and 60 mg every-4 weeks [36]. Significant reductions in total cholesterol, apolipoprotein B, non-HDL-C, VLDL-C and increases in HDL-C were also observed. With the lower doses used with this optimized ASO, no significant elevations in liver tests or reductions in platelet count were noted. A phase 2 trial in patients with CVD and triglyceride levels greater than 200 mg/dl is currently underway [Study of ISIS 678354 (AKCEA-APOCIII-$L_{Rx}$) in patients with hypertriglyceridemia and established cardiovascular disease, NCT03385239].

## Angiopoietin-like 3

Complete loss-of-function mutations in *ANGPTL3* result in familial combined hypolipidemia, with reductions in LDL-C, triglycerides and HDL-C [37]. In a recent phase 1 study in 44 human volunteers with elevated triglyceride levels, multiple doses (10, 20, 40, or 60 mg per week for 6 weeks) of a GalNac-modified ASO targeted to ANGPTL3 resulted in reductions of 46.6–84.5% in ANGPTL3 protein, 33.2–63.1% in triglycerides, 1.3–32.9% in LDL-C, 27.9–60% in VLDL-C, 10.0–36.6% in non-HDL-C, 3.4–25.7% in apolipoprotein B-100 and 18.9–58.8% in apolipoprotein C-III (Table 2) [12▪▪]. In preclinical studies, there was also a reduction in liver triglyceride content and extent of atherosclerosis, as well as improvement in insulin sensitivity [12▪▪]. The broad improvement in apoB-containing atherogenic lipoproteins and its effects on insulin sensitivity suggest that targeting ANGPTL3 may be useful for patients with metabolic syndrome and elevated remnant cholesterol at additional risk for CVD despite controlled LDL-C, as well as in nonalcoholic steatohepatitis. No significant elevations in liver tests or reductions in platelet count were noted. A phase 2 trial in patients with diabetes mellitus, fatty liver disease and triglyceride levels greater than 200 mg/dl is currently underway [Study of ISIS 703802 (AKCEA-ANGPTL3-$L_{Rx}$) in patients with hypertriglyceridemia, type 2 diabetes mellitus, and nonalcoholic fatty liver disease, NCT03371355].

## Lipoprotein(a)

There are currently no specific therapies to reduce circulating Lp(a) levels. Lp(a) is an independent, genetic risk factor for CVD [38]. Although Lp(a) levels can be reduced by niacin and PCSK9 inhibitors, the effect is modest (20–30%) and patients with highly elevated Lp(a) levels do not achieve adequate absolute reductions in Lp(a) [39–41]. Novel approaches to lower Lp(a) include ASOs, which specifically target apolipoprotein(a) mRNA and reduce the ability hepatocytes to assemble Lp(a) particles by limiting availability of apolipoprotein(a). The first clinical demonstration of a specific Lp(a)-lowering drug was with ISIS-apo$(a)_{Rx}$, which treated healthy volunteers with modest elevations in Lp(a). Significant mean decreases in plasma Lp(a) of 39.6% in the 100 mg group, 59.0% in the 200 mg group, and 77.8% in the 300 mg group were noted (Table 2), along with similar reductions were observed in the amount of oxidized phospholipids associated with apolipoprotein B-100 and apolipoprotein(a) [42]. This study was followed up by a phase 2 trial in patients with baseline Lp(a) 50–175 mg/dl (cohort A) or greater than 175 mg/dl [cohort B; the drug was renamed as IONIS-APO$(a)_{Rx}$] at doses of 100 mg weekly for 1 month, 200 mg weekly for 1 month and 300 mg weekly for 3 months [13▪▪]. Significant mean Lp(a) reductions of 66.8% in cohort A and 71.6% in cohort B were noted.

With advances in hepatic targeting ASOs to hepatocytes using the GalNAc approach, the development of this compound was terminated, and a

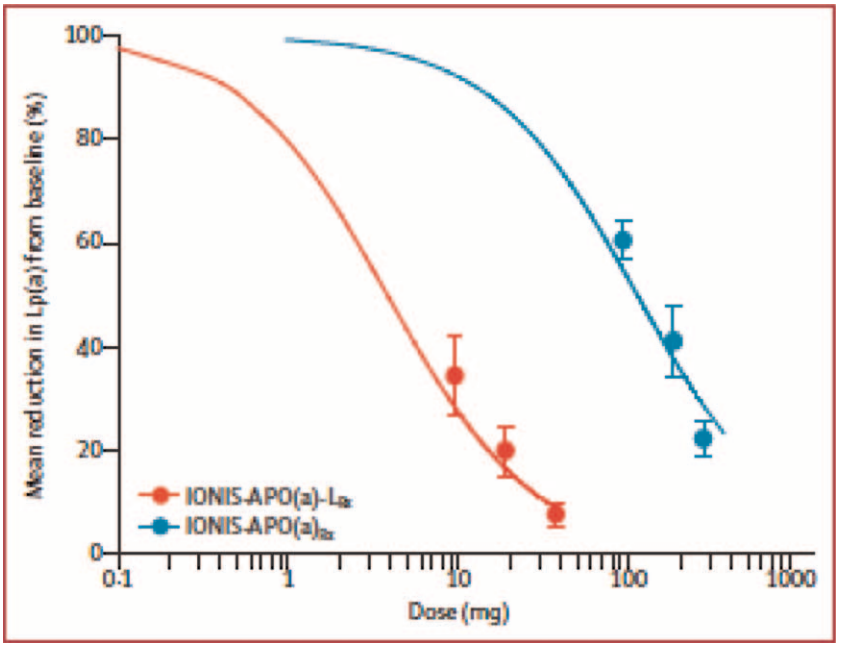


**FIGURE 3.** Comparison of dose–response curves of IONIS-APO(a)$_{Rx}$ and IONIS-APO(a)-L$_{Rx}$ after 4 weeks of subcutaneous administration. Graphs are modelled from clinical trial data and demonstrated a 30-fold higher potency of IONIS-APO(a)-L$_{Rx}$ versus IONIS-APO(a)$_{Rx}$. Error bars are SEM. The upper left side of the curve was extrapolated based on the curve fit of the data because of the fact that lower doses were not tested. Reprinted with permission from Viney *et al.* [13▪▪].

new compound was studied in patients with modest elevations of Lp(a). IONIS-APO(a)-L$_{Rx}$ contains the same nucleotide sequence as IONIS-APO(a)$_{Rx}$ but in addition has the GalNAc moiety for targeting to the hepatocyte asialoglycoprotein receptor as well as a reduced content of PS moieties [13▪▪]. IONIS-APO(a)-L$_{Rx}$ was shown to be 30-fold more potent than the parent molecule, with an effective dose to lower plasma Lp(a) by 50% of 3.9 versus 122 mg (30-fold lower) for the parent molecule (Fig. 3). Significant dose-dependent reductions in mean Lp(a) concentrations were noted in Lp(a) of 66% in the 10 mg group, 80% in the 20 mg group, and 92% in the 40 mg group. This level of potency may allow achievement on normal levels of Lp(a) (<30 or <75 nmol/l) in most patients with elevated Lp(a). No significant elevations in liver tests or reductions in platelet count were noted. A dose-ranging and safety phase 2 trial of AKCEA-APO(a)-L$_{Rx}$ in patients with established CVD and baseline Lp(a) greater than 60 mg/dl (>150 nmol/l) is currently underway [phase 2 study of ISIS 681257 (AKCEA-APO(a)-L$_{Rx}$] in patients with hyperlipoproteinemia(a) and cardiovascular disease, NCT03070782).

## CONCLUSION

Over the last 5 years, significant progress has been made in drug development using RNA therapeutics to treat previously difficult to target lipid disorders. Phase 1–3 studies have been performed and many of these drugs are moving towards regulatory approval over the next 1–5 years. These drugs are not only highly specific to their targets but also they demonstrate unparalleled potency and efficacy in reducing plasma levels of atherogenic lipoproteins. The safety and tolerability profile of these drugs will require additional experience and larger patient cohorts, but very early experience with the GalNac-modified drugs shows no significant signals in liver test abnormalities or platelet count reductions. As these RNA therapeutics are approved for clinical use, they will provide clinicians with powerful tools to reduce lipid levels at unprecedented levels. With the ability to bring lipid levels to normal in many if not most patients, it is anticipated that the rate of CVD events will be significantly reduced and the prognosis of patients with lipid disorders improved to levels seen in individuals without risk factors.

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*None.*

### Conflicts of interest

*S.T. is a co-inventor of and receives royalties from patents owned by the University of California San Diego*

*on oxidation-specific antibodies, is a co-founder of Oxitope, Inc, consultant to Boston Heart Diagnsotics and has a dual appointment at UCSD and Ionis Pharmaceuticals, Inc.*

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# Baseline and on-statin treatment lipoprotein(a) levels for prediction of cardiovascular events: individual patient-data meta-analysis of statin outcome trials

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## Summary

**Background** Elevated lipoprotein(a) is a genetic risk factor for cardiovascular disease in general population studies. However, its contribution to risk for cardiovascular events in patients with established cardiovascular disease or on statin therapy is uncertain.

**Methods** Patient-level data from seven randomised, placebo-controlled, statin outcomes trials were collated and harmonised to calculate hazard ratios (HRs) for cardiovascular events, defined as fatal or non-fatal coronary heart disease, stroke, or revascularisation procedures. HRs for cardiovascular events were estimated within each trial across predefined lipoprotein(a) groups (15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL, *vs* <15 mg/dL), before pooling estimates using multivariate random-effects meta-analysis.

**Findings** Analyses included data for 29 069 patients with repeat lipoprotein(a) measurements (mean age 62 years [SD 8]; 8064 [28%] women; 5751 events during 95 576 person-years at risk). Initiation of statin therapy reduced LDL cholesterol (mean change −39% [95% CI −43 to −35]) without a significant change in lipoprotein(a). Associations of baseline and on-statin treatment lipoprotein(a) with cardiovascular disease risk were approximately linear, with increased risk at lipoprotein(a) values of 30 mg/dL or greater for baseline lipoprotein(a) and 50 mg/dL or greater for on-statin lipoprotein(a). For baseline lipoprotein(a), HRs adjusted for age and sex (*vs* <15 mg/dL) were 1·04 (95% CI 0·91–1·18) for 15 mg/dL to less than 30 mg/dL, 1·11 (1·00–1·22) for 30 mg/dL to less than 50 mg/dL, and 1·31 (1·08–1·58) for 50 mg/dL or higher; respective HRs for on-statin lipoprotein(a) were 0·94 (0·81–1·10), 1·06 (0·94–1·21), and 1·43 (1·15–1·76). HRs were almost identical after further adjustment for previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL cholesterol, and HDL cholesterol. The association of on-statin lipoprotein(a) with cardiovascular disease risk was stronger than for on-placebo lipoprotein(a) (interaction p=0·010) and was more pronounced at younger ages (interaction p=0·008) without effect-modification by any other patient-level or study-level characteristics.

**Interpretation** In this individual-patient data meta-analysis of statin-treated patients, elevated baseline and on-statin lipoprotein(a) showed an independent approximately linear relation with cardiovascular disease risk. This study provides a rationale for testing the lipoprotein(a) lowering hypothesis in cardiovascular disease outcomes trials.

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## Introduction

Lipoprotein(a) is composed of apolipoprotein(a) bound covalently to apolipoprotein B of an LDL-like particle.[1,2] It mediates atherogenicity via its LDL moiety, which has a similar proportion of cholesterol content as traditional LDL particles. Furthermore, lipoprotein(a) induces pro-inflammatory responses[3,4] via accumulation of oxidised phospholipids[5] and potentially exerts prothrombotic effects via the plasminogen-like apolipoprotein(a) moiety.[6] By contrast with other major lipoproteins, there is no approved specific therapy to lower circulating plasma levels of lipoprotein(a).

Epidemiological[7] and genetic[8,9] evidence has accumulated over the past decade showing that elevated lipoprotein(a), driven primarily by the *LPA* gene,[10] is associated with increased risk of coronary heart disease, stroke, peripheral arterial disease, and calcific aortic valve stenosis.[1,2,11] These data have established lipoprotein(a) as a cardiovascular disease risk factor, but the bulk of evidence is based on studies including individuals without previous cardiovascular disease and without intensive secondary prevention therapies. By contrast, the role of elevated lipoprotein(a) in patients with previous cardiovascular disease events, on statin therapy or on other guideline-recommended treatments, is less clear. Previous studies in such patient populations have yielded inconsistent results, with findings ranging from significant positive associations to null associations (eg, after acute coronary syndromes).[2] Moreover, findings of several studies—including JUPITER[12] and AIM-HIGH[13]—have shown that elevated

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## Research in context

### Evidence before this study

We searched PubMed for clinical trials published up to July 9, 2018, with the terms "Lipoprotein(a)" or "Lp(a)" plus "statin" and "cardiovascular diseases"[MeSH]. Our review identified seven statin trials (4D, 4S, FLARE, JUPITER, LIPID, MIRACL, and TNT) that reported on the association of lipoprotein(a) with cardiovascular risk. Interpretation of the available evidence is complicated by inconsistent findings across trials (positive *vs* null associations), limited statistical power of single trials, scant availability of follow-up lipoprotein(a) measurements, and differing definitions of lipoprotein(a) categories across trials.

### Added value of this study

We obtained patient-level data from seven placebo-controlled statin trials encompassing 29 069 patients and analysed the relation of baseline and on-treatment lipoprotein(a) to risk of major adverse cardiovascular events. Elevated lipoprotein(a) of 50 mg/dL or higher, at baseline or on-treatment, was associated with an increased hazard ratio of cardiovascular events independent of other cardiovascular risk factors and evident on treatment with either statin or placebo.

### Implications of all the available evidence

These data suggest that residual risk is present in patients with elevated lipoprotein(a) that is not addressed by statins and supports the rationale for outcomes trials to test specific therapies to lower lipoprotein(a).

lipoprotein(a) remains predictive for cardiovascular disease risk at LDL cholesterol levels less than 70 mg/dL,[1] but other studies suggest a positive association only when LDL cholesterol is raised.[14] Furthermore, a major limitation of all post-hoc studies reporting lipoprotein(a) levels and outcomes is that they included few patients with lipoprotein(a) values above 50 mg/dL and, therefore, were uniformly underpowered to test the hypothesis that elevated lipoprotein(a) levels are associated with increased cardiovascular event risk in the setting of statin therapy or previous history of cardiovascular disease.

To test this hypothesis with adequate statistical power, we established the Lipoprotein(a) Studies Collaboration, a consortium of patient-level data from placebo-controlled trials of statins with patient-level data for cardiovascular disease outcomes and lipoprotein(a) measurements at baseline and follow-up (ie, under statin treatment). We report the results of this analysis in documenting the associations of baseline and on-treatment lipoprotein(a) with cardiovascular risk.

## Methods

### Trials included in the meta-analysis

To be eligible for the meta-analysis, randomised placebo-controlled statin trials were required to have assayed lipoprotein(a) concentration at baseline and follow-up, have recorded incidence of cardiovascular disease outcomes using well-defined criteria, and be willing to share patient data at the individual level. We included data from AFCAPS,[15] CARDS,[16] 4D,[17] JUPITER,[12] LIPID,[18] MIRACL,[19] and 4S[20] trials. Study design, target population, and entry criteria are summarised in table 1; more detailed descriptions of trial designs[15,21–26] and lipoprotein(a) methodology and data[12,16–20] were reported previously by each trial. Trials not included in the meta-analysis were either not allowed or unwilling to provide individual-patient data. Because of contractual agreements on sharing individual-patient data, other eligible trials could not be included in the meta-analysis. All contributing trials have obtained ethics approval and patients' informed consent.

### Statistical analysis

We did analyses according to a prespecified plan, developed before any combined analyses. We $\log_e$-transformed lipoprotein(a) values. In all trials except 4S, the on-statin concentration of lipoprotein(a) during follow-up was measured at one timepoint. In the 4S trial, the on-statin amount of lipoprotein(a) was estimated as the geometric mean of lipoprotein(a) values assessed at up to four distinct timepoints. In the JUPITER trial, lipoprotein(a) values were provided in nmol/L, which we divided by 2·4 to convert to mg/dL.[27] In 4S, lipoprotein(a) values were provided in IU/L, which we divided by 19·07 to convert to mg/dL. When information on lipoprotein(a) was missing either at baseline (0·5%) or at follow-up (5·5%), the lipoprotein(a) value was mean-imputed from study-specific mixed-effects models, which predicted lipoprotein(a) values using fixed effects for assigned treatment, time in study, and the interaction of the two variables, plus a random intercept allowed to vary at the patient level.

Because conventional LDL cholesterol assays capture cholesterol both in LDL and lipoprotein(a) particles, we corrected LDL cholesterol values for lipoprotein(a) cholesterol. Lipoprotein(a) mass is composed of about 30–45% cholesterol.[28] We used a conservative measurement of the content of lipoprotein(a) cholesterol by multiplying lipoprotein(a) mass (mg/dL) by 0·30 to derive lipoprotein(a) cholesterol, then we subtracted this value from the measured LDL cholesterol to obtain LDL cholesterol corrected for lipoprotein(a) cholesterol (referred to herein as LDL-$C_{corr}$).[28]

We defined the combined cardiovascular disease endpoint as the occurrence of fatal or non-fatal coronary heart disease, stroke, or any coronary or carotid revascularisation procedures. In quantifying associations of on-treatment lipoprotein(a) with cardiovascular risk, we considered all

| | Years of baseline | Target population | Lipid entry criteria (mmol/L) | Comparator to placebo | Included in cardiovascular disease outcome definition | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Myocardial infarction | Stable angina | Stroke | Revascular-isation | Other |
| AFCAPS[15] | 1990–93 | Primary prevention | Total cholesterol 4·65–6·82, LDL cholesterol 3·36–4·91, triglycerides ≤4·52, HDL cholesterol ≤1·16 (men) and ≤1·22 (women) | Lovastatin 20 mg | Yes | Yes | Yes | Yes | Yes* |
| CARDS[16] | 1997–2001 | Type 2 diabetes | LDL cholesterol ≤4·14, triglycerides ≤6·78 | Atorvastatin 10 mg | Yes | No | Yes | Yes | No |
| 4D[17] | 1998–2002 | Type 2 diabetes and haemodialysis | LDL cholesterol 2·07–4·92, triglycerides ≤11·3 | Atorvastatin 20 mg | Yes | No | Yes | Yes | No |
| JUPITER[12] | 2003–06 | Primary prevention with C-reactive protein >2 mg/dL | LDL cholesterol <3·4, triglycerides <5·65 | Rosuvastatin 20 mg | Yes | No | Yes | Yes | Yes† |
| LIPID[18] | 1990–92 | Previous myocardial infarction or unstable angina | Total cholesterol 4·0–7·0, triglycerides <5·0 | Pravastatin 40 mg | Yes | No | Yes | Yes | No |
| MIRACL[19] | 1997–99 | Acute coronary syndrome | Total cholesterol <7·0 | Atorvastatin 80 mg | Yes | No | Yes | Yes | No |
| 4S[20] | 1989–90 | Previous myocardial infarction or angina | Total cholesterol 5·5–8·0, triglycerides ≤2·5 | Simvastatin 20 mg | Yes | No | No | Yes | No |

*Transient ischaemic attack, peripheral vascular disease, sudden death, and deaths from other cardiovascular causes. †Deaths from other cardiovascular causes.

***Table 1:*** **Design features of contributing trials**

cardiovascular events that occurred after randomisation because any change in lipoprotein(a) under statin therapy is anticipated to occur within a short period (sensitivity analyses omitted the initial period of follow-up).[12]

We estimated associations of lipoprotein(a) with cardiovascular disease risk using a two-step approach: we first calculated estimates within each study separately, then pooled these estimates across studies using multivariate random-effects meta-analysis.[29] We calculated hazard ratios (HRs) using Cox proportional hazard regression models; these models used time on study as a timescale, were stratified by trial arm, and compared prespecified lipoprotein(a) groups (<15 mg/dL, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL). We tested the assumption for the proportionality of hazards using Schoenfeld residuals, and the assumption was met. The analysis had four inter-related principal aims. First, to analyse shapes of associations, we calculated pooled HRs over lipoprotein(a) groups and plotted them against pooled geometric means of lipoprotein(a) concentrations within each category.[29] Second, to ascertain the extent of confounding, we adjusted HRs progressively for age, sex, previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL-$C_{corr}$, and HDL cholesterol (multivariable adjusted model). We made further adjustments for body-mass index (BMI) and estimated glomerular filtration rate (eGFR) in the subset of patients in whom these data were available. Third, to investigate whether the predictive value of lipoprotein(a) concentrations at follow-up differed between patients randomly allocated statin or placebo, we fit interaction models by trial arm. Fourth, to investigate effect-modification by individual-patient and study-level characteristics, we did formal tests of interaction and meta-regression analyses with these variables. Little variability was noted within each trial of the proportion of patients with previous cardiovascular disease and with a history of diabetes at baseline (eg, secondary *vs* primary cardiovascular disease prevention trials, diabetes as inclusion or exclusion criterion) and, hence, we investigated effect-modification by these characteristics at the study level instead of at the patient level. We assessed between-trial heterogeneity with the $I^2$ statistic.[30] We did analyses with Stata version 14.1 MP. We used two-sided statistical tests and calculated 95% CIs. We judged p values less than 0·05 significant for principal analyses; for subgroup analyses, we used a Bonferroni-corrected significance level of p<0·007 (for seven subgroups).

### Role of the funding source

The funders had no role in study design, data collection, data analysis, or writing of the report. AL is an employee of one of the funders and secured funding for the meta-analysis and provided input on data interpretation. PW and ST had full access to all data in the study and had final responsibility for the decision to submit for publication.

## Results

Of 45 044 patients enrolled in the seven trials, 15 975 (35%) were excluded because of missing lipoprotein(a) measurements at both baseline and follow-up, leaving 29 069 patients for analysis (appendix). Few differences were noted in baseline characteristics of patients with or without available lipoprotein(a) measurements (appendix). Baseline characteristics of the 29 069 patients are shown in table 2. At trial entry, mean age was 62 years (SD 8), 8064 (28%) patients were women, 15 252 (52%) had previous cardiovascular disease, 5177 (18%) had diabetes, 4847 (17%) were current smokers, mean systolic blood pressure was 137 mm Hg (SD 18), and mean LDL-$C_{corr}$ was 3·30 mmol/L (SD 0·67). The concentration of lipoprotein(a) at baseline was low-to-normal (median 11 mg/dL [IQR 5–29]). In cross-sectional analyses, the baseline lipoprotein(a) concentration was higher in women (% mean difference adjusted for age, 12% [95% CI 3 to 21]),

See Online for appendix

| | AFCAPS[15] | CARDS[16] | 4D[17] | JUPITER[12] | LIPID[18] | MIRACL[19] | 4S[20] | Total |
|---|---|---|---|---|---|---|---|---|
| **Baseline** | | | | | | | | |
| Patients (n) | 1005 | 2470 | 1249 | 9612 | 7863 | 2431 | 4439 | 29 069 |
| Lipoprotein(a) (mg/dL) | 7 (3–17) | 9 (5–22) | 12 (5–42) | 11 (5–23) | 14 (7–44) | 10 (5–29) | 10 (4–28) | 11 (5–29) |
| <15 | 733 (73%) | 1658 (67%) | 709 (57%) | 5896 (61%) | 4118 (52%) | 1481 (61%) | 2654 (60%) | 17 249 (59%) |
| 15 to <30 | 134 (13%) | 310 (13%) | 129 (10%) | 1867 (19%) | 1147 (15%) | 362 (15%) | 781 (18%) | 4730 (16%) |
| 30 to <50 | 84 (8%) | 212 (9%) | 140 (11%) | 851 (9%) | 877 (11%) | 223 (9%) | 714 (16%) | 3101 (11%) |
| ≥50 | 54 (5%) | 290 (12%) | 271 (22%) | 998 (10%) | 1721 (22%) | 365 (15%) | 290 (7%) | 3989 (14%) |
| Age (years) | 59 (7) | 62 (8) | 66 (8) | 66 (8) | 61 (8) | 65 (11) | 59 (7) | 62 (8) |
| Sex | | | | | | | | |
| Women | 173 (17%) | 779 (32%) | 576 (46%) | 3556 (37%) | 1333 (17%) | 820 (34%) | 827 (19%) | 8064 (28%) |
| Men | 832 (83%) | 1691 (68%) | 673 (54%) | 6056 (63%) | 6530 (83%) | 1611 (66%) | 3612 (81%) | 21 005 (72%) |
| Previous cardiovascular disease | 0 | 6 (<1%) | 513 (41%) | 0 | 7863 (100%) | 2431 (100%) | 4439 (100%) | 15 252 (52%) |
| Diabetes | 32 (3%) | 2470 (100%) | 1249 (100%) | 0 | 676 (9%) | 548 (23%) | 202 (5%) | 5177 (18%) |
| Current smoking | 130 (13%) | 551 (22%) | 108 (9%) | 1492 (16%) | 735 (9%) | 693 (29%) | 1138 (26%) | 4847 (17%) |
| Systolic blood pressure (mm Hg) | 136 (17) | 144 (16) | 146 (22) | 136 (17) | 134 (19) | 128 (20) | 139 (20) | 137 (18) |
| LDL-$C_{corr}$ (mmol/L) | .. | 2·75 (0·78) | 3·00 (0·86) | 2·57 (0·49) | 3·68 (0·74) | 3·04 (0·86) | 4·74 (0·66) | 3·30 (0·67) |
| HDL cholesterol (mmol/L) | .. | 1·64 (0·50) | 0·94 (0·34) | 1·35 (0·40) | 0·96 (0·24) | 1·20 (0·31) | 1·19 (0·30) | 1·21 (0·35) |
| Body-mass index (kg/m²) | 26 (3) | 29 (4) | 28 (5) | 29 (6) | .. | 28 (5) | 26 (3) | 28 (5) |
| eGFR (mL/min) | .. | .. | .. | 75 (17) | 71 (17) | .. | .. | 73 (17) |
| Apolipoprotein B (g/L) | .. | 1·16 (0·24) | 1·10 (0·30) | 1·08 (0·21) | 1·33 (0·25) | .. | 1·16 (0·18) | 1·17 (0·23) |
| **On-statin** | | | | | | | | |
| Patients (n) | 504 | 1255 | 616 | 4802 | 3941 | 1200 | 2218 | 14 536 |
| Time to lipoprotein(a) repeat (years) | 1·0 (1·0–1·0) | 2·5 (2·0–2·8) | 0·5 (0·5–0·5) | 1·0 (1·0–1·0) | 1·0 (1·0–1·0) | 0·2 (0·2–0·2) | 2·5 (2·5–2·5) | 1·0 (1·0–1·0) |
| Lipoprotein(a) (mg/dL) | 7 (3–19) | 8 (4–22) | 11 (5–40) | 11 (4–25) | 13 (6–43) | 11 (5–33) | 11 (4–33) | 11 (5–32) |
| <15 | 366 (73%) | 864 (69%) | 351 (57%) | 2912 (61%) | 2106 (53%) | 707 (59%) | 1268 (57%) | 8574 (59%) |
| 15 to <30 | 59 (12%) | 134 (11%) | 60 (10%) | 868 (18%) | 548 (14%) | 175 (15%) | 321 (15%) | 2165 (15%) |
| 30 to <50 | 43 (9%) | 103 (8%) | 73 (12%) | 417 (9%) | 439 (11%) | 96 (8%) | 375 (17%) | 1546 (11%) |
| ≥50 | 36 (7%) | 154 (12%) | 132 (21%) | 605 (13%) | 848 (22%) | 222 (19%) | 254 (12%) | 2251 (15%) |
| % change in lipoprotein(a) *vs* baseline (95% CI) | –1% (–6 to 4) | –13% (–15 to –10) | –6% (–9 to –3) | 2% (1 to 3) | –7% (–8 to –5) | 9% (6 to 12) | 15% (13 to 17) | –0·4% (–7 to 7) |
| LDL-$C_{corr}$ (mmol/L) | .. | 1·68 (0·58) | 1·73 (0·78) | 1·43 (0·70) | 2·57 (0·71) | 1·56 (0·77) | 2·97 (0·70) | 1·99 (0·70) |
| % change in LDL-$C_{corr}$ *vs* baseline (95% CI) | .. | –37% (–38 to –36) | –41% (–43 to –39) | –43% (–44 to –42) | –29% (–30 to –29) | –47% (–49 to –46) | –37% (–37 to –36) | –39% (–43 to –35) |
| **Cardiovascular disease incidence** | | | | | | | | |
| Follow-up (years) | 5·6 (4·8–6·2) | 4·1 (3·1–4·8) | 2·4 (1·4–3·7) | 2·0 (1·5–2·4) | 5·4 (3·1–6·0) | 0·3 (0·3–0·3) | 5·3 (3·9–5·5) | 3·0 (1·5–5·3) |
| Events, overall (n) | 68 | 170 | 338 | 234 | 3040 | 537 | 1364 | 5751 |
| Events, statin arm (n) | 31 | 71 | 166 | 81 | 1428 | 258 | 568 | 2603 |

Data are mean (SD), number of patients (%), or median (IQR), unless stated otherwise. Percentages might not total 100% because of rounding. Total mean (SD) and % change (95% CI) were calculated by pooling study-specific estimates with random-effects meta-analysis. eGFR=estimated glomerular filtration rate. LDL-$C_{corr}$=LDL cholesterol corrected for lipoprotein(a) cholesterol.

***Table 2:*** **Patients' characteristics**

lower in patients with diabetes, and unrelated to smoking (respective % mean differences adjusted for age and sex, –17% [–24 to –9] and 2% [–3 to 8]). Furthermore, LDL-$C_{corr}$, $\log_e$ triglycerides, BMI, and systolic blood pressure were associated with a lower concentration of lipoprotein(a), and HDL cholesterol was associated with higher lipoprotein(a) concentrations (respective % mean differences adjusted for age and sex per SD, –16% [95% CI –23 to –8], –12% [–15 to –9], –7% [–10 to –5], –2% [–5 to 0], and 7% [3 to 11]). Baseline lipoprotein(a) was not associated with age (% mean difference adjusted for sex per SD, –1% [95% CI –2 to 1]).

14 536 patients were randomly allocated statin treatment (table 2). Initiation of statin therapy reduced LDL-$C_{corr}$ by 39% (95% CI 35–43). The effect of statin treatment on lipoprotein(a) concentration was heterogeneous across trials; the pooled percentage change was –0·4% (95% CI –7 to 7), with three trials showing a mean increase (between 2% and 15%) and four trials showing a mean decrease (between –1% and –13%) in lipoprotein(a) (table 2). The median concentration of lipoprotein(a) on statin therapy was 11 mg/dL (IQR 5–32). The age-adjusted and sex-adjusted correlation between baseline and follow-up $\log_e$ lipoprotein(a) was comparable in patients

assigned statin treatment and those allocated placebo ($r$=0·948 *vs* $r$=0·952).

During 95 576 person-years at risk (median follow-up 3·0 years [IQR 1·5–5·3]), 5751 cardiovascular events were recorded, of which 2603 occurred in patients allocated statin treatment (table 2). When patients were grouped by lipoprotein(a) concentration (categories <15 mg/dL, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL), incidence of cardiovascular events per 1000 person-years was, respectively, 55·3 (95% CI 53·4–57·3), 56·3 (52·6–60·2), 66·7 (62·0–71·8), and 80·0 (75·3–84·9) for baseline lipoprotein(a), and 49·0 (46·5–51·6), 46·4 (41·6–51·7), 56·2 (50·3–62·8), and 77·2 (71·1–83·8) for on-statin lipoprotein(a).

In analyses adjusted for age and sex, associations of baseline and on-statin lipoprotein(a) values with risk for cardiovascular disease were positive and roughly linear, with a possible threshold effect in the group with lipoprotein(a) values of 50 mg/dL or higher (figure 1). Compared with patients with baseline lipoprotein(a) values lower than 15 mg/dL, risk for cardiovascular disease was similar with lipoprotein(a) values of 15 mg/dL to less than 30 mg/dL (HR 1·04, 95% CI 0·91–1·18) and increased with lipoprotein(a) values of 30 mg/dL to less than 50 mg/dL (1·11, 1·00–1·22) and with values of 50 mg/dL or higher (1·31, 1·08–1·58; table 3). For analyses of on-statin lipoprotein(a), corresponding HRs were 0·94 (95% CI 0·81–1·10), 1·06 (0·94–1·21), and 1·43 (1·15–1·76). Associations remained robust after additional adjustment for previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL-$C_{corr}$, and HDL cholesterol concentration (figure 1; table 3). Compared with patients with baseline lipoprotein(a) values lower than 15 mg/dL, multivariable-adjusted HRs for cardiovascular disease were 1·04 (95% CI 0·91–1·20) in patients with lipoprotein(a) values of 15 mg/dL to less than 30 mg/dL, 1·13 (1·02–1·25) in patients with lipoprotein(a) values of 30 mg/dL to less than 50 mg/dL, and 1·35 (1·11–1·66) in patients with lipoprotein(a) values of 50 mg/dL or higher. For analyses of on-statin lipoprotein(a), respective multivariable-adjusted HRs were 0·95 (95% CI 0·82–1·11), 1·08 (0·95–1·23), and 1·42 (1·16–1·74).

In a sensitivity analysis of patients with information on triglycerides, BMI, or eGFR, further adjustment for these variables did not change the magnitude of association between lipoprotein(a) measurements and cardiovascular risk (appendix). Effect sizes comparable with those in the principal analysis were recorded when further categorising the highest lipoprotein(a) group into patients with concentrations of 50 mg/dL to less than 75 mg/dL and 75 mg/dL or higher (appendix), and in the on-statin analysis when omitting events that occurred in the initial period between randomisation and on-statin measurement of lipoprotein(a) (appendix). Trial-specific findings are provided in the appendix.

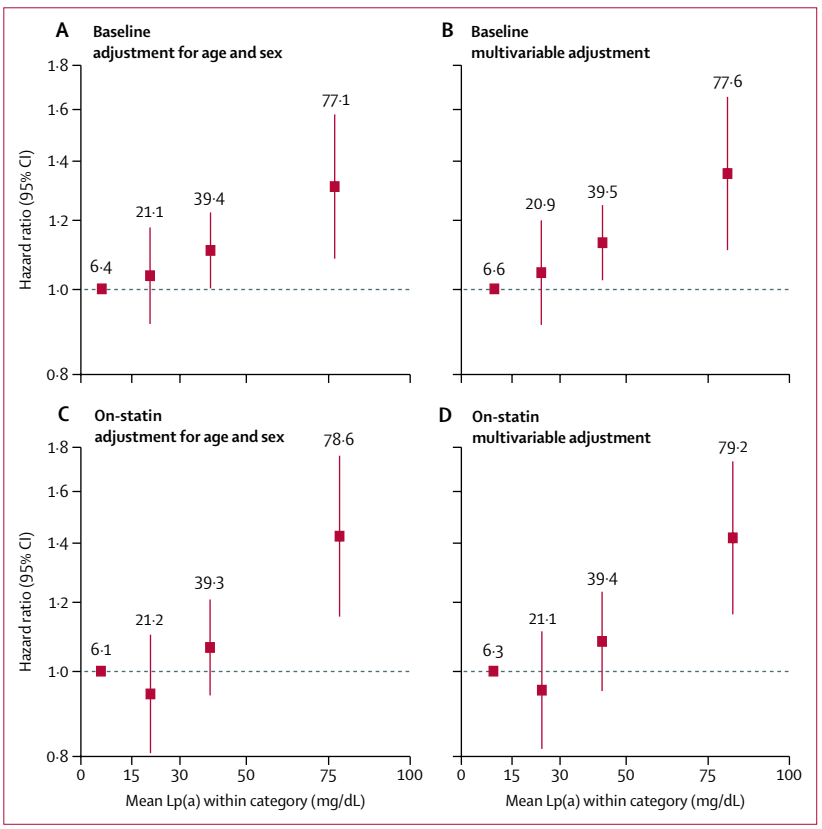


***Figure 1:*** **Associations of baseline and on-statin lipoprotein(a) concentrations with incident cardiovascular disease**

Squares represent hazard ratios and vertical lines 95% CIs. Lipoprotein(a) categories were <15 mg/dL, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL; numbers above each vertical line are mean lipoprotein(a) values within each category. Lipoprotein(a) <15 mg/dL was the reference group. (A) and (C) adjusted for age and sex. (B) and (D) adjusted for age, sex, previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL cholesterol corrected for lipoprotein(a) cholesterol, and HDL cholesterol. Lp(a)=lipoprotein(a).

Lipoprotein(a) concentration during follow-up was associated more strongly with risk for cardiovascular disease in patients assigned statins than in those allocated placebo (figure 2). When comparing individuals with lipoprotein(a) concentrations of 50 mg/dL or higher with patients with lipoprotein(a) concentrations lower than 50 mg/dL, the HRs adjusted for age and sex for cardiovascular disease were 1·48 (95% CI 1·23–1·78) when allocated statin and 1·23 (1·04–1·45) when allocated placebo (interaction p=0·010). Corresponding multivariable-adjusted HRs were 1·47 (95% CI 1·25–1·73) and 1·26 (1·06–1·50; interaction p=0·031). The median time from randomisation to measurement of lipoprotein(a) during follow-up was 1·0 years in both trial arms (IQR 1·0–1·0 in both arms).

Some heterogeneity was noted between trials in HRs for cardiovascular disease, which was most pronounced in the group with lipoprotein(a) concentrations of 50 mg/dL or higher—eg, $I^2$ statistics for HRs adjusted for age and sex were 73% (95% CI 43–88) for baseline lipoprotein(a) and 62% (13–83) for on-statin lipoprotein(a) in this group (table 3).

| | Lipoprotein(a) 15 to <30 mg/dL | | | Lipoprotein(a) 30 to <50 mg/dL | | | Lipoprotein(a) ≥50 mg/dL | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | p value | $I^2$ (95% CI) | HR (95% CI) | p value | $I^2$ (95% CI) | HR (95% CI) | p value | $I^2$ (95% CI) |
| **Baseline lipoprotein(a)** | | | | | | | | | |
| Basic adjustment: seven trials, 29 069 patients, 5751 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 1·04 (0·91–1·18) | 0·59 | 43% (0–76) | 1·11 (1·00–1·22) | 0·047 | 0% (0–71) | 1·31 (1·08–1·58) | 0·005 | 73% (43–88) |
| Progressive adjustment: six trials, 27 764 patients, 5649 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 1·03 (0·90–1·18) | 0·64 | 54% (0–81) | 1·10 (1·00–1·22) | 0·053 | 0% (0–75) | 1·30 (1·06–1·59) | 0·010 | 78% (52–90) |
| Plus previous cardiovascular disease | 1·04 (0·90–1·19) | 0·61 | 53% (0–81) | 1·10 (1·00–1·22) | 0·049 | 0% (0–75) | 1·31 (1·07–1·60) | 0·009 | 78% (52–90) |
| Plus diabetes | 1·04 (0·91–1·19) | 0·60 | 52% (0–81) | 1·11 (1·01–1·23) | 0·036 | 0% (0–75) | 1·32 (1·08–1·61) | 0·007 | 78% (51–90) |
| Plus smoking | 1·03 (0·91–1·18) | 0·61 | 50% (0–80) | 1·11 (1·01–1·22) | 0·034 | 0% (0–75) | 1·31 (1·08–1·59) | 0·007 | 77% (48–90) |
| Plus systolic blood pressure | 1·03 (0·90–1·18) | 0·64 | 53% (0–81) | 1·11 (1·01–1·22) | 0·031 | 0% (0–75) | 1·31 (1·07–1·59) | 0·008 | 77% (49–90) |
| Plus LDL-$C_{corr}$ | 1·04 (0·90–1·19) | 0·61 | 55% (0–82) | 1·12 (1·02–1·24) | 0·019 | 0% (0–75) | 1·34 (1·09–1·65) | 0·005 | 78% (53–90) |
| Plus HDL cholesterol | 1·04 (0·91–1·20) | 0·54 | 54% (0–82) | 1·13 (1·02–1·25) | 0·016 | 0% (0–75) | 1·35 (1·11–1·66) | 0·003 | 77% (49–90) |
| **On-statin lipoprotein(a)** | | | | | | | | | |
| Basic adjustment: seven trials, 14 536 patients, 2603 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 0·94 (0·81–1·10) | 0·45 | 18% (0–62) | 1·06 (0·94–1·21) | 0·33 | 0% (0–71) | 1·43 (1·15–1·76) | 0·001 | 62% (13–83) |
| Progressive adjustment: six trials, 13 883 patients, 2561 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 0·93 (0·79–1·09) | 0·37 | 18% (0–63) | 1·06 (0·93–1·21) | 0·35 | 0% (0–75) | 1·39 (1·12–1·72) | 0·002 | 64% (13–85) |
| Plus previous cardiovascular disease | 0·93 (0·79–1·09) | 0·37 | 18% (0–63) | 1·06 (0·93–1·21) | 0·36 | 0% (0–75) | 1·39 (1·12–1·72) | 0·002 | 64% (13–85) |
| Plus diabetes | 0·94 (0·80–1·10) | 0·43 | 17% (0–62) | 1·07 (0·94–1·22) | 0·31 | 0% (0–75) | 1·39 (1·13–1·71) | 0·002 | 62% (7–84) |
| Plus smoking | 0·94 (0·81–1·09) | 0·42 | 8% (0–77) | 1·07 (0·94–1·22) | 0·30 | 0% (0–75) | 1·39 (1·13–1·71) | 0·002 | 62% (8–84) |
| Plus systolic blood pressure | 0·94 (0·81–1·09) | 0·41 | 9% (0–77) | 1·07 (0·94–1·22) | 0·30 | 0% (0–75) | 1·39 (1·13–1·71) | 0·002 | 61% (6–84) |
| Plus LDL-$C_{corr}$ | 0·94 (0·81–1·10) | 0·47 | 13% (0–78) | 1·08 (0·95–1·23) | 0·26 | 0% (0–75) | 1·41 (1·15–1·73) | 0·001 | 61% (3–84) |
| Plus HDL cholesterol | 0·95 (0·82–1·11) | 0·53 | 13% (0–78) | 1·08 (0·95–1·23) | 0·24 | 0% (0–75) | 1·42 (1·16–1·74) | 0·001 | 58% (0–83) |

Patients with lipoprotein(a) concentration <15 mg/dL served as the reference group. HR=hazard ratio. LDL-$C_{corr}$=LDL cholesterol corrected for lipoprotein(a) cholesterol.

***Table 3:*** **Associations of baseline and on-statin lipoprotein(a) with incident cardiovascular disease according to different levels of adjustment**

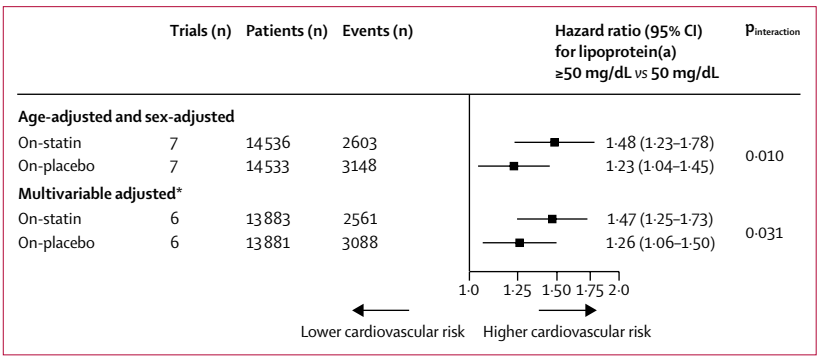


***Figure 2:*** **Comparative predictive value of on-statin versus on-placebo lipoprotein(a) concentrations for incident cardiovascular disease**

*Adjusted for age, sex, previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL cholesterol corrected for lipoprotein(a) cholesterol, and HDL cholesterol.

With the exception of stronger associations of on-statin lipoprotein(a) concentrations with cardiovascular disease risk at younger ages (<60 years *vs* 60 to <70 years *vs* ≥70 years; interaction p=0·008), HRs did not vary across clinically relevant subgroups—eg, by sex, smoking, systolic blood pressure, lipid variables, or BMI (figure 3). Furthermore, the magnitude of association was independent of a study's proportion of patients with previous cardiovascular disease or diabetes, the length of follow-up for clinical events, and the time between study baseline and follow-up measurement of on-statin lipoprotein(a) (appendix). Contributing trials used differing statin interventions, precluding a subgroup analysis by statin type or statin dose.

## Discussion

The findings of our meta-analysis of lipoprotein(a) and cardiovascular events show that patients with raised concentrations of lipoprotein(a) on statin treatment (mainly people with concentrations >50 mg/dL) are at a significantly higher risk of cardiovascular disease. The association with cardiovascular events was independent of conventional cardiovascular disease risk factors. This observation was further underpinned by very weak or null cross-sectional correlations of lipoprotein(a) with these risk factors. Importantly, HRs for high lipoprotein(a) at baseline and while taking statins were of similar magnitude, suggesting that statin treatment might not affect lipoprotein(a)-mediated risk appreciably in people with increased amounts of lipoprotein(a). Overall, these data suggest that patients with raised concentrations of lipoprotein(a), representing about 25% of those with previous cardiovascular disease or an indication for statins,[1] are at substantial residual risk—even while taking statins. In this patient population, treatments that specifically lower lipoprotein(a) might mitigate lipoprotein(a)-mediated risk. An appropriately

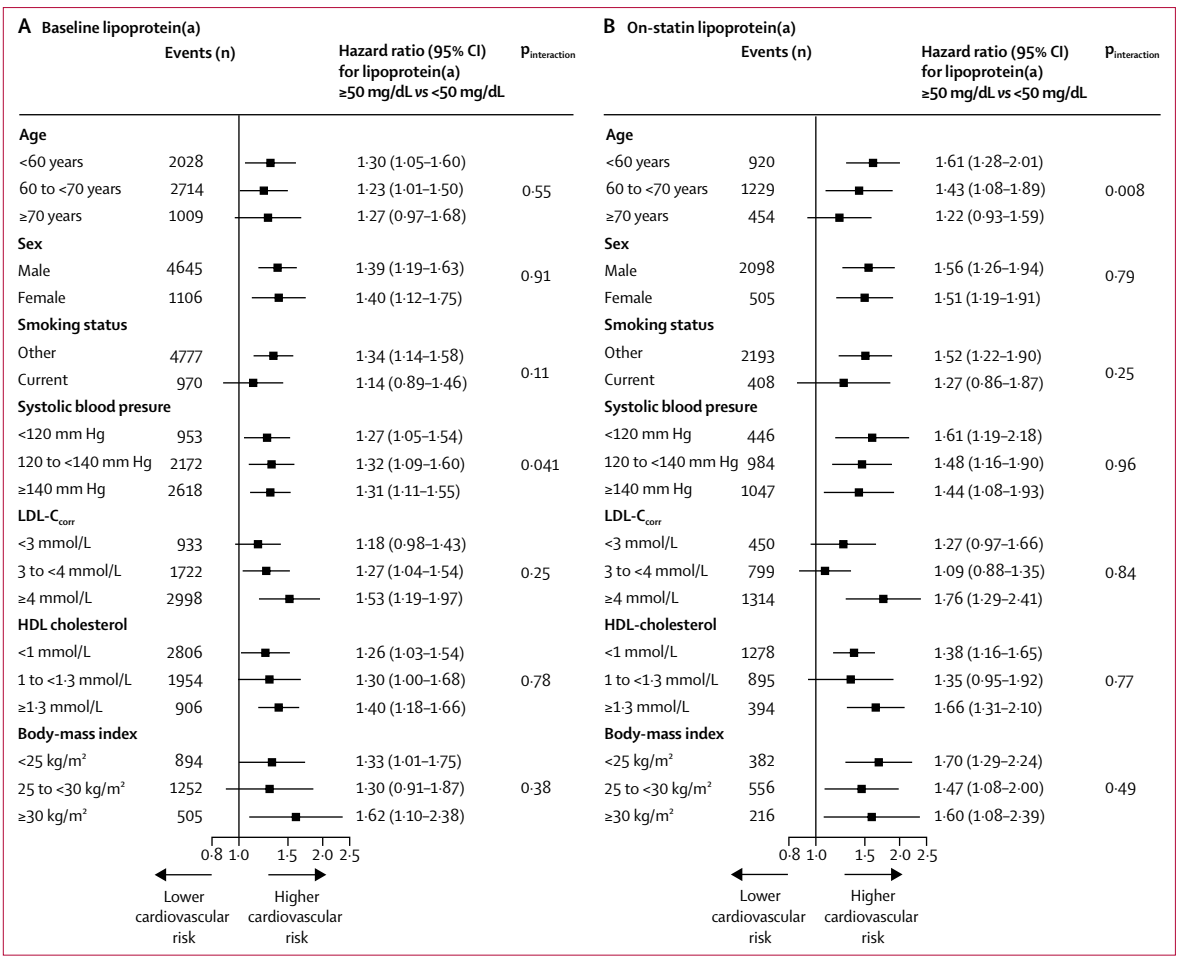


***Figure 3:*** **Associations of baseline and on-statin lipoprotein(a) with incident cardiovascular disease by individual patient characteristics**
Hazard ratios are comparing patients with lipoprotein(a) concentrations of 50 mg/dL or higher with values lower than 50 mg/dL. LDL-$C_{corr}$=LDL cholesterol corrected for lipoprotein(a) cholesterol.

designed cardiovascular disease outcomes trial with robust lowering of lipoprotein(a) is, therefore, justified to test the hypothesis that cutting levels of lipoprotein(a) reduces cardiovascular events, independent of statin treatment.

At baseline, amounts of lipoprotein(a) were associated weakly with demographic and laboratory variables. The most significant (but nevertheless weak) correlations were inverse with diabetes mellitus and triglycerides. The observation of an inverse association of lipoprotein(a) with incident diabetes has been made previously,[31] and the inverse association is most pronounced at very low levels of lipoprotein(a) (≤5 mg/dL), which corresponds approximately to the bottom tenth of the lipoprotein(a) distribution in the global population.[1,2] Whether these findings are causal, or whether they are due to reverse causality, is unknown.[32] Although the underlying mechanisms are not well understood, fasting and postprandial insulin levels are associated inversely with lipoprotein(a).[33] Lipoprotein(a) correlated weakly with LDL cholesterol, but this relation became inversely associated after subtracting the estimated cholesterol content in lipoprotein(a) from the laboratory measurement of LDL cholesterol.[28]

Studies in which modern lipoprotein(a) assays were used to assess the role of lipoprotein(a) in predicting risk for cardiovascular events in patients without cardiovascular disease have been almost uniformly positive.[7] However, results of studies in patients with previous cardiovascular disease or who were taking statins either have been mixed or have suggested the effect is present mainly in people with increased LDL cholesterol.[2] A major limitation of all substudies reporting lipoprotein(a) and outcomes has been power. All studies have enrolled patients with lipoprotein(a) concentrations in the mid-to-low-normal range (10–15 mg/dL; normal <30 mg/dL), as confirmed in our meta-analysis; thus, statistical power to assess risk in patients with highly elevated lipoprotein(a) (ie, >50 mg/dL) was low. Our study is well powered, with 5751 total events and 2603 events in patients allocated statins, making it equivalent to (or larger than) most individual, randomised

controlled, cardiovascular outcome trials in the modern era. By contrast with findings of a previous analysis of individual-patient data,[34] our study had higher statistical power because it included at least ten times more cardiovascular events and, hence, could characterise associations with high lipoprotein(a) concentrations more precisely. Moreover, our analysis used clinically relevant lipoprotein(a) categories informed by guideline recommendations rather than trial-specific quintiles.

Our meta-analysis is highly representative of clinical care in patients treated with statins. First, the studies we included represent patients who were treated with moderate-to-high doses of five major statins used clinically. Second, the studies reflect the variety of patients treated clinically, including primary prevention, high-risk primary prevention with elevated C-reactive protein or diabetes, secondary prevention, stable coronary artery disease, acute coronary syndromes, patients on dialysis, and those with highly elevated LDL cholesterol in the familial hypercholesterolaemia range. Therefore, the studies we included in our meta-analysis broadly reflect patients with high residual risk despite statin treatment, potentially due to other unmodified risk factors such as increased amounts of lipoprotein(a).

The categories of lipoprotein(a) concentrations chosen by us in our meta-analysis reflect clinical risk as suggested by findings of epidemiological and genetic studies. The cutoff of less than 15 mg/dL we used to define the reference group corresponds roughly to the median global concentration of lipoprotein(a).[35,36] Concentrations lower than 30 mg/dL represent the usual cutoff in US laboratories, which is regarded as the normal concentration and is based on data showing that risk for myocardial infarction starts to accrue at concentrations of lipoprotein(a) higher than 25–30 mg/dL.[7,37] The range of 30–50 mg/dL was chosen because it is the grey zone between concentrations considered pathophysiologically relevant and concentrations judged by the European Atherosclerosis Society as high risk (>50 mg/dL), based on the European population prevalence of 20%.

In our study, elevation of cardiovascular disease risk became evident at baseline in patients with concentrations of lipoprotein(a) ranging between 30 mg/dL and lower than 50 mg/dL, and risk was further pronounced when lipoprotein(a) concentration exceeded 50 mg/dL at baseline, including for patients treated with statins. The HRs for lipoprotein(a) concentrations of 50 mg/dL or higher are consistent with those reported in studies of PCSK9 inhibitors in patients with background statin therapy.[38] Additional analyses at even greater concentrations of lipoprotein(a)—ie, 75 mg/dL or higher—were limited by low power because of few patients with lipoprotein(a) concentrations in this range, but the findings support a graded relation between lipoprotein(a) and cardiovascular risk. Outcome trials of lipoprotein(a) lowering are likely to include patients with mean baseline lipoprotein(a) substantially greater than 50 mg/dL; therefore, extrapolation to event reduction with lipoprotein(a) lowering from these data could be an underestimate.

A key observation of our study is that lipoprotein(a) concentrations were associated more strongly with cardiovascular disease risk in patients assigned statins than in people allocated placebo. Findings of a small angiographic study suggested initially that risk associated with lipoprotein(a) is attenuated when LDL cholesterol is well controlled.[39] By contrast, findings of our study—utilising a far larger body of data—support the opposite conclusion, that risk is associated independently with both LDL cholesterol and lipoprotein(a). When LDL-attributable risk is reduced with statin treatment, lipoprotein(a)-associated risk becomes an even stronger predictor of residual risk. This observation is especially evident at lipoprotein(a) concentrations exceeding 50 mg/dL. In support of our observation in this study, the trials FOURIER (NCT01764633) and ODYSSEY OUTCOMES (NCT01663402) have presented preliminary findings of their data, both showing that elevated baseline lipoprotein(a) remains a risk factor for cardiovascular disease, even with on-treatment LDL cholesterol lower than 50 mg/dL in patients treated with statins and PCSK9 inhibitors. The findings highlight the importance of determining whether there is a cardiovascular benefit of treatment to reduce lipoprotein(a) when initial concentrations exceed this threshold, irrespective of concurrent treatment with statin. A second important observation is that all major subgroups of patients seemed to be at risk of elevated lipoprotein(a), including those older than 70 years, women, smokers, people with low and high LDL-$C_{corr}$, low HDL cholesterol, and all categories of BMI.

It is important to emphasise that the lipoprotein(a) hypothesis remains to be tested. To do so requires a randomised trial that compares cardiovascular outcomes in patients treated with an agent that specifically lowers lipoprotein(a) versus placebo. Such a trial might be possible with an antisense oligonucleotide targeting *LPA* messenger RNA, thereby reducing plasma lipoprotein(a) levels. Phase 1 and 2 trials with such an agent have shown the potential to lower lipoprotein(a) levels by more than 90%.[27,40]

Our study has several limitations. First, individual patient data could not be obtained from several other statin trials that reported lipoprotein(a) concentrations and outcomes. It is possible that inclusion of other data would have modified the reported effect sizes. Second, the relation of lipoprotein(a) to residual cardiovascular risk in patients receiving treatment with non-statin lipid-modifying agents (eg, ezetimibe, PCSK9 inhibitors) remains undetermined. Third, lipoprotein(a) assays were heterogeneous and most were in lipoprotein(a) mass (mg/dL) rather than molar (nmol/L) concentration, and the timepoints at which lipoprotein(a) was measured in each trial were not uniform. Therefore, assays not reported in mg/dL had to be converted mathematically

to mg/dL, which might have introduced imprecision into the lipoprotein(a) measurement. A National Heart, Lung, and Blood Institute Working Group on lipoprotein(a) recommended global standardisation of lipoprotein(a) assays to address this limitation.[2] Fourth, we cannot rule out that index event bias could have attenuated effect sizes in secondary prevention trials, although the scope of this bias was reduced by employment of multivariable adjustment. Fifth, our analysis identified moderate-to-high between-study heterogeneity, which could not be accounted for by baseline disease status (ie, previous cardiovascular disease or previous diabetes) nor by differing lengths of follow-up.

In conclusion, our meta-analysis shows an approximately linear relation between cardiovascular risk and concentrations of lipoprotein(a), which is evident at lipoprotein(a) concentrations of 30 mg/dL to less than 50 mg/dL and pronounced at concentrations of 50 mg/dL or higher and persists despite statin treatment. These data provide a rationale for evaluating drugs that can lower lipoprotein(a) specifically and might have the potential to reduce residual cardiovascular risk independent of statin treatment.

#### Contributors

PW and ST wrote the analysis plan, collected and harmonised data, had access to all raw data, and wrote the first draft of the report. PW did the statistical analysis. PMR, PJN, JS, AMT, TRP, GGS, AGO, HMC, FK, CD, CW, and SM obtained patient data in statin trials and provided cleaned data to the coordinating centre. AL secured funding for the study and contributed to data interpretation. All authors contributed to writing the final report and approved the version submitted.

#### Declaration of interests

PW reports consultancy fees from Novartis Pharmaceuticals during the conduct of the study; and travel expenses from Bayer, Daiichi Sankyo, and Sanofi-Aventis outside the submitted work. PMR reports grants from AstraZeneca during the conduct of the study; grants from Novartis, Kowa, Pfizer, and the National Heart, Lung, and Blood Institute outside the submitted work; and personal fees from Novartis and Sanofi outside the submitted work. AMT reports personal fees from Amgen, Bayer, Merck, and Pfizer outside the submitted work; and non-financial support from Bayer outside the submitted work. TRP reports personal fees from Amgen and Sanofi Regeneron outside the submitted work. GGS reports grants from Pfizer during the conduct of the study; and grants from Cerenis, Roche, Sanofi, and The Medicines Company outside the submitted work. HMC reports grants from AstraZeneca, Boehringer Ingelheim, and Roche Pharmaceuticals during the conduct of the study; grants, non-financial support, and travel expenses from Eli Lilly and Regeneron during the conduct of the study; personal fees from Eli Lilly during the conduct of the study; institutional fees from Novartis Pharmaceuticals during the conduct of the study; grants and speaker fees from Pfizer during the conduct of the study; grants and travel expenses from Sanofi Aventis and Novo Nordisk during the conduct of the study; honorarium and speakers' bureau fees from Sanofi during the conduct of the study; and holds shares in Bayer and Roche Pharmaceuticals. CW reports personal fees from Boehringer Ingelheim and Sanofi-Genzyme outside the submitted work. SM reports institutional support from the National Institutes of Health (NIH; grants R01 HL117861, R01 HL134811, and K24 HL136852) outside the submitted work; non-financial support from Quest Diagnostics for measuring lipoprotein(a) in the JUPITER trial outside the submitted work; personal fees from Quest Diagnostics outside the submitted work; and an institutional research grant from Atherotech Diagnostics outside the submitted work. The JUPITER trial was funded by AstraZeneca. AL is an employee of Novartis Pharma AG. ST declares research support from the NIH (grants R01-HL119828, R01-HL078610, R01 HL106579, R01 HL128550, R01 HL136098, P01 HL136275, and R35 HL135737) and is supported by a grant to the Leducq Epigenetics of Atherosclerosis Network from the Fondation Leducq; currently has a dual appointment at the University of California San Diego and Ionis Pharmaceuticals; is a co-inventor and receives royalties from patents owned by the University of California San Diego on oxidation-specific antibodies; and is a co-founder of Oxitope. PJN, JS, AGO, FK, and CD declare no competing interests.

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# Predicting the Effect of Fenofibrate on Cardiovascular Risk for Individual Patients With Type 2 Diabetes Mellitus

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## OBJECTIVE

**In clinical trials, treatment with fenofibrate did not reduce the incidence of major cardiovascular events (MCVE) in patients with type 2 diabetes mellitus (T2DM). However, treatment effects reported by trials comprise patients who respond poorly and patients who respond well to fenofibrate. Our aim was to use statistical modeling to estimate the expected treatment effect of fenofibrate for individual patients with T2DM.**

## RESEARCH DESIGN AND METHODS

**To estimate individual risk, the FIELD risk model, with 5-year MCVE as primary outcome, was externally validated in T2DM patients from ACCORD and the SMART observational cohort. Fenofibrate treatment effect was estimated in 17,142 T2DM patients from FIELD, ACCORD, and SMART. Individual treatment effect, expressed as absolute risk reduction (ARR), is the difference between treated and untreated MCVE risk. Results were stratified for patients with and without dyslipidemia (i.e., high triglycerides and low LDL cholesterol).**

## RESULTS

**External validation of the FIELD risk model showed good calibration and moderate discrimination in ACCORD (C-statistic 0.67 [95% CI 0.65–0.69]) and SMART (C-statistic 0.66 [95% CI 0.63–0.69]). Median 5-year MCVE risk in all three studies combined was 6.7% (interquartile range [IQR] 4.0–11.7) in patients without (*N* = 13,224), and 9.4% (IQR 5.4–16.1%) in patients with (*N* = 3,918), dyslipidemia. The median ARR was 2.15% (IQR 1.23–3.68) in patients with dyslipidemia, corresponding with a number needed to treat (NNT) of 47, and 0.22% (IQR 0.13–0.38) in patients without dyslipidemia (NNT 455).**

## CONCLUSIONS

**In individual patients with T2DM, there is a wide range of absolute treatment effect of fenofibrate, and overall the fenofibrate treatment effect was larger in patients with dyslipidemia. The method of individualized treatment effect prediction of fenofibrate on MCVE risk reduction in T2DM can be used to guide clinical decision-making.**


Patients with type 2 diabetes mellitus (T2DM) have a high risk of cardiovascular disease (CVD) (1). Although statins reduce cardiovascular morbidity and mortality significantly by reducing LDL cholesterol (LDL-C), the remaining residual risk underscores the clinical need for additional treatment options (2,3). Fibrates specifically target the dyslipidemia

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seen in T2DM by reducing plasma triglycerides (TG) by 30% and increasing HDL cholesterol (HDL-C) by 10% (4). The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trials, both placebo-controlled randomized clinical trials, investigated the effect of fenofibrate in patients with T2DM and showed no overall reduction in CVD (5,6). However, although the benefit of fenofibrate was not statistically significant, it is conceivable that there are patients in whom fenofibrate does convey clinical benefit (3). Subgroup analyses are a method frequently used to address this issue. Based on subgroup analysis in FIELD and ACCORD, the American Diabetes Association guideline recommends statin/fibrate combination therapy in T2DM in men with TG >2.3 mmol/L and HDL-C <0.9 mmol/L (7).

Individualized treatment effect prediction considers both favorable and unfavorable risk factors simultaneously and takes relative effects presented by randomized trials one step further by expressing treatment effect in terms of absolute risk reduction (ARR) for individual patients (8–13).

The aim of the current study was to estimate individual treatment effect of fenofibrate on major cardiovascular events (MCVEs) for T2DM patients from the FIELD and ACCORD randomized clinical trials and the Second manifestations of ARTerial disease (SMART) observational cohort study.

## RESEARCH DESIGN AND METHODS

### FIELD, ACCORD, and SMART

FIELD evaluated the effect of fenofibrate compared with placebo in 9,795 patients with T2DM between the age of 50 and 75 years who were not taking statin therapy at study entry. Detailed information about the study design has previously been published (3). The primary outcomes were coronary events (coronary disease mortality and nonfatal myocardial infarction), which occurred in 544 patients during a 5-year median follow-up. FIELD reported an 11% reduction in coronary events in patients using fenofibrate (hazard ratio [HR] 0.89 [95% CI 0.75–1.05]) (5). In total, 859 patients suffered an MCVE, defined as nonfatal myocardial infarction, nonfatal stroke, or cardiovascular mortality.

The ACCORD lipid trial consisted of 5,518 patients with T2DM and investigated the effect of fenofibrate in combination with atorvastatin compared with atorvastatin plus placebo (6,14). The primary outcome was MCVE, which occurred 601 times after a median follow-up of 4.7 years. The ACCORD trial reported an 8% reduction in MCVEs (i.e., nonfatal myocardial infarction, nonfatal stroke, and cardiovascular death) (HR 0.92 [95% CI 0.79–1.08]) in patients using fenofibrate on a background of statin therapy.

Although the primary end points used in FIELD and ACCORD differed, we used the MCVE composite end point for the analyses in the present manuscript.

The SMART study is an ongoing longitudinal cohort study that started in 1996 and included 1,829 T2DM patients with and without CVD (15). In the SMART cohort, the composite end point MCVE was observed in 334 patients, with a median follow-up time of 5.8 years. In all three studies, written informed consent was obtained from all participants and the ethics boards of the institutions approved the studies.

### Missing Data

In FIELD, 0.2% of data were missing for duration of T2DM and 0.3% for urinary albumin-to-creatinine ratio. In ACCORD, missing data ranged from 0.1% for current smoking to 4.3% in UACR. In SMART, missing data ranged from 0.05% for TG and HDL-C to 7% for UACR. Single imputation by bootstrapping and predictive mean matching was used (areglmpute in R, Hmisc package) to account for missing data in the predictors (16).

### Model Derivation and Validation

For development of the FIELD model for the prediction of 5-year ARR for MCVE by fenofibrate, Cox proportional hazards models for time to MCVE were used, with time to event in years. For prevention of overfitting, predictors were prespecified based on the presence in three or more CVD prediction models for patients with T2DM (17). The initial model included the following variables at baseline: fenofibrate treatment, age, sex, ethnicity, diabetes duration, current smoking, previous CVD, use of antihypertensive medication, $HbA_{1c}$, systolic blood pressure, non-HDL-C, HDL-C, TG, UACR, dyslipidemia, estimated glomerular filtration rate (eGFR) (MDRD formula), and interaction terms between these predictors and fenofibrate. Metabolic syndrome was not added as a separate categorical variable because its individual components were included as single predictors. In eight of the nine prognostic models that were used for predictor selection, a marker for body weight was initially included but fell out of the model owing to inadequate (additional) prognostic value. Therefore, we did not add BMI or waist circumference to our set of predictors. Fenofibrate use was forced in the model. Model selection was based on backward selection using the Wald $\chi^2$ statistic for removal of variables, based on which ethnicity, TG, and dyslipidemia dropped out of the model. TG probably fell out of the model because their prognostic value is already captured in non-HDL-C, which is a stronger predictor for CVD. Continuous variables were checked for nonnormality by visual inspection of the Martingale residual distribution, which led to log transformation of UACR and HDL-C. The Cox proportional hazard assumption was tested and met for all predictors. The final model is shown in Supplementary Box 1.

Model validation was performed in the ACCORD lipid trial and the SMART observational cohort study. To ensure adequate risk stratification, we recalibrated the final model for baseline survival in ACCORD and SMART to take differences in baseline survival (e.g., by a difference in background lipid-lowering therapy) into account. Model performance was tested with calculation of the C-statistic for discrimination and with calibration plots for predicted versus observed MCVE risk (expressed as event-free survival). For comparison, the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) risk model (18) was validated in these three data sets (Supplementary Material).

### Individualized Treatment Effect Prediction

For demonstration of the application of the prediction model in a large and heterogeneous group of patients that resembles clinical practice, patient data from FIELD, ACCORD, and SMART were combined ($N$ = 17,142). Results were stratified for the presence of dyslipidemia, which was defined as TG >2.3 mmol/L and HDL-C <1.00 mmol/L for men and <1.30 mmol/L for women. Results stratified for TG ≤2.3 mmol/L and >2.3 mmol/L are shown in Supplementary Material. First, 5-year MCVE risk was calculated by setting treatment status to zero in the

FIELD risk model for all patients (irrespective of their actual treatment status). Second, ARR was calculated as MCVE risk minus on-treatment risk. ARR (%) was also expressed as number needed to treat (NNT = 100/ARR). An example of how ARR is calculated can be found in Box 2 (Supplementary Material). Subgroup-specific HRs for the effect of fenofibrate on 5-year MCVE risk in patients with and without dyslipidemia were derived from the combined FIELD and ACCORD data using Cox proportional hazards models. These were HR 0.97 (95% CI 0.86–1.09) for patients without dyslipidemia and HR 0.77 (95% CI 0.64–0.94) for patients with dyslipidemia. On-treatment risk was calculated by multiplying 5-year MCVE risk with the subgroup-specific HR for patients with and without dyslipidemia. Third, patients were stratified according to treatment effect. The analyses were performed in R, version 3.2.2 (package 'rms'; R Core Team, Vienna, Austria), and SAS, version 9.3. Fourth, a calculation sheet was made (Microsoft Office Excel 2007).

## RESULTS

### Baseline Characteristics

FIELD included 9,795 patients, ACCORD 5,518 patients, and SMART 1,829 patients with T2DM. The mean age was 62.2 ± 7.3 years in the total population, and 66% were men (Table 1). Of the FIELD participants, 22% had a history of CVD and the median duration of T2DM was 5 years (interquartile range [IQR] 2–10). In ACCORD, 37% of the patients had a history of CVD with a median duration of T2DM of 9 years (IQR 5–15). In SMART, 66% had a history of CVD and the median T2DM duration was 10 years (IQR 5–15 years).

### External Validation of the Prediction Model

The FIELD risk model for the 5-year treatment effect of fenofibrate on MCVE in patients with T2DM is provided in Supplementary Box 1. External validation showed moderate discrimination, with a C-statistic of 0.67 (95% CI 0.65–0.69) and 0.68 (95% 0.64–0.72) in ACCORD and SMART, respectively. Calibration between observed and predicted 5-year MCVE risk was well balanced in both studies (Fig. 1). The ADVANCE risk model was less well calibrated and had a lower C-statistic in ACCORD and SMART compared with the FIELD risk model (Supplementary Figs. 1–3).

### Five-Year MCVE-Risk and Treatment Effect of Fenofibrate

In a pooled analysis of FIELD, ACCORD, and SMART, the median 5-year MCVE risk was 9.4% (IQR 5.4–16.2) and 6.7% (IQR 4.0–11.7) in patients with and patients without dyslipidemia, respectively (Fig. 2). There was a wide range in absolute treatment effect of fenofibrate, with a median ARR of 2.15% (IQR 1.24–3.69, NNT 47) in patients with dyslipidemia and 0.22% (IQR 0.13–0.38, NNT = 455) in patients without dyslipidemia (Fig. 2). Results were similar when stratified for TG ≤2.3 mmol/L and >2.3 mmol/L (Tables 1 and 2 and Supplementary Figs. 4 and 5). For calculation of individual ARR, a calculation sheet is provided in Supplementary Material.

Of patients with dyslipidemia, 54% had an ARR ≥2% (NNT ≤50). Patients with dyslipidemia and an ARR ≥2% (NNT ≤50) and had a median MCVE risk of 15.4% (IQR 11.7–22.5) and a median ARR of 3.52% (IQR 2.37–5.13) (Table 2). In patients with dyslipidemia and ARR <2%, the median MCVE risk was 5.2% (IQR 3.8–6.8) and the median ARR was 1.18% (0.86–1.55). Of patients with dyslipidemia, 16% (*N* = 634) had an ARR <1.0%. The median MCVE risk in these patients was 3.26% (IQR 2.63–3.90) and median ARR 0.74% (IQR 0.60–0.89, NNT = 135).

Of patients without dyslipidemia, 97% had ARR by fenofibrate of <1% (NNT >100). In these patients, the median MCVE risk was 6.6% (IQR 3.9–11.2) and

**Table 1—Baseline characteristics**

| | FIELD | ACCORD | SMART | Total |
|---|---|---|---|---|
| *N* | 9,795 | 5,518 | 1,829 | 17,142 |
| Age (years) | 62.2 ± 6.9 | 62.8 ± 6.6 | 60.3 ± 10.2 | 62.2 ± 7.3 |
| Males (%, *N*) | 63 (6,138) | 69 (3,824) | 70 (1,272) | 66 (11,234) |
| Duration of T2DM (years) | 5 (2–10) | 9 (5–15) | 4 (1–9) | 6 (3–11) |
| Previous CVD | 22 (2,131) | 37 (2,016) | 69 (1,260) | 32 (5,407) |
| Current smoking | 9 (922) | 15 (803) | 25 (456) | 13 (2,181) |
| Antihypertension medication | 58 (5,659) | 82 (4,530) | 62 (1,130) | 66 (11,319) |
| Systolic blood pressure (mmHg) | 140 ± 15 | 134 ± 17 | 145 ± 21 | 139 ± 17 |
| Diastolic blood pressure (mmHg) | 82 ± 9 | 74 ± 10 | 83 ± 12 | 79 ± 10 |
| BMI (kg/m$^2$) | 30.7 ± 5.5 | 32.3 ± 5.3 | 29.0 ± 5.0 | 31.0 ± 5.5 |
| Waist circumference (cm) | 104 ± 13 | 108 ± 14 | 101 ± 13 | 105 ± 13 |
| Total cholesterol (mmol/L) | 5.0 ± 0.7 | 4.5 ± 1.0 | 4.8 ± 1.4 | 4.9 ± 0.9 |
| HDL-C (mmol/L) | 1.10 ± 0.26 | 0.99 ± 0.20 | 1.13 ± 0.33 | 1.06 ± 0.26 |
| Non-HDL-C (mmol/L) | 3.9 ± 0.7 | 3.5 ± 1.0 | 3.7 ± 1.4 | 3.9 ± 0.9 |
| TG (mmol/L) | 1.73 (1.34–2.32) | 1.82 (1.28–2.59) | 1.7 (1.2–2.5) | 1.75 (1.30–2.42) |
| LDL-C (mmol/L) | 3.1 ± 0.7 | 2.6 ± 0.8 | 2.8 ± 1.1 | 2.9 ± 0.8 |
| $HbA_{1c}$ (%) | 6.9 (6.1–7.8) | 8.1 (7.6–8.8) | 6.8 (6.2–7.7) | 7.4 (6.5–8.3) |
| $HbA_{1c}$ (mmol/mol) | 52 (43–62) | 65 (60–73) | 51 (44–61) | 57 (48–67) |
| Glucose (mmol/L) | 8.91 ± 2.60 | 9.71 ± 2.90 | 8.71 ± 2.92 | 9.15 ± 2.76 |
| eGFR (mL/min/1.73 m$^2$) | 88 ± 18 | 90 ± 22 | 78 ± 22 | 87 ± 20 |
| UACR (mg/mmol) | 1.10 (0.60–2.95) | 1.58 (0.79–5.20) | 1.43 (0.85–3.06) | 1.25 (0.68–3.50) |

Data are mean ± SD, median (IQR), or % (*N*) unless otherwise indicated. eGFR calculated with the MDRD formula.

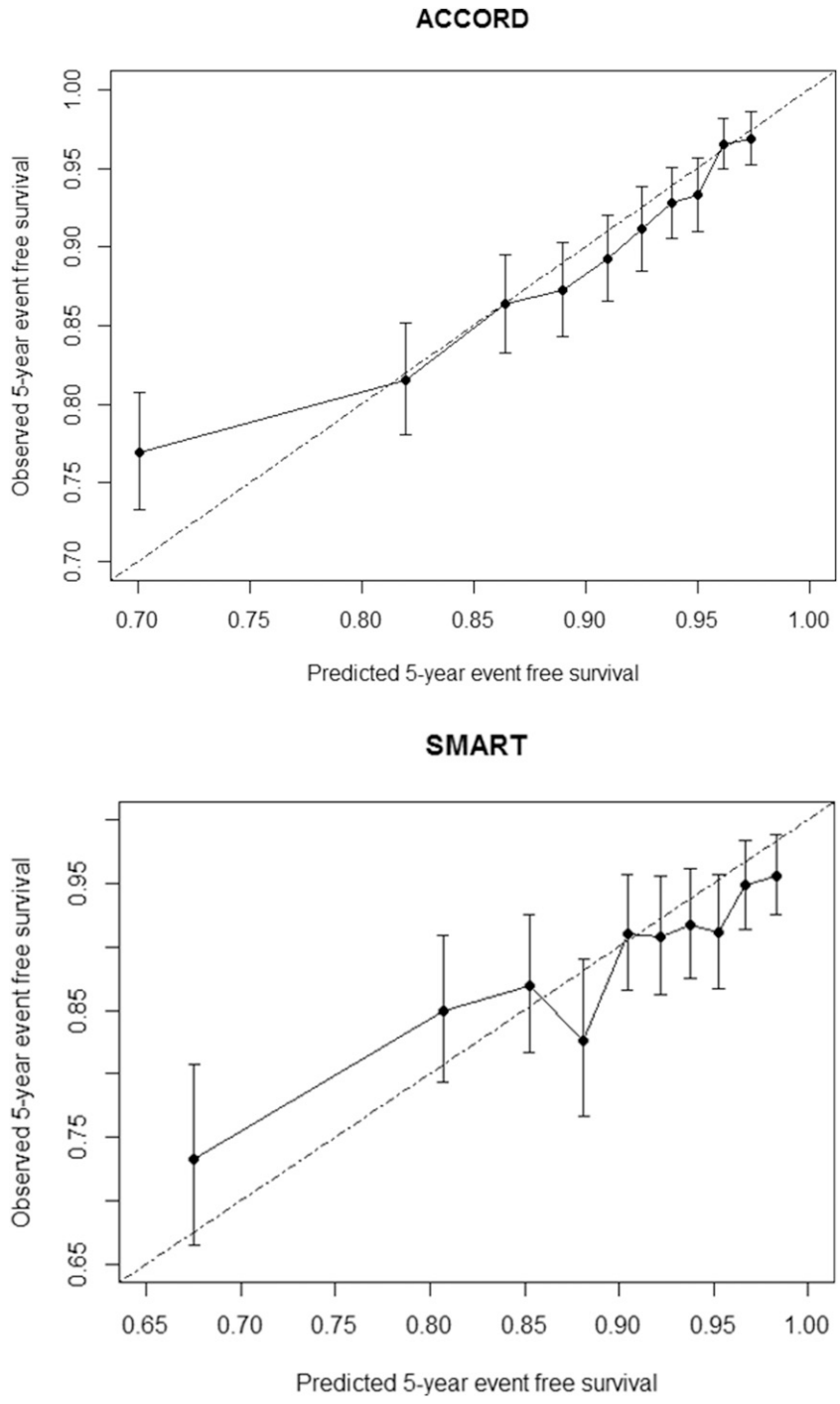


**Figure 1**—External calibration of the FIELD risk score in the ACCORD and SMART studies. Calibration of observed vs. predicted 5-year event-free survival for MCVE within deciles of predicted risk.

ARR 0.21% (IQR 0.13–0.36) (Table 2). The median MCVE risk in patients without dyslipidemia with an ARR ≥1% (NNT ≤100) was 36.8% (IQR 33.6–42.9).

## CONCLUSIONS

In this study, we used statistical modeling to estimate the individual treatment effect of fenofibrate for 17,142 patients with T2DM from the FIELD and ACCORD randomized trials and the SMART observational cohort study. We found a wide range of individual treatment effects. More than half (54%) of the patients with dyslipidemia had a substantial treatment effect (ARR >2%, NNT <50) and nearly all (97%) patients without dyslipidemia had a small treatment effect (<1%, NNT >100).

Individualized treatment prediction is a method to overcome disadvantages of subgroup analyses and facilitate the translation from randomized clinical trials to individual patients (8–13).

Subgroup analyses often overestimate treatment effect (19) and study only one patient characteristic at the time (17), thereby not taking into account that treatment effect usually depends on several, often correlated, patient characteristics. This can lead to confounded interpretations (20). Individualized treatment prediction uses multivariate models to estimate individual, absolute treatment effects, thereby contributing to the improvement of personalized medicine.

As mentioned before, patients with T2DM have a high residual cardiovascular risk, even when risk factors are adequately treated according to guidelines (2), and additional treatment options to reduce cardiovascular risk are needed (3). On the other hand, overtreatment is undesirable, especially in T2DM patients who usually use many medications including oral hyperglycemic drugs and/or insulin. Before adding additional drugs, such as fenofibrate, both physician and patient need to know what the expected effect of fenofibrate will be. The multivariable prediction model presented in this article can be used to translate group-level evidence to individual patients in clinical practice. Presenting an individual treatment effect can benefit communication with a patient because benefits can be weighed against expected harms such as costs and side effects. This will facilitate shared decision-making and improve treatment adherence (21,22). Although the use of individualized treatment effect prediction in clinical practice might seem complicated, we provide an easy-to-use calculation sheet that only requires a physician to fill in the patient's clinical information. This way physicians are able to practice evidence-based personalized medicine in daily practice.

External validation of the FIELD risk model showed good calibration and moderate discrimination in both the ACCORD randomized clinical trial and in "real-life patients" from the SMART observational cohort study after recalibration of baseline survival. This means that the FIELD risk model can be used to estimate 5-year MCVE risk for individual patients with T2DM. For individualized treatment effect prediction, it is especially important that the predicted risks are in agreement with the observed risks, and therefore

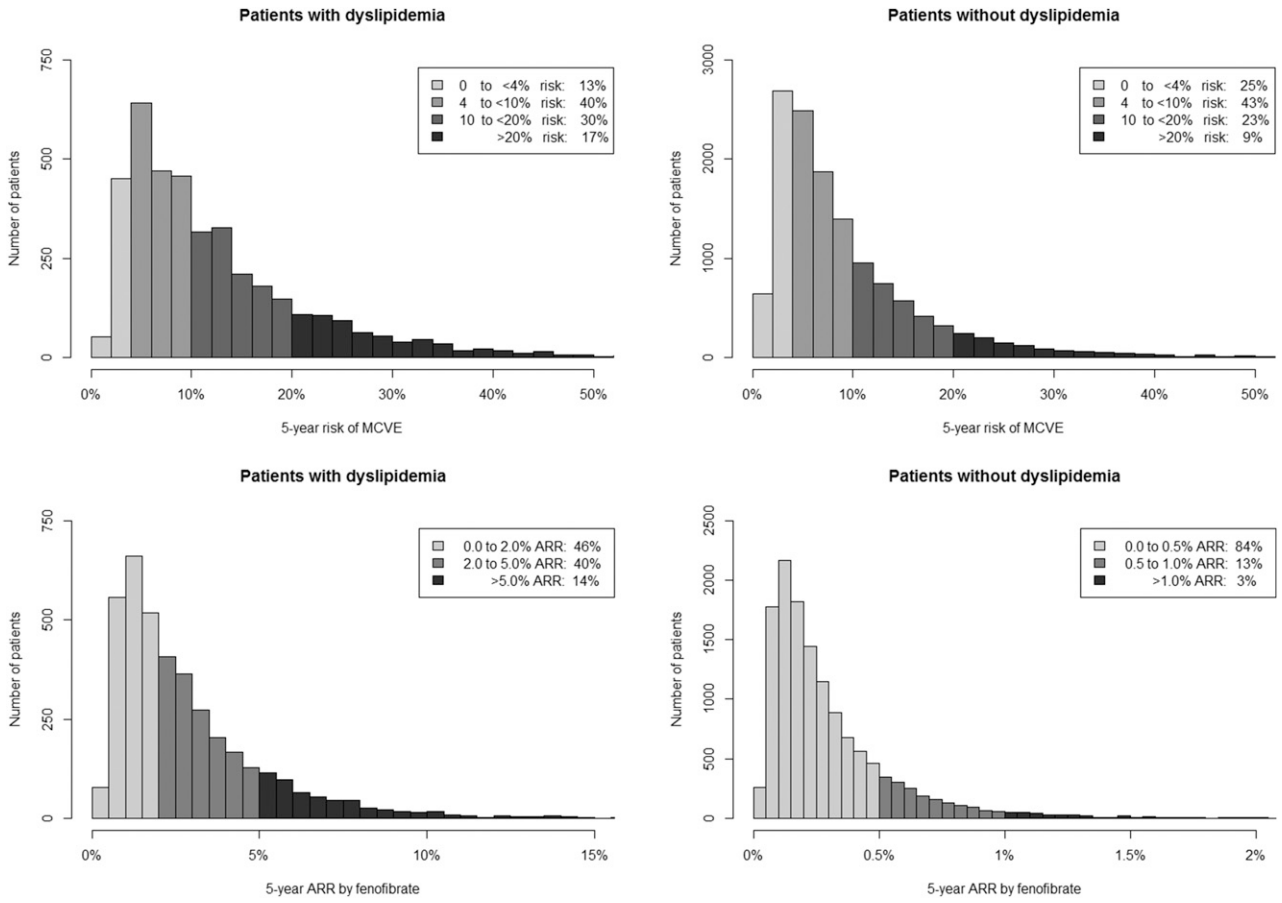


**Figure 2**—Top: distribution of predicted 5-year MCVE risk in T2DM patients with and T2DM patients without dyslipidemia. Bottom: distribution of individual treatment effect of fenofibrate on MCVE risk in T2DM patients with and T2DM patients without dyslipidemia. Treatment effect is expressed as ARR.

calibration is the most important performance measure in this regard (10). One of the reasons that there was a difference in baseline survival might be the fact that FIELD patients were not on background therapy with statins. However, the well-balanced calibration of the FIELD model in ACCORD after baseline risk adjustment indicates that the difference in background statin therapy did not influence treatment effect predictions.

We found that, overall, patients with dyslipidemia had a larger treatment effect of fenofibrate than patients without dyslipidemia. This is due to a larger relative treatment effect, which is in line with two meta-analyses that found a higher relative benefit of fibrate in patients with dyslipidemia (23,24). Furthermore, patients with dyslipidemia generally have a higher 5-year MCVE risk than patients without dyslipidemia because both low HDL-C and high TG levels are independently related to increased CVD risk (25–27). That fenofibrate did not show an overall treatment effect in FIELD and ACCORD is probably because the majority of patients did not have dyslipidemia at baseline. Although we generally found a higher treatment effect in patients with dyslipidemia, we also found that some patients without dyslipidemia had a considerable fenofibrate treatment effect (ARR $\geq$1%, NNT $<$100). Furthermore, 16% of patients with dyslipidemia had a small treatment effect (ARR $<$1%, NNT $>$100). These results indicate that simply treating all patients with dyslipidemia and none without dyslipidemia with fenofibrate does not necessarily tailor the right treatment to the right patient. Therefore, treatment recommendations should go beyond group level and use individualized treatment effect prediction to guide clinical decision-making.

Future projects to develop this method and add to the development of personalized medicine should be aimed at estimating the combined effect of different types of medication, e.g., glycemic control, statins, and blood pressure–lowering medication, on clinical end points because the combined effect of these medications might be different than each effect separately. For development of a model like this, trials that investigate the combined effect of these medications should be performed. Furthermore, the effect on microvascular T2DM end point such as nephropathy or retinopathy can be estimated, just like quality of life. A recent development is to predict gain in healthy life expectancy instead of risk because risk is in very large part determined by age (3). Another interesting future project would be to determine which lipid effect of fenofibrate (TG lowering, HDL increasing, or other) is most important for the observed treatment effect. Finally, as clinical practice changes over time, it would be valuable to validate and update the presented model regularly.

Some limitations with regard to this study should be considered. First, the prediction model presented here was developed using data from clinical trials, which might limit generalizability to patients

**Table 2—Patient characteristics according to treatment effect in T2DM patients with and T2DM patients without dyslipidemia**

| | No dyslipidemia (*N* = 13,224) | | Dyslipidemia (*N* = 3,918) | |
|---|---|---|---|---|
| | ARR <1.0% | ARR ≥1.0% | ARR <2.0% | ARR ≥2.0% |
| *N* | 12,891 | 333 | 1,814 | 2,104 |
| 5-year MCVE risk (%) | 6.6 (3.9–11.2) | 36.9 (33.5–42.6) | 5.2 (3.8–6.9) | 15.4 (11.7–22.5) |
| ARR (%) | 0.21 (0.13–0.36) | 1.19 (1.08–1.38) | 1.18 (0.87–1.56) | 3.51 (2.66–5.13) |
| Age (years) | 62.3 ± 7.2 | 70.1 ± 5.8 | 58.4 ± 6.3 | 63.7 ± 6.9 |
| Male sex | 66 (8,543) | 93 (308) | 45 (814) | 75 (1,569) |
| Duration of T2DM (years) | 6 (2–11) | 14 (7–21) | 4 (2–8) | 8 (4–13) |
| Previous CVD | 29 (3,765) | 95 (316) | 9 (162) | 55 (1,164) |
| Current smoking | 11 (1,458) | 36 (120) | 8 (144) | 22 (459) |
| Antihypertension medication | 64 (8,244) | 91 (302) | 58 (1,052) | 82 (1,721) |
| Systolic blood pressure (mmHg) | 139 ± 17 | 153 ± 19 | 134 ± 15 | 142 ± 18 |
| Diastolic blood pressure (mmHg) | 80 ± 10 | 77 ± 13 | 80 ± 9 | 79 ± 11 |
| BMI (kg/m$^2$) | 30.7 ± 5.6 | 29.2 ± 4.4 | 32.5 ± 5.5 | 31.8 ± 5.1 |
| Waist circumference (cm) | 104 ± 14 | 105 ± 13 | 106 ± 13 | 108 ± 13 |
| Total cholesterol (mmol/L) | 4.8 ± 0.9 | 4.9 ± 1.0 | 5.1 ± 0.9 | 5.2 ± 1.1 |
| HDL-C (mmol/L) | 1.12 ± 0.26 | 0.95 ± 0.18 | 0.93 ± 0.16 | 0.84 ± 0.14 |
| Non-HDL-C (mmol/L) | 3.7 ± 0.8 | 3.9 ± 1.0 | 4.2 ± 0.8 | 4.3 ± 1.0 |
| TG (mmol/L) | 1.54 (1.21–1.92) | 1.70 (1.35–2.07) | 2.90 (2.55–3.47) | 3.10 (2.61–3.90) |
| LDL-C (mmol/L) | 2.9 ± 0.8 | 3.1 ± 0.9 | 2.7 ± 0.8 | 2.8 ± 0.9 |
| $HbA_{1c}$ (%) | 7.3 (6.4–8.2) | 8.3 (7.5–9.3) | 7.2 (6.3–8.1) | 8.0 (7.2–8.9) |
| $HbA_{1c}$ (mmol/mol) | 56 (46–66) | 67 (58–78) | 55 (45–65) | 64 (55–74) |
| Glucose (mmol/L) | 8.93 ± 2.68 | 9.98 ± 3.06 | 9.15 ± 2.59 | 10.31 ± 3.02 |
| eGFR (mL/min/1.73 m$^2$) | 88 ± 20 | 72 ± 18 | 92 ± 20 | 82 ± 22 |
| UACR (mg/mmol) | 1.15 (0.65–2.92) | 11.75 (4.40–49.60) | 1.01 (0.60–2.03) | 2.70 (1.13–10.38) |

Numbers are mean ± SD, median (IQR), or % (*N*) unless otherwise indicated. eGFR calculated with the MDRD formula.

with T2DM in clinical practice because clinical trial patients tend to have less comorbidity, better adherence, and a larger expected treatment benefit (28). However, validation of the FIELD risk model in the SMART observational cohort showed good risk calibration, indicating that generalization to real-life patients is probably fairly accurate. Furthermore, by using pooled data from FIELD, ACCORD, and SMART, we created a large and heterogeneous group of T2DM patients that resembles the wide variety of patients seen in clinical practice. Also, in FIELD there was a 23% statin drop-in rate; however, a calibration plot showed a good balance between predicted and observed risks in FIELD, indicating that the statin drop-in did not influence the precision of the predictions made at baseline (Supplementary Material). Second, the cutoffs for TG and HDL-C to define dyslipidemia have not been generally established. We used sex-specific HDL-C cutoffs (HDL-C <1.00 mmol/L for men and <1.30 mmol/L for women) as in the definition for metabolic syndrome (29) and as used in FIELD (5) and a TG cutoff of >2.3 mmol/L based on the definition for moderately increased TG of the 2012 Endocrine Society guideline (30), as well as the 2016 ESC/EAS (European Society of Cardiology and European Atherosclerosis Society) guidelines that recommend considering lipid-lowering treatment when TG levels are >2.3 mmol/L (31). The two meta-analyses that investigated the effect of fibrates on CVD also used the TG cutoff of >2.3 mmol/L, but one used an HDL-C cutoff of <0.9 mmol/L, and the other of <1.0 mmol/L, to define dyslipidemia (23,24). These differences in dyslipidemia definition might complicate the generalizability of our results. Third, predictions are made for a 5-year period, while patients and physicians might want information for a longer period or even for over the lifetime. The 5-year time period was chosen owing to the available follow-up data in the derivation and validation sets, but longer-term predictions would have been of added value.

In conclusion, in individual patients with T2DM there is a wide range in the individual treatment effect of fenofibrate on MCVE risk. We found a wide range of individual treatment effect of fenofibrate in patients with T2DM, and overall, patients with dyslipidemia had a larger fenofibrate treatment effect than patients without dyslipidemia. The method of individualized treatment effect prediction of fenofibrate on MCVE risk reduction in T2DM can be used to guide clinical decision-making.

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# Baseline and on-statin treatment lipoprotein(a) levels for prediction of cardiovascular events: individual patient-data meta-analysis of statin outcome trials

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## Summary

**Background** Elevated lipoprotein(a) is a genetic risk factor for cardiovascular disease in general population studies. However, its contribution to risk for cardiovascular events in patients with established cardiovascular disease or on statin therapy is uncertain.

**Methods** Patient-level data from seven randomised, placebo-controlled, statin outcomes trials were collated and harmonised to calculate hazard ratios (HRs) for cardiovascular events, defined as fatal or non-fatal coronary heart disease, stroke, or revascularisation procedures. HRs for cardiovascular events were estimated within each trial across predefined lipoprotein(a) groups (15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL, *vs* <15 mg/dL), before pooling estimates using multivariate random-effects meta-analysis.

**Findings** Analyses included data for 29 069 patients with repeat lipoprotein(a) measurements (mean age 62 years [SD 8]; 8064 [28%] women; 5751 events during 95 576 person-years at risk). Initiation of statin therapy reduced LDL cholesterol (mean change −39% [95% CI −43 to −35]) without a significant change in lipoprotein(a). Associations of baseline and on-statin treatment lipoprotein(a) with cardiovascular disease risk were approximately linear, with increased risk at lipoprotein(a) values of 30 mg/dL or greater for baseline lipoprotein(a) and 50 mg/dL or greater for on-statin lipoprotein(a). For baseline lipoprotein(a), HRs adjusted for age and sex (*vs* <15 mg/dL) were 1·04 (95% CI 0·91–1·18) for 15 mg/dL to less than 30 mg/dL, 1·11 (1·00–1·22) for 30 mg/dL to less than 50 mg/dL, and 1·31 (1·08–1·58) for 50 mg/dL or higher; respective HRs for on-statin lipoprotein(a) were 0·94 (0·81–1·10), 1·06 (0·94–1·21), and 1·43 (1·15–1·76). HRs were almost identical after further adjustment for previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL cholesterol, and HDL cholesterol. The association of on-statin lipoprotein(a) with cardiovascular disease risk was stronger than for on-placebo lipoprotein(a) (interaction p=0·010) and was more pronounced at younger ages (interaction p=0·008) without effect-modification by any other patient-level or study-level characteristics.

**Interpretation** In this individual-patient data meta-analysis of statin-treated patients, elevated baseline and on-statin lipoprotein(a) showed an independent approximately linear relation with cardiovascular disease risk. This study provides a rationale for testing the lipoprotein(a) lowering hypothesis in cardiovascular disease outcomes trials.

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## Introduction

Lipoprotein(a) is composed of apolipoprotein(a) bound covalently to apolipoprotein B of an LDL-like particle.[1,2] It mediates atherogenicity via its LDL moiety, which has a similar proportion of cholesterol content as traditional LDL particles. Furthermore, lipoprotein(a) induces pro-inflammatory responses[3,4] via accumulation of oxidised phospholipids[5] and potentially exerts prothrombotic effects via the plasminogen-like apolipoprotein(a) moiety.[6] By contrast with other major lipoproteins, there is no approved specific therapy to lower circulating plasma levels of lipoprotein(a).

Epidemiological[7] and genetic[8,9] evidence has accumulated over the past decade showing that elevated lipoprotein(a), driven primarily by the *LPA* gene,[10] is associated with increased risk of coronary heart disease, stroke, peripheral arterial disease, and calcific aortic valve stenosis.[1,2,11] These data have established lipoprotein(a) as a cardiovascular disease risk factor, but the bulk of evidence is based on studies including individuals without previous cardiovascular disease and without intensive secondary prevention therapies. By contrast, the role of elevated lipoprotein(a) in patients with previous cardiovascular disease events, on statin therapy or on other guideline-recommended treatments, is less clear. Previous studies in such patient populations have yielded inconsistent results, with findings ranging from significant positive associations to null associations (eg, after acute coronary syndromes).[2] Moreover, findings of several studies—including JUPITER[12] and AIM-HIGH[13]—have shown that elevated

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## Research in context

### Evidence before this study

We searched PubMed for clinical trials published up to July 9, 2018, with the terms "Lipoprotein(a)" or "Lp(a)" plus "statin" and "cardiovascular diseases"[MeSH]. Our review identified seven statin trials (4D, 4S, FLARE, JUPITER, LIPID, MIRACL, and TNT) that reported on the association of lipoprotein(a) with cardiovascular risk. Interpretation of the available evidence is complicated by inconsistent findings across trials (positive *vs* null associations), limited statistical power of single trials, scant availability of follow-up lipoprotein(a) measurements, and differing definitions of lipoprotein(a) categories across trials.

### Added value of this study

We obtained patient-level data from seven placebo-controlled statin trials encompassing 29 069 patients and analysed the relation of baseline and on-treatment lipoprotein(a) to risk of major adverse cardiovascular events. Elevated lipoprotein(a) of 50 mg/dL or higher, at baseline or on-treatment, was associated with an increased hazard ratio of cardiovascular events independent of other cardiovascular risk factors and evident on treatment with either statin or placebo.

### Implications of all the available evidence

These data suggest that residual risk is present in patients with elevated lipoprotein(a) that is not addressed by statins and supports the rationale for outcomes trials to test specific therapies to lower lipoprotein(a).

lipoprotein(a) remains predictive for cardiovascular disease risk at LDL cholesterol levels less than 70 mg/dL,[1] but other studies suggest a positive association only when LDL cholesterol is raised.[14] Furthermore, a major limitation of all post-hoc studies reporting lipoprotein(a) levels and outcomes is that they included few patients with lipoprotein(a) values above 50 mg/dL and, therefore, were uniformly underpowered to test the hypothesis that elevated lipoprotein(a) levels are associated with increased cardiovascular event risk in the setting of statin therapy or previous history of cardiovascular disease.

To test this hypothesis with adequate statistical power, we established the Lipoprotein(a) Studies Collaboration, a consortium of patient-level data from placebo-controlled trials of statins with patient-level data for cardiovascular disease outcomes and lipoprotein(a) measurements at baseline and follow-up (ie, under statin treatment). We report the results of this analysis in documenting the associations of baseline and on-treatment lipoprotein(a) with cardiovascular risk.

## Methods

### Trials included in the meta-analysis

To be eligible for the meta-analysis, randomised placebo-controlled statin trials were required to have assayed lipoprotein(a) concentration at baseline and follow-up, have recorded incidence of cardiovascular disease outcomes using well-defined criteria, and be willing to share patient data at the individual level. We included data from AFCAPS,[15] CARDS,[16] 4D,[17] JUPITER,[12] LIPID,[18] MIRACL,[19] and 4S[20] trials. Study design, target population, and entry criteria are summarised in table 1; more detailed descriptions of trial designs[15,21–26] and lipoprotein(a) methodology and data[12,16–20] were reported previously by each trial. Trials not included in the meta-analysis were either not allowed or unwilling to provide individual-patient data. Because of contractual agreements on sharing individual-patient data, other eligible trials could not be included in the meta-analysis. All contributing trials have obtained ethics approval and patients' informed consent.

### Statistical analysis

We did analyses according to a prespecified plan, developed before any combined analyses. We $\log_e$-transformed lipoprotein(a) values. In all trials except 4S, the on-statin concentration of lipoprotein(a) during follow-up was measured at one timepoint. In the 4S trial, the on-statin amount of lipoprotein(a) was estimated as the geometric mean of lipoprotein(a) values assessed at up to four distinct timepoints. In the JUPITER trial, lipoprotein(a) values were provided in nmol/L, which we divided by 2·4 to convert to mg/dL.[27] In 4S, lipoprotein(a) values were provided in IU/L, which we divided by 19·07 to convert to mg/dL. When information on lipoprotein(a) was missing either at baseline (0·5%) or at follow-up (5·5%), the lipoprotein(a) value was mean-imputed from study-specific mixed-effects models, which predicted lipoprotein(a) values using fixed effects for assigned treatment, time in study, and the interaction of the two variables, plus a random intercept allowed to vary at the patient level.

Because conventional LDL cholesterol assays capture cholesterol both in LDL and lipoprotein(a) particles, we corrected LDL cholesterol values for lipoprotein(a) cholesterol. Lipoprotein(a) mass is composed of about 30–45% cholesterol.[28] We used a conservative measurement of the content of lipoprotein(a) cholesterol by multiplying lipoprotein(a) mass (mg/dL) by 0·30 to derive lipoprotein(a) cholesterol, then we subtracted this value from the measured LDL cholesterol to obtain LDL cholesterol corrected for lipoprotein(a) cholesterol (referred to herein as LDL-$C_{corr}$).[28]

We defined the combined cardiovascular disease endpoint as the occurrence of fatal or non-fatal coronary heart disease, stroke, or any coronary or carotid revascularisation procedures. In quantifying associations of on-treatment lipoprotein(a) with cardiovascular risk, we considered all

| | Years of baseline | Target population | Lipid entry criteria (mmol/L) | Comparator to placebo | Included in cardiovascular disease outcome definition | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Myocardial infarction | Stable angina | Stroke | Revascular-isation | Other |
| AFCAPS[15] | 1990–93 | Primary prevention | Total cholesterol 4·65–6·82, LDL cholesterol 3·36–4·91, triglycerides ≤4·52, HDL cholesterol ≤1·16 (men) and ≤1·22 (women) | Lovastatin 20 mg | Yes | Yes | Yes | Yes | Yes* |
| CARDS[16] | 1997–2001 | Type 2 diabetes | LDL cholesterol ≤4·14, triglycerides ≤6·78 | Atorvastatin 10 mg | Yes | No | Yes | Yes | No |
| 4D[17] | 1998–2002 | Type 2 diabetes and haemodialysis | LDL cholesterol 2·07–4·92, triglycerides ≤11·3 | Atorvastatin 20 mg | Yes | No | Yes | Yes | No |
| JUPITER[12] | 2003–06 | Primary prevention with C-reactive protein >2 mg/dL | LDL cholesterol <3·4, triglycerides <5·65 | Rosuvastatin 20 mg | Yes | No | Yes | Yes | Yes† |
| LIPID[18] | 1990–92 | Previous myocardial infarction or unstable angina | Total cholesterol 4·0–7·0, triglycerides <5·0 | Pravastatin 40 mg | Yes | No | Yes | Yes | No |
| MIRACL[19] | 1997–99 | Acute coronary syndrome | Total cholesterol <7·0 | Atorvastatin 80 mg | Yes | No | Yes | Yes | No |
| 4S[20] | 1989–90 | Previous myocardial infarction or angina | Total cholesterol 5·5–8·0, triglycerides ≤2·5 | Simvastatin 20 mg | Yes | No | No | Yes | No |

*Transient ischaemic attack, peripheral vascular disease, sudden death, and deaths from other cardiovascular causes. †Deaths from other cardiovascular causes.

***Table 1:*** **Design features of contributing trials**

cardiovascular events that occurred after randomisation because any change in lipoprotein(a) under statin therapy is anticipated to occur within a short period (sensitivity analyses omitted the initial period of follow-up).[12]

We estimated associations of lipoprotein(a) with cardiovascular disease risk using a two-step approach: we first calculated estimates within each study separately, then pooled these estimates across studies using multivariate random-effects meta-analysis.[29] We calculated hazard ratios (HRs) using Cox proportional hazard regression models; these models used time on study as a timescale, were stratified by trial arm, and compared prespecified lipoprotein(a) groups (<15 mg/dL, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL). We tested the assumption for the proportionality of hazards using Schoenfeld residuals, and the assumption was met. The analysis had four inter-related principal aims. First, to analyse shapes of associations, we calculated pooled HRs over lipoprotein(a) groups and plotted them against pooled geometric means of lipoprotein(a) concentrations within each category.[29] Second, to ascertain the extent of confounding, we adjusted HRs progressively for age, sex, previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL-$C_{corr}$, and HDL cholesterol (multivariable adjusted model). We made further adjustments for body-mass index (BMI) and estimated glomerular filtration rate (eGFR) in the subset of patients in whom these data were available. Third, to investigate whether the predictive value of lipoprotein(a) concentrations at follow-up differed between patients randomly allocated statin or placebo, we fit interaction models by trial arm. Fourth, to investigate effect-modification by individual-patient and study-level characteristics, we did formal tests of interaction and meta-regression analyses with these variables. Little variability was noted within each trial of the proportion of patients with previous cardiovascular disease and with a history of diabetes at baseline (eg, secondary *vs* primary cardiovascular disease prevention trials, diabetes as inclusion or exclusion criterion) and, hence, we investigated effect-modification by these characteristics at the study level instead of at the patient level. We assessed between-trial heterogeneity with the $I^2$ statistic.[30] We did analyses with Stata version 14.1 MP. We used two-sided statistical tests and calculated 95% CIs. We judged p values less than 0·05 significant for principal analyses; for subgroup analyses, we used a Bonferroni-corrected significance level of p<0·007 (for seven subgroups).

### Role of the funding source

The funders had no role in study design, data collection, data analysis, or writing of the report. AL is an employee of one of the funders and secured funding for the meta-analysis and provided input on data interpretation. PW and ST had full access to all data in the study and had final responsibility for the decision to submit for publication.

## Results

Of 45 044 patients enrolled in the seven trials, 15 975 (35%) were excluded because of missing lipoprotein(a) measurements at both baseline and follow-up, leaving 29 069 patients for analysis (appendix). Few differences were noted in baseline characteristics of patients with or without available lipoprotein(a) measurements (appendix). Baseline characteristics of the 29 069 patients are shown in table 2. At trial entry, mean age was 62 years (SD 8), 8064 (28%) patients were women, 15 252 (52%) had previous cardiovascular disease, 5177 (18%) had diabetes, 4847 (17%) were current smokers, mean systolic blood pressure was 137 mm Hg (SD 18), and mean LDL-$C_{corr}$ was 3·30 mmol/L (SD 0·67). The concentration of lipoprotein(a) at baseline was low-to-normal (median 11 mg/dL [IQR 5–29]). In cross-sectional analyses, the baseline lipoprotein(a) concentration was higher in women (% mean difference adjusted for age, 12% [95% CI 3 to 21]),

See Online for appendix

| | AFCAPS[15] | CARDS[16] | 4D[17] | JUPITER[12] | LIPID[18] | MIRACL[19] | 4S[20] | Total |
|---|---|---|---|---|---|---|---|---|
| **Baseline** | | | | | | | | |
| Patients (n) | 1005 | 2470 | 1249 | 9612 | 7863 | 2431 | 4439 | 29 069 |
| Lipoprotein(a) (mg/dL) | 7 (3–17) | 9 (5–22) | 12 (5–42) | 11 (5–23) | 14 (7–44) | 10 (5–29) | 10 (4–28) | 11 (5–29) |
| <15 | 733 (73%) | 1658 (67%) | 709 (57%) | 5896 (61%) | 4118 (52%) | 1481 (61%) | 2654 (60%) | 17 249 (59%) |
| 15 to <30 | 134 (13%) | 310 (13%) | 129 (10%) | 1867 (19%) | 1147 (15%) | 362 (15%) | 781 (18%) | 4730 (16%) |
| 30 to <50 | 84 (8%) | 212 (9%) | 140 (11%) | 851 (9%) | 877 (11%) | 223 (9%) | 714 (16%) | 3101 (11%) |
| ≥50 | 54 (5%) | 290 (12%) | 271 (22%) | 998 (10%) | 1721 (22%) | 365 (15%) | 290 (7%) | 3989 (14%) |
| Age (years) | 59 (7) | 62 (8) | 66 (8) | 66 (8) | 61 (8) | 65 (11) | 59 (7) | 62 (8) |
| Sex | | | | | | | | |
| Women | 173 (17%) | 779 (32%) | 576 (46%) | 3556 (37%) | 1333 (17%) | 820 (34%) | 827 (19%) | 8064 (28%) |
| Men | 832 (83%) | 1691 (68%) | 673 (54%) | 6056 (63%) | 6530 (83%) | 1611 (66%) | 3612 (81%) | 21 005 (72%) |
| Previous cardiovascular disease | 0 | 6 (<1%) | 513 (41%) | 0 | 7863 (100%) | 2431 (100%) | 4439 (100%) | 15 252 (52%) |
| Diabetes | 32 (3%) | 2470 (100%) | 1249 (100%) | 0 | 676 (9%) | 548 (23%) | 202 (5%) | 5177 (18%) |
| Current smoking | 130 (13%) | 551 (22%) | 108 (9%) | 1492 (16%) | 735 (9%) | 693 (29%) | 1138 (26%) | 4847 (17%) |
| Systolic blood pressure (mm Hg) | 136 (17) | 144 (16) | 146 (22) | 136 (17) | 134 (19) | 128 (20) | 139 (20) | 137 (18) |
| LDL-$C_{corr}$ (mmol/L) | .. | 2·75 (0·78) | 3·00 (0·86) | 2·57 (0·49) | 3·68 (0·74) | 3·04 (0·86) | 4·74 (0·66) | 3·30 (0·67) |
| HDL cholesterol (mmol/L) | .. | 1·64 (0·50) | 0·94 (0·34) | 1·35 (0·40) | 0·96 (0·24) | 1·20 (0·31) | 1·19 (0·30) | 1·21 (0·35) |
| Body-mass index (kg/m²) | 26 (3) | 29 (4) | 28 (5) | 29 (6) | .. | 28 (5) | 26 (3) | 28 (5) |
| eGFR (mL/min) | .. | .. | .. | 75 (17) | 71 (17) | .. | .. | 73 (17) |
| Apolipoprotein B (g/L) | .. | 1·16 (0·24) | 1·10 (0·30) | 1·08 (0·21) | 1·33 (0·25) | .. | 1·16 (0·18) | 1·17 (0·23) |
| **On-statin** | | | | | | | | |
| Patients (n) | 504 | 1255 | 616 | 4802 | 3941 | 1200 | 2218 | 14 536 |
| Time to lipoprotein(a) repeat (years) | 1·0 (1·0–1·0) | 2·5 (2·0–2·8) | 0·5 (0·5–0·5) | 1·0 (1·0–1·0) | 1·0 (1·0–1·0) | 0·2 (0·2–0·2) | 2·5 (2·5–2·5) | 1·0 (1·0–1·0) |
| Lipoprotein(a) (mg/dL) | 7 (3–19) | 8 (4–22) | 11 (5–40) | 11 (4–25) | 13 (6–43) | 11 (5–33) | 11 (4–33) | 11 (5–32) |
| <15 | 366 (73%) | 864 (69%) | 351 (57%) | 2912 (61%) | 2106 (53%) | 707 (59%) | 1268 (57%) | 8574 (59%) |
| 15 to <30 | 59 (12%) | 134 (11%) | 60 (10%) | 868 (18%) | 548 (14%) | 175 (15%) | 321 (15%) | 2165 (15%) |
| 30 to <50 | 43 (9%) | 103 (8%) | 73 (12%) | 417 (9%) | 439 (11%) | 96 (8%) | 375 (17%) | 1546 (11%) |
| ≥50 | 36 (7%) | 154 (12%) | 132 (21%) | 605 (13%) | 848 (22%) | 222 (19%) | 254 (12%) | 2251 (15%) |
| % change in lipoprotein(a) *vs* baseline (95% CI) | –1% (–6 to 4) | –13% (–15 to –10) | –6% (–9 to –3) | 2% (1 to 3) | –7% (–8 to –5) | 9% (6 to 12) | 15% (13 to 17) | –0·4% (–7 to 7) |
| LDL-$C_{corr}$ (mmol/L) | .. | 1·68 (0·58) | 1·73 (0·78) | 1·43 (0·70) | 2·57 (0·71) | 1·56 (0·77) | 2·97 (0·70) | 1·99 (0·70) |
| % change in LDL-$C_{corr}$ *vs* baseline (95% CI) | .. | –37% (–38 to –36) | –41% (–43 to –39) | –43% (–44 to –42) | –29% (–30 to –29) | –47% (–49 to –46) | –37% (–37 to –36) | –39% (–43 to –35) |
| **Cardiovascular disease incidence** | | | | | | | | |
| Follow-up (years) | 5·6 (4·8–6·2) | 4·1 (3·1–4·8) | 2·4 (1·4–3·7) | 2·0 (1·5–2·4) | 5·4 (3·1–6·0) | 0·3 (0·3–0·3) | 5·3 (3·9–5·5) | 3·0 (1·5–5·3) |
| Events, overall (n) | 68 | 170 | 338 | 234 | 3040 | 537 | 1364 | 5751 |
| Events, statin arm (n) | 31 | 71 | 166 | 81 | 1428 | 258 | 568 | 2603 |

Data are mean (SD), number of patients (%), or median (IQR), unless stated otherwise. Percentages might not total 100% because of rounding. Total mean (SD) and % change (95% CI) were calculated by pooling study-specific estimates with random-effects meta-analysis. eGFR=estimated glomerular filtration rate. LDL-$C_{corr}$=LDL cholesterol corrected for lipoprotein(a) cholesterol.

***Table 2:*** **Patients' characteristics**

lower in patients with diabetes, and unrelated to smoking (respective % mean differences adjusted for age and sex, –17% [–24 to –9] and 2% [–3 to 8]). Furthermore, LDL-$C_{corr}$, $\log_e$ triglycerides, BMI, and systolic blood pressure were associated with a lower concentration of lipoprotein(a), and HDL cholesterol was associated with higher lipoprotein(a) concentrations (respective % mean differences adjusted for age and sex per SD, –16% [95% CI –23 to –8], –12% [–15 to –9], –7% [–10 to –5], –2% [–5 to 0], and 7% [3 to 11]). Baseline lipoprotein(a) was not associated with age (% mean difference adjusted for sex per SD, –1% [95% CI –2 to 1]).

14 536 patients were randomly allocated statin treatment (table 2). Initiation of statin therapy reduced LDL-$C_{corr}$ by 39% (95% CI 35–43). The effect of statin treatment on lipoprotein(a) concentration was heterogeneous across trials; the pooled percentage change was –0·4% (95% CI –7 to 7), with three trials showing a mean increase (between 2% and 15%) and four trials showing a mean decrease (between –1% and –13%) in lipoprotein(a) (table 2). The median concentration of lipoprotein(a) on statin therapy was 11 mg/dL (IQR 5–32). The age-adjusted and sex-adjusted correlation between baseline and follow-up $\log_e$ lipoprotein(a) was comparable in patients

assigned statin treatment and those allocated placebo ($r$=0·948 *vs* $r$=0·952).

During 95 576 person-years at risk (median follow-up 3·0 years [IQR 1·5–5·3]), 5751 cardiovascular events were recorded, of which 2603 occurred in patients allocated statin treatment (table 2). When patients were grouped by lipoprotein(a) concentration (categories <15 mg/dL, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL), incidence of cardiovascular events per 1000 person-years was, respectively, 55·3 (95% CI 53·4–57·3), 56·3 (52·6–60·2), 66·7 (62·0–71·8), and 80·0 (75·3–84·9) for baseline lipoprotein(a), and 49·0 (46·5–51·6), 46·4 (41·6–51·7), 56·2 (50·3–62·8), and 77·2 (71·1–83·8) for on-statin lipoprotein(a).

In analyses adjusted for age and sex, associations of baseline and on-statin lipoprotein(a) values with risk for cardiovascular disease were positive and roughly linear, with a possible threshold effect in the group with lipoprotein(a) values of 50 mg/dL or higher (figure 1). Compared with patients with baseline lipoprotein(a) values lower than 15 mg/dL, risk for cardiovascular disease was similar with lipoprotein(a) values of 15 mg/dL to less than 30 mg/dL (HR 1·04, 95% CI 0·91–1·18) and increased with lipoprotein(a) values of 30 mg/dL to less than 50 mg/dL (1·11, 1·00–1·22) and with values of 50 mg/dL or higher (1·31, 1·08–1·58; table 3). For analyses of on-statin lipoprotein(a), corresponding HRs were 0·94 (95% CI 0·81–1·10), 1·06 (0·94–1·21), and 1·43 (1·15–1·76). Associations remained robust after additional adjustment for previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL-$C_{corr}$, and HDL cholesterol concentration (figure 1; table 3). Compared with patients with baseline lipoprotein(a) values lower than 15 mg/dL, multivariable-adjusted HRs for cardiovascular disease were 1·04 (95% CI 0·91–1·20) in patients with lipoprotein(a) values of 15 mg/dL to less than 30 mg/dL, 1·13 (1·02–1·25) in patients with lipoprotein(a) values of 30 mg/dL to less than 50 mg/dL, and 1·35 (1·11–1·66) in patients with lipoprotein(a) values of 50 mg/dL or higher. For analyses of on-statin lipoprotein(a), respective multivariable-adjusted HRs were 0·95 (95% CI 0·82–1·11), 1·08 (0·95–1·23), and 1·42 (1·16–1·74).

In a sensitivity analysis of patients with information on triglycerides, BMI, or eGFR, further adjustment for these variables did not change the magnitude of association between lipoprotein(a) measurements and cardiovascular risk (appendix). Effect sizes comparable with those in the principal analysis were recorded when further categorising the highest lipoprotein(a) group into patients with concentrations of 50 mg/dL to less than 75 mg/dL and 75 mg/dL or higher (appendix), and in the on-statin analysis when omitting events that occurred in the initial period between randomisation and on-statin measurement of lipoprotein(a) (appendix). Trial-specific findings are provided in the appendix.

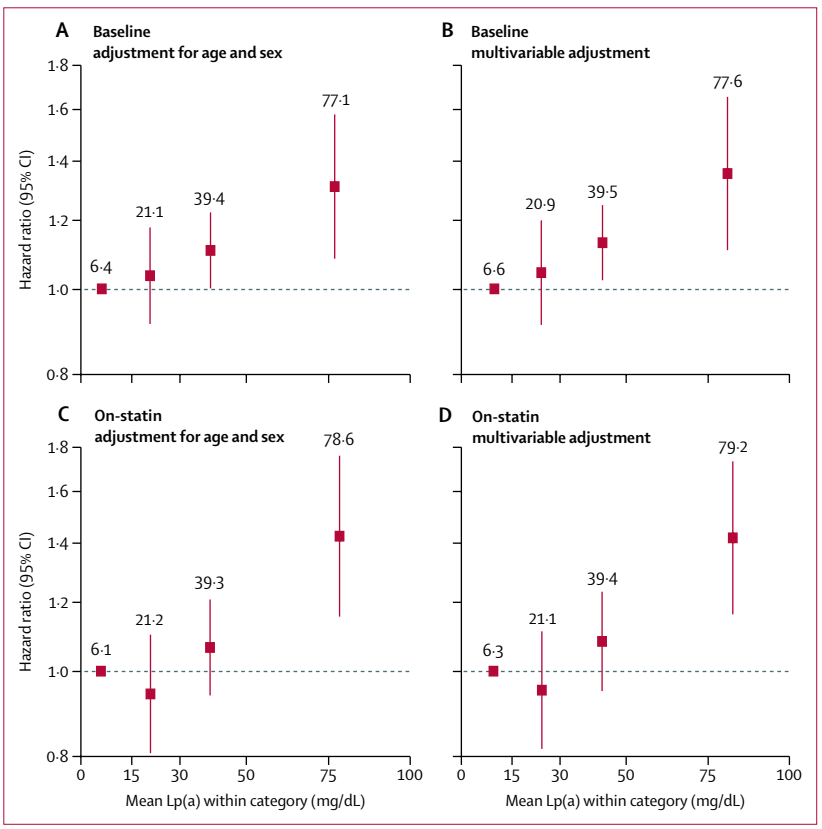


***Figure 1:*** **Associations of baseline and on-statin lipoprotein(a) concentrations with incident cardiovascular disease**

Squares represent hazard ratios and vertical lines 95% CIs. Lipoprotein(a) categories were <15 mg/dL, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL; numbers above each vertical line are mean lipoprotein(a) values within each category. Lipoprotein(a) <15 mg/dL was the reference group. (A) and (C) adjusted for age and sex. (B) and (D) adjusted for age, sex, previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL cholesterol corrected for lipoprotein(a) cholesterol, and HDL cholesterol. Lp(a)=lipoprotein(a).

Lipoprotein(a) concentration during follow-up was associated more strongly with risk for cardiovascular disease in patients assigned statins than in those allocated placebo (figure 2). When comparing individuals with lipoprotein(a) concentrations of 50 mg/dL or higher with patients with lipoprotein(a) concentrations lower than 50 mg/dL, the HRs adjusted for age and sex for cardiovascular disease were 1·48 (95% CI 1·23–1·78) when allocated statin and 1·23 (1·04–1·45) when allocated placebo (interaction p=0·010). Corresponding multivariable-adjusted HRs were 1·47 (95% CI 1·25–1·73) and 1·26 (1·06–1·50; interaction p=0·031). The median time from randomisation to measurement of lipoprotein(a) during follow-up was 1·0 years in both trial arms (IQR 1·0–1·0 in both arms).

Some heterogeneity was noted between trials in HRs for cardiovascular disease, which was most pronounced in the group with lipoprotein(a) concentrations of 50 mg/dL or higher—eg, $I^2$ statistics for HRs adjusted for age and sex were 73% (95% CI 43–88) for baseline lipoprotein(a) and 62% (13–83) for on-statin lipoprotein(a) in this group (table 3).

| | Lipoprotein(a) 15 to <30 mg/dL | | | Lipoprotein(a) 30 to <50 mg/dL | | | Lipoprotein(a) ≥50 mg/dL | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | p value | $I^2$ (95% CI) | HR (95% CI) | p value | $I^2$ (95% CI) | HR (95% CI) | p value | $I^2$ (95% CI) |
| **Baseline lipoprotein(a)** | | | | | | | | | |
| Basic adjustment: seven trials, 29 069 patients, 5751 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 1·04 (0·91–1·18) | 0·59 | 43% (0–76) | 1·11 (1·00–1·22) | 0·047 | 0% (0–71) | 1·31 (1·08–1·58) | 0·005 | 73% (43–88) |
| Progressive adjustment: six trials, 27 764 patients, 5649 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 1·03 (0·90–1·18) | 0·64 | 54% (0–81) | 1·10 (1·00–1·22) | 0·053 | 0% (0–75) | 1·30 (1·06–1·59) | 0·010 | 78% (52–90) |
| Plus previous cardiovascular disease | 1·04 (0·90–1·19) | 0·61 | 53% (0–81) | 1·10 (1·00–1·22) | 0·049 | 0% (0–75) | 1·31 (1·07–1·60) | 0·009 | 78% (52–90) |
| Plus diabetes | 1·04 (0·91–1·19) | 0·60 | 52% (0–81) | 1·11 (1·01–1·23) | 0·036 | 0% (0–75) | 1·32 (1·08–1·61) | 0·007 | 78% (51–90) |
| Plus smoking | 1·03 (0·91–1·18) | 0·61 | 50% (0–80) | 1·11 (1·01–1·22) | 0·034 | 0% (0–75) | 1·31 (1·08–1·59) | 0·007 | 77% (48–90) |
| Plus systolic blood pressure | 1·03 (0·90–1·18) | 0·64 | 53% (0–81) | 1·11 (1·01–1·22) | 0·031 | 0% (0–75) | 1·31 (1·07–1·59) | 0·008 | 77% (49–90) |
| Plus LDL-$C_{corr}$ | 1·04 (0·90–1·19) | 0·61 | 55% (0–82) | 1·12 (1·02–1·24) | 0·019 | 0% (0–75) | 1·34 (1·09–1·65) | 0·005 | 78% (53–90) |
| Plus HDL cholesterol | 1·04 (0·91–1·20) | 0·54 | 54% (0–82) | 1·13 (1·02–1·25) | 0·016 | 0% (0–75) | 1·35 (1·11–1·66) | 0·003 | 77% (49–90) |
| **On-statin lipoprotein(a)** | | | | | | | | | |
| Basic adjustment: seven trials, 14 536 patients, 2603 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 0·94 (0·81–1·10) | 0·45 | 18% (0–62) | 1·06 (0·94–1·21) | 0·33 | 0% (0–71) | 1·43 (1·15–1·76) | 0·001 | 62% (13–83) |
| Progressive adjustment: six trials, 13 883 patients, 2561 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 0·93 (0·79–1·09) | 0·37 | 18% (0–63) | 1·06 (0·93–1·21) | 0·35 | 0% (0–75) | 1·39 (1·12–1·72) | 0·002 | 64% (13–85) |
| Plus previous cardiovascular disease | 0·93 (0·79–1·09) | 0·37 | 18% (0–63) | 1·06 (0·93–1·21) | 0·36 | 0% (0–75) | 1·39 (1·12–1·72) | 0·002 | 64% (13–85) |
| Plus diabetes | 0·94 (0·80–1·10) | 0·43 | 17% (0–62) | 1·07 (0·94–1·22) | 0·31 | 0% (0–75) | 1·39 (1·13–1·71) | 0·002 | 62% (7–84) |
| Plus smoking | 0·94 (0·81–1·09) | 0·42 | 8% (0–77) | 1·07 (0·94–1·22) | 0·30 | 0% (0–75) | 1·39 (1·13–1·71) | 0·002 | 62% (8–84) |
| Plus systolic blood pressure | 0·94 (0·81–1·09) | 0·41 | 9% (0–77) | 1·07 (0·94–1·22) | 0·30 | 0% (0–75) | 1·39 (1·13–1·71) | 0·002 | 61% (6–84) |
| Plus LDL-$C_{corr}$ | 0·94 (0·81–1·10) | 0·47 | 13% (0–78) | 1·08 (0·95–1·23) | 0·26 | 0% (0–75) | 1·41 (1·15–1·73) | 0·001 | 61% (3–84) |
| Plus HDL cholesterol | 0·95 (0·82–1·11) | 0·53 | 13% (0–78) | 1·08 (0·95–1·23) | 0·24 | 0% (0–75) | 1·42 (1·16–1·74) | 0·001 | 58% (0–83) |

Patients with lipoprotein(a) concentration <15 mg/dL served as the reference group. HR=hazard ratio. LDL-$C_{corr}$=LDL cholesterol corrected for lipoprotein(a) cholesterol.

***Table 3:*** **Associations of baseline and on-statin lipoprotein(a) with incident cardiovascular disease according to different levels of adjustment**

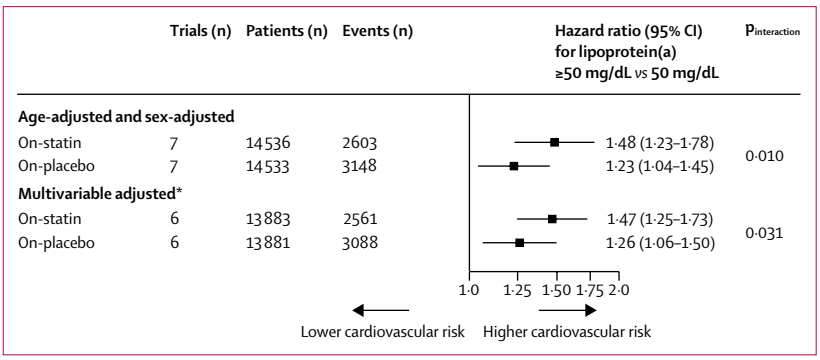


***Figure 2:*** **Comparative predictive value of on-statin versus on-placebo lipoprotein(a) concentrations for incident cardiovascular disease**

*Adjusted for age, sex, previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL cholesterol corrected for lipoprotein(a) cholesterol, and HDL cholesterol.

With the exception of stronger associations of on-statin lipoprotein(a) concentrations with cardiovascular disease risk at younger ages (<60 years *vs* 60 to <70 years *vs* ≥70 years; interaction p=0·008), HRs did not vary across clinically relevant subgroups—eg, by sex, smoking, systolic blood pressure, lipid variables, or BMI (figure 3). Furthermore, the magnitude of association was independent of a study's proportion of patients with previous cardiovascular disease or diabetes, the length of follow-up for clinical events, and the time between study baseline and follow-up measurement of on-statin lipoprotein(a) (appendix). Contributing trials used differing statin interventions, precluding a subgroup analysis by statin type or statin dose.

## Discussion

The findings of our meta-analysis of lipoprotein(a) and cardiovascular events show that patients with raised concentrations of lipoprotein(a) on statin treatment (mainly people with concentrations >50 mg/dL) are at a significantly higher risk of cardiovascular disease. The association with cardiovascular events was independent of conventional cardiovascular disease risk factors. This observation was further underpinned by very weak or null cross-sectional correlations of lipoprotein(a) with these risk factors. Importantly, HRs for high lipoprotein(a) at baseline and while taking statins were of similar magnitude, suggesting that statin treatment might not affect lipoprotein(a)-mediated risk appreciably in people with increased amounts of lipoprotein(a). Overall, these data suggest that patients with raised concentrations of lipoprotein(a), representing about 25% of those with previous cardiovascular disease or an indication for statins,[1] are at substantial residual risk—even while taking statins. In this patient population, treatments that specifically lower lipoprotein(a) might mitigate lipoprotein(a)-mediated risk. An appropriately

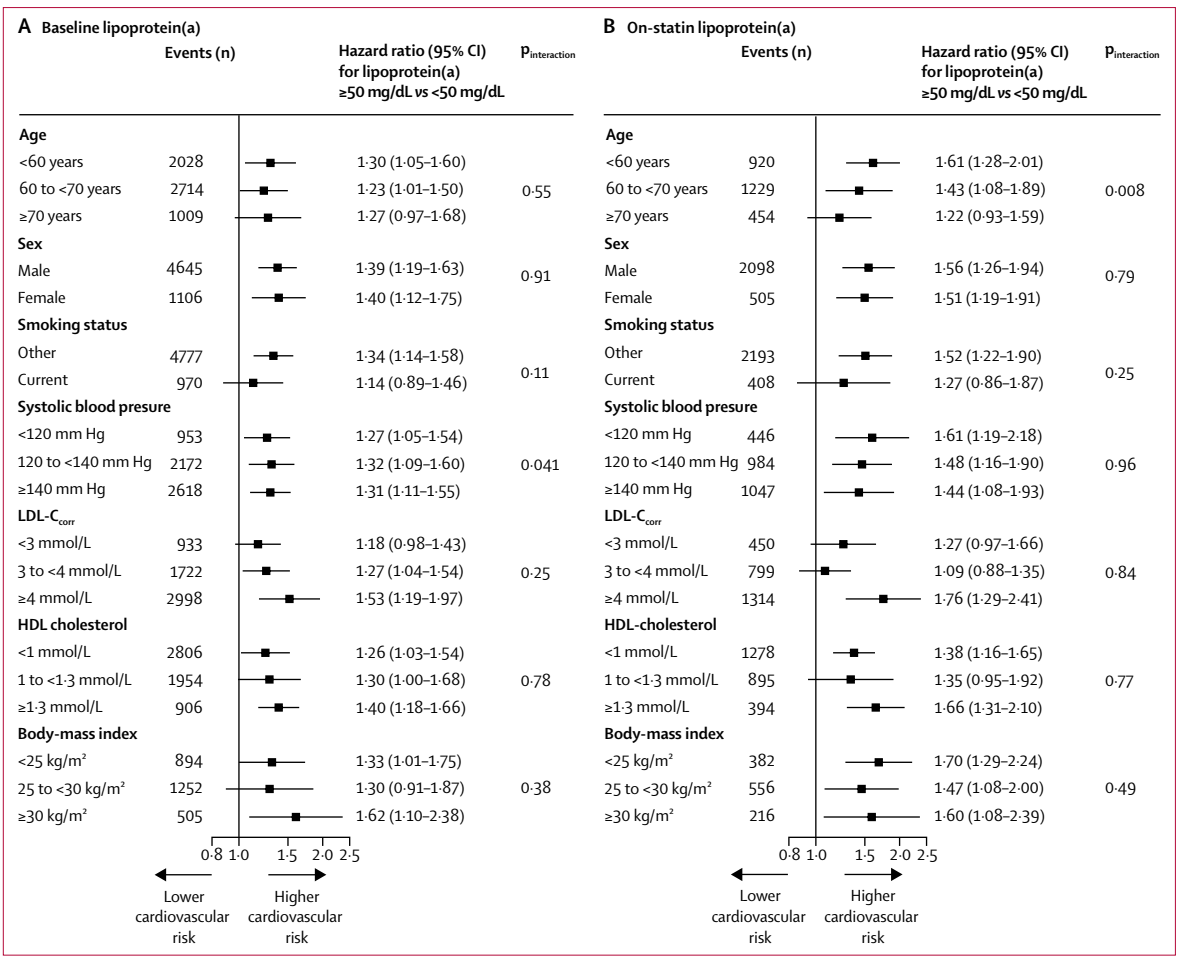


***Figure 3:*** **Associations of baseline and on-statin lipoprotein(a) with incident cardiovascular disease by individual patient characteristics**
Hazard ratios are comparing patients with lipoprotein(a) concentrations of 50 mg/dL or higher with values lower than 50 mg/dL. LDL-$C_{corr}$=LDL cholesterol corrected for lipoprotein(a) cholesterol.

designed cardiovascular disease outcomes trial with robust lowering of lipoprotein(a) is, therefore, justified to test the hypothesis that cutting levels of lipoprotein(a) reduces cardiovascular events, independent of statin treatment.

At baseline, amounts of lipoprotein(a) were associated weakly with demographic and laboratory variables. The most significant (but nevertheless weak) correlations were inverse with diabetes mellitus and triglycerides. The observation of an inverse association of lipoprotein(a) with incident diabetes has been made previously,[31] and the inverse association is most pronounced at very low levels of lipoprotein(a) (≤5 mg/dL), which corresponds approximately to the bottom tenth of the lipoprotein(a) distribution in the global population.[1,2] Whether these findings are causal, or whether they are due to reverse causality, is unknown.[32] Although the underlying mechanisms are not well understood, fasting and postprandial insulin levels are associated inversely with lipoprotein(a).[33] Lipoprotein(a) correlated weakly with LDL cholesterol, but this relation became inversely associated after subtracting the estimated cholesterol content in lipoprotein(a) from the laboratory measurement of LDL cholesterol.[28]

Studies in which modern lipoprotein(a) assays were used to assess the role of lipoprotein(a) in predicting risk for cardiovascular events in patients without cardiovascular disease have been almost uniformly positive.[7] However, results of studies in patients with previous cardiovascular disease or who were taking statins either have been mixed or have suggested the effect is present mainly in people with increased LDL cholesterol.[2] A major limitation of all substudies reporting lipoprotein(a) and outcomes has been power. All studies have enrolled patients with lipoprotein(a) concentrations in the mid-to-low-normal range (10–15 mg/dL; normal <30 mg/dL), as confirmed in our meta-analysis; thus, statistical power to assess risk in patients with highly elevated lipoprotein(a) (ie, >50 mg/dL) was low. Our study is well powered, with 5751 total events and 2603 events in patients allocated statins, making it equivalent to (or larger than) most individual, randomised

controlled, cardiovascular outcome trials in the modern era. By contrast with findings of a previous analysis of individual-patient data,[34] our study had higher statistical power because it included at least ten times more cardiovascular events and, hence, could characterise associations with high lipoprotein(a) concentrations more precisely. Moreover, our analysis used clinically relevant lipoprotein(a) categories informed by guideline recommendations rather than trial-specific quintiles.

Our meta-analysis is highly representative of clinical care in patients treated with statins. First, the studies we included represent patients who were treated with moderate-to-high doses of five major statins used clinically. Second, the studies reflect the variety of patients treated clinically, including primary prevention, high-risk primary prevention with elevated C-reactive protein or diabetes, secondary prevention, stable coronary artery disease, acute coronary syndromes, patients on dialysis, and those with highly elevated LDL cholesterol in the familial hypercholesterolaemia range. Therefore, the studies we included in our meta-analysis broadly reflect patients with high residual risk despite statin treatment, potentially due to other unmodified risk factors such as increased amounts of lipoprotein(a).

The categories of lipoprotein(a) concentrations chosen by us in our meta-analysis reflect clinical risk as suggested by findings of epidemiological and genetic studies. The cutoff of less than 15 mg/dL we used to define the reference group corresponds roughly to the median global concentration of lipoprotein(a).[35,36] Concentrations lower than 30 mg/dL represent the usual cutoff in US laboratories, which is regarded as the normal concentration and is based on data showing that risk for myocardial infarction starts to accrue at concentrations of lipoprotein(a) higher than 25–30 mg/dL.[7,37] The range of 30–50 mg/dL was chosen because it is the grey zone between concentrations considered pathophysiologically relevant and concentrations judged by the European Atherosclerosis Society as high risk (>50 mg/dL), based on the European population prevalence of 20%.

In our study, elevation of cardiovascular disease risk became evident at baseline in patients with concentrations of lipoprotein(a) ranging between 30 mg/dL and lower than 50 mg/dL, and risk was further pronounced when lipoprotein(a) concentration exceeded 50 mg/dL at baseline, including for patients treated with statins. The HRs for lipoprotein(a) concentrations of 50 mg/dL or higher are consistent with those reported in studies of PCSK9 inhibitors in patients with background statin therapy.[38] Additional analyses at even greater concentrations of lipoprotein(a)—ie, 75 mg/dL or higher—were limited by low power because of few patients with lipoprotein(a) concentrations in this range, but the findings support a graded relation between lipoprotein(a) and cardiovascular risk. Outcome trials of lipoprotein(a) lowering are likely to include patients with mean baseline lipoprotein(a) substantially greater than 50 mg/dL; therefore, extrapolation to event reduction with lipoprotein(a) lowering from these data could be an underestimate.

A key observation of our study is that lipoprotein(a) concentrations were associated more strongly with cardiovascular disease risk in patients assigned statins than in people allocated placebo. Findings of a small angiographic study suggested initially that risk associated with lipoprotein(a) is attenuated when LDL cholesterol is well controlled.[39] By contrast, findings of our study—utilising a far larger body of data—support the opposite conclusion, that risk is associated independently with both LDL cholesterol and lipoprotein(a). When LDL-attributable risk is reduced with statin treatment, lipoprotein(a)-associated risk becomes an even stronger predictor of residual risk. This observation is especially evident at lipoprotein(a) concentrations exceeding 50 mg/dL. In support of our observation in this study, the trials FOURIER (NCT01764633) and ODYSSEY OUTCOMES (NCT01663402) have presented preliminary findings of their data, both showing that elevated baseline lipoprotein(a) remains a risk factor for cardiovascular disease, even with on-treatment LDL cholesterol lower than 50 mg/dL in patients treated with statins and PCSK9 inhibitors. The findings highlight the importance of determining whether there is a cardiovascular benefit of treatment to reduce lipoprotein(a) when initial concentrations exceed this threshold, irrespective of concurrent treatment with statin. A second important observation is that all major subgroups of patients seemed to be at risk of elevated lipoprotein(a), including those older than 70 years, women, smokers, people with low and high LDL-$C_{corr}$, low HDL cholesterol, and all categories of BMI.

It is important to emphasise that the lipoprotein(a) hypothesis remains to be tested. To do so requires a randomised trial that compares cardiovascular outcomes in patients treated with an agent that specifically lowers lipoprotein(a) versus placebo. Such a trial might be possible with an antisense oligonucleotide targeting *LPA* messenger RNA, thereby reducing plasma lipoprotein(a) levels. Phase 1 and 2 trials with such an agent have shown the potential to lower lipoprotein(a) levels by more than 90%.[27,40]

Our study has several limitations. First, individual patient data could not be obtained from several other statin trials that reported lipoprotein(a) concentrations and outcomes. It is possible that inclusion of other data would have modified the reported effect sizes. Second, the relation of lipoprotein(a) to residual cardiovascular risk in patients receiving treatment with non-statin lipid-modifying agents (eg, ezetimibe, PCSK9 inhibitors) remains undetermined. Third, lipoprotein(a) assays were heterogeneous and most were in lipoprotein(a) mass (mg/dL) rather than molar (nmol/L) concentration, and the timepoints at which lipoprotein(a) was measured in each trial were not uniform. Therefore, assays not reported in mg/dL had to be converted mathematically

to mg/dL, which might have introduced imprecision into the lipoprotein(a) measurement. A National Heart, Lung, and Blood Institute Working Group on lipoprotein(a) recommended global standardisation of lipoprotein(a) assays to address this limitation.[2] Fourth, we cannot rule out that index event bias could have attenuated effect sizes in secondary prevention trials, although the scope of this bias was reduced by employment of multivariable adjustment. Fifth, our analysis identified moderate-to-high between-study heterogeneity, which could not be accounted for by baseline disease status (ie, previous cardiovascular disease or previous diabetes) nor by differing lengths of follow-up.

In conclusion, our meta-analysis shows an approximately linear relation between cardiovascular risk and concentrations of lipoprotein(a), which is evident at lipoprotein(a) concentrations of 30 mg/dL to less than 50 mg/dL and pronounced at concentrations of 50 mg/dL or higher and persists despite statin treatment. These data provide a rationale for evaluating drugs that can lower lipoprotein(a) specifically and might have the potential to reduce residual cardiovascular risk independent of statin treatment.

#### Contributors

PW and ST wrote the analysis plan, collected and harmonised data, had access to all raw data, and wrote the first draft of the report. PW did the statistical analysis. PMR, PJN, JS, AMT, TRP, GGS, AGO, HMC, FK, CD, CW, and SM obtained patient data in statin trials and provided cleaned data to the coordinating centre. AL secured funding for the study and contributed to data interpretation. All authors contributed to writing the final report and approved the version submitted.

#### Declaration of interests

PW reports consultancy fees from Novartis Pharmaceuticals during the conduct of the study; and travel expenses from Bayer, Daiichi Sankyo, and Sanofi-Aventis outside the submitted work. PMR reports grants from AstraZeneca during the conduct of the study; grants from Novartis, Kowa, Pfizer, and the National Heart, Lung, and Blood Institute outside the submitted work; and personal fees from Novartis and Sanofi outside the submitted work. AMT reports personal fees from Amgen, Bayer, Merck, and Pfizer outside the submitted work; and non-financial support from Bayer outside the submitted work. TRP reports personal fees from Amgen and Sanofi Regeneron outside the submitted work. GGS reports grants from Pfizer during the conduct of the study; and grants from Cerenis, Roche, Sanofi, and The Medicines Company outside the submitted work. HMC reports grants from AstraZeneca, Boehringer Ingelheim, and Roche Pharmaceuticals during the conduct of the study; grants, non-financial support, and travel expenses from Eli Lilly and Regeneron during the conduct of the study; personal fees from Eli Lilly during the conduct of the study; institutional fees from Novartis Pharmaceuticals during the conduct of the study; grants and speaker fees from Pfizer during the conduct of the study; grants and travel expenses from Sanofi Aventis and Novo Nordisk during the conduct of the study; honorarium and speakers' bureau fees from Sanofi during the conduct of the study; and holds shares in Bayer and Roche Pharmaceuticals. CW reports personal fees from Boehringer Ingelheim and Sanofi-Genzyme outside the submitted work. SM reports institutional support from the National Institutes of Health (NIH; grants R01 HL117861, R01 HL134811, and K24 HL136852) outside the submitted work; non-financial support from Quest Diagnostics for measuring lipoprotein(a) in the JUPITER trial outside the submitted work; personal fees from Quest Diagnostics outside the submitted work; and an institutional research grant from Atherotech Diagnostics outside the submitted work. The JUPITER trial was funded by AstraZeneca. AL is an employee of Novartis Pharma AG. ST declares research support from the NIH (grants R01-HL119828, R01-HL078610, R01 HL106579, R01 HL128550, R01 HL136098, P01 HL136275, and R35 HL135737) and is supported by a grant to the Leducq Epigenetics of Atherosclerosis Network from the Fondation Leducq; currently has a dual appointment at the University of California San Diego and Ionis Pharmaceuticals; is a co-inventor and receives royalties from patents owned by the University of California San Diego on oxidation-specific antibodies; and is a co-founder of Oxitope. PJN, JS, AGO, FK, and CD declare no competing interests.

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# Baseline and on-statin treatment lipoprotein(a) levels for prediction of cardiovascular events: individual patient-data meta-analysis of statin outcome trials

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## Summary

**Background** Elevated lipoprotein(a) is a genetic risk factor for cardiovascular disease in general population studies. However, its contribution to risk for cardiovascular events in patients with established cardiovascular disease or on statin therapy is uncertain.

**Methods** Patient-level data from seven randomised, placebo-controlled, statin outcomes trials were collated and harmonised to calculate hazard ratios (HRs) for cardiovascular events, defined as fatal or non-fatal coronary heart disease, stroke, or revascularisation procedures. HRs for cardiovascular events were estimated within each trial across predefined lipoprotein(a) groups (15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL, *vs* <15 mg/dL), before pooling estimates using multivariate random-effects meta-analysis.

**Findings** Analyses included data for 29 069 patients with repeat lipoprotein(a) measurements (mean age 62 years [SD 8]; 8064 [28%] women; 5751 events during 95 576 person-years at risk). Initiation of statin therapy reduced LDL cholesterol (mean change −39% [95% CI −43 to −35]) without a significant change in lipoprotein(a). Associations of baseline and on-statin treatment lipoprotein(a) with cardiovascular disease risk were approximately linear, with increased risk at lipoprotein(a) values of 30 mg/dL or greater for baseline lipoprotein(a) and 50 mg/dL or greater for on-statin lipoprotein(a). For baseline lipoprotein(a), HRs adjusted for age and sex (*vs* <15 mg/dL) were 1·04 (95% CI 0·91–1·18) for 15 mg/dL to less than 30 mg/dL, 1·11 (1·00–1·22) for 30 mg/dL to less than 50 mg/dL, and 1·31 (1·08–1·58) for 50 mg/dL or higher; respective HRs for on-statin lipoprotein(a) were 0·94 (0·81–1·10), 1·06 (0·94–1·21), and 1·43 (1·15–1·76). HRs were almost identical after further adjustment for previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL cholesterol, and HDL cholesterol. The association of on-statin lipoprotein(a) with cardiovascular disease risk was stronger than for on-placebo lipoprotein(a) (interaction p=0·010) and was more pronounced at younger ages (interaction p=0·008) without effect-modification by any other patient-level or study-level characteristics.

**Interpretation** In this individual-patient data meta-analysis of statin-treated patients, elevated baseline and on-statin lipoprotein(a) showed an independent approximately linear relation with cardiovascular disease risk. This study provides a rationale for testing the lipoprotein(a) lowering hypothesis in cardiovascular disease outcomes trials.

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## Introduction

Lipoprotein(a) is composed of apolipoprotein(a) bound covalently to apolipoprotein B of an LDL-like particle.[1,2] It mediates atherogenicity via its LDL moiety, which has a similar proportion of cholesterol content as traditional LDL particles. Furthermore, lipoprotein(a) induces pro-inflammatory responses[3,4] via accumulation of oxidised phospholipids[5] and potentially exerts prothrombotic effects via the plasminogen-like apolipoprotein(a) moiety.[6] By contrast with other major lipoproteins, there is no approved specific therapy to lower circulating plasma levels of lipoprotein(a).

Epidemiological[7] and genetic[8,9] evidence has accumulated over the past decade showing that elevated lipoprotein(a), driven primarily by the *LPA* gene,[10] is associated with increased risk of coronary heart disease, stroke, peripheral arterial disease, and calcific aortic valve stenosis.[1,2,11] These data have established lipoprotein(a) as a cardiovascular disease risk factor, but the bulk of evidence is based on studies including individuals without previous cardiovascular disease and without intensive secondary prevention therapies. By contrast, the role of elevated lipoprotein(a) in patients with previous cardiovascular disease events, on statin therapy or on other guideline-recommended treatments, is less clear. Previous studies in such patient populations have yielded inconsistent results, with findings ranging from significant positive associations to null associations (eg, after acute coronary syndromes).[2] Moreover, findings of several studies—including JUPITER[12] and AIM-HIGH[13]—have shown that elevated

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## Research in context

### Evidence before this study

We searched PubMed for clinical trials published up to July 9, 2018, with the terms "Lipoprotein(a)" or "Lp(a)" plus "statin" and "cardiovascular diseases"[MeSH]. Our review identified seven statin trials (4D, 4S, FLARE, JUPITER, LIPID, MIRACL, and TNT) that reported on the association of lipoprotein(a) with cardiovascular risk. Interpretation of the available evidence is complicated by inconsistent findings across trials (positive *vs* null associations), limited statistical power of single trials, scant availability of follow-up lipoprotein(a) measurements, and differing definitions of lipoprotein(a) categories across trials.

### Added value of this study

We obtained patient-level data from seven placebo-controlled statin trials encompassing 29 069 patients and analysed the relation of baseline and on-treatment lipoprotein(a) to risk of major adverse cardiovascular events. Elevated lipoprotein(a) of 50 mg/dL or higher, at baseline or on-treatment, was associated with an increased hazard ratio of cardiovascular events independent of other cardiovascular risk factors and evident on treatment with either statin or placebo.

### Implications of all the available evidence

These data suggest that residual risk is present in patients with elevated lipoprotein(a) that is not addressed by statins and supports the rationale for outcomes trials to test specific therapies to lower lipoprotein(a).

lipoprotein(a) remains predictive for cardiovascular disease risk at LDL cholesterol levels less than 70 mg/dL,[1] but other studies suggest a positive association only when LDL cholesterol is raised.[14] Furthermore, a major limitation of all post-hoc studies reporting lipoprotein(a) levels and outcomes is that they included few patients with lipoprotein(a) values above 50 mg/dL and, therefore, were uniformly underpowered to test the hypothesis that elevated lipoprotein(a) levels are associated with increased cardiovascular event risk in the setting of statin therapy or previous history of cardiovascular disease.

To test this hypothesis with adequate statistical power, we established the Lipoprotein(a) Studies Collaboration, a consortium of patient-level data from placebo-controlled trials of statins with patient-level data for cardiovascular disease outcomes and lipoprotein(a) measurements at baseline and follow-up (ie, under statin treatment). We report the results of this analysis in documenting the associations of baseline and on-treatment lipoprotein(a) with cardiovascular risk.

## Methods

### Trials included in the meta-analysis

To be eligible for the meta-analysis, randomised placebo-controlled statin trials were required to have assayed lipoprotein(a) concentration at baseline and follow-up, have recorded incidence of cardiovascular disease outcomes using well-defined criteria, and be willing to share patient data at the individual level. We included data from AFCAPS,[15] CARDS,[16] 4D,[17] JUPITER,[12] LIPID,[18] MIRACL,[19] and 4S[20] trials. Study design, target population, and entry criteria are summarised in table 1; more detailed descriptions of trial designs[15,21–26] and lipoprotein(a) methodology and data[12,16–20] were reported previously by each trial. Trials not included in the meta-analysis were either not allowed or unwilling to provide individual-patient data. Because of contractual agreements on sharing individual-patient data, other eligible trials could not be included in the meta-analysis. All contributing trials have obtained ethics approval and patients' informed consent.

### Statistical analysis

We did analyses according to a prespecified plan, developed before any combined analyses. We $\log_e$-transformed lipoprotein(a) values. In all trials except 4S, the on-statin concentration of lipoprotein(a) during follow-up was measured at one timepoint. In the 4S trial, the on-statin amount of lipoprotein(a) was estimated as the geometric mean of lipoprotein(a) values assessed at up to four distinct timepoints. In the JUPITER trial, lipoprotein(a) values were provided in nmol/L, which we divided by 2·4 to convert to mg/dL.[27] In 4S, lipoprotein(a) values were provided in IU/L, which we divided by 19·07 to convert to mg/dL. When information on lipoprotein(a) was missing either at baseline (0·5%) or at follow-up (5·5%), the lipoprotein(a) value was mean-imputed from study-specific mixed-effects models, which predicted lipoprotein(a) values using fixed effects for assigned treatment, time in study, and the interaction of the two variables, plus a random intercept allowed to vary at the patient level.

Because conventional LDL cholesterol assays capture cholesterol both in LDL and lipoprotein(a) particles, we corrected LDL cholesterol values for lipoprotein(a) cholesterol. Lipoprotein(a) mass is composed of about 30–45% cholesterol.[28] We used a conservative measurement of the content of lipoprotein(a) cholesterol by multiplying lipoprotein(a) mass (mg/dL) by 0·30 to derive lipoprotein(a) cholesterol, then we subtracted this value from the measured LDL cholesterol to obtain LDL cholesterol corrected for lipoprotein(a) cholesterol (referred to herein as LDL-$C_{corr}$).[28]

We defined the combined cardiovascular disease endpoint as the occurrence of fatal or non-fatal coronary heart disease, stroke, or any coronary or carotid revascularisation procedures. In quantifying associations of on-treatment lipoprotein(a) with cardiovascular risk, we considered all

| | Years of baseline | Target population | Lipid entry criteria (mmol/L) | Comparator to placebo | Included in cardiovascular disease outcome definition | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Myocardial infarction | Stable angina | Stroke | Revascular-isation | Other |
| AFCAPS[15] | 1990–93 | Primary prevention | Total cholesterol 4·65–6·82, LDL cholesterol 3·36–4·91, triglycerides ≤4·52, HDL cholesterol ≤1·16 (men) and ≤1·22 (women) | Lovastatin 20 mg | Yes | Yes | Yes | Yes | Yes* |
| CARDS[16] | 1997–2001 | Type 2 diabetes | LDL cholesterol ≤4·14, triglycerides ≤6·78 | Atorvastatin 10 mg | Yes | No | Yes | Yes | No |
| 4D[17] | 1998–2002 | Type 2 diabetes and haemodialysis | LDL cholesterol 2·07–4·92, triglycerides ≤11·3 | Atorvastatin 20 mg | Yes | No | Yes | Yes | No |
| JUPITER[12] | 2003–06 | Primary prevention with C-reactive protein >2 mg/dL | LDL cholesterol <3·4, triglycerides <5·65 | Rosuvastatin 20 mg | Yes | No | Yes | Yes | Yes† |
| LIPID[18] | 1990–92 | Previous myocardial infarction or unstable angina | Total cholesterol 4·0–7·0, triglycerides <5·0 | Pravastatin 40 mg | Yes | No | Yes | Yes | No |
| MIRACL[19] | 1997–99 | Acute coronary syndrome | Total cholesterol <7·0 | Atorvastatin 80 mg | Yes | No | Yes | Yes | No |
| 4S[20] | 1989–90 | Previous myocardial infarction or angina | Total cholesterol 5·5–8·0, triglycerides ≤2·5 | Simvastatin 20 mg | Yes | No | No | Yes | No |

*Transient ischaemic attack, peripheral vascular disease, sudden death, and deaths from other cardiovascular causes. †Deaths from other cardiovascular causes.

***Table 1:*** **Design features of contributing trials**

cardiovascular events that occurred after randomisation because any change in lipoprotein(a) under statin therapy is anticipated to occur within a short period (sensitivity analyses omitted the initial period of follow-up).[12]

We estimated associations of lipoprotein(a) with cardiovascular disease risk using a two-step approach: we first calculated estimates within each study separately, then pooled these estimates across studies using multivariate random-effects meta-analysis.[29] We calculated hazard ratios (HRs) using Cox proportional hazard regression models; these models used time on study as a timescale, were stratified by trial arm, and compared prespecified lipoprotein(a) groups (<15 mg/dL, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL). We tested the assumption for the proportionality of hazards using Schoenfeld residuals, and the assumption was met. The analysis had four inter-related principal aims. First, to analyse shapes of associations, we calculated pooled HRs over lipoprotein(a) groups and plotted them against pooled geometric means of lipoprotein(a) concentrations within each category.[29] Second, to ascertain the extent of confounding, we adjusted HRs progressively for age, sex, previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL-$C_{corr}$, and HDL cholesterol (multivariable adjusted model). We made further adjustments for body-mass index (BMI) and estimated glomerular filtration rate (eGFR) in the subset of patients in whom these data were available. Third, to investigate whether the predictive value of lipoprotein(a) concentrations at follow-up differed between patients randomly allocated statin or placebo, we fit interaction models by trial arm. Fourth, to investigate effect-modification by individual-patient and study-level characteristics, we did formal tests of interaction and meta-regression analyses with these variables. Little variability was noted within each trial of the proportion of patients with previous cardiovascular disease and with a history of diabetes at baseline (eg, secondary *vs* primary cardiovascular disease prevention trials, diabetes as inclusion or exclusion criterion) and, hence, we investigated effect-modification by these characteristics at the study level instead of at the patient level. We assessed between-trial heterogeneity with the $I^2$ statistic.[30] We did analyses with Stata version 14.1 MP. We used two-sided statistical tests and calculated 95% CIs. We judged p values less than 0·05 significant for principal analyses; for subgroup analyses, we used a Bonferroni-corrected significance level of p<0·007 (for seven subgroups).

### Role of the funding source

The funders had no role in study design, data collection, data analysis, or writing of the report. AL is an employee of one of the funders and secured funding for the meta-analysis and provided input on data interpretation. PW and ST had full access to all data in the study and had final responsibility for the decision to submit for publication.

## Results

Of 45 044 patients enrolled in the seven trials, 15 975 (35%) were excluded because of missing lipoprotein(a) measurements at both baseline and follow-up, leaving 29 069 patients for analysis (appendix). Few differences were noted in baseline characteristics of patients with or without available lipoprotein(a) measurements (appendix). Baseline characteristics of the 29 069 patients are shown in table 2. At trial entry, mean age was 62 years (SD 8), 8064 (28%) patients were women, 15 252 (52%) had previous cardiovascular disease, 5177 (18%) had diabetes, 4847 (17%) were current smokers, mean systolic blood pressure was 137 mm Hg (SD 18), and mean LDL-$C_{corr}$ was 3·30 mmol/L (SD 0·67). The concentration of lipoprotein(a) at baseline was low-to-normal (median 11 mg/dL [IQR 5–29]). In cross-sectional analyses, the baseline lipoprotein(a) concentration was higher in women (% mean difference adjusted for age, 12% [95% CI 3 to 21]),

See Online for appendix

| | AFCAPS[15] | CARDS[16] | 4D[17] | JUPITER[12] | LIPID[18] | MIRACL[19] | 4S[20] | Total |
|---|---|---|---|---|---|---|---|---|
| **Baseline** | | | | | | | | |
| Patients (n) | 1005 | 2470 | 1249 | 9612 | 7863 | 2431 | 4439 | 29 069 |
| Lipoprotein(a) (mg/dL) | 7 (3–17) | 9 (5–22) | 12 (5–42) | 11 (5–23) | 14 (7–44) | 10 (5–29) | 10 (4–28) | 11 (5–29) |
| <15 | 733 (73%) | 1658 (67%) | 709 (57%) | 5896 (61%) | 4118 (52%) | 1481 (61%) | 2654 (60%) | 17 249 (59%) |
| 15 to <30 | 134 (13%) | 310 (13%) | 129 (10%) | 1867 (19%) | 1147 (15%) | 362 (15%) | 781 (18%) | 4730 (16%) |
| 30 to <50 | 84 (8%) | 212 (9%) | 140 (11%) | 851 (9%) | 877 (11%) | 223 (9%) | 714 (16%) | 3101 (11%) |
| ≥50 | 54 (5%) | 290 (12%) | 271 (22%) | 998 (10%) | 1721 (22%) | 365 (15%) | 290 (7%) | 3989 (14%) |
| Age (years) | 59 (7) | 62 (8) | 66 (8) | 66 (8) | 61 (8) | 65 (11) | 59 (7) | 62 (8) |
| Sex | | | | | | | | |
| Women | 173 (17%) | 779 (32%) | 576 (46%) | 3556 (37%) | 1333 (17%) | 820 (34%) | 827 (19%) | 8064 (28%) |
| Men | 832 (83%) | 1691 (68%) | 673 (54%) | 6056 (63%) | 6530 (83%) | 1611 (66%) | 3612 (81%) | 21 005 (72%) |
| Previous cardiovascular disease | 0 | 6 (<1%) | 513 (41%) | 0 | 7863 (100%) | 2431 (100%) | 4439 (100%) | 15 252 (52%) |
| Diabetes | 32 (3%) | 2470 (100%) | 1249 (100%) | 0 | 676 (9%) | 548 (23%) | 202 (5%) | 5177 (18%) |
| Current smoking | 130 (13%) | 551 (22%) | 108 (9%) | 1492 (16%) | 735 (9%) | 693 (29%) | 1138 (26%) | 4847 (17%) |
| Systolic blood pressure (mm Hg) | 136 (17) | 144 (16) | 146 (22) | 136 (17) | 134 (19) | 128 (20) | 139 (20) | 137 (18) |
| LDL-$C_{corr}$ (mmol/L) | .. | 2·75 (0·78) | 3·00 (0·86) | 2·57 (0·49) | 3·68 (0·74) | 3·04 (0·86) | 4·74 (0·66) | 3·30 (0·67) |
| HDL cholesterol (mmol/L) | .. | 1·64 (0·50) | 0·94 (0·34) | 1·35 (0·40) | 0·96 (0·24) | 1·20 (0·31) | 1·19 (0·30) | 1·21 (0·35) |
| Body-mass index (kg/m²) | 26 (3) | 29 (4) | 28 (5) | 29 (6) | .. | 28 (5) | 26 (3) | 28 (5) |
| eGFR (mL/min) | .. | .. | .. | 75 (17) | 71 (17) | .. | .. | 73 (17) |
| Apolipoprotein B (g/L) | .. | 1·16 (0·24) | 1·10 (0·30) | 1·08 (0·21) | 1·33 (0·25) | .. | 1·16 (0·18) | 1·17 (0·23) |
| **On-statin** | | | | | | | | |
| Patients (n) | 504 | 1255 | 616 | 4802 | 3941 | 1200 | 2218 | 14 536 |
| Time to lipoprotein(a) repeat (years) | 1·0 (1·0–1·0) | 2·5 (2·0–2·8) | 0·5 (0·5–0·5) | 1·0 (1·0–1·0) | 1·0 (1·0–1·0) | 0·2 (0·2–0·2) | 2·5 (2·5–2·5) | 1·0 (1·0–1·0) |
| Lipoprotein(a) (mg/dL) | 7 (3–19) | 8 (4–22) | 11 (5–40) | 11 (4–25) | 13 (6–43) | 11 (5–33) | 11 (4–33) | 11 (5–32) |
| <15 | 366 (73%) | 864 (69%) | 351 (57%) | 2912 (61%) | 2106 (53%) | 707 (59%) | 1268 (57%) | 8574 (59%) |
| 15 to <30 | 59 (12%) | 134 (11%) | 60 (10%) | 868 (18%) | 548 (14%) | 175 (15%) | 321 (15%) | 2165 (15%) |
| 30 to <50 | 43 (9%) | 103 (8%) | 73 (12%) | 417 (9%) | 439 (11%) | 96 (8%) | 375 (17%) | 1546 (11%) |
| ≥50 | 36 (7%) | 154 (12%) | 132 (21%) | 605 (13%) | 848 (22%) | 222 (19%) | 254 (12%) | 2251 (15%) |
| % change in lipoprotein(a) *vs* baseline (95% CI) | –1% (–6 to 4) | –13% (–15 to –10) | –6% (–9 to –3) | 2% (1 to 3) | –7% (–8 to –5) | 9% (6 to 12) | 15% (13 to 17) | –0·4% (–7 to 7) |
| LDL-$C_{corr}$ (mmol/L) | .. | 1·68 (0·58) | 1·73 (0·78) | 1·43 (0·70) | 2·57 (0·71) | 1·56 (0·77) | 2·97 (0·70) | 1·99 (0·70) |
| % change in LDL-$C_{corr}$ *vs* baseline (95% CI) | .. | –37% (–38 to –36) | –41% (–43 to –39) | –43% (–44 to –42) | –29% (–30 to –29) | –47% (–49 to –46) | –37% (–37 to –36) | –39% (–43 to –35) |
| **Cardiovascular disease incidence** | | | | | | | | |
| Follow-up (years) | 5·6 (4·8–6·2) | 4·1 (3·1–4·8) | 2·4 (1·4–3·7) | 2·0 (1·5–2·4) | 5·4 (3·1–6·0) | 0·3 (0·3–0·3) | 5·3 (3·9–5·5) | 3·0 (1·5–5·3) |
| Events, overall (n) | 68 | 170 | 338 | 234 | 3040 | 537 | 1364 | 5751 |
| Events, statin arm (n) | 31 | 71 | 166 | 81 | 1428 | 258 | 568 | 2603 |

Data are mean (SD), number of patients (%), or median (IQR), unless stated otherwise. Percentages might not total 100% because of rounding. Total mean (SD) and % change (95% CI) were calculated by pooling study-specific estimates with random-effects meta-analysis. eGFR=estimated glomerular filtration rate. LDL-$C_{corr}$=LDL cholesterol corrected for lipoprotein(a) cholesterol.

***Table 2:*** **Patients' characteristics**

lower in patients with diabetes, and unrelated to smoking (respective % mean differences adjusted for age and sex, –17% [–24 to –9] and 2% [–3 to 8]). Furthermore, LDL-$C_{corr}$, $\log_e$ triglycerides, BMI, and systolic blood pressure were associated with a lower concentration of lipoprotein(a), and HDL cholesterol was associated with higher lipoprotein(a) concentrations (respective % mean differences adjusted for age and sex per SD, –16% [95% CI –23 to –8], –12% [–15 to –9], –7% [–10 to –5], –2% [–5 to 0], and 7% [3 to 11]). Baseline lipoprotein(a) was not associated with age (% mean difference adjusted for sex per SD, –1% [95% CI –2 to 1]).

14 536 patients were randomly allocated statin treatment (table 2). Initiation of statin therapy reduced LDL-$C_{corr}$ by 39% (95% CI 35–43). The effect of statin treatment on lipoprotein(a) concentration was heterogeneous across trials; the pooled percentage change was –0·4% (95% CI –7 to 7), with three trials showing a mean increase (between 2% and 15%) and four trials showing a mean decrease (between –1% and –13%) in lipoprotein(a) (table 2). The median concentration of lipoprotein(a) on statin therapy was 11 mg/dL (IQR 5–32). The age-adjusted and sex-adjusted correlation between baseline and follow-up $\log_e$ lipoprotein(a) was comparable in patients

assigned statin treatment and those allocated placebo ($r$=0·948 *vs* $r$=0·952).

During 95 576 person-years at risk (median follow-up 3·0 years [IQR 1·5–5·3]), 5751 cardiovascular events were recorded, of which 2603 occurred in patients allocated statin treatment (table 2). When patients were grouped by lipoprotein(a) concentration (categories <15 mg/dL, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL), incidence of cardiovascular events per 1000 person-years was, respectively, 55·3 (95% CI 53·4–57·3), 56·3 (52·6–60·2), 66·7 (62·0–71·8), and 80·0 (75·3–84·9) for baseline lipoprotein(a), and 49·0 (46·5–51·6), 46·4 (41·6–51·7), 56·2 (50·3–62·8), and 77·2 (71·1–83·8) for on-statin lipoprotein(a).

In analyses adjusted for age and sex, associations of baseline and on-statin lipoprotein(a) values with risk for cardiovascular disease were positive and roughly linear, with a possible threshold effect in the group with lipoprotein(a) values of 50 mg/dL or higher (figure 1). Compared with patients with baseline lipoprotein(a) values lower than 15 mg/dL, risk for cardiovascular disease was similar with lipoprotein(a) values of 15 mg/dL to less than 30 mg/dL (HR 1·04, 95% CI 0·91–1·18) and increased with lipoprotein(a) values of 30 mg/dL to less than 50 mg/dL (1·11, 1·00–1·22) and with values of 50 mg/dL or higher (1·31, 1·08–1·58; table 3). For analyses of on-statin lipoprotein(a), corresponding HRs were 0·94 (95% CI 0·81–1·10), 1·06 (0·94–1·21), and 1·43 (1·15–1·76). Associations remained robust after additional adjustment for previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL-$C_{corr}$, and HDL cholesterol concentration (figure 1; table 3). Compared with patients with baseline lipoprotein(a) values lower than 15 mg/dL, multivariable-adjusted HRs for cardiovascular disease were 1·04 (95% CI 0·91–1·20) in patients with lipoprotein(a) values of 15 mg/dL to less than 30 mg/dL, 1·13 (1·02–1·25) in patients with lipoprotein(a) values of 30 mg/dL to less than 50 mg/dL, and 1·35 (1·11–1·66) in patients with lipoprotein(a) values of 50 mg/dL or higher. For analyses of on-statin lipoprotein(a), respective multivariable-adjusted HRs were 0·95 (95% CI 0·82–1·11), 1·08 (0·95–1·23), and 1·42 (1·16–1·74).

In a sensitivity analysis of patients with information on triglycerides, BMI, or eGFR, further adjustment for these variables did not change the magnitude of association between lipoprotein(a) measurements and cardiovascular risk (appendix). Effect sizes comparable with those in the principal analysis were recorded when further categorising the highest lipoprotein(a) group into patients with concentrations of 50 mg/dL to less than 75 mg/dL and 75 mg/dL or higher (appendix), and in the on-statin analysis when omitting events that occurred in the initial period between randomisation and on-statin measurement of lipoprotein(a) (appendix). Trial-specific findings are provided in the appendix.

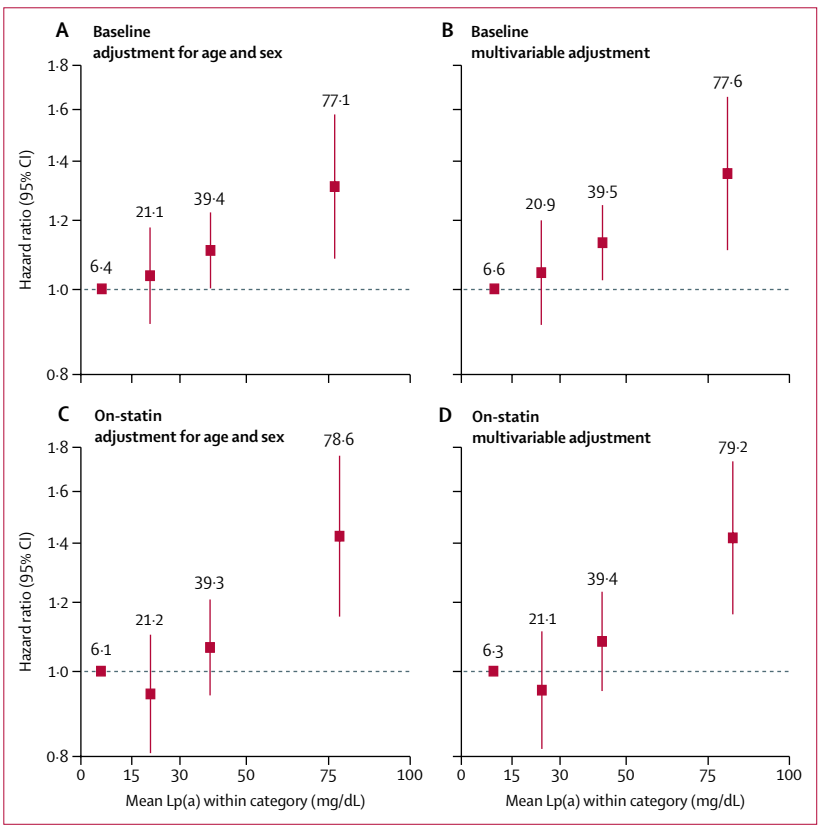


***Figure 1:*** **Associations of baseline and on-statin lipoprotein(a) concentrations with incident cardiovascular disease**

Squares represent hazard ratios and vertical lines 95% CIs. Lipoprotein(a) categories were <15 mg/dL, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL; numbers above each vertical line are mean lipoprotein(a) values within each category. Lipoprotein(a) <15 mg/dL was the reference group. (A) and (C) adjusted for age and sex. (B) and (D) adjusted for age, sex, previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL cholesterol corrected for lipoprotein(a) cholesterol, and HDL cholesterol. Lp(a)=lipoprotein(a).

Lipoprotein(a) concentration during follow-up was associated more strongly with risk for cardiovascular disease in patients assigned statins than in those allocated placebo (figure 2). When comparing individuals with lipoprotein(a) concentrations of 50 mg/dL or higher with patients with lipoprotein(a) concentrations lower than 50 mg/dL, the HRs adjusted for age and sex for cardiovascular disease were 1·48 (95% CI 1·23–1·78) when allocated statin and 1·23 (1·04–1·45) when allocated placebo (interaction p=0·010). Corresponding multivariable-adjusted HRs were 1·47 (95% CI 1·25–1·73) and 1·26 (1·06–1·50; interaction p=0·031). The median time from randomisation to measurement of lipoprotein(a) during follow-up was 1·0 years in both trial arms (IQR 1·0–1·0 in both arms).

Some heterogeneity was noted between trials in HRs for cardiovascular disease, which was most pronounced in the group with lipoprotein(a) concentrations of 50 mg/dL or higher—eg, $I^2$ statistics for HRs adjusted for age and sex were 73% (95% CI 43–88) for baseline lipoprotein(a) and 62% (13–83) for on-statin lipoprotein(a) in this group (table 3).

| | Lipoprotein(a) 15 to <30 mg/dL | | | Lipoprotein(a) 30 to <50 mg/dL | | | Lipoprotein(a) ≥50 mg/dL | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | p value | $I^2$ (95% CI) | HR (95% CI) | p value | $I^2$ (95% CI) | HR (95% CI) | p value | $I^2$ (95% CI) |
| **Baseline lipoprotein(a)** | | | | | | | | | |
| Basic adjustment: seven trials, 29 069 patients, 5751 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 1·04 (0·91–1·18) | 0·59 | 43% (0–76) | 1·11 (1·00–1·22) | 0·047 | 0% (0–71) | 1·31 (1·08–1·58) | 0·005 | 73% (43–88) |
| Progressive adjustment: six trials, 27 764 patients, 5649 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 1·03 (0·90–1·18) | 0·64 | 54% (0–81) | 1·10 (1·00–1·22) | 0·053 | 0% (0–75) | 1·30 (1·06–1·59) | 0·010 | 78% (52–90) |
| Plus previous cardiovascular disease | 1·04 (0·90–1·19) | 0·61 | 53% (0–81) | 1·10 (1·00–1·22) | 0·049 | 0% (0–75) | 1·31 (1·07–1·60) | 0·009 | 78% (52–90) |
| Plus diabetes | 1·04 (0·91–1·19) | 0·60 | 52% (0–81) | 1·11 (1·01–1·23) | 0·036 | 0% (0–75) | 1·32 (1·08–1·61) | 0·007 | 78% (51–90) |
| Plus smoking | 1·03 (0·91–1·18) | 0·61 | 50% (0–80) | 1·11 (1·01–1·22) | 0·034 | 0% (0–75) | 1·31 (1·08–1·59) | 0·007 | 77% (48–90) |
| Plus systolic blood pressure | 1·03 (0·90–1·18) | 0·64 | 53% (0–81) | 1·11 (1·01–1·22) | 0·031 | 0% (0–75) | 1·31 (1·07–1·59) | 0·008 | 77% (49–90) |
| Plus LDL-$C_{corr}$ | 1·04 (0·90–1·19) | 0·61 | 55% (0–82) | 1·12 (1·02–1·24) | 0·019 | 0% (0–75) | 1·34 (1·09–1·65) | 0·005 | 78% (53–90) |
| Plus HDL cholesterol | 1·04 (0·91–1·20) | 0·54 | 54% (0–82) | 1·13 (1·02–1·25) | 0·016 | 0% (0–75) | 1·35 (1·11–1·66) | 0·003 | 77% (49–90) |
| **On-statin lipoprotein(a)** | | | | | | | | | |
| Basic adjustment: seven trials, 14 536 patients, 2603 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 0·94 (0·81–1·10) | 0·45 | 18% (0–62) | 1·06 (0·94–1·21) | 0·33 | 0% (0–71) | 1·43 (1·15–1·76) | 0·001 | 62% (13–83) |
| Progressive adjustment: six trials, 13 883 patients, 2561 events | | | | | | | | | |
| Age-adjusted and sex-adjusted | 0·93 (0·79–1·09) | 0·37 | 18% (0–63) | 1·06 (0·93–1·21) | 0·35 | 0% (0–75) | 1·39 (1·12–1·72) | 0·002 | 64% (13–85) |
| Plus previous cardiovascular disease | 0·93 (0·79–1·09) | 0·37 | 18% (0–63) | 1·06 (0·93–1·21) | 0·36 | 0% (0–75) | 1·39 (1·12–1·72) | 0·002 | 64% (13–85) |
| Plus diabetes | 0·94 (0·80–1·10) | 0·43 | 17% (0–62) | 1·07 (0·94–1·22) | 0·31 | 0% (0–75) | 1·39 (1·13–1·71) | 0·002 | 62% (7–84) |
| Plus smoking | 0·94 (0·81–1·09) | 0·42 | 8% (0–77) | 1·07 (0·94–1·22) | 0·30 | 0% (0–75) | 1·39 (1·13–1·71) | 0·002 | 62% (8–84) |
| Plus systolic blood pressure | 0·94 (0·81–1·09) | 0·41 | 9% (0–77) | 1·07 (0·94–1·22) | 0·30 | 0% (0–75) | 1·39 (1·13–1·71) | 0·002 | 61% (6–84) |
| Plus LDL-$C_{corr}$ | 0·94 (0·81–1·10) | 0·47 | 13% (0–78) | 1·08 (0·95–1·23) | 0·26 | 0% (0–75) | 1·41 (1·15–1·73) | 0·001 | 61% (3–84) |
| Plus HDL cholesterol | 0·95 (0·82–1·11) | 0·53 | 13% (0–78) | 1·08 (0·95–1·23) | 0·24 | 0% (0–75) | 1·42 (1·16–1·74) | 0·001 | 58% (0–83) |

Patients with lipoprotein(a) concentration <15 mg/dL served as the reference group. HR=hazard ratio. LDL-$C_{corr}$=LDL cholesterol corrected for lipoprotein(a) cholesterol.

***Table 3:*** **Associations of baseline and on-statin lipoprotein(a) with incident cardiovascular disease according to different levels of adjustment**

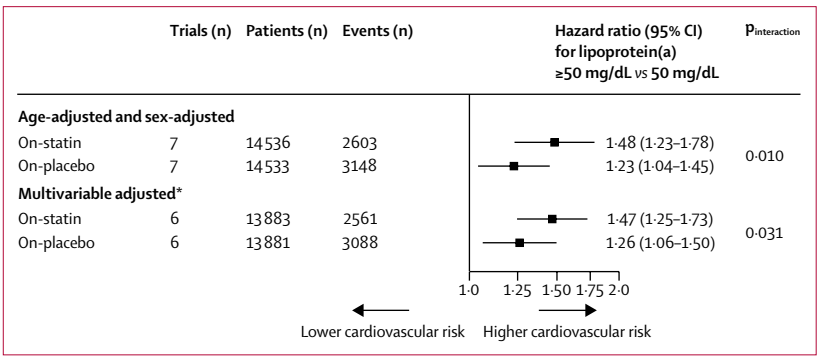


***Figure 2:*** **Comparative predictive value of on-statin versus on-placebo lipoprotein(a) concentrations for incident cardiovascular disease**

*Adjusted for age, sex, previous cardiovascular disease, diabetes, smoking, systolic blood pressure, LDL cholesterol corrected for lipoprotein(a) cholesterol, and HDL cholesterol.

With the exception of stronger associations of on-statin lipoprotein(a) concentrations with cardiovascular disease risk at younger ages (<60 years *vs* 60 to <70 years *vs* ≥70 years; interaction p=0·008), HRs did not vary across clinically relevant subgroups—eg, by sex, smoking, systolic blood pressure, lipid variables, or BMI (figure 3). Furthermore, the magnitude of association was independent of a study's proportion of patients with previous cardiovascular disease or diabetes, the length of follow-up for clinical events, and the time between study baseline and follow-up measurement of on-statin lipoprotein(a) (appendix). Contributing trials used differing statin interventions, precluding a subgroup analysis by statin type or statin dose.

## Discussion

The findings of our meta-analysis of lipoprotein(a) and cardiovascular events show that patients with raised concentrations of lipoprotein(a) on statin treatment (mainly people with concentrations >50 mg/dL) are at a significantly higher risk of cardiovascular disease. The association with cardiovascular events was independent of conventional cardiovascular disease risk factors. This observation was further underpinned by very weak or null cross-sectional correlations of lipoprotein(a) with these risk factors. Importantly, HRs for high lipoprotein(a) at baseline and while taking statins were of similar magnitude, suggesting that statin treatment might not affect lipoprotein(a)-mediated risk appreciably in people with increased amounts of lipoprotein(a). Overall, these data suggest that patients with raised concentrations of lipoprotein(a), representing about 25% of those with previous cardiovascular disease or an indication for statins,[1] are at substantial residual risk—even while taking statins. In this patient population, treatments that specifically lower lipoprotein(a) might mitigate lipoprotein(a)-mediated risk. An appropriately

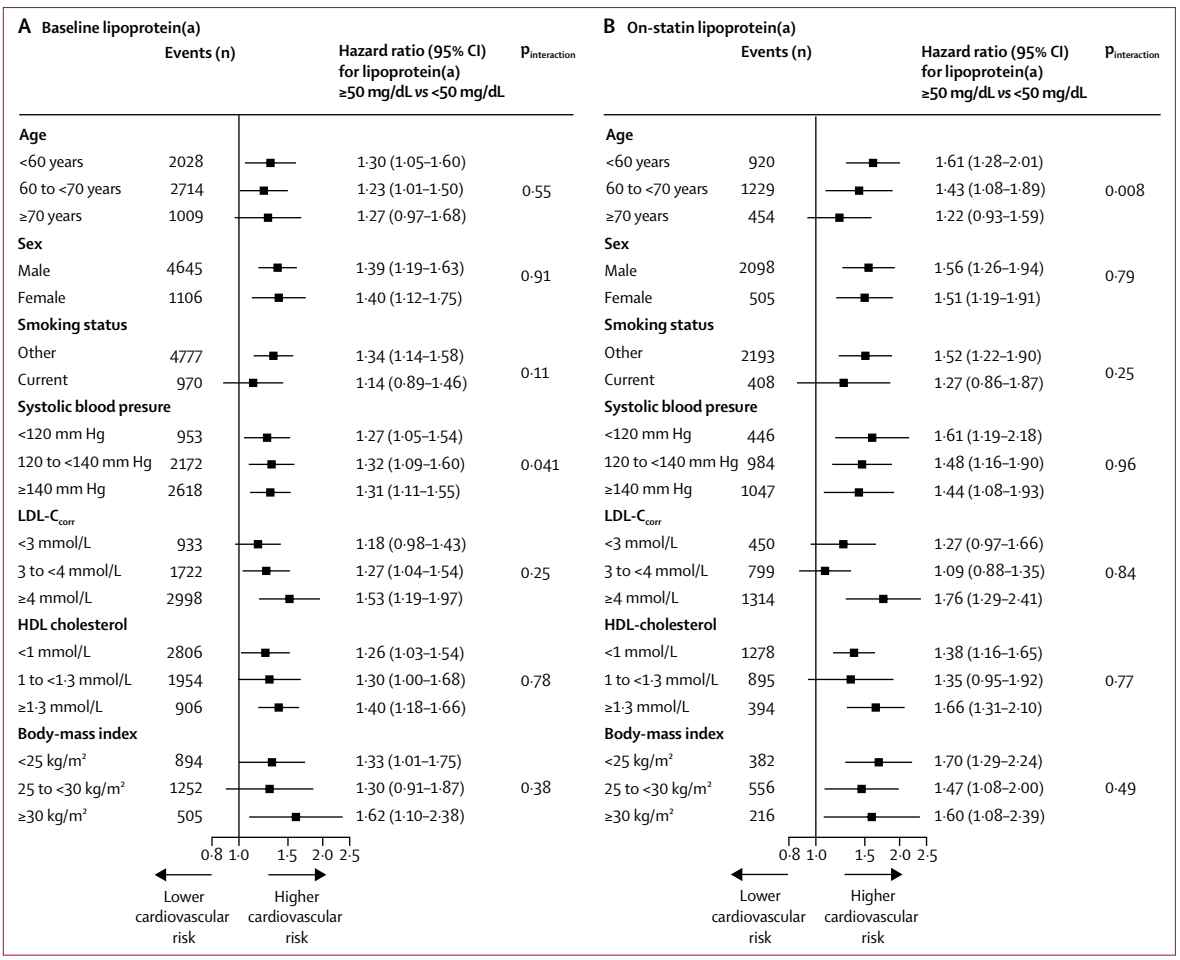


***Figure 3:*** **Associations of baseline and on-statin lipoprotein(a) with incident cardiovascular disease by individual patient characteristics**
Hazard ratios are comparing patients with lipoprotein(a) concentrations of 50 mg/dL or higher with values lower than 50 mg/dL. LDL-$C_{corr}$=LDL cholesterol corrected for lipoprotein(a) cholesterol.

designed cardiovascular disease outcomes trial with robust lowering of lipoprotein(a) is, therefore, justified to test the hypothesis that cutting levels of lipoprotein(a) reduces cardiovascular events, independent of statin treatment.

At baseline, amounts of lipoprotein(a) were associated weakly with demographic and laboratory variables. The most significant (but nevertheless weak) correlations were inverse with diabetes mellitus and triglycerides. The observation of an inverse association of lipoprotein(a) with incident diabetes has been made previously,[31] and the inverse association is most pronounced at very low levels of lipoprotein(a) (≤5 mg/dL), which corresponds approximately to the bottom tenth of the lipoprotein(a) distribution in the global population.[1,2] Whether these findings are causal, or whether they are due to reverse causality, is unknown.[32] Although the underlying mechanisms are not well understood, fasting and postprandial insulin levels are associated inversely with lipoprotein(a).[33] Lipoprotein(a) correlated weakly with LDL cholesterol, but this relation became inversely associated after subtracting the estimated cholesterol content in lipoprotein(a) from the laboratory measurement of LDL cholesterol.[28]

Studies in which modern lipoprotein(a) assays were used to assess the role of lipoprotein(a) in predicting risk for cardiovascular events in patients without cardiovascular disease have been almost uniformly positive.[7] However, results of studies in patients with previous cardiovascular disease or who were taking statins either have been mixed or have suggested the effect is present mainly in people with increased LDL cholesterol.[2] A major limitation of all substudies reporting lipoprotein(a) and outcomes has been power. All studies have enrolled patients with lipoprotein(a) concentrations in the mid-to-low-normal range (10–15 mg/dL; normal <30 mg/dL), as confirmed in our meta-analysis; thus, statistical power to assess risk in patients with highly elevated lipoprotein(a) (ie, >50 mg/dL) was low. Our study is well powered, with 5751 total events and 2603 events in patients allocated statins, making it equivalent to (or larger than) most individual, randomised

controlled, cardiovascular outcome trials in the modern era. By contrast with findings of a previous analysis of individual-patient data,[34] our study had higher statistical power because it included at least ten times more cardiovascular events and, hence, could characterise associations with high lipoprotein(a) concentrations more precisely. Moreover, our analysis used clinically relevant lipoprotein(a) categories informed by guideline recommendations rather than trial-specific quintiles.

Our meta-analysis is highly representative of clinical care in patients treated with statins. First, the studies we included represent patients who were treated with moderate-to-high doses of five major statins used clinically. Second, the studies reflect the variety of patients treated clinically, including primary prevention, high-risk primary prevention with elevated C-reactive protein or diabetes, secondary prevention, stable coronary artery disease, acute coronary syndromes, patients on dialysis, and those with highly elevated LDL cholesterol in the familial hypercholesterolaemia range. Therefore, the studies we included in our meta-analysis broadly reflect patients with high residual risk despite statin treatment, potentially due to other unmodified risk factors such as increased amounts of lipoprotein(a).

The categories of lipoprotein(a) concentrations chosen by us in our meta-analysis reflect clinical risk as suggested by findings of epidemiological and genetic studies. The cutoff of less than 15 mg/dL we used to define the reference group corresponds roughly to the median global concentration of lipoprotein(a).[35,36] Concentrations lower than 30 mg/dL represent the usual cutoff in US laboratories, which is regarded as the normal concentration and is based on data showing that risk for myocardial infarction starts to accrue at concentrations of lipoprotein(a) higher than 25–30 mg/dL.[7,37] The range of 30–50 mg/dL was chosen because it is the grey zone between concentrations considered pathophysiologically relevant and concentrations judged by the European Atherosclerosis Society as high risk (>50 mg/dL), based on the European population prevalence of 20%.

In our study, elevation of cardiovascular disease risk became evident at baseline in patients with concentrations of lipoprotein(a) ranging between 30 mg/dL and lower than 50 mg/dL, and risk was further pronounced when lipoprotein(a) concentration exceeded 50 mg/dL at baseline, including for patients treated with statins. The HRs for lipoprotein(a) concentrations of 50 mg/dL or higher are consistent with those reported in studies of PCSK9 inhibitors in patients with background statin therapy.[38] Additional analyses at even greater concentrations of lipoprotein(a)—ie, 75 mg/dL or higher—were limited by low power because of few patients with lipoprotein(a) concentrations in this range, but the findings support a graded relation between lipoprotein(a) and cardiovascular risk. Outcome trials of lipoprotein(a) lowering are likely to include patients with mean baseline lipoprotein(a) substantially greater than 50 mg/dL; therefore, extrapolation to event reduction with lipoprotein(a) lowering from these data could be an underestimate.

A key observation of our study is that lipoprotein(a) concentrations were associated more strongly with cardiovascular disease risk in patients assigned statins than in people allocated placebo. Findings of a small angiographic study suggested initially that risk associated with lipoprotein(a) is attenuated when LDL cholesterol is well controlled.[39] By contrast, findings of our study—utilising a far larger body of data—support the opposite conclusion, that risk is associated independently with both LDL cholesterol and lipoprotein(a). When LDL-attributable risk is reduced with statin treatment, lipoprotein(a)-associated risk becomes an even stronger predictor of residual risk. This observation is especially evident at lipoprotein(a) concentrations exceeding 50 mg/dL. In support of our observation in this study, the trials FOURIER (NCT01764633) and ODYSSEY OUTCOMES (NCT01663402) have presented preliminary findings of their data, both showing that elevated baseline lipoprotein(a) remains a risk factor for cardiovascular disease, even with on-treatment LDL cholesterol lower than 50 mg/dL in patients treated with statins and PCSK9 inhibitors. The findings highlight the importance of determining whether there is a cardiovascular benefit of treatment to reduce lipoprotein(a) when initial concentrations exceed this threshold, irrespective of concurrent treatment with statin. A second important observation is that all major subgroups of patients seemed to be at risk of elevated lipoprotein(a), including those older than 70 years, women, smokers, people with low and high LDL-$C_{corr}$, low HDL cholesterol, and all categories of BMI.

It is important to emphasise that the lipoprotein(a) hypothesis remains to be tested. To do so requires a randomised trial that compares cardiovascular outcomes in patients treated with an agent that specifically lowers lipoprotein(a) versus placebo. Such a trial might be possible with an antisense oligonucleotide targeting *LPA* messenger RNA, thereby reducing plasma lipoprotein(a) levels. Phase 1 and 2 trials with such an agent have shown the potential to lower lipoprotein(a) levels by more than 90%.[27,40]

Our study has several limitations. First, individual patient data could not be obtained from several other statin trials that reported lipoprotein(a) concentrations and outcomes. It is possible that inclusion of other data would have modified the reported effect sizes. Second, the relation of lipoprotein(a) to residual cardiovascular risk in patients receiving treatment with non-statin lipid-modifying agents (eg, ezetimibe, PCSK9 inhibitors) remains undetermined. Third, lipoprotein(a) assays were heterogeneous and most were in lipoprotein(a) mass (mg/dL) rather than molar (nmol/L) concentration, and the timepoints at which lipoprotein(a) was measured in each trial were not uniform. Therefore, assays not reported in mg/dL had to be converted mathematically

to mg/dL, which might have introduced imprecision into the lipoprotein(a) measurement. A National Heart, Lung, and Blood Institute Working Group on lipoprotein(a) recommended global standardisation of lipoprotein(a) assays to address this limitation.[2] Fourth, we cannot rule out that index event bias could have attenuated effect sizes in secondary prevention trials, although the scope of this bias was reduced by employment of multivariable adjustment. Fifth, our analysis identified moderate-to-high between-study heterogeneity, which could not be accounted for by baseline disease status (ie, previous cardiovascular disease or previous diabetes) nor by differing lengths of follow-up.

In conclusion, our meta-analysis shows an approximately linear relation between cardiovascular risk and concentrations of lipoprotein(a), which is evident at lipoprotein(a) concentrations of 30 mg/dL to less than 50 mg/dL and pronounced at concentrations of 50 mg/dL or higher and persists despite statin treatment. These data provide a rationale for evaluating drugs that can lower lipoprotein(a) specifically and might have the potential to reduce residual cardiovascular risk independent of statin treatment.

#### Contributors

PW and ST wrote the analysis plan, collected and harmonised data, had access to all raw data, and wrote the first draft of the report. PW did the statistical analysis. PMR, PJN, JS, AMT, TRP, GGS, AGO, HMC, FK, CD, CW, and SM obtained patient data in statin trials and provided cleaned data to the coordinating centre. AL secured funding for the study and contributed to data interpretation. All authors contributed to writing the final report and approved the version submitted.

#### Declaration of interests

PW reports consultancy fees from Novartis Pharmaceuticals during the conduct of the study; and travel expenses from Bayer, Daiichi Sankyo, and Sanofi-Aventis outside the submitted work. PMR reports grants from AstraZeneca during the conduct of the study; grants from Novartis, Kowa, Pfizer, and the National Heart, Lung, and Blood Institute outside the submitted work; and personal fees from Novartis and Sanofi outside the submitted work. AMT reports personal fees from Amgen, Bayer, Merck, and Pfizer outside the submitted work; and non-financial support from Bayer outside the submitted work. TRP reports personal fees from Amgen and Sanofi Regeneron outside the submitted work. GGS reports grants from Pfizer during the conduct of the study; and grants from Cerenis, Roche, Sanofi, and The Medicines Company outside the submitted work. HMC reports grants from AstraZeneca, Boehringer Ingelheim, and Roche Pharmaceuticals during the conduct of the study; grants, non-financial support, and travel expenses from Eli Lilly and Regeneron during the conduct of the study; personal fees from Eli Lilly during the conduct of the study; institutional fees from Novartis Pharmaceuticals during the conduct of the study; grants and speaker fees from Pfizer during the conduct of the study; grants and travel expenses from Sanofi Aventis and Novo Nordisk during the conduct of the study; honorarium and speakers' bureau fees from Sanofi during the conduct of the study; and holds shares in Bayer and Roche Pharmaceuticals. CW reports personal fees from Boehringer Ingelheim and Sanofi-Genzyme outside the submitted work. SM reports institutional support from the National Institutes of Health (NIH; grants R01 HL117861, R01 HL134811, and K24 HL136852) outside the submitted work; non-financial support from Quest Diagnostics for measuring lipoprotein(a) in the JUPITER trial outside the submitted work; personal fees from Quest Diagnostics outside the submitted work; and an institutional research grant from Atherotech Diagnostics outside the submitted work. The JUPITER trial was funded by AstraZeneca. AL is an employee of Novartis Pharma AG. ST declares research support from the NIH (grants R01-HL119828, R01-HL078610, R01 HL106579, R01 HL128550, R01 HL136098, P01 HL136275, and R35 HL135737) and is supported by a grant to the Leducq Epigenetics of Atherosclerosis Network from the Fondation Leducq; currently has a dual appointment at the University of California San Diego and Ionis Pharmaceuticals; is a co-inventor and receives royalties from patents owned by the University of California San Diego on oxidation-specific antibodies; and is a co-founder of Oxitope. PJN, JS, AGO, FK, and CD declare no competing interests.

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JAMA Cardiology | Original Investigation

# Association of *LPA* Variants With Risk of Coronary Disease and the Implications for Lipoprotein(a)-Lowering Therapies
## A Mendelian Randomization Analysis

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**IMPORTANCE** Human genetic studies have indicated that plasma lipoprotein(a) (Lp[a]) is causally associated with the risk of coronary heart disease (CHD), but randomized trials of several therapies that reduce Lp(a) levels by 25% to 35% have not provided any evidence that lowering Lp(a) level reduces CHD risk.

**OBJECTIVE** To estimate the magnitude of the change in plasma Lp(a) levels needed to have the same evidence of an association with CHD risk as a 38.67-mg/dL (ie, 1-mmol/L) change in low-density lipoprotein cholesterol (LDL-C) level, a change that has been shown to produce a clinically meaningful reduction in the risk of CHD.

**DESIGN, SETTING, AND PARTICIPANTS** A mendelian randomization analysis was conducted using individual participant data from 5 studies and with external validation using summarized data from 48 studies. Population-based prospective cohort and case-control studies featured 20 793 individuals with CHD and 27 540 controls with individual participant data, whereas summarized data included 62 240 patients with CHD and 127 299 controls. Data were analyzed from November 2016 to March 2018.

**EXPOSURES** Genetic *LPA* score and plasma Lp(a) mass concentration.

**MAIN OUTCOMES AND MEASURES** Coronary heart disease.

**RESULTS** Of the included study participants, 53% were men, all were of white European ancestry, and the mean age was 57.5 years. The association of genetically predicted Lp(a) with CHD risk was linearly proportional to the absolute change in Lp(a) concentration. A 10-mg/dL lower genetically predicted Lp(a) concentration was associated with a 5.8% lower CHD risk (odds ratio [OR], 0.942; 95% CI, 0.933-0.951; $P = 3 \times 10^{-37}$), whereas a 10-mg/dL lower genetically predicted LDL-C level estimated using an LDL-C genetic score was associated with a 14.5% lower CHD risk (OR, 0.855; 95% CI, 0.818-0.893; $P = 2 \times 10^{-12}$). Thus, a 101.5-mg/dL change (95% CI, 71.0-137.0) in Lp(a) concentration had the same association with CHD risk as a 38.67-mg/dL change in LDL-C level. The association of genetically predicted Lp(a) concentration with CHD risk appeared to be independent of changes in LDL-C level owing to genetic variants that mimic the relationship of statins, PCSK9 inhibitors, and ezetimibe with CHD risk.

**CONCLUSIONS AND RELEVANCE** The clinical benefit of lowering Lp(a) is likely to be proportional to the absolute reduction in Lp(a) concentration. Large absolute reductions in Lp(a) of approximately 100 mg/dL may be required to produce a clinically meaningful reduction in the risk of CHD similar in magnitude to what can be achieved by lowering LDL-C level by 38.67 mg/dL (ie, 1 mmol/L).

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Editor's Note
Supplemental content

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Apolipoprotein(a), which is encoded by the *LPA* gene, covalently binds to a cholesterol-rich low-density lipoprotein (LDL) particle to form lipoprotein(a) (Lp[a]).[1] Meta-analyses of prospective observational studies have reported that higher plasma Lp(a) concentration is associated with dose-dependent higher risk of coronary heart disease (CHD).[2] Furthermore, mendelian randomization analyses have provided strong evidence that the association between Lp(a) and risk of CHD is likely to be causal.[3-5] However, several large randomized trials evaluating therapies that lower Lp(a) concentration by between 20% and 35% (including niacin, cholesterol ester transfer protein inhibitors, and PCSK9 inhibitors) have not provided clear evidence that lowering plasma Lp(a) concentration reduces the risk of cardiovascular events beyond that which would be expected from the observed LDL-lowering effect of these therapies.[6-11] Although these trials were not specifically designed to assess the Lp(a)-lowering effect of these agents, these trials raise the question of how much Lp(a) concentration must be lowered to produce a clinically meaningful reduction in cardiovascular events. Therapies that more specifically and potently lower Lp(a) concentrations by up to 90% by inhibiting apolipoprotein(a) synthesis are in development.[12] Whether lowering Lp(a) concentrations with these new therapies will reduce the risk of cardiovascular events is unknown.

Owing to the skewed distribution of plasma Lp(a) concentration, prior studies have reported the association between log-transformed concentrations of Lp(a) and CHD risk.[2,13-15] Changes in log-transformed Lp(a) concentrations represent proportional changes in Lp(a) concentrations. However, proportional reduction is not a useful metric for assessing the potential clinical benefit of lowering Lp(a) level because concentrations can vary by as much as 1000-fold among members of the same population, and therefore, the same proportional change in Lp(a) concentration can result in markedly different absolute changes, depending on the initial Lp(a) concentration.[16]

Importantly, statins and other therapies that reduce LDL particle concentrations are associated with a dose-dependent reduction in the risk of cardiovascular events that is determined by the absolute (rather than the proportional) change in LDL cholesterol (LDL-C) level.[17-19] Because Lp(a) contains an LDL particle, we hypothesized that there would be evidence to support a clinical association of Lp(a) with the risk of CHD that may also be proportional to the absolute change in circulating Lp(a) mass concentration. To test this hypothesis, we created a genetic score to estimate the magnitude and shape of the relationship of Lp(a) with the risk of CHD. We then estimated the absolute change in plasma Lp(a) concentration required to achieve the equivalent change in CHD risk as a 38.67-mg/dL (ie, 1-mmol/L) change in LDL-C level (to convert to millimoles per liter, multiply by 0.0259), a change in LDL-C that has been demonstrated to produce a clinically meaningful 20% to 25% reduction in the risk of cardiovascular events in short-term trials.[17-19] Our objective was to make inferences about how much Lp(a) concentration must be reduced pharmacologically to produce a clinically meaningful reduction in CHD risk and thereby determine who is most likely to benefit from treatment with Lp(a)-lowering therapy to inform clinical guidelines and the design of randomized trials evaluating Lp(a)-lowering therapies.

### Key Points

**Question** How much does plasma lipoprotein(a) need to be lowered to produce a clinically meaningful reduction in the risk of coronary heart disease?

**Findings** In a mendelian randomization analysis involving more than 80 000 patients and more than 150 000 controls, coronary heart disease risk was proportionally associated with the absolute change in plasma lipoprotein(a) mass concentration; a 101.5-mg/dL change in lipoprotein(a) concentration was associated with the same coronary heart disease risk as a 38.67-mg/dL (ie, 1-mmol/L) change in low-density lipoprotein cholesterol level.

**Meaning** Lipoprotein(a) concentration must be lowered by approximately 100 mg/dL to achieve the same reduction in coronary heart disease risk as can be achieved by lowering low-density lipoprotein cholesterol level by 38.67 mg/dL.

## Methods

### Study Population and Outcomes

We studied 48 333 participants of European descent (including 20 793 with CHD) from 5 studies for whom individual participant-level data were available as part of the CHD Exome+ Consortium. Descriptions of the included studies are provided in eMethods 1 of the Supplement. The primary outcome was CHD, defined as CHD death, nonfatal myocardial infarction, or (for 3 of the studies) other coronary events with *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision* codes I20-25. Participants provided written informed consent for genetic studies. As this was an analysis of anonymized data that had already been collected, ethical approval was not sought for this particular investigation.

### *LPA* Genetic Score

All CHD Exome+ Consortium participants were genotyped using a customized version of the Illumina Exome Beadchip array, which included ultrafine mapping of the *LPA* gene region involving 2426 variants genotyped within a 660-kb window (eFigure 1 in the Supplement). To select variants for inclusion in the genetic score, we identified variants in the *LPA* gene region that were conditionally associated with Lp(a) concentrations at a genome-wide level of significance ($P < 5 \times 10^{-8}$) using forward stepwise regression among participants free from CHD at baseline in each study. We adjusted for study, age, sex, and 5 principal components of ancestry. Genetic variants correlated with a selected variant at $r^2$ greater than 0.4 were excluded from further steps of the procedure (eFigure 2 in the Supplement). For each participant, we calculated a weighted genetic score by summing the number of Lp(a)-raising alleles inherited at each variant included in the score, weighted by each variant's association with absolute change in Lp(a) mass

Table 1. Baseline Characteristics of Participants

| Source | No. | Patients With CHD, No. | Lp(a) Measured, No.[a] | Age, Mean (SD), y | Men, No. | Lp(a) Concentration, mg/dL | |
|---|---|---|---|---|---|---|---|
| | | | | | | Mean (SD) | Median |
| CCHS | 7808 | 1943 | 7396 | 58 (15) | 3463 | 29.3 (33.6) | 16.9 |
| CGPS-CIHDS | 17 120 | 7740 | 9964 | 59 (13) | 9635 | 25.0 (28.1) | 13.6 |
| EPIC-CVD | 20 780 | 9810 | 15 899 | 55 (10) | 9792 | 52.6 (37.1) | 43.3 |
| PROSPER | 1279 | 641 | 0 | 76 (4) | 708 | NA | NA |
| WOSCOPS | 1346 | 659 | 1017 | 56 (6) | 1346 | 36.0 (39.3) | 19.0 |

Abbreviations: CCHS, Copenhagen City Heart study; CGPS, Copenhagen General Population study; CHD, coronary heart disease; CIHDS, Copenhagen Ischemic Heart Disease study; EPIC-CVD, European Prospective Investigation Into Cancer and Nutrition-Cardiovascular Disease study; Lp(a), lipoprotein (a); PROSPER, Prospective Study of Pravastatin in the Elderly at Risk study; WOSCOPS, West of Scotland Coronary Prevention study.

[a] Values of Lp(a) concentration were winsorized at 130 mg/dL.

concentration (measured in milligrams per deciliter). In sensitivity analyses, we repeated the primary analyses using different choices of variants in the genetic scores, as described in eMethods 2 in the Supplement.

## Study Design

To assess the dose-response shape of the association between genetically predicted Lp(a) and CHD risk, we divided participants into deciles of the genetic score and measured the association between each decile of genetically predicted Lp(a) concentration and the risk of CHD using the first decile as the reference group. Informed by the shape of the association, we estimated the association between the *LPA* score and the risk of CHD for absolute changes in Lp(a) concentration.

To estimate the absolute reduction in Lp(a) concentration required to have the same change in CHD risk as a 38.67-mg/dL decrease in LDL-C level, we used the following protocol (eFigure 3 in the Supplement). First, we measured the association between the *LPA* score and the risk of CHD per 10-mg/dL decrease in genetically predicted Lp(a) concentration. Next, we measured the association between a genetic score consisting of variants in or near genes that encode the targets of currently available LDL-C–lowering therapies and CHD risk per 10-mg/dL decrease in genetically predicted LDL-C (eTable 1 in the Supplement).[20] We then calculated the ratio between these 2 estimates to obtain the change in Lp(a) concentration that has an equivalent association with CHD risk as a 1-mg/dL change in LDL-C level. To estimate the amount Lp(a) concentration must be reduced to have the same association with CHD risk as a 38.67-mg/dL reduction in LDL-C level, we multiplied this ratio by 38.67. Finally, we estimated the predicted short-term change associated with different magnitudes of pharmacological lowering of Lp(a) concentration by converting the change in Lp(a) concentration into a change in LDL-C level having an equivalent predicted effect on CHD risk and using the estimated change associated with statin treatment per 38.67-mg/dL reduction in LDL-C level, as reported by the Cholesterol Treatment Trialists' Collaboration.[17]

## Statistical Analyses

We estimated the association of each variant with Lp(a) or LDL-C concentration using linear regression and with CHD risk using logistic regression. All regression analyses were performed separately in each of the studies, adjusting for age, sex, and the first 5 principal components of ancestry; these estimates were combined across studies in a fixed-effects inverse variance-weighted meta-analysis. Heterogeneity was assessed using the $I^2$ statistic. Mendelian randomization estimates were then obtained from these variant-specific estimates using a previously reported method that accounts for correlation between variants.[21] Nonlinearity in the mendelian randomization estimates of the shape of the association of Lp(a) change with the risk of CHD was assessed using fractional polynomials, as described elsewhere.[22] For external replication in an independent sample, we performed the same analyses using summarized genetic associations with CHD risk from the Coronary Artery Disease Genome Wide Replication and Meta-analysis (CARDIOGRAM) plus The Coronary Artery Disease (C4D) Genetics (CARDIOGRAMplusC4D) consortium in up to 62 240 patients and 127 299 controls.[23]

All analyses were performed using the statistical software platform R version 3.4.1 (R Programming). A detailed description of the methods is provided in eMethods 2 of the Supplement.

# Results

## Participant Characteristics

The baseline characteristics of participants are presented in **Table 1**. Across the 5 studies contributing to the initial sample, the median Lp(a) concentration varied from 13.6 mg/dL to 43.3 mg/dL (eTable 2 and eFigure 4 in the Supplement).

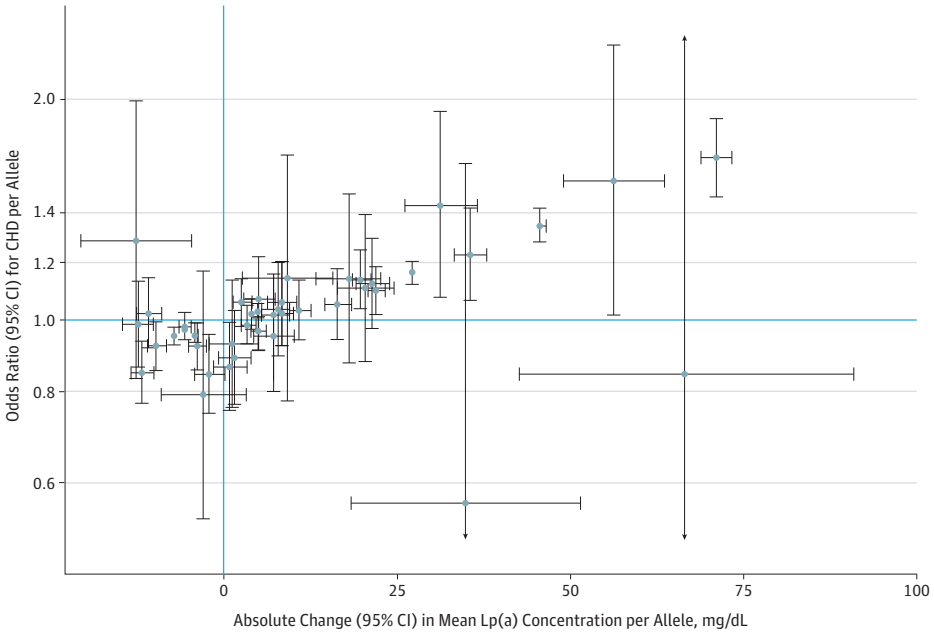
## *LPA* Genetic Score

The stepwise selection procedure identified 43 genetic variants conditionally associated with Lp(a) (eTable 3 in the Supplement). The genetic score comprising these variants explained 51% to 63% of the variance in Lp(a) concentration in each study (eFigure 5 in the Supplement). This explanatory ability is lower than observed previously[24] because our genetic score was constructed conservatively to minimize bias owing to overfitting. Associations of each variant with Lp(a) concentration and CHD risk are displayed in **Figure 1**.

## Association of *LPA* Genetic Score With CHD

In analyses dividing the population into deciles of genetically predicted absolute Lp(a) mass concentration, the exposure–

Figure 1. Association of *LPA* Variants With Lipoprotein(a) (Lp[a]) Concentration and Coronary Heart Disease (CHD) Risk




Marginal genetic associations with Lp(a) and CHD risk (error bars indicate 95% confidence intervals) obtained in the CHD Exome+ consortium for 43 variants included in the *LPA* genetic score. Associations are orientated to the minor allele.

outcome association for log-transformed CHD risk was approximately linear, ie, fixed changes in absolute Lp(a) concentrations led to equal odds ratios (ORs) for CHD regardless of the starting Lp(a) concentration (**Figure 2**A). By contrast, the exposure-outcome association for deciles of log-transformed Lp(a) concentration was curvilinear (Figure 2B), with fixed proportional changes in Lp(a) concentrations leading to greater log-ORs for individuals with higher baseline Lp(a) concentrations (and hence, increasingly greater absolute changes in Lp[a] concentrations). These findings are consistent and support the hypothesis that the risk of CHD is log-linearly proportional to absolute changes in Lp(a) concentration.

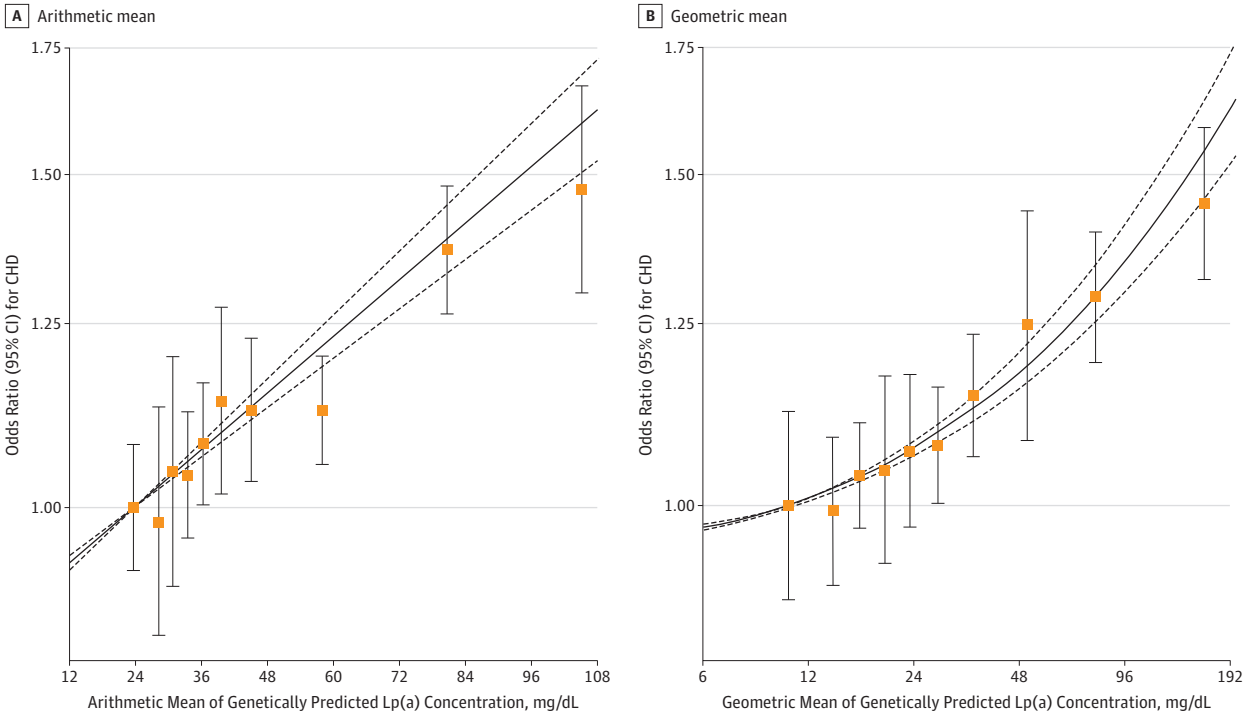
Overall, each 10-mg/dL lower genetically predicted Lp(a) level was associated with a 5.8% lower risk of CHD (OR, 0.942; 95% CI, 0.933-0.951; $P = 3 \times 10^{-37}$). There was no evidence of heterogeneity with similar genetic association estimates obtained across all studies independent of the type of Lp(a) assay used (eFigures 6 and 7 in the Supplement). Estimates were also similar in sensitivity analyses that varied the number of genetic variants included in the *LPA* score (eTable 4 in the Supplement). In external replication analyses involving participants from CARDIOGRAMplusC4D, a 10-mg/dL lower genetically predicted Lp(a) level was associated with a 5.2% lower risk of CHD (OR, 0.948; 95% CI, 0.941-0.955; $P = 1 \times 10^{-47}$).

## Expected Clinical Benefit of Lowering Lp(a) Concentration

Using the LDL-C genetic score, a 10-mg/dL genetically predicted lower LDL-C level was associated with a 14.5% lower risk of CHD (OR, 0.855; 95% CI, 0.818-0.893; $P = 2 \times 10^{-12}$) (eFigure 8 in the Supplement). This finding suggests that a 1-mg/dL difference in LDL-C level has the same association with CHD risk as a 2.63-mg/dL difference in Lp(a) concentration (ie, log[0.855] / log[0.942] = 2.63), and therefore, a 38.67-mg/dL difference in LDL-C level has the same association as a 101.5-mg/dL (95% CI, 71.0-137.0) difference in Lp(a) concentration. In external replication analyses using data from CARDIOGRAMplusC4D, a 10-mg/dL lower LDL-C level was associated with a 14.0% lower CHD risk (OR, 0.860; 95% CI, 0.841-0.879; $P = 3 \times 10^{-40}$), suggesting that a 109.1-mg/dL (95% CI, 89.0-133.1) difference in Lp(a) concentration has the same association with CHD risk as a 38.67-mg/dL difference in LDL-C level.

Changes in genetically predicted Lp(a) and LDL-C concentrations represent lifelong exposure to these lipoproteins. Hence, to estimate the effect of lowering Lp(a) concentration in a short-term trial, we assumed that if lifelong exposure to 101.5-mg/dL lower Lp(a) concentration has the same association with CHD risk as lifelong exposure to 38.67-mg/dL lower LDL-C level, then short-term exposure to 101.5-mg/dL lower Lp(a) concentration should have the same association with CHD risk as short-term exposure to 38.67-mg/dL lower LDL-C levels observed in randomized trials. This assumption is valid only if changes in Lp(a) concentration and LDL-C level have similar cumulative associations with CHD over time. It is further supported by the observation that the ratio of the association of lifelong exposure to Lp(a) with CHD risk estimated from mendelian randomization to the association of intermediate-term exposure to Lp(a), estimated from observational studies in the Emerging Risk Factors Consortium,[2] is very similar to the ratio of the association of lifelong exposure to LDL-C with CHD risk estimated from mendelian randomization to the association of intermediate-term exposure to LDL-C in the Emerging Risk Factors Consortium (**Figure 3**; eFigure 9 in the

Figure 2. Shape of Association Between Genetically Predicted Lipoprotein(a) (Lp[a]) and Coronary Heart Disease (CHD) Risk




A, Arithmetic mean of Lp(a) in each decile (untransformed, linear scale). B, Geometric mean of Lp(a) in each decile (log-transformed, log-scale). Points on the curve indicate mendelian randomization estimates in each decile of genetically predicted Lp(a) (error bars indicate 95% confidence intervals; first decile is reference group). The solid line indicates the best-fitting fractional polynomial (left, linear term only; right, square root and cubic terms) to model the dose-dependent relationship; the dotted lines indicate the 95% confidence intervals for the relationship.

Supplement).[25] Therefore, Lp(a) and LDL-C appear to have similar cumulative associations with the risk of CHD over time.

**Table 2** shows the expected clinical benefit in CHD risk from both lifelong and short-term exposure to absolute differences in Lp(a) concentration. Lifelong estimates are conventional mendelian randomization estimates, while short-term estimates are calculated using the difference in Lp(a) concentration needed to achieve the same change for a given reduction in LDL-C level over a median of 5 years of treatment with a statin, as reported by the Cholesterol Treatment Trialists' Collaboration.[18]

### Independent Association of Lp(a) and LDL-C–Lowering Therapies

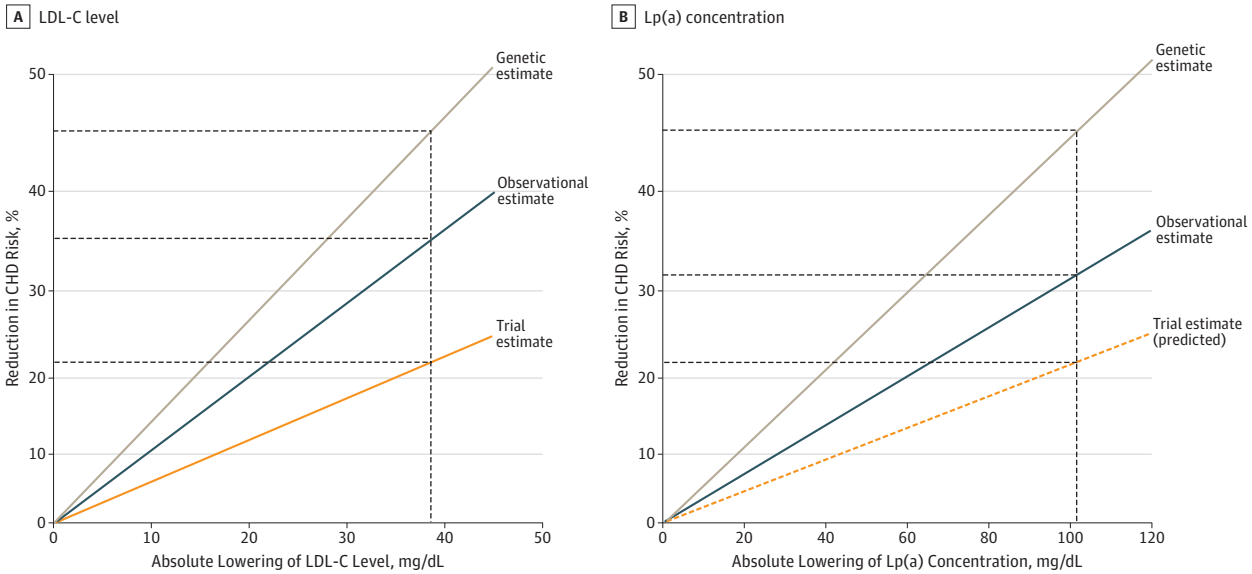
To assess whether the association of lowering Lp(a) concentration with the risk of CHD is likely to be independent of lowering LDL-C level with statins, we divided the population into 3 groups based on the number of LDL-C–lowering alleles each participant inherited at a common variant (rs12916) in the 3-hydroxy-3-methyl-glutaryl-CoA reductase (*HMGCR*) gene, which encodes the target of statins.[26,27] The *LPA* score had nearly identical associations per 10-mg/dL lower Lp(a) concentration in each of these 3 groups (CC genotype group: OR, 0.945; 95% CI, 0.927-0.964; CT genotype group: OR, 0.939; 95% CI, 0.927-0.952; TT genotype group: OR, 0.945; 95% CI, 0.932-0.957; $P = .79$ for difference) (eFigure 10 in the Supplement), suggesting that the relative risk reduction of lowering Lp(a) concentration is likely to be independent of lowering LDL-C level with statins. Similar findings were observed for genetic variants in the *PCSK9* and *NPC1L1* gene regions that mimic the changes associated with PCSK9 inhibitors and ezetimibe, respectively.

## Discussion

We found that the association of genetically predicted plasma Lp(a) with the risk of CHD was linearly proportional to the absolute difference in Lp(a) concentration. Absolute differences in Lp(a) concentration of approximately 100 mg/dL had an equivalent association with CHD risk as a 38.67-mg/dL difference in LDL-C level. The results of this study may have important implications for informing clinical practice guidelines on the use of Lp(a)-lowering therapies, for designing randomized trials to evaluate Lp(a)-lowering therapies currently in development, and for designing screening programs to reduce the global burden of CHD.

Because a 100-mg/dL difference in Lp(a) concentration had the same association with CHD risk as a 38.67-mg/dL difference in LDL-C level, the results of this study suggest that pharmacologically lowering Lp(a) concentration by approximately 100 mg/dL should reduce the risk of CHD (CHD death or nonfatal myocardial infarction) by approximately 22% to 25% in a 3- to 5-year randomized trial, similar to the association that has been observed for a 38.67-mg/dL reduction in

Figure 3. Estimates of Coronary Heart Disease (CHD) Risk Reduction With Lowering of Low-Density Lipoprotein Cholesterol (LDL-C) Level and Lipoprotein(a) (Lp[a]) Concentration




Genetic estimates of lifelong lowering from mendelian randomization (brown line), observational estimates from prospective cohort studies (blue line), and (A) trial estimate from short-term statin trials (for LDL-C) or (B) predicted trial estimate (for Lp[a]) (orange line). The vertical line is at 38.67 mg/dL (ie, 1 mmol/L) for LDL-C level and at 101.5 mg/dL for Lp(a) concentration, the estimated equivalent lowering in Lp(a) for the same reduction in CHD risk. To convert LDL-C to millimoles per liter, multiply by 0.0259.

Table 2. Expected Clinical Benefit of Lowering Lp(a)

| Reduction in Lp(a) Concentration, mg/dL | Reduction in LDL-C Level for Equivalent CHD Risk Reduction, mg/dL (95% CI)[a] | Estimated Lifelong Proportional Risk Reduction Owing to Genetically Decreased Exposure, % (95% CI)[b] | Estimated Short-term Proportional Risk Reduction in Randomized Trial, % (95% CI)[c] |
|---|---|---|---|
| 120 | 45.7 (34.1-65.4) | 51.1 (45.5-56.2) | 27.7 (20.9-37.5) |
| 100 | 38.1 (28.4-54.5) | 44.9 (39.7-49.8) | 23.7 (17.8-32.4) |
| 80 | 30.5 (22.7-43.6) | 38.0 (33.2-42.3) | 19.4 (14.5-26.9) |
| 50 | 19.0 (14.2-27.3) | 25.8 (22.3-29.1) | 12.6 (9.3-17.8) |
| 30 | 11.4 (8.5-16.4) | 16.4 (14.1-18.7) | 7.8 (5.7-11.1) |
| 20 | 7.6 (5.7-10.9) | 11.3 (9.6-12.9) | 5.3 (3.8-7.5) |
| 10 | 3.8 (2.8-5.5) | 5.8 (4.9-6.7) | 2.7 (1.9-3.9) |
| 5 | 1.9 (1.4-2.7) | 2.9 (2.5-3.4) | 1.3 (1.0-1.9) |

Abbreviations: CHD, coronary heart disease; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a).

SI conversion factor: To convert LDL-C to millimoles per liter, multiply by 0.0259.

[a] Each mg/dL lower Lp(a) has an association with CHD risk that is equivalent to a 0.38-mg/dL reduction in LDL-C based on the ratio of the associations of the genetic scores with CHD risk.

[b] Effect size (95% confidence interval) for Lp(a) reduction obtained from mendelian randomization approach.

[c] Effect size (95% confidence interval) for Lp(a) reduction obtained by considering equivalent lowering of LDL-C and in comparison with estimate from randomized trials of statins on major coronary events (risk ratio, 0.76; 95% CI, 0.73-0.78).

LDL-C level during treatment with a statin.[17-19] Therefore, it follows that lowering Lp(a) concentration by 80 mg/dL might be expected to reduce the risk of CHD events by approximately 18% to 20%, while lowering Lp(a) concentration by 50 mg/dL might reduce CHD events by 10% to 12% (Table 2), assuming that there are no unrecognized competing risks associated with lowering Lp(a) concentration. Therefore, only persons with very high Lp(a) concentrations are likely to benefit substantially from therapies that reduce Lp(a) concentration.

This finding likely explains why therapies that reduce Lp(a) concentration by 20% to 35% have failed to provide clear evidence that lowering Lp(a) concentration reduces the risk of cardiovascular events in previous randomized trials even though Lp(a) is a genetically supported target. The median Lp(a) concentration among participants enrolled in these trials was approximately 12 to 20 mg/dL.[7-11] Therefore, a 30% reduction in Lp(a) concentration would translate into only a 3- to 6-mg/dL absolute reduction in circulating plasma Lp(a) concentration, a small absolute reduction that was likely far too modest to reduce the risk of cardiovascular events appreciably in a short-term randomized trial.

The results of this study suggest that randomized trials evaluating new, more potent Lp(a)-lowering therapies in development should be designed to enroll individuals with very high baseline Lp(a) concentrations of 90 to 100 mg/dL or more. Reducing Lp(a) concentration by 80% to 90% in such individuals should translate into large absolute reductions in Lp(a) concentrations of 70 to 90 mg/dL, which should in turn translate into approximately a 15% to 20% proportional reduction in the risk of CHD events. Enrolling patients at high risk of CHD owing to markedly elevated Lp(a) concentration in the initial proof-of-concept clinical trials is similar to the strategy used by the Scandinavian Simvastatin Survival Study trial,[28] which enrolled high-risk patients with markedly elevated LDL-C concentrations and was the first trial to demonstrate that treatment with statins led to large, clinically meaningful reductions in the risk of cardiovascular events.

The magnitude of the pharmacologic reduction in Lp(a) mass that is likely needed to produce clinically meaningful reductions in CHD risk estimated in this study is larger than estimated in a 2018 study evaluating changes in Lp(a) during treatment with niacin.[29] However, whereas that previous study involved informal estimates of the reversible CHD risk by lowering Lp(a) concentrations in a short-term trial,[29] we used a more systematic approach. In particular, our study estimated the differences in genetically predicted Lp(a) and LDL-C concentrations needed to have the same change in lifetime CHD risk and incorporated an assessment of the differential cumulative associations of Lp(a) and LDL-C with CHD risk over time to estimate how much Lp(a) concentration must be lowered pharmacologically to produce the same change as lowering LDL-C level by 38.67 mg/dL (ie, 1 mmol/L) with a statin. This approach has been successfully used to accurately anticipate the results of several recent trials.[26,27,30] Similar analyses to those used in the current study are needed before it would be possible to accurately anticipate the potential effect of pharmacologically lowering Lp(a) on the risk of stroke, peripheral vascular disease, aortic stenosis, or composite end points that include these outcomes.[31]

Finally, it should be noted that plasma Lp(a) concentration is largely heritable. Therefore, if the linear relationship with CHD risk continues at very high absolute Lp(a) concentrations (as occurs for LDL-C), then Lp(a) concentrations in excess of 200 mg/dL may be associated with a 3- to 4-fold increased lifetime risk of CHD (OR, 3.30; 95% CI, 2.75-3.96) and thus may represent an inherited lipoprotein disorder that is associated with a similar lifetime risk of CHD as heterozygous familial hypercholesterolemia but with a prevalence that may be 2-fold higher than that of heterozygous familial hypercholesterolemia.[32,33] Therefore, screening for individuals with extremely elevated Lp(a) concentrations and treating them with one of the new Lp(a)-lowering therapies in development could potentially have the same effect on reducing the global burden of CHD as current screening programs designed to detect and treat individuals with familial hypercholesterolemia.

### Limitations

Our study has limitations. Multiple different assays were used to measure Lp(a) concentrations in the included studies. However, we focused only on absolute differences in Lp(a) associated with genetic variants, which were very similar across all included studies, regardless of assay used or baseline Lp(a) concentrations. In addition, our estimate of the effect of lowering Lp(a) is agnostic to the mechanism of action, and hence our use of plasma Lp(a) mass concentration to estimate the dose-response relationship does not imply that our estimates are solely via changes in plasma Lp(a) mass concentration. If pharmacologic Lp(a) lowering has associations not adequately captured by the genetic variants (eg, antithrombotic associations), then smaller absolute reductions in Lp(a) than estimated here may produce clinically meaningful reductions in CHD risk.[34]

## Conclusions

The association of genetically predicted Lp(a) with CHD risk was linearly proportional to the absolute change in Lp(a) mass concentration. Large absolute reductions in Lp(a) concentration of approximately 100 mg/dL are likely necessary to achieve clinically meaningful reductions in the risk of CHD similar in magnitude to what can be achieved by lowering LDL-C level by 38.67 mg/dL (ie, 1 mmol/L) with a statin.

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**Author Contributions:** Dr Burgess had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Burgess and Ference contributed equally.
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WILEY

ORIGINAL ARTICLE

# Increased residual cardiovascular risk in patients with diabetes and high versus normal triglycerides despite statin-controlled LDL cholesterol

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**Aim:** To determine whether high triglycerides (TG) in the presence of statin-controlled LDL-C influence the risk of cardiovascular disease (CVD) among patients with diabetes in real-world clinical practice.

**Materials and methods:** We identified adults with diabetes from the Southern California and Pacific Northwest regions of Kaiser Permanente. We included patients undergoing statin therapy with LDL-C from 40-100 mg/dL who were not undergoing other lipid-lowering therapies and had a prior diagnosis of atherosclerotic CVD or at least one other CVD risk factor. We grouped patients into high TG (200-499 mg/dL; n = 5542) or normal TG (<150 mg/dL, n = 22 411) from January 2010 through December 2016 to compare incidence rates and rate ratios of first non-fatal myocardial infarction (MI), non-fatal stroke, unstable angina and coronary revascularization. We adjusted multivariable analyses for age, sex, race/ethnicity, smoking status, blood pressure, HbA1c, serum creatinine, presence of ischaemic heart disease and study site.

**Results:** Adjusted rate ratios for the four outcomes were all statistically significantly different. The incidence rate for non-fatal MI was 30% higher in the high TG group (rate ratio, 1.30; 95% CI, 1.08-1.58; $P = 0.006$). The rate was 23% higher for non-fatal stroke (1.23, 1.01-1.49, $P = 0.037$), 21% higher for coronary revascularization (rate ratio, 1.21; 95% CI, 1.02-1.43; $P = 0.027$) and was, non-significantly, 33% higher for unstable angina (rate ratio, 1.33; 95% CI, 0.87-2.03; $P = 0.185$).

**Conclusions:** Despite statin-controlled LDL-C levels, CV events were greater among patients with diabetes and high TG levels. Because we controlled for cardiometabolic risk factors, it is likely that the difference in TG levels contributed to the excess risk observed in patients with high TGs.

KEYWORDS

cardiovascular risk, epidemiology, hypertriglyceridaemia

## 1 | INTRODUCTION

Low-density lipoprotein cholesterol (LDL-C) is the primary lipid target for prevention of cardiovascular disease (CVD) in patients with diabetes.[1] Nevertheless, elevated triglyceride (TG) levels are common in diabetes, occurring in approximately 50% of all patients,[2] and in up to 60% of those with LDL-C levels below 100 mg/dL.[3] Current guidelines from the American Diabetes Association (ADA) recommend medical therapy when TG levels are ≥500 mg/dL for prevention of pancreatitis, but *post-hoc* analyses of clinical trials concerning LDL-C lowering suggest that TG levels are associated with CVD and mortality, independent of other lipid fractions.[4–8] Genetic studies also

suggest a causal role of TG in the development and progression of atherosclerotic CVD.[9] Thus, further reduction in CVD risk with agents that lower TG levels may be achievable.[10] Indeed, there is evidence that even moderate TG elevation (200-499 mg/dL) is associated with CVD risk among patients who have achieved LDL-C control.[11–13] However, real-world evidence of the relationship between elevated TG levels and CVD among statin-treated patients with diabetes who have succeeded in attaining LDL-C control is lacking. Therefore, we conducted an observational longitudinal cohort study based on electronic health records (EHR) of patients with diabetes in an integrated delivery system who were at high risk of CVD events and had statin-controlled LDL-C. Our objective was to determine whether high TG in the presence of statin-controlled LDL-C influences CVD risk in real-world clinical practice.

## 2 | MATERIALS AND METHODS

Kaiser Permanente (KP) is an integrated delivery system that provides medical care to individuals in eight semi-autonomous regions, including the Pacific Northwest (KPNW) and Southern California (KPSC) regions that were used for this study. Both organizations use an EPIC-based electronic health record (EHR) of both inpatient and outpatient contacts. These data are combined with enrollment, laboratory and pharmacy information systems to develop a comprehensive dataset of all types of health care utilization that is standardized into a common data model.[14] The KPNW Institutional Review Board (IRB) approved the study with a waiver of informed consent, and the KPSC's IRB ceded review to KPNW.

To provide real-world context for the potential benefit of treating high TG levels in high-risk patients whose LDL is well controlled with statin therapy, the sample for the current study was selected to mimic the inclusion and exclusion criteria of patients participating in the Reduction of Cardiovascular Events with EPA - Intervention Trial (REDUCE-IT). We identified all KPNW and KPSC patients 45 years of age and older with atherosclerotic CVD (ASCVD) or diabetes, with at least one other risk factor, with a TG level less than 500 mg/dL in 2010 while undergoing statin therapy but no other anti-hyperlipidaemic therapy, and with LDL-C values between 40 and 100 mg/dL (n = 170 590). We then selected all patients who had a diagnosis of diabetes (ICD-9-CM 250.x) in 2010 and a diagnosis of ASCVD (myocardial infarction (MI) [410.x or 412], ischemic stroke [434.x], acute coronary syndrome [411.1], or peripheral arterial disease (PAD) [443.8x, 443.9x]), or diagnosis of diabetes and age ≥ 50 and receiving an anti-hyperglycemic agent with at least one of the following additional risk factors: cigarette smoking, hypertension diagnosis (401.x-405.x) or blood pressure ≥ 140/90 mm Hg, high density lipoprotein cholesterol (HDL-C) > 40 mg/dL in men or > 50 mg/dL in women, high-sensitivity C reactive protein (hs-CRP) > 3.0, estimated glomerular filtration rate (eGFR) from 30-59 mL/min/1.73 $m^2$, urine albumin creatine ratio (UACR) ≥ 30 mg/g, or ≥ 55 years of age in men and ≥ 65 years of age in women, resulting in a sample size, before exclusions, of 65 496. We divided patients into a high TG group (200-499 mg/dL; n = 11 797) and a normal TG group (<150 mg/dL; n = 42 320). Because a point estimate of TG has a wide range of inherent variability, we excluded 11 339 patients with levels of 150-199 mg/dL from further analysis to ensure that we created two analysis groups with distinctly different TG levels. Other exclusion criteria, again following REDUCE-IT guidelines, were: AIDS/HIV (ICD-9-CM 042.x, 043.x, 044.x), malignant cancer (140.xx-239.xx), end-stage renal disease (585.6), planned surgery (defined for this study as any surgery within 6 months of the date of TG testing), and liver disease (diagnoses of cirrhosis or hepatitis, alanine aminotransferase [ALT] or aspartate aminotransferase [AST] >3x upper limit of normal [ULN], bilirubin >2x ULN), kidney dysfunction (albumin level < 3.4 g/dL, blood urea nitrogen level > 20 mg/dL, or a serum creatinine >1.3 mg/dL in men or 1.1 mg/dL in women), or thyroid function abnormalities (thyroid stimulating hormone values <0.4 mU/L or > 4.2 mU/L with or without treatment). REDUCE-IT guidelines excluded New York Heart Association (NYHA) Class IV heart failure only, but our data did not include a heart failure class; thus, we excluded all individuals with a diagnosis of heart failure (ICD-9-CM 428.x). These criteria resulted in the exclusion of 6255 patients from the high TG group and 19 909 from the normal TG group, giving final sample sizes of 5542 and 22 411 patients, respectively, in the high and normal TG groups. A consort diagram of sample selection is displayed in Supporting Information Figure S1.

### 2.1 | Index date and follow-up period

If multiple TG results were available in 2010, all must have been in the same TG group range, that is, <150 mg/dL for the normal TG group and 200-499 mg/dL for the high TG group. We used the first available TG level in 2010 as the index value and defined the baseline period, for baseline data collection, as 6 months before and 6 months after the index TG level. We defined the index date for beginning follow-up as the date of the index TG level plus 182 days, to avoid immortal time bias that would result from including the 6-month post index TG level as follow-up time. The follow-up period for each patient extended from the index date through December 2016 (maximum follow-up period of 6.5 years) with censoring on 31 December 2016 or when a patient died or left the health plan.

### 2.2 | Study outcomes and covariates

Our CVD outcomes of interest were non-fatal MI, non-fatal stroke, coronary revascularization or unstable angina based on events recorded in the inpatient or emergency room setting. We compared baseline demographics (age, sex, race/ethnicity), clinical characteristics (haemoglobin A1c [A1C], smoking status, body mass index [BMI], systolic and diastolic blood pressure, lipid fractions) and comorbidities (including MI, stroke, unstable angina and chronic kidney disease [CKD]) between the high and normal TG groups, using t-tests for continuous variables and $\chi^2$ tests for dichotomous and categorical variables. We also compared the crude prevalence (number and proportion of each group with each outcome) that occurred any time during follow-up using $\chi^2$ tests.

### 2.3 | Statistical analyses

We compared multivariable adjusted incidence rates and rate ratios of the outcomes between TG groups using generalized linear models with Poisson errors (log-link), with follow-up time as an offset variable to account for differential follow-up. Based on univariate analyses of the association between covariates and outcomes, we adjusted the multivariable analyses for age, sex, race/ethnicity, low HDL-C (<40 mg/dL in men, <50 mg/dL in women), smoking status, A1C, blood pressure, serum creatinine, presence of ischaemic heart disease and study site. We assessed the robustness of the reported rate ratios using the recently proposed E-value, a measure of the minimum association that an unmeasured confounder must have with respect to both treatment and outcome to explain the significant associations.[15] A large E-value implies that a large amount of residual confounding would be needed to explain the effect estimate, and it is a sensitivity technique for observational data that has been used in recent studies.[16,17] All analyses were conducted using SAS version 9.4 (Cary, North Carolina).

## 3 | RESULTS

Compared with patients in the normal TG group, patients in the high TG group were 1.5 years younger (63.8 vs 65.3 years; $P < 0.001$), were more likely to be men (47.0% vs 44.5%; $P < 0.001$) and of a white race (52.1% vs 38.3%; $P < 0.001$) and were more likely to smoke (7.4% vs 5.3%; $P < 0.001$) (Table 1). Almost one third of the patients in both groups were of Hispanic ethnicity. Cardiometabolic risk factors including BMI, A1C and blood pressure were lower in the normal TG group, but the differences were of limited clinical significance. HDL-C was significantly lower in the high TG group (41 vs 50 mg/dL; $P < 0.001$). Patients in the high TG group were more likely to have a history of MI (7.2% vs 6.4%; $P = 0.037$) and to have CKD (23.0% vs 18.1%; $P < 0.001$).

Although statistically significantly different (5.28 years in the high TG group vs 5.37 years in the low TG group; $P < 0.001$), follow-up time was nearly identical in the two groups (Table 2). The crude prevalence of non-fatal MI was greater in the high TG group compared with the normal TG group (3.3% vs 2.5%; $P < 0.001$), as was the prevalence of coronary revascularization (4.1% vs 3.1%; $P < 0.001$). The crude prevalence of non-fatal stroke (2.0% vs 2.7%; $P = 0.468$) and unstable angina (0.7% vs 0.5%; $P = 0.124$) was not statistically significantly different.

As shown in Figure 1, the adjusted incidence rates per 1000 person-years of all four outcomes were greater in the high vs normal TG groups, but the 95% confidence intervals for non-fatal stroke and unstable angina slightly overlapped. The lower 95% confidence limit for non-fatal MI in the high TG group was identical to the upper limit in the low TG group. However, the rate ratios for three of the four outcomes were statistically significantly different. The incidence rate was 30% higher in the high TG group for non-fatal MI (rate ratio, 1.30; 95% CI, 1.08-1.58; $P = 0.006$), was 23% higher for non-fatal stroke (rate ratio, 1.23; 95% CI, 1.01-1.49; $P = 0.037$) and was 21% higher for coronary revascularization (rate ratio, 1.21; 95% CI, 1.02-1.43; $P = 0.027$). Although numerically greater, the rate ratio for unstable angina was not statistically significant (rate ratio, 1.33; 95% CI, 0.87-2.03; $P = 0.185$). E-values for the risk ratios were 1.92, 1.76 and 1.71 for non-fatal MI, non-fatal stroke and revascularization, respectively. Thus, the observed risk ratios could be explained by an unmeasured confounder that was associated with both TG group and outcome by the amount of the E-value above and beyond the measured confounders, but weaker confounding could not do so.

**TABLE 1** Baseline characteristics of patients with high vs normal triglycerides

| | TG 200–499 mg/dL | TG < 150 mg/dL | *P* value |
|---|---|---|---|
| n | 5542 | 22 411 | – |
| Age, y | 63.8 (8.7) | 65.3 (9.0) | <0.001 |
| Men | 47.0% | 44.5% | <0.001 |
| White | 52.1% | 38.3% | <0.001 |
| Black | 3.0% | 16.3% | |
| Hispanic | 30.0% | 29.2% | |
| Asian | 12.6% | 14.4% | |
| Other non-white | 2.3% | 1.8% | |
| Current smoker | 7.4% | 5.3% | <0.001 |
| BMI, kg/m$^2$ | 32.7 (6.6) | 30.8 (6.7) | <0.001 |
| A1C | 7.4% (0.9) | 7.2% (0.9) | <0.001 |
| Systolic blood pressure, mm Hg | 130 (12) | 129 (12) | <0.001 |
| Diastolic blood pressure, mm Hg | 72 (8) | 70 (8) | <0.001 |
| Triglycerides, mg/dL | 251 (61) | 100 (29) | <0.001 |
| LDL-C, mg/dL | 74 (16) | 74 (15) | 0.024 |
| HDL-C, mg/dL | 41 (9) | 50 (12) | <0.001 |
| Myocardial infarction | 7.2% | 6.4% | 0.037 |
| Stroke | 0.6% | 0.9% | 0.025 |
| Unstable angina | 0.4% | 0.5% | 0.112 |
| Other ischaemic heart disease | 12.9% | 12.1% | 0.106 |
| CKD (eGFR < 60 mL/min/1.73 m$^2$) | 23.0% | 18.1% | <0.001 |

**TABLE 2** Crude prevalence of study outcomes during follow-up

| | | TG 200–499 mg/dL | TG < 150 mg/dL | *P* value |
|---|---|---|---|---|
| Years of follow-up | Mean | 5.28 | 5.37 | <0.001 |
| | Std dev | 1.73 | 1.66 | |
| Non-fatal MI | n | 181 | 554 | <0.001 |
| | % | 3.3% | 2.5% | |
| Non-fatal stroke | n | 162 | 615 | 0.468 |
| | % | 2.9% | 2.7% | |
| Unstable angina | n | 37 | 112 | 0.124 |
| | % | 0.7% | 0.5% | |
| Coronary revascularization | n | 225 | 691 | <0.001 |
| | % | 4.1% | 3.1% | |

## 4 | DISCUSSION

In this observational longitudinal cohort study of 27 953 patients with diabetes who were at high risk of CVD but with statin-controlled

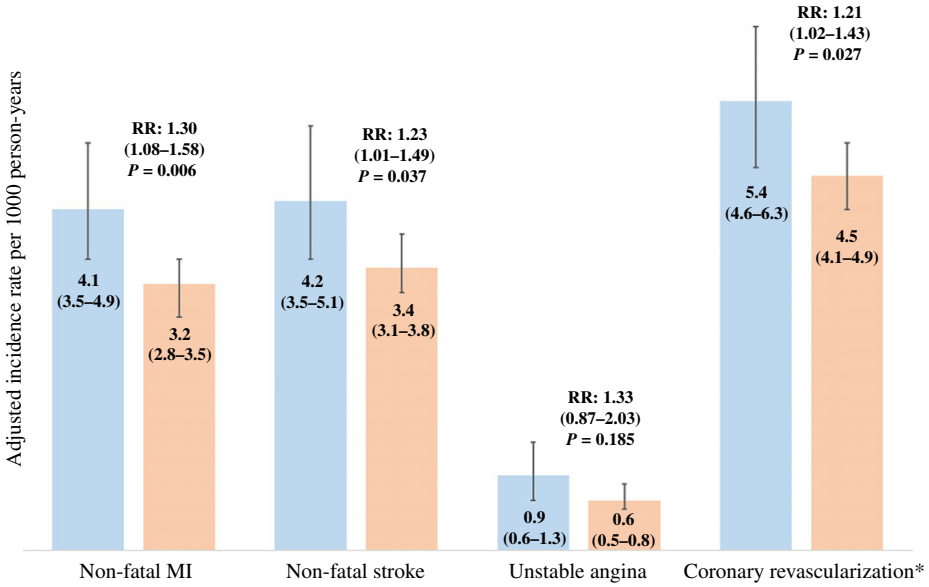


**FIGURE 1** Adjusted incidence rates per 1000 person-years (95% confidence limits) and incidence rate ratios comparing patients with high vs normal triglycerides. Models are adjusted for age, sex, smoking status, HbA1c, blood pressure, serum creatinine and history of ischaemic heart disease, RR: rare ratio. (■) TG 200-499 mg/dL, and (■) TG < 150 mg/dL

LDL-C, we found that TG levels in the 200-499 mg/dL range were statistically significantly associated with CVD events over a mean follow-up period of more than 5 years when compared with otherwise similar patients with TG levels <150 mg/dL. Both TG groups had LDL-C levels ranging from 40 to100 mg/dL while undergoing statin therapy, and we statistically controlled for minor differences in other cardiometabolic risk factors. Thus, our results suggest that the differences in residual CVD risk may be explained, at least in part, by the difference in TG levels even after adjustment for differences in demographic and clinical characteristics including HDL-C. Recent publications, including observational real-world data, have also reported increased residual CVD risk in patients with elevated and high TG levels, even after adjustment for HDL-C levels.[13,18]

Although statins are recommended for primary CVD prevention in diabetes patients 40 years of age and older, and for secondary CVD prevention among all adults,[1] they are prescribed in cardiology practices for only approximately 62% of patients aged 40-75 years,[19] among whom just over 50% maintain statin usage over an extended period of time.[20] As a result, only 40%-60% of diabetes patients attain LDL-C levels <100 mg/dL.[2,20–22] To account for CVD risk that could be attributed to the absence of statin use or LDL-C control, we focused our analyses on patients with a current statin prescription and LDL-C levels from 40 to100 mg/dL. Among these patients, 35.4% had TG levels greater than 150 mg/dL, similar to the nearly 40% with levels ≥150 mg/dL despite statin use according to an analysis of National Health and Nutrition Examination Survey (NHANES) data and the 40% among diabetes patients with LDL-C < 100 mg/dL with unspecified statin use, but somewhat lower than the 56% reported in another NHANES analysis.[2,3,23] Despite the large proportion of diabetes patients with high TG levels, studies concerning the association between TGs and CVD in diabetes are scant. One Italian study of patients with diabetes undergoing lipid-lowering therapy revealed that mean TG levels were significantly associated with all-cause mortality, independent of other cardiometabolic risk factors.[24] Another study involving 28 218 adults with diabetes with LDL-C < 100 mg/dL, conducted in a setting similar to that of ours, revealed a strong independent association between high TG and coronary heart disease (CHD) events in men that was significant among women only if HDL-C was low.[3] Similarly, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial did not find a significant CVD benefit of fibrate therapy overall, but did report a possible benefit among the subgroup of diabetes patients with both high TG levels and low HDL-C.[25] The Strong Heart Study recently reported an association between high TG levels and CHD events that was particularly significant among patients with diabetes.[26] Two other observational studies involving high-risk statin-treated patients, with and without diabetes, revealed an elevated risk of CVD events, especially non-fatal MI, among patients with high TGs after controlling for HDL-C and other risk factors.[12,13] Unlike any of the above studies, ours focused on statin-treated patients with diabetes who had attained good LDL-C control. Thus, our finding of an association between high TG levels and a substantially increased risk of CVD fills an important knowledge gap.

Three meta-analyses have demonstrated that TG levels are independently associated with CVD in general populations.[27–29] However, the ADA does not currently recommend treating hypertriglyceridaemia for CVD risk reduction because clinical trials of agents that lower TG, specifically fenofibrates and niacin, have failed to demonstrate a reduction in CVD outcomes.[25,30,31] The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study did not find a significant reduction in the primary outcome of non-fatal MI or death from CHD, but did show a reduction in total CVD events, mainly because of fewer non-fatal MIs and revascularizations.[32] Stronger evidence is

needed. In addition to the REDUCE-IT study, the Outcomes Study to Assess Statin Residual Risk Reduction With EpaNova in High CV Risk Patients With Hypertriglyceridemia (STRENGTH) and the Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides In Patients With Diabetes (PROMINENT) studies are ongoing large CV outcomes trials involving high-risk CVD patients, including a large percentage of patients with diabetes, undergoing statin therapy, with the results from the REDUCE-IT study (EPA vs placebo) to be presented first in 2018.[33–36]

Our study has important limitations. We used observational laboratory data, from which we could not accurately determine fasting status, and which probably include a combnation of fasting and non-fasting TG results. Fasting values may be better for diagnosing hypertriglyceridaemia,[37] but non-fasting values better predict CVD risk.[38–40] Furthermore, because non-fasting TG levels are higher than fasting TG levels,[37,41] any resulting misclassification would bias our results toward the null, suggesting that our estimates of excess CVD risk in the high TG group may be conservative. We assessed TG levels and other cardiometabolic risk factors at baseline and did not assess the impact of changes during follow-up. Observational data cannot establish causality, but real-world data provide important information concerning risk and associations as seen in clinical practice. Finally, although the E-value is a relatively new tool for sensitivity analysis and may be unfamiliar to many readers, it is rapidly becoming a widely accepted method for assessing unmeasured confounding.

In conclusion, we found that, despite statin-controlled LDL-C levels and adjustment for HDL-C, CV event rates were greater among diabetes patients with high compared to normal TG levels. Because we controlled for group differences, including other cardiometabolic risk factors, it is probable that the difference in TG level contributed to the excess risk observed in patients with high TG levels.

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## CONFLICTS OF INTEREST

G. A. N. has received funding unrelated to this study from Boehringer-Ingelheim, Janssen Pharmaceuticals, Merck & Co. and Sanofi. S. P. and C. B. G. are employees of Amarin Pharma. K. R. has received funding unrelated to this study from Merck & Co., Amgen and Regeneron. S. F. has consulted for Amarin, Amgen, Kowa, Aegerion and Akcea.

## Author contributions

G. A. N. contributed to the study conception and design and the interpretation of results, and developed the first draft of the manuscript. S. P., K. R., C. B. G. and S. F. contributed to the study conception and design and the interpretation of results, and reviewed/edited the manuscript. The final draft for submission was approved by all authors. G. A. N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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# Remnant-Like Particle Cholesterol, Low-Density Lipoprotein Triglycerides, and Incident Cardiovascular Disease

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## ABSTRACT

**BACKGROUND** Hypertriglyceridemia is associated with increased remnant-like particle cholesterol (RLP-C) and triglycerides in low-density lipoprotein (LDL-TG). Recent studies have focused on atherogenicity of RLP-C, with few data on LDL-TG.

**OBJECTIVES** The aim of this study was to examine associations of RLP-C and LDL-TG with incident cardiovascular disease (CVD) events and genetic variants in the ARIC (Atherosclerosis Risk In Communities) study.

**METHODS** Fasting plasma RLP-C and LDL-TG levels were measured in 9,334 men and women without prevalent CVD. Participants were followed for incident CVD events (coronary heart disease and ischemic stroke) for up to 16 years. Associations between LDL-TG and RLP-C levels and genetic variants were assessed by whole-exome sequencing using single-variant analysis for common variants and gene-based burden tests for rare variants; both an unbiased and a candidate gene approach were explored.

**RESULTS** RLP-C and LDL-TG levels were correlated with triglyceride levels ($r = 0.85$ and $r = 0.64$, $p < 0.0001$). In minimally adjusted analyses, RLP-C and LDL-TG were associated with CVD risk, but in models adjusted for traditional risk factors including lipids, only LDL-TG was associated with incident CHD (hazard ratio: 1.28; 95% confidence interval: 1.10 to 1.50) and stroke (hazard ratio: 1.47; 95% confidence interval: 1.13 to 1.92). A common *APOE* variant, rs7412, had the strongest association with LDL-TG and RLP-C ($p < 5 \times 10^{-8}$).

**CONCLUSIONS** RLP-C and LDL-TG levels were predictive of CVD and associated with *APOE* variants. LDL-TG may represent a marker of dysfunctional remnant lipoprotein metabolism associated with increased CVD risk. Further research is needed to determine whether LDL-TG plays a causal role in CVD and may be a target for therapy.
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Although the association between elevated plasma triglycerides (TGs) and cardiovascular disease (CVD) has been known for decades (1,2), genetic studies provide new evidence that genes associated with TG-rich lipoprotein (TGRL) metabolism are related to development of atherosclerotic CVD (3,4).

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Genetic variants associated with TG metabolism indicate the importance of lipases (e.g., lipoprotein lipase [LPL] and hepatic lipase), their activators (e.g., apolipoprotein [apo] CII and apoAV) and inhibitors (e.g., apoCIII and angiopoietin-like protein [ANGPTL] 4), and ligands for cellular receptors involved in clearance of TGRLs (apoB and apoE) in CVD (5). However, these variants affect multiple lipoproteins, complicating investigations into direct pathophysiology. Increased production and delayed catabolism of TGRLs lead to increased TG-enriched remnant lipoproteins, with increased levels of remnant-like particle cholesterol (RLP-C). In hypertriglyceridemia, cholesteryl ester transfer protein-mediated transfer of TGs from chylomicrons and very low-density lipoprotein (VLDL) to low-density lipoprotein (LDL) and high-density lipoprotein in exchange for cholesteryl esters from LDL and high-density lipoprotein leads to TG-enriched VLDL remnants, intermediate-density lipoprotein, and LDL and to small dense LDL. Numerous studies have focused on the atherogenic potential of remnant lipoproteins and RLP-C (6–8). However, few data describe the association between TGs in LDL (LDL-TG) and future CVD risk.

We examined these 2 lipoprotein measures linked to hypertriglyceridemia—LDL-TG and RLP-C—and their association with CVD in the ARIC (Atherosclerosis Risk in Communities) study. We hypothesized that elevated LDL-TG and RLP-C levels were associated with increased CVD risk. We also used genetic array analysis to investigate associations of genetic variants with LDL-TG and RLP-C levels.

## METHODS

See the Online Appendix for details.

**STUDY POPULATION.** ARIC is a prospective study of CVD in 15,792 middle-aged adults recruited from 4 U.S. communities from 1987 to 1989 (9). **Figure 1** describes the selection and demographics of the 9,334 subjects included in this analysis.

Incident CVD events were a composite of incident coronary heart disease (CHD) and incident ischemic stroke after visit 4 and through December 31, 2013. Methods of assessing incident CHD events and ischemic strokes in ARIC have been described (10,11). Median follow-up for CVD, CHD, and ischemic stroke events was 15.6 years (25th percentile, 75th percentile: 10.8, 16.6 years), 15.6 years (25th percentile, 75th percentile: 11.5, 16.6 years), and 15.8 years (25th percentile, 75th percentile: 13.8, 16.7 years), respectively.

**LIPOPROTEIN AND LIPID ASSAYS.** Lipids were measured in 12-h fasting plasma stored at −70°C with ethylenediaminetetraacetic acid. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and TGs were measured using enzymatic measures (12). RLP-C (13) and LDL-TG (14) were determined by fully automated detergent-based homogeneous methods (Denka Seiken, Tokyo, Japan).

**STATISTICAL ANALYSIS.** LDL-TG and RLP-C were modeled as continuous and categorical variables. Associations between exposure variables and outcomes were determined using Cox proportional hazards modeling. Linear terms representing quartile number were used to obtain p values for trend. Model 1 was adjusted for age, sex, and race. Model 2 included model 1 plus risk factors in the Pooled Cohort Equation (PCE). Kaplan-Meier survival curves were calculated for each outcome across RLP-C and LDL-TG quartiles.

**GENETIC METHODS AND ANALYSIS.** In a targeted gene approach, we investigated candidate genes and well-established variants within those genes (*LPL*, *LIPC*, *LIPG*, *APOC3*, *APOA5*, *ANGPTL3*, and *ANGPTL4*) and *APOE* haplotypes with respect to LDL-TG and RLP-C.

In an unbiased approach, genotypes were obtained from the Illumina HumanExome BeadChip. Genes with cumulative minor allele count ≥3 in both European Americans and African Americans (13,690 genes) were included.

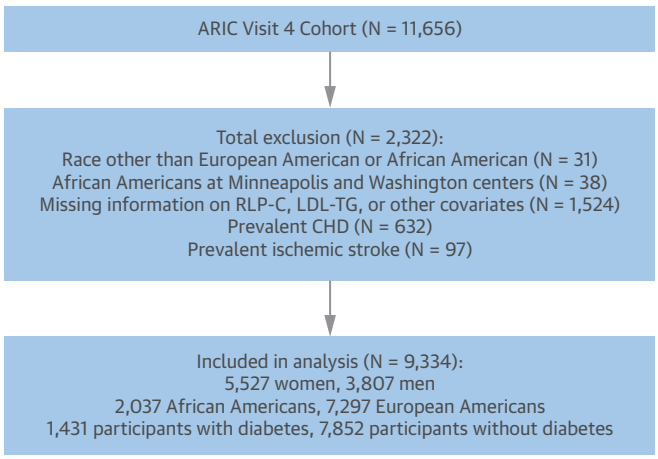
Whole-exome sequencing for 5,847 European Americans and 1,915 African Americans was completed at Baylor College of Medicine Human Genome Sequencing Center. Exomes were captured using HGSC VCRome 2.1 reagent (15); samples were paired-end sequenced using Illumina GAII or HiSeq instruments. Variant calling was done using Atlas2 (16). Whole-exome variants were annotated using ANNOVAR (17) and dbNSFP version 2.0 (18).

Both exome-chip and whole-exome sequencing were available in 5,767 European Americans and 1,857 African Americans.

### ABBREVIATIONS AND ACRONYMS

**ANGPTL** = angiopoietin-like protein
**apo** = apolipoprotein
**CHD** = coronary heart disease
**CI** = confidence interval
**CVD** = cardiovascular disease
**HDL-C** = high-density lipoprotein cholesterol
**HR** = hazard ratio
**hs-CRP** = high-sensitivity C-reactive protein
**LDL** = low-density lipoprotein
**LDL-TG** = low-density lipoprotein triglycerides
**LPL** = lipoprotein lipase
**MAF** = minor allele frequency
**PCE** = Pooled Cohort Equation
**RLP-C** = remnant-like particle cholesterol
**TG** = triglycerides
**TGRL** = triglyceride-rich lipoprotein
**VLDL** = very low-density lipoprotein

**FIGURE 1 Study Population**




The ARIC (Atherosclerosis Risk In Communities) study is a prospective study of cardiovascular disease (CVD) in 15,792 middle-aged adults recruited from 4 U.S. communities from 1987 to 1989. The present study was conducted among participants in ARIC visit 4 (1996 to 1998). Of 11,656 eligible subjects, we excluded those with self-reported race neither white nor black (n = 31) and African American participants at the Minnesota and Washington County field centers (n = 38) because of small enrollment numbers; subjects missing data for low-density lipoprotein triglyceride (LDL-TG), remnant-like particle cholesterol (RLP-C), or other covariates (n = 1,524); and those with prevalent coronary heart disease (CHD) (n = 632) or ischemic stroke (n = 97) at visit 4. Therefore, 9,334 subjects were included in this analysis.

## RESULTS

In the 9,334 participants, RLP-C levels were higher in European Americans than African Americans (median 6.7 mg/dl [25th percentile, 75th percentile: 3.4, 13.5 mg/dl] vs. 3.9 mg/dl [25th percentile, 75th percentile: 2.2, 7.2 mg/dl], $p = 0.0001$, Wilcoxon rank sum test). Subjects with RLP-C and LDL-TG levels in the highest quartile (**Tables 1 and 2**) had proatherogenic lipid profiles, were more likely to have diabetes and hypertension, and had higher body mass index, fasting blood glucose, and plasma levels of the inflammatory markers high-sensitivity C-reactive protein (hs-CRP) and white blood cell count. Statin use was higher in subjects with RLP-C or LDL-TG levels in the third and fourth quartiles.

**ASSOCIATION OF RLP-C AND LDL-TG WITH OTHER LIPIDS.** As expected, RLP-C and LDL-TG showed strong positive correlations with TGs ($r = 0.85$ and $r = 0.65$, respectively, $p < 0.0001$) (**Table 3**). RLP-C and LDL-TG were also positively associated with the cholesterol in small dense LDL and with non-HDL-C and were negatively correlated with HDL-C. RLP-C and LDL-TG were also correlated with each other ($r = 0.5108$, $p < 0.0001$).

**ASSOCIATION OF RLP-C AND LDL-TG WITH INCIDENT CVD.** In quartile analyses (**Figure 2**), RLP-C showed a graded association with incident CVD but no association with incident ischemic stroke. LDL-TG also showed a graded association with incident CVD, but its association with incident ischemic stroke was driven largely by LDL-TG levels in the highest quartile.

In the categorical analysis of RLP-C, risk for CHD, ischemic stroke, and CVD was significantly higher across increasing quartiles of RLP-C in model 1, but not after adjustment for PCE risk factors in model 2 (**Table 4**). Similarly, RLP-C analyzed as a continuous variable was significantly associated with incident CHD (hazard ratio [HR]: 1.26; 95% confidence interval [CI]: 1.19 to 1.34; $p < 0.001$) and ischemic stroke (HR: 1.18; 95% CI: 1.07 to 1.30; $p < 0.001$) in model 1, but not with any outcome after adjustment for PCE risk factors (**Table 5**). Additional adjustment for log TGs (model 3) resulted in an inverse association of RLP-C with CVD risk (**Table 5**). However, given the extremely high correlation between TG and RLP-C levels (Spearman $r = 0.8535$), our risk prediction modeling was most likely affected by multicollinearity.

For LDL-TG, risk for CHD, ischemic stroke, and CVD was significantly higher across increasing quartiles of LDL-TG in the categorical analysis, and the associations with ischemic stroke and CVD risk persisted after adjustment for PCE risk factors (**Table 6**). In the continuous analysis, even after adjustment for PCE risk factors, LDL-TG was significantly associated with all outcomes: CHD (HR: 1.28; 95% CI: 1.10 to 1.50; $p < 0.002$), ischemic stroke (HR: 1.47; 95% CI: 1.13 to 1.92; $p < 0.005$), and CVD (HR: 1.35; 95% CI: 1.17 to 1.55; $p < 0.001$) (**Table 5**). Further adjustment for log TGs (model 3) did not have a significant impact on the association of LDL-TG with CVD outcomes (**Table 5**).

To assess the extent to which LDL-TG provides incremental value in the prediction of future CVD risk beyond circulating TG and apoB levels, we determined the area under the curve, net reclassification index, and integrated discrimination improvement (Online Table 1). Although improvements in the C-statistics were generally modest for each lipid trait added separately, LDL-TG did show greater improvement in the area under the curve (with significant effects on continuous net reclassification index and integrated discrimination improvement) compared with apoB and TGs. Furthermore, addition of LDL-TG to a PCE model including both apoB and TGs resulted in further improvement in the area under the curve for CVD risk prediction. The overall modest improvement in the C-statistic of each of these lipid measures is not surprising given the

traditional CVD lipid risk factors already included in the PCE model and the well-described phenomenon of pleiotropy affecting various lipid traits.

**EXOME ANALYSIS: UNBIASED APPROACH.** Using an unbiased approach, we assessed the association of nonsynonymous common variants by race (minor allele frequency [MAF] >1%) and performed a meta-analysis. In the meta-analysis, 11 detected single variant-trait associations with RLP-C and LDL-TG reached pre-defined significance ($p < 2.5 \times 10^{-8}$) (Online Table 2), all in genes previously associated with other lipid traits, including small dense LDL (19). Genetic variants associated with both RLP-C and LDL-TG tended to have the same direction of effect on both traits, except rs7412 in *APOE*.

We also assessed the association of nonsynonymous rare variants by race (MAF <1%) and performed a meta-analysis. A total of 13,690 genes contained ≥1 annotated nonsynonymous variant (MAF ≤1%) and cumulative minor allele count ≥3 in each race. Two aggregate gene-based tests, *APOC3* for RLP-C and *TARM1* for LDL-TG, reached pre-defined significance in the meta-analysis ($p \leq 2.5 \times 10^{-6}$) (Online Table 3). The association with *APOC3* was in a consistent direction in both races, with 3 nonsynonymous variants in *APOC3* leading the association in the meta-analysis ($p < 0.05$) (Online Table 4). The single nonsynonymous variant (rs2361558) that was monomorphic in African Americans led to the association between the aggregated rare variants in *TARM1* and LDL-TG levels. The association of LDL-TG with genetic variants in *TARM1* (20) may be important because of the potential link between remnant lipoproteins and the inflammatory response in the etiology of atherosclerotic CVD.

**EXOME ANALYSIS: CANDIDATE GENE APPROACH.** Associations between RLP-C and LDL-TG levels and coding nonsynonymous and splicing common variants belonging to 7 candidate genes (*LPL*, *LIPC*, *LIPG*, *APOC3*, *APOA5*, *ANGPTL3*, and *ANGPTL4*) were evaluated using single-variant analysis of whole-exome sequencing data (**Tables 7 and 8**). These candidate genes were selected because lipases and their activators and inhibitors play a key role in remnant lipoprotein metabolism. Not surprisingly, multiethnic meta-analysis showed significant associations between 2 common variants—rs3135506 (*APOA5*) and rs328 (*LPL*)—and both RLP-C and LDL-TG levels, in a consistent direction in both races ($p < 0.05$ in both races), as well as between RLP-C and LDL-TG. Multiethnic meta-analysis showed relatively weak associations between a common *LIPC* variant (rs6078) and both RLP-C and LDL-TG levels, in a consistent direction in both races but different directions for RLP-C and LDL-TG. Because it was previously reported that rs2070895 in the promotor region of the hepatic lipase gene was the lead single-nucleotide polymorphism associated with decreased hepatic lipase activity, we imputed rs2070895 in ARIC participants using the 1000 Genomes Project reference panel (21). Multiethnic meta-analysis showed a strong association between rs2070895 and higher LDL-TG levels in both races but no significant association between rs2070895 and RLP-C levels (**Tables 7 and 8**).

**TABLE 1 Baseline Characteristics and Distribution of Cardiovascular Disease Risk Factors Across Remnant-Like Particle Cholesterol Quartiles**

| | RLP-C Quartile (mg/dl) | | | | |
|---|---|---|---|---|---|
| | Quartile 1 (0.4–3.1) | Quartile 2 (3.2–5.9) | Quartile 3 (6.0–12.2) | Quartile 4 (12.3–259.1) | p Value for Trend |
| Age, yrs | 62.7 ± 5.8 | 62.6 ± 5.7 | 62.8 ± 5.6 | 62.8 ± 5.6 | 0.275 |
| Female | 63.6 | 62.7 | 57.2 | 53.2 | <0.001 |
| African American | 34.3 | 25.0 | 16.6 | 11.0 | <0.001 |
| BMI, kg/m$^2$ | 27.5 ± 6.0 | 28.7 ± 5.8 | 29.3 ± 5.6 | 29.6 ± 4.9 | <0.001 |
| SBP, mm Hg | 125.9 ± 19.8 | 126.8 ± 19.1 | 127.5 ± 18.3 | 128.9 ± 18.2 | <0.001 |
| Hypertension | 42.0 | 44.2 | 46.3 | 50.5 | <0.001 |
| Hypertensive medication user | 36.4 | 40.4 | 41.6 | 45.5 | <0.001 |
| Diabetes | 10.6 | 13.4 | 15.8 | 22.1 | <0.001 |
| Current smoking | 15.6 | 14.7 | 14.2 | 12.5 | 0.002 |
| HDL-C, mg/dl | 59.7 ± 17.0 | 54.0 ± 16.7 | 48.2 ± 14.5 | 41.2 ± 12.3 | <0.001 |
| LDL-C, mg/dl | 115.5 ± 30.8 | 124.8 ± 32.7 | 126.6 ± 32.7 | 124.9 ± 35.9 | <0.001 |
| Total cholesterol, mg/dl | 190.6 ± 32.5 | 200.6 ± 35.0 | 203.8 ± 34.9 | 211.9 ± 40.8 | <0.001 |
| Triglycerides, mg/dl | 74 (61, 90) | 107 (91, 125) | 141 (119, 167) | 216 (176, 275) | <0.001 |
| Fasting glucose, mg/dl | 102.3 ± 24.9 | 104.9 ± 25.3 | 107.6 ± 30.1 | 114.6 ± 38.8 | <0.001 |
| Statin user | 6.2 | 8.9 | 9.9 | 12.8 | <0.001 |
| Cholesterol-lowering medication user | 7.9 | 10.7 | 12.7 | 17.5 | <0.001 |
| WBC | 5.7 (4.9, 6.9) | 5.9 (5.0, 7.2) | 6.3 (5.3, 7.4) | 6.4 (5.5, 7.6) | <0.001 |
| hs-CRP, mg/l | 1.94 (0.87, 5.11) | 2.32 (1.04, 5.44) | 2.63 (1.20, 5.50) | 2.78 (1.29, 5.67) | <0.001 |

Values are mean ± SD, %, or median (25th percentile, 75th percentile).

BMI = body mass index; HDL-C = high-density lipoprotein cholesterol; hs-CRP = high-sensitivity C-reactive protein; LDL-C = low-density lipoprotein cholesterol; RLP-C = remnant-like particle cholesterol; SBP = systolic blood pressure; WBC = white blood cell count.

**ASSOCIATION OF RLP-C AND LDL-TG WITH GENETIC VARIANTS OF *APOE*.** Our unbiased approach showed the most significant associations with genetic variants at the *APOE* locus, particularly rs7412. ApoE has high affinity for the LDL (apoB/E) receptor as well as other hepatic receptors and plays an important role in clearance of remnant lipoproteins from the circulation (22). The 3 common allelic variants of *APOE* (*APOE* ε2, *APOE* ε3, and *APOE* ε4) have genotype-specific effects on TG and total cholesterol levels (23).

**TABLE 2 Baseline Characteristics and Distribution of Cardiovascular Disease Risk Factors Across Low-Density Lipoprotein Triglyceride Quartiles**

| | LDL-TG Quartile (mg/dl) | | | | |
|---|---|---|---|---|---|
| | Quartile 1 (0.7-17.0) | Quartile 2 (17.1-22.6) | Quartile 3 (22.7-29.6) | Quartile 4 (29.7-104.0) | p Value for Trend |
| Age, yrs | 62.5 ± 5.7 | 62.6 ± 5.8 | 62.8 ± 5.6 | 63.0 ± 5.6 | 0.001 |
| Female | 57.1 | 56.4 | 56.9 | 66.4 | <0.001 |
| African American | 31.7 | 22.3 | 17.9 | 15.2 | <0.001 |
| BMI, kg/m$^2$ | 27.8 ± 5.8 | 28.6 ± 5.7 | 28.9 ± 5.5 | 29.6 ± 5.4 | <0.001 |
| SBP, mm Hg | 126.0 ± 19.6 | 126.4 ± 19.0 | 127.1 ± 18.2 | 129.5 ± 18.6 | <0.001 |
| Hypertension | 42.4 | 44.3 | 46.2 | 50.2 | <0.001 |
| Hypertensive medication user | 36.4 | 39.6 | 42.3 | 45.4 | <0.001 |
| Diabetes | 11.6 | 14.5 | 15.1 | 20.6 | <0.001 |
| Current smoking | 11.7 | 15.2 | 16.2 | 14.0 | 0.014 |
| HDL-C, mg/dl | 58.7 ± 18.2 | 52.2 ± 16.5 | 47.4 ± 15.1 | 45.0 ± 13.4 | <0.001 |
| LDL-C, mg/dl | 108.0 ± 29.7 | 119.0 ± 29.7 | 126.9 ± 31.2 | 138.4 ± 34.8 | <0.001 |
| Total cholesterol, mg/dl | 184.6 ± 32.7 | 194.7 ± 32.0 | 204.6 ± 33.0 | 222.9 ± 37.3 | <0.001 |
| Triglycerides, mg/dl | 79 (62, 105) | 105 (83, 137) | 134 (108, 177) | 182 (142, 240) | <0.001 |
| Fasting glucose, mg/dl | 102.5 ± 24.5 | 106.6 ± 30.6 | 108.1 ± 29.9 | 111.9 ± 35.8 | <0.001 |
| Statin user | 6.4 | 7.8 | 10.6 | 12.9 | <0.001 |
| Cholesterol-lowering medication user | 8.2 | 9.8 | 13.8 | 16.9 | <0.001 |
| WBC | 5.7 (4.8, 6.8) | 6.0 (5.1, 7.2) | 6.3 (5.3, 7.4) | 6.5 (5.4, 7.7) | <0.001 |
| hs-CRP, mg/l | 1.67 (0.79, 4.44) | 2.10 (1.04, 4.97) | 2.58 (1.20, 5.45) | 3.53 (1.56, 6.74) | <0.001 |

Values are mean ± SD, %, or median (25th percentile, 75th percentile).
LDL-TG = low-density lipoprotein-triglyceride; other abbreviations as in Table 1.

Because rs7412 defines *APOE* ε2 allele status, we assessed *APOE* haplotypes and found that *APOE* ε2/2 was associated with reduced LDL-TG and increased RLP-C ($p < 0.0001$ vs. any other haplotype) (Figure 3). Furthermore, rs7412 was significantly associated with increased TG and HDL-C levels and with decreased LDL-TG, LDL-C, total cholesterol, non-HDL-C, and lipoprotein(a) levels (Table 9).

## DISCUSSION

Although both RLP-C and LDL-TG were strongly associated with TGs, as expected, they had different associations with incident CVD events in up to 16 years of follow-up in the ARIC study. Both RLP-C and LDL-TG were associated with incident CVD events in minimally adjusted models, but only LDL-TG remained significantly associated with incident CHD and ischemic stroke in models adjusted for traditional PCE risk factors. With further adjustment for TGs and hs-CRP, LDL-TG remained significantly associated with CVD events (HR: 1.26; 95% CI: 1.08 to 1.47; $p = 0.003$). In the genetic analyses, a common *APOE* variant had the strongest association with both RLP-C and LDL-TG, but subjects with ε2/2 had decreased LDL-TG and increased RLP-C.

**RLP-C AND CVD.** Unlike in previous studies, RLP-C was quantified directly using a fully automated detergent-based homogenous assay. Numerous studies suggest that high RLP-C concentrations increase risk for atherosclerosis and CHD (24,25).

As in prior studies (24,26,27), in ARIC, RLP-C was significantly correlated with elevated TGs and diabetes at baseline. Although RLP-C was also significantly associated with incident CVD in a basic model adjusted for age, sex, and race, after adjustment for traditional CVD risk factors, including total cholesterol, HDL-C, diabetes, and antihypertensive medication use, RLP-C was not significantly associated with incident CVD events.

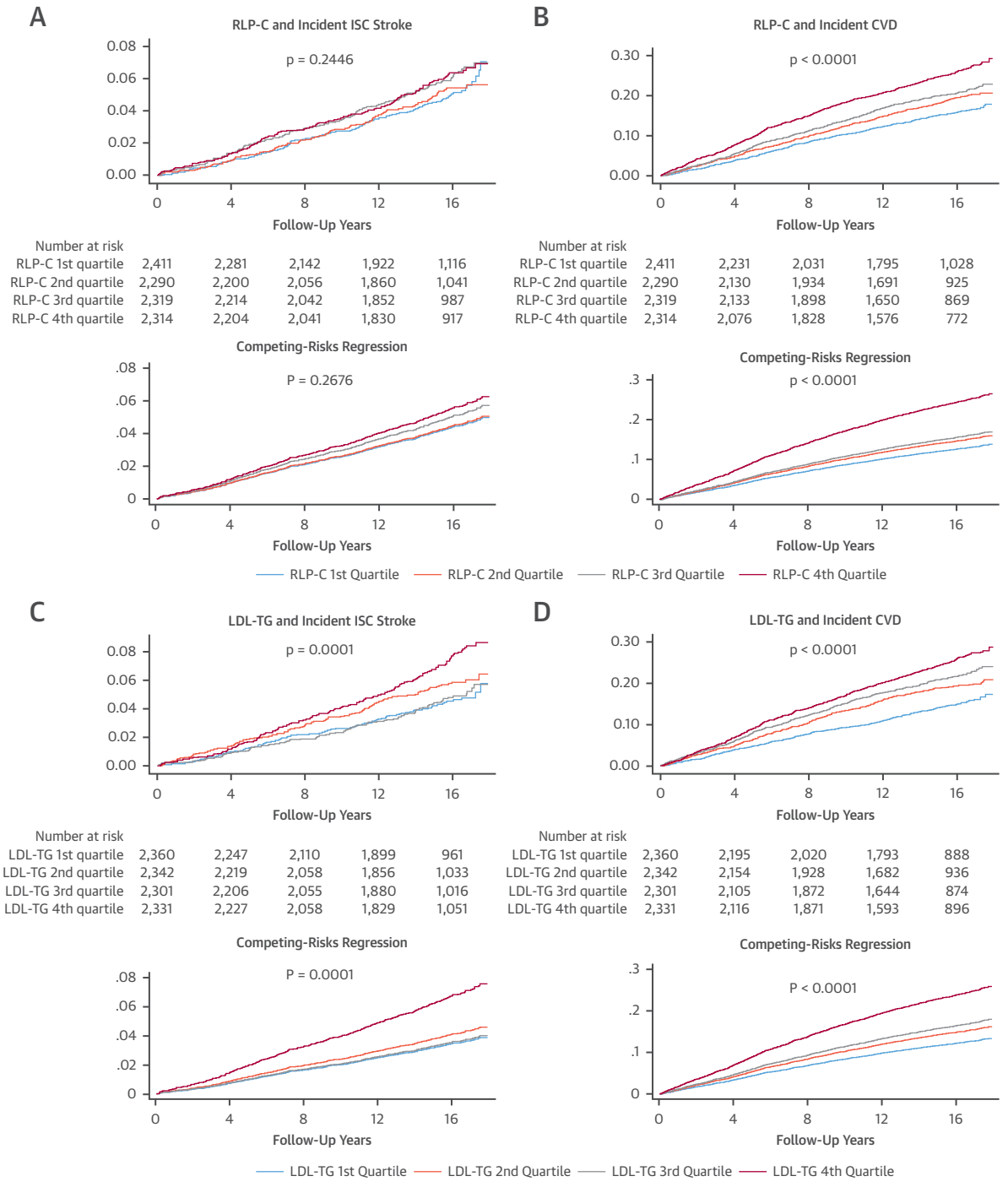
In an analysis of genetic variants affecting single lipoprotein classes, including nonfasting remnants, HDL-C, and LDL-C, the causal odds ratio for ischemic heart disease was 2.8 per 39-mg/dl increase in nonfasting remnant cholesterol levels (7). However, in

**TABLE 3 Spearman's Correlation Coefficients, Including Regression Coefficients and Intercepts, Among Cardiovascular Disease Risk Factors**

| | RLP-C (mg/dl) | | | LDL-TG (mg/dl) | | |
|---|---|---|---|---|---|---|
| | Spearman R (p Value) | Regression Coefficient (95% CI) | Intercept (95% CI) | Spearman R (p Value) | Regression Coefficient (95% CI) | Intercept (95% CI) |
| Triglycerides, mg/dl | 0.8535 (<0.0001) | 0.103 (0.101 to 0.105) | −4.860 (−5.137 to −4.583) | 0.6425 (<0.0001) | 0.065 (0.063 to 0.067) | 15.14 (14.79 to 15.48) |
| Small dense LDL-C, mg/dl | 0.5879 (<0.0001) | 0.256 (0.246 to 0.266) | −1.303 (−1.783 to −0.824) | 0.6968 (<0.0001) | 0.341 (0.333 to 0.348) | 9.632 (9.262 to 10.003) |
| Total cholesterol, mg/dl | 0.2055 (<0.0001) | 0.067 (0.061 to 0.074) | −3.798 (−5.064 to −2.532) | 0.3947 (<0.0001) | 0.120 (0.114 to 0.125) | 0.267 (−0.817 to 1.352) |
| LDL-C, mg/dl | 0.1083 (<0.0001) | 0.015 (0.010 to 0.021) | 7.228 (6.478 to 7.977) | 0.3491 (<0.0001) | 0.107 (0.101 to 0.113) | 11.04 (10.29 to 11.78) |
| HDL-C, mg/dl | −0.4429 (<0.0001) | −0.227 (−0.240 to −0.214) | 21.31 (20.61 to 22.01) | −0.3117 (<0.0001) | −0.178 (−0.191 to −0.166) | 33.44 (32.79 to 34.10) |
| Non-HDL-C, mg/dl | 0.3957 (<0.0001) | 0.108 (0.103 to 0.114) | −6.536 (−7.429 to −5.644) | 0.5316 (<0.0001) | 0.148 (0.143 to 0.153) | 2.060 (1.316 to 2.805) |
| Lp(a), mg/dl | −0.2231 (<0.0001) | −0.052 (−0.059 to −0.044) | 11.11 (10.81 to 11.42) | −0.0290 (0.0057) | −0.001 (−0.008 to 0.006) | 24.41 (24.13 to 24.69) |

CI = confidence interval; Lp(a) = lipoprotein(a); other abbreviations as in Tables 1 and 2.

**FIGURE 2** **Kaplan-Meier Survival Analyses**




**(A)** Incident ischemic (ISC) stroke and remnant-like particle cholesterol (RLP-C). **(B)** Incident cardiovascular disease (CVD) and RLP-C. **(C)** Incident ischemic stroke and low-density lipoprotein triglycerides (LDL-TG). **(D)** Incident CVD and LDL-TG. P values are from log rank tests.

**TABLE 4 Association of Coronary Heart Disease, Ischemic Stroke, and Cardiovascular Disease Events Competing With Nonevent Death Across Quartiles of Remnant-Like Particle Cholesterol**

| | | RLP-C (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| Incident Event | Model | Quartile 1 (0.4–3.1) (n = 2,411) | Quartile 2 (3.2–5.9) (n = 2,290) | Quartile 3 (6.0–12.2) (n = 2,319) | Quartile 4 (12.3–259.1) (n = 2,314) | p for Trend of Linearity |
| CHD | CHD | 267 (11.07) | 335 (14.63) | 357 (15.39) | 475 (20.53) | <0.001 |
| | Non-CHD death | 526 (21.82) | 469 (20.48) | 498 (21.47) | 447 (19.32) | NA |
| | Model 1 | Reference | 1.35 (1.15–1.59) | 1.38 (1.17–1.62) | 1.86 (1.60–2.17) | <0.0001 |
| | Model 2 | Reference | 1.10 (0.94–1.30) | 0.98 (0.82–1.16) | 1.06 (0.88–1.27) | 0.41 |
| Ischemic stroke | Ischemic stroke | 113 (4.69) | 110 (4.80) | 130 (5.61) | 130 (5.62) | 0.31 |
| | Non-ischemic stroke death | 599 (24.84) | 544 (23.76) | 580 (25.01) | 600 (25.93) | NA |
| | Model 1 | Reference | 1.12 (0.86–1.46) | 1.38 (1.07–1.79) | 1.43 (1.10–1.86) | 0.02 |
| | Model 2 | Reference | 0.96 (0.73–1.26) | 1.16 (0.88–1.53) | 1.07 (0.78–1.45) | 0.54 |
| CVD | CVD | 355 (14.72) | 414 (18.08) | 450 (19.40) | 566 (24.46) | <0.001 |
| | Non-CVD death | 482 (19.99) | 426 (18.60) | 451 (19.45) | 408 (17.63) | NA |
| | Model 1 | Reference | 1.28 (1.11–1.47) | 1.36 (1.18–1.57) | 1.77 (1.55–2.03) | <0.0001 |
| | Model 2 | Reference | 1.05 (0.91–1.22) | 0.99 (0.85–1.16) | 1.05 (0.89–1.23) | 0.77 |

Values are n (%) or hazard ratio (95% confidence interval) using the first (lowest) quartile as the referent. Model 1 was adjusted by age, sex, and race; model 2 (Pooled Cohort Equation model) was model 1 plus total cholesterol, HDL-C, systolic blood pressure, antihypertensive medication use, current smoking, and diabetic status.

CHD = coronary heart disease; NA = not applicable; other abbreviations as in Table 1.

contrast to our study, remnant cholesterol was calculated as total cholesterol minus HDL-C and directly measured LDL-C.

In a biracial cohort from the Jackson Heart Study and Framingham Offspring Cohort Study, RLP-C was positively associated with incident CHD in unadjusted models (8). Lipoproteins were classified by ultracentrifugation, and RLP-C was determined by the sum of cholesterol in the densest VLDL subfraction ($VLDL_3$ cholesterol) and intermediate-density lipoprotein cholesterol. After adjustment for HDL-C and LDL-C levels, the association of RLP-C with CHD was not significant, similar to in our study, which directly quantified fasting RLP-C.

**TABLE 5 Association of Coronary Heart Disease, Ischemic Stroke, and Cardiovascular Disease Events With Remnant-Like Particle Cholesterol and Low-Density Lipoprotein Triglyceride as Continuous Variables**

| | | RLP-C | | LDL-TG | |
|---|---|---|---|---|---|
| Incident Event | Model | Hazard Ratio (95% CI) | p Value | Hazard Ratio (95% CI) | p Value |
| CHD | Model 1 | 1.26 (1.19–1.34) | <0.001 | 1.97 (1.73–2.24) | <0.001 |
| | Model 2 | 0.99 (0.92–1.06) | 0.73 | 1.28 (1.10–1.50) | 0.002 |
| | Model 3 | 0.85 (0.76–0.96) | 0.008 | 1.27 (1.07–1.50) | 0.006 |
| Ischemic stroke | Model 1 | 1.18 (1.07–1.30) | 0.001 | 1.64 (1.32–2.04) | <0.001 |
| | Model 2 | 1.05 (0.93–1.18) | 0.46 | 1.47 (1.13–1.92) | 0.005 |
| | Model 3 | 0.82 (0.68–1.01) | 0.058 | 1.36 (1.01–1.82) | 0.040 |
| CVD | Model 1 | 1.25 (1.19–1.32) | <0.001 | 1.94 (1.73–2.17) | <0.001 |
| | Model 2 | 1.00 (0.94–1.06) | 0.97 | 1.35 (1.17–1.55) | <0.001 |
| | Model 3 | 0.84 (0.76–0.93) | 0.001 | 1.31 (1.13–1.53) | <0.001 |

Values are hazard ratio (per natural log unit increase for RLP-C and LDL-TG) and 95% confidence interval. Exposure values were assessed as continuous variables. Model 1 was adjusted by age, sex, and race; model 2 (Pooled Cohort Equation model) was model 1 plus total cholesterol, HDL-C, systolic blood pressure, antihypertensive medication use, current smoking, and diabetes mellitus; model 3 was model 2 plus log triglycerides.

Abbreviations as in Tables 1 to 4.

**LDL-TG AND CVD.** To our knowledge, our study is the first to report significant associations of LDL-TG with both ischemic stroke and CHD. Few data are available on the clinical utility of LDL-TG levels in CVD risk prediction, possibly because of the complexity of measuring LDL-TG (26). In a cross-sectional study of patients with stable CHD, in which LDL-TG was measured after fractionation of LDL by equilibrium density-gradient centrifugation, altered LDL metabolism characterized by high LDL-TG was correlated with prevalent CHD and systemic low-grade inflammation independent of LDL-C (28). Our results corroborate and extend these findings in a large population without clinical CHD and demonstrate that high LDL-TG levels measured by a validated automated assay (14) are associated with incident stroke and CHD after adjustment for traditional risk factors, including total cholesterol and HDL-C. Furthermore, in our study we found that individuals with elevated LDL-TG and RLP-C levels also had increased levels of the inflammatory markers hs-CRP and white blood cell count.

In a secondary analysis of the AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides and Impact on Global Health Outcomes) trial (26), LDL-TG failed to predict CVD events, including stroke. AIM-HIGH was a secondary prevention trial in 3,094 patients on statin therapy, predominantly white men, with a mean 3-year follow-up. By comparison, the ARIC cohort is

**TABLE 6 Association of Coronary Heart Disease, Ischemic Stroke, and Cardiovascular Disease Events Competing With Nonevent Death Across Quartiles of Low-Density Lipoprotein Triglyceride**

| | | LDL-TG (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| Incident Event | Model | Quartile 1 (0.7–17.0) (n = 2,360) | Quartile 2 (17.1–22.6) (n = 2,342) | Quartile 3 (22.7–29.6) (n = 2,301) | Quartile 4 (29.7–104) (n = 2,331) | p for Trend of Linearity |
| CHD | CHD | 257 (10.89) | 326 (13.92) | 403 (17.51) | 448 (19.22) | <0.001 |
| | Non-CHD death | 506 (21.44) | 479 (20.45) | 469 (20.38) | 486 (20.85) | NA |
| | Model 1 | Reference | 1.30 (1.10–1.53) | 1.70 (1.46–2.00) | 2.00 (1.72–2.34) | <0.0001 |
| | Model 2 | Reference | 1.07 (0.91–1.27) | 1.23 (1.04–1.46) | 1.20 (1.00–1.45) | 0.07 |
| Ischemic stroke | Ischemic stroke | 98 (4.15) | 123 (5.25) | 101 (4.39) | 161 (6.91) | <0.001 |
| | Non-ischemic stroke death | 571 (24.19) | 568 (24.25) | 589 (25.60) | 595 (25.53) | NA |
| | Model 1 | Reference | 1.34 (1.03–1.75) | 1.14 (0.86–1.50) | 1.84 (1.42–2.37) | <0.0001 |
| | Model 2 | Reference | 1.28 (0.97–1.69) | 1.03 (0.76–1.39) | 1.57 (1.15–2.15) | 0.005 |
| CVD | CVD | 327 (13.86) | 420 (17.93) | 472 (20.51) | 566 (24.28) | <0.001 |
| | Non-CVD death | 469 (19.87) | 432 (18.45) | 438 (19.04) | 428 (18.36) | NA |
| | Model 1 | Reference | 1.35 (1.17–1.56) | 1.60 (1.39–1.84) | 2.04 (1.77–2.34) | <0.0001 |
| | Model 2 | Reference | 1.15 (0.99–1.34) | 1.20 (1.03–1.41) | 1.31 (1.11–1.55) | 0.014 |

Values are n (%) or hazard ratio (95% confidence interval) using the first (lowest) quartile as the referent. Model 1 was adjusted by age, sex, and race; model 2 (Pooled Cohort Equation model) was model 1 plus total cholesterol, HDL-C, systolic blood pressure, antihypertensive medication use, current smoking, and diabetic status.

Abbreviations as in Tables 1, 2, and 4.

larger and biracial, with a longer follow-up of up to 16 years.

Prior studies evaluating the relationship between lipids and stroke risk have shown varied associations (29–31). Interestingly, data now suggest that TG level is independently associated with the stroke risk and that this association is stronger in women than men (32). Although men have higher TG levels than

**TABLE 7 Log (Remnant-Like Particle Cholesterol), Single-Variant Meta-Analysis of Candidate Genes**

| | | | Meta-Analysis | | | | African Americans | | | | | European Americans | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gene | Name | rs | p Value | Beta | SE | MAC | p Value | Beta | SE | MAF | MAC | p Value | Beta | SE | MAF | MAC |
| *ANGPTL3* | 1:63063472:G:A | NA | 3.08E-02 | 1.985 | 0.919 | 1 | NA | NA | NA | NA | NA | 3.08E-02 | 1.985 | 0.919 | 0.0001 | 1 |
| *ANGPTL3* | 1:63064415:G:A | rs144284900 | 3.68E-02 | −1.257 | 0.602 | 2 | 3.68E-02 | −1.257 | 0.602 | 0.0005 | 2 | NA | NA | NA | NA | NA |
| *APOA5* | 11:116662407:G:C | rs3135506 | 6.73E-19 | 0.267 | 0.030 | 963 | 3.45E-03 | 0.180 | 0.062 | 0.0555 | 208 | 1.34E-17 | 0.295 | 0.035 | 0.0659 | 755 |
| *APOA5* | 11:116661392:C:A | rs2075291 | 1.79E-04 | 0.837 | 0.223 | 15 | 7.29E-04 | 0.832 | 0.246 | 0.0032 | 12 | 1.05E-01 | 0.862 | 0.531 | 0.0003 | 3 |
| *APOA5* | 11:116661001:G:A | rs143292359 | 1.52E-02 | 0.845 | 0.348 | 7 | NA | NA | NA | NA | NA | 1.52E-02 | 0.845 | 0.348 | 0.0006 | 7 |
| *APOA5* | 11:116661346:C:T | rs780433260 | 4.35E-02 | −1.717 | 0.850 | 1 | 4.35E-02 | −1.717 | 0.850 | 0.0003 | 1 | NA | NA | NA | NA | NA |
| *APOA5* | 11:116661656:G:A | rs201079485 | 4.55E-02 | 1.300 | 0.650 | 2 | NA | NA | NA | NA | NA | 4.55E-02 | 1.300 | 0.650 | 0.0002 | 2 |
| *APOC3* | 11:116701354:G:A | rs138326449 | 1.76E-10 | −1.164 | 0.182 | 25 | 1.28E-01 | −0.747 | 0.491 | 0.0008 | 3 | 3.76E-10 | −1.230 | 0.196 | 0.0019 | 22 |
| *APOC3* | 11:116701353:C:T | rs76353203 | 3.29E-03 | −0.998 | 0.340 | 7 | 2.96E-01 | −0.629 | 0.602 | 0.0005 | 2 | 4.44E-03 | −1.170 | 0.411 | 0.0004 | 5 |
| *APOC3* | 11:116701613:G:T | rs140621530 | 1.41E-02 | −0.863 | 0.352 | 6 | 1.25E-02 | −0.951 | 0.381 | 0.0013 | 5 | 7.04E-01 | −0.349 | 0.920 | 0.0001 | 1 |
| *APOC3* | 11:116701608:G:T | NA | 4.77E-02 | −1.820 | 0.919 | 1 | NA | NA | NA | NA | NA | 4.77E-02 | −1.820 | 0.919 | 0.0001 | 1 |
| *LIPC* | 15:58723939:G:A | rs2070895 | 0.0732 | −0.033 | 0.018 | 4,765 | 0.0297 | −0.068 | 0.031 | 0.52 | 1,788 | 0.0608 | −0.036 | 0.019 | 0.216 | 2,977 |
| *LIPC* | 15:58855760:A:C | rs142036980 | 4.79E-03 | 0.735 | 0.261 | 11 | 8.55E-03 | 0.747 | 0.284 | 0.0024 | 9 | 3.05E-01 | 0.670 | 0.653 | 0.0002 | 2 |
| *LIPC* | 15:58833993:G:A | rs6078 | 1.77E-02 | −0.089 | 0.038 | 578 | 1.05E-01 | −0.089 | 0.055 | 0.0664 | 250 | 8.32E-02 | −0.089 | 0.052 | 0.0289 | 328 |
| *LIPG* | 18:47091689:A:G | NA | 2.45E-02 | 2.068 | 0.920 | 1 | NA | NA | NA | NA | NA | 2.45E-02 | 2.068 | 0.920 | 0.0001 | 1 |
| *LIPG* | 18:47110110:C:T | rs777816384 | 3.22E-02 | 1.970 | 0.920 | 1 | NA | NA | NA | NA | NA | 3.22E-02 | 1.970 | 0.920 | 0.0001 | 1 |
| *LIPG* | 18:47113195:G:A | NA | 3.98E-02 | 1.237 | 0.602 | 2 | 3.98E-02 | 1.237 | 0.602 | 0.0005 | 2 | NA | NA | NA | NA | NA |
| *LIPG* | 18:47109933:C:T | rs144717284 | 4.06E-02 | 1.232 | 0.602 | 2 | 4.06E-02 | 1.232 | 0.602 | 0.0005 | 2 | NA | NA | NA | NA | NA |
| *LPL* | 8:19819724:C:G | rs328 | 7.16E-16 | −0.206 | 0.026 | 1,392 | 9.47E-03 | −0.141 | 0.054 | 0.0712 | 270 | 8.38E-15 | −0.225 | 0.029 | 0.0989 | 1,122 |
| *LPL* | 8:19811733:G:A | rs118204057 | 4.35E-04 | 1.447 | 0.411 | 5 | NA | NA | NA | NA | NA | 4.35E-04 | 1.447 | 0.411 | 0.0004 | 5 |
| *LPL* | 8:19818441:C:T | rs141502542 | 8.14E-04 | 2.851 | 0.852 | 1 | 8.14E-04 | 2.851 | 0.852 | 0.0003 | 1 | NA | NA | NA | NA | NA |
| *LPL* | 8:19805708:G:A | rs1801177 | 1.96E-03 | 0.144 | 0.046 | 372 | 7.65E-02 | 0.115 | 0.065 | 0.0482 | 183 | 8.91E-03 | 0.173 | 0.066 | 0.0165 | 189 |
| *LPL* | 8:19809322:G:A | rs145657341 | 1.51E-02 | 2.235 | 0.920 | 1 | NA | NA | NA | NA | NA | 1.51E-02 | 2.235 | 0.920 | 0.0001 | 1 |

Meta-analysis p ≤ 0.05.

MAC = minor allele count; MAF = minor allele frequency; NA = not applicable.

**TABLE 8** Log (Low-Density Lipoprotein Triglyceride), Single-Variant Meta-Analysis of Candidate Genes

| | | | Meta-Analysis | | | | African Americans | | | | | European Americans | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gene | Name | rs | p Value | Beta | SE | MAC | p Value | Beta | SE | MAF | MAC | p Value | Beta | SE | MAF | MAC |
| *ANGPTL3* | 1:63067964:T:C | rs776441268 | 4.61E-03 | −1.168 | 0.412 | 1 | NA | NA | NA | NA | NA | 4.61E-03 | −1.168 | 0.412 | 0.0001 | 1 |
| *ANGPTL4* | 19:8438715:C:T | rs769769905 | 2.26E-02 | 1.009 | 0.442 | 1 | 2.26E-02 | 1.009 | 0.442 | 0.0003 | 1 | NA | NA | NA | NA | NA |
| *APOA5* | 11:116662407:G:C | rs3135506 | 1.86E-07 | 0.073 | 0.014 | 960 | 3.84E-02 | 0.067 | 0.033 | 0.0550 | 201 | 1.69E-06 | 0.074 | 0.015 | 0.0663 | 759 |
| *APOA5* | 11:116661656:G:A | rs201079485 | 5.60E-03 | 0.808 | 0.292 | 2 | NA | NA | NA | NA | NA | 5.60E-03 | 0.808 | 0.292 | 0.0002 | 2 |
| *APOA5* | 11:116660870:C:T | rs774294731 | 3.70E-02 | 0.923 | 0.443 | 1 | 3.70E-02 | 0.923 | 0.443 | 0.0003 | 1 | NA | NA | NA | NA | NA |
| *APOA5* | 11:116661338:C:G | NA | 4.10E-02 | −0.640 | 0.313 | 2 | 4.10E-02 | −0.640 | 0.313 | 0.0006 | 2 | NA | NA | NA | NA | NA |
| *APOC3* | 11:116701354:G:A | rs138326449 | 7.96E-04 | −0.279 | 0.083 | 25 | 7.85E-02 | −0.450 | 0.256 | 0.0008 | 3 | 3.27E-03 | −0.259 | 0.088 | 0.0019 | 22 |
| *APOC3* | 11:116701613:G:T | rs140621530 | 3.62E-03 | −0.520 | 0.179 | 6 | 4.00E-03 | −0.570 | 0.198 | 0.0014 | 5 | 4.67E-01 | −0.300 | 0.413 | 0.0001 | 1 |
| *APOC3* | 11:116701353:C:T | rs76353203 | 3.16E-02 | −0.342 | 0.159 | 7 | 6.59E-02 | −0.576 | 0.313 | 0.0005 | 2 | 1.58E-01 | −0.260 | 0.184 | 0.0004 | 5 |
| *APOC3* | 11:116703578:CA:C | rs750185333 | 3.25E-02 | −0.946 | 0.443 | 1 | 3.25E-02 | −0.946 | 0.443 | 0.0003 | 1 | NA | NA | NA | NA | NA |
| *LIPC* | 15:58723939:G:A | rs2070895 | 3.48E-08 | 0.047 | 0.009 | 4,705 | 0.0665 | 0.029 | 0.016 | 0.518 | 1,744 | 1.84E-08 | 0.048 | 0.009 | 0.215 | 2,961 |
| *LIPC* | 15:58837989:G:A | rs200684324 | 5.52E-04 | 0.822 | 0.238 | 3 | NA | NA | NA | NA | NA | 5.52E-04 | 0.822 | 0.238 | 0.0003 | 3 |
| *LIPC* | 15:58860927:T:A | rs374799133 | 1.62E-03 | 1.300 | 0.412 | 1 | NA | NA | NA | NA | NA | 1.62E-03 | 1.300 | 0.412 | 0.0001 | 1 |
| *LIPC* | 15:58840562:G:A | rs200613217 | 9.60E-03 | 1.149 | 0.444 | 1 | 9.60E-03 | 1.149 | 0.444 | 0.0003 | 1 | NA | NA | NA | NA | NA |
| *LIPC* | 15:58833993:G:A | rs6078 | 2.10E-02 | 0.042 | 0.018 | 570 | 3.36E-02 | 0.061 | 0.029 | 0.0668 | 245 | 2.11E-01 | 0.029 | 0.023 | 0.0287 | 325 |
| *LIPC* | 15:58853143:G:A | rs746042863 | 3.04E-02 | 0.893 | 0.412 | 1 | NA | NA | NA | NA | NA | 3.04E-02 | 0.893 | 0.412 | 0.0001 | 1 |
| *LIPC* | 15:58855778:T:C | rs761668960 | 4.95E-02 | 0.811 | 0.413 | 1 | NA | NA | NA | NA | NA | 4.95E-02 | 0.811 | 0.413 | 0.0001 | 1 |
| *LIPC* | 15:58861013:G:A | NA | 4.95E-02 | −0.810 | 0.412 | 1 | NA | NA | NA | NA | NA | 4.95E-02 | −0.810 | 0.412 | 0.0001 | 1 |
| *LPL* | 8:19819724:C:G | rs328 | 1.44E-06 | −0.057 | 0.012 | 1,378 | 3.92E-03 | −0.082 | 0.028 | 0.0720 | 266 | 6.92E-05 | −0.052 | 0.013 | 0.0982 | 1,112 |
| *LPL* | 8:19818441:C:T | rs141502542 | 6.15E-04 | 1.518 | 0.443 | 1 | 6.15E-04 | 1.518 | 0.443 | 0.0003 | 1 | NA | NA | NA | NA | NA |
| *LPL* | 8:19805708:G:A | rs1801177 | 3.02E-03 | 0.067 | 0.022 | 367 | 2.03E-01 | 0.044 | 0.035 | 0.0473 | 175 | 4.93E-03 | 0.083 | 0.029 | 0.0168 | 192 |
| *LPL* | 8:19811796:GA:G | NA | 1.09E-02 | 1.050 | 0.412 | 1 | NA | NA | NA | NA | NA | 1.09E-02 | 1.050 | 0.412 | 0.0001 | 1 |
| *LPL* | 8:19811733:G:A | rs118204057 | 1.64E-02 | 0.443 | 0.184 | 5 | NA | NA | NA | NA | NA | 1.64E-02 | 0.443 | 0.184 | 0.0004 | 5 |
| *LPL* | 8:19805745:A:T | rs367924602 | 2.57E-02 | −0.460 | 0.206 | 4 | NA | NA | NA | NA | NA | 2.57E-02 | −0.460 | 0.206 | 0.0003 | 4 |
| *LPL* | 8:19819645:G:A | rs149089920 | 3.70E-02 | 0.923 | 0.443 | 1 | 3.70E-02 | 0.923 | 0.443 | 0.0003 | 1 | NA | NA | NA | NA | NA |
| *LPL* | 8:19797000:A:G | rs756418111 | 3.93E-02 | −0.914 | 0.444 | 1 | 3.93E-02 | −0.914 | 0.444 | 0.0003 | 1 | NA | NA | NA | NA | NA |
| *LPL* | 8:19816887:A:G | rs300 | 4.80E-02 | 0.151 | 0.076 | 34 | 4.78E-02 | 0.154 | 0.078 | 0.0091 | 33 | 8.59E-01 | 0.073 | 0.412 | 0.0001 | 1 |

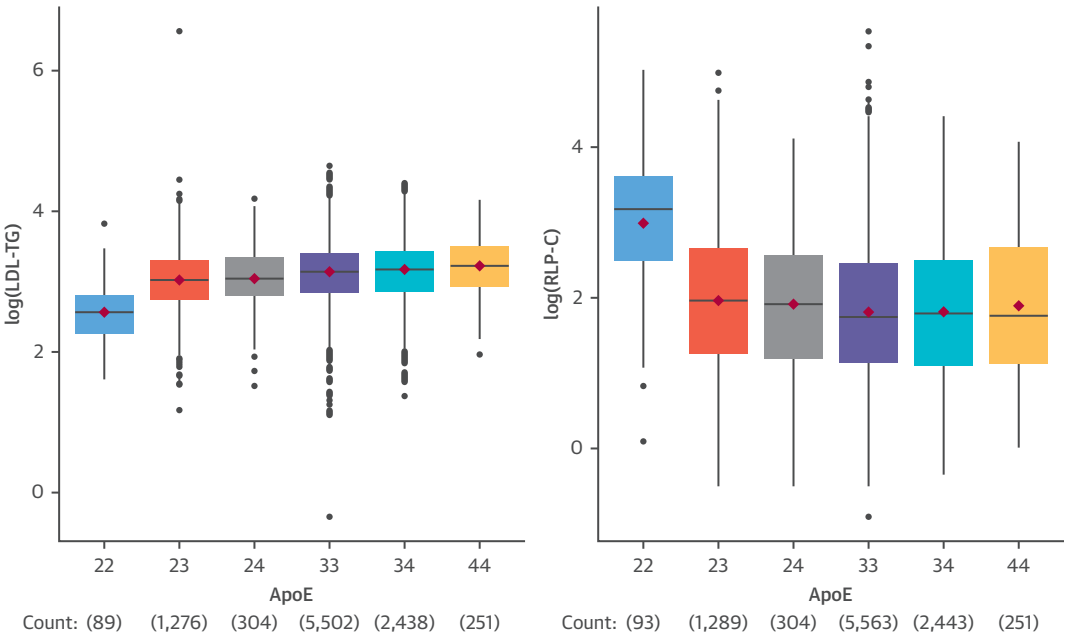
Meta-analysis $p \leq 0.05$.
Abbreviations as in Table 7.

women, we observed that women had higher LDL-TG levels than men, which may be one reason high TG level had a stronger association with stroke in women than in men.

Arterial disease may differ among vascular beds, particularly smaller arteries and arterioles (33). Plaque composition in the smaller cerebral arteries suggests a more fibrotic process than in the coronary arteries, which have more lipid-rich cores and typical atheromatous lesions (34). In addition, arteriolar lesions are characterized by hyalinosis instead of lipid. The associations of higher LDL-TG level with increased hs-CRP level and white blood cell count may reflect an adverse impact on inflammation, which may lead to more cerebrovascular disease.

**EXOME ANALYSIS: UNBIASED APPROACH.** Our exome-chip survey showed 11 common (MAF >1%) nonsynonymous variant-trait associations, all detected variants of genes previously associated with lipid CVD risk factors, including TG, total cholesterol, HDL-C, LDL-C, and small dense LDL. In exome analysis of rare variants, aggregated variants of *TARM1* and *APOC3* were also associated with decreased levels of LDL-TG and RLP-C, respectively. The association of LDL-TG levels with genetic variants in *TARM1* has not been previously reported. *TARM1* encodes a novel costimulator of proinflammatory cytokine secretion by macrophages and neutrophils (20) and may provide a link between LDL-TG and chronic low-grade inflammation underlying CVD progression. ApoCIII inhibits lipolysis by LPL and can delay clearance of atherogenic lipoproteins (35). *APOC3* loss-of-function variants are associated with lower TG and small dense LDL levels, higher HDL-C levels, reduced postprandial lipemia, and reduced CHD risk (36). Our findings that *APOC3* loss-of-function variants are associated with decreased RLP-C and LDL-TG levels support these previous reports. Notably, a gain-of-function variant of *LPL* (rs328), identified by both the unbiased approach and the candidate gene approach, was strongly associated with lower RLP-C and LDL-TG levels in our study. This well-known missense variant has been associated with lower TG and increased HDL-C levels (37) and reduced CHD (38).

**FIGURE 3** Associations of ApoE Haplotypes With Log Low-Density Lipoprotein Triglycerides and Remnant-Like Particle Cholesterol




Associations between low-density lipoprotein triglycerides (LDL-TG) **(left)** and remnant-like particle cholesterol (RLP-C) **(right)** levels and genetic variants were assessed by whole-exome sequencing. A common *APOE* variant, rs7412, had the strongest association with LDL-TG and RLP-C ($p < 5 \times 10^{-8}$). Because rs7412 defines *APOE* ε2 allele status, we assessed *APOE* haplotypes and found that *APOE* ε2/2 was associated with reduced LDL-TG and increased RLP-C ($p < 0.0001$ vs. any other haplotype). The different relationships of RLP-C and LDL-TG with *APOE* ε2/2 may be partly explained by the low affinity of apolipoprotein (apo) E2 for the low-density lipoprotein (LDL) (apoB/E) receptor, potentially leading to delayed clearance of remnant particles. This delayed clearance may lead to increased RLP-C levels while lowering LDL and LDL-TG levels via up-regulation of cellular LDL receptors in these subjects.

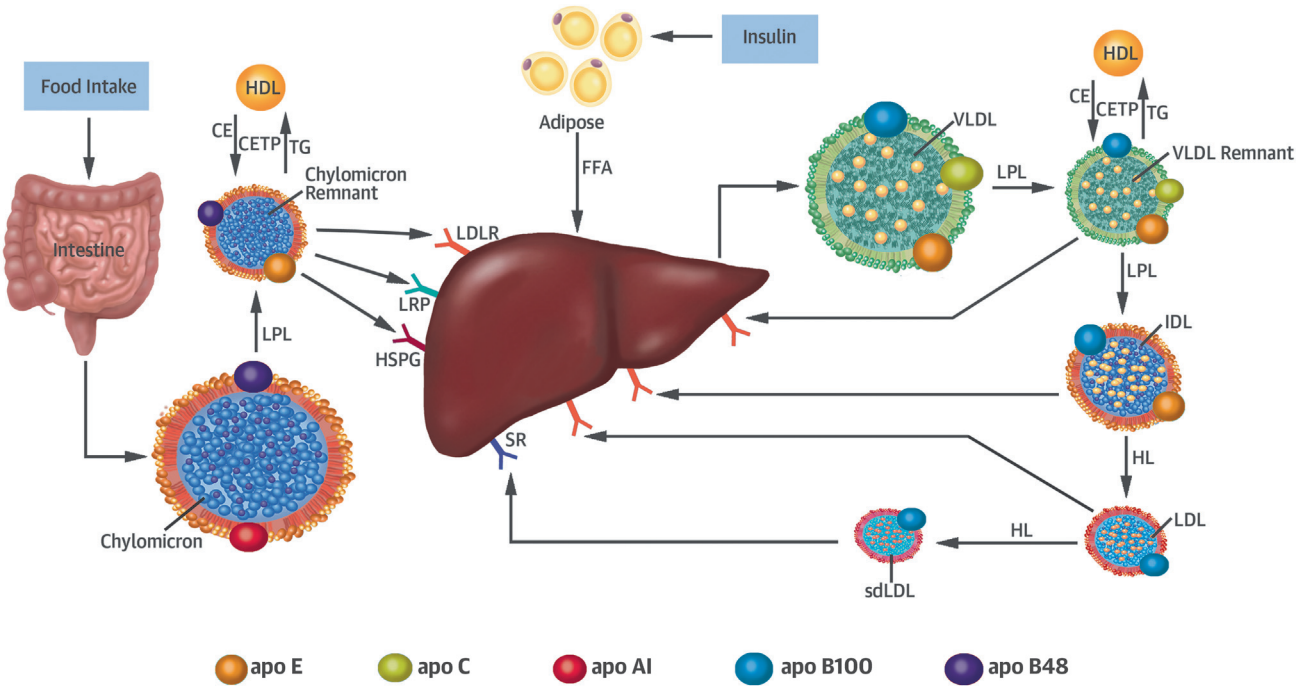
**EXOME ANALYSIS: CANDIDATE GENE APPROACH.** Our candidate gene approach showed significant associations between common variants in *APOA5* and *LPL* and circulating RLP-C and LDL-TG levels. ApoAV is postulated to regulate plasma TG levels by enhancing TGRL catabolism by LPL (39) or by inhibiting VLDL synthesis (40). The highly statistically significant variant-trait associations for *LPL* variants in both

**TABLE 9** Associations Between rs7412 (Reference/Alternative Alleles: C/T, MAF = 0.08 in European Americans, MAF = 0.11 in African Americans) and Lipids

| | Meta-Analysis | | | European Americans | | | African Americans | | |
|---|---|---|---|---|---|---|---|---|---|
| Trait | Beta | SE | p Value | Beta | SE | p Value | Beta | SE | p Value |
| Log RLP-C | 0.267 | 0.02 | 2.64E-32 | 0.264 | 0.027 | 6.01E-23 | 0.275 | 0.042 | 6.10E-11 |
| Log TG | 0.062 | 0.01 | 3.27E-09 | 0.080 | 0.01 | 3.88E-10 | 0.025 | 0.018 | 0.177279 |
| HDL-C | 1.170 | 0.32 | 0.0003 | 0.827 | 0.38 | 0.0273 | 2.193 | 0.647 | 7.07E-4 |
| HDL-2 | 0.044 | 0.01 | 0.0003 | 0.032 | 0.02 | 0.00901 | 0.070 | 0.022 | 9.00E-05 |
| HDL-3 | 0.463 | 0.22 | 0.0337 | 0.315 | 0.25 | 0.2113 | 0.909 | 0.436 | 0.0372 |
| Log LDL-TG | −0.139 | 0.01 | 5.68E-39 | −0.138 | 0.012 | 3.39E-30 | −0.140 | 0.022 | 2.35E-10 |
| LDL-C | −16.581 | 0.817 | 1.20E-91 | −16.170 | 0.94 | 5.78E-66 | −17.815 | 1.64 | 1.22E-27 |
| TC | −0.327 | 0.02 | 5.39E-49 | −0.304 | 0.03 | 3.22E-32 | −0.393 | 0.04 | 4.07E-19 |
| Non-HDL-C | −0.358 | 0.02 | 1.83E-52 | −0.326 | 0.03 | 1.22E-32 | −0.447 | 0.05 | 1.15E-22 |
| Lp(a) | −0.139 | 0.03 | 3.40E-10 | −0.12 | 0.03 | 3.66E-05 | −0.165 | 0.03 | 1.32E-06 |

TC = total cholesterol; other abbreviations as in Tables 1 to 3.

**CENTRAL ILLUSTRATION** **Remnant Lipoprotein Metabolism**




**Saeed, A. et al. J Am Coll Cardiol. 2018;72(2):156–69.**

Chylomicrons secreted from the intestine and very low-density lipoprotein (VLDL) secreted from the liver are lipolyzed by lipoprotein lipase (LPL), leading to triglyceride-rich lipoprotein (TGRL) remnants. Chylomicron secretion is regulated largely by food intake, whereas VLDL secretion is controlled by insulin. Remnant particles undergo remodeling via the enzymatic action of cholesteryl ester transfer protein (CETP) with high-density lipoprotein (HDL), hepatic lipase (HL), and the exchange of soluble apolipoproteins (apos) such as E, C-I, C-II, and C-III. TGRL remnants are cleared from the circulation via receptor-mediated uptake involving the low-density lipoprotein (LDL) receptor (LDLR), LDL receptor–like protein (LRP), and heparan sulfate proteoglycans (HSPGs). Chylomicron remnants and VLDL remnants compete for the same lipolytic pathway, a process mediated by apoE. Although chylomicron remnant clearance may be mediated by LDLR, LRP, or HSPG, VLDL remnants are believed to be predominantly cleared via LDLR. Subjects with apoE2 isoforms have reduced remnant clearance and are postulated to have compensatory up-regulation of cellular LDLR expression that may lead to decreased LDL-triglyceride (TG) and LDL-C levels. The purported role of HL in the lipolytic conversion of intermediate-density lipoprotein (IDL) to LDL may at least partly explain why subjects with decreased HL activity due to genetic variation in the *LIPC* gene (e.g., rs2070895) have elevated LDL-TG levels. CE = cholesteryl ester; FFA = free fatty acid; sdLDL = small dense low-density lipoprotein; SR = scavenger receptor.

unbiased and candidate gene approaches may indicate the importance of LPL as the rate-limiting enzyme for hydrolysis of circulating TGs. We found weaker associations between a common *LIPC* variant (rs6078) and RLP-C and LDL-TG levels. However, a strong association was found between rs2070895 and LDL-TG levels. rs2070895 is located in the promotor region of the hepatic lipase gene and was previously found to be associated with decreased hepatic lipase activity (41). Hepatic lipase plays an important role in the lipolytic conversion of VLDL to LDL, a process modulated by high-density lipoprotein composition (42). Mutations in the hepatic lipase gene were associated with increased ischemic heart disease risk in the Copenhagen City Heart Study (43). Complete deficiency of hepatic lipase has also been linked with impaired catabolism and accumulation of remnant particle RLP-C as well as increased TG content of LDL (41).

**ApoE AND CVD.** A novel aspect of this study is the identification of genetic variants associated with RLP-C and LDL-TG, including the *APOE* variant rs7412. A review of epidemiological studies of *APOE* polymorphism and CHD estimated that about 6% of the variation in CHD risk in North Americans is attributable to this locus (44). Most genotyping assays used in population studies did not include rs7412, but the

CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) consortium recently demonstrated that *APOE* ε2 was associated with reduced subclinical atherosclerosis assessed by carotid intima-media thickness and coronary calcium scores and also with clinical CHD (45). Previous studies also suggest a protective effect of *APOE* ε2 on atherosclerosis (46,47), despite the association between apoE2/2 and type III hyperlipoproteinemia (46), which is characterized by accumulated remnant lipoproteins with resulting increased blood TG and cholesterol levels.

In our cohort, the *APOE* variant rs7412 was significantly associated with LDL-TG and RLP-C in both races. Furthermore, *APOE* ε2/2 was associated with higher RLP-C and TG levels but lower LDL-TG levels. The different relationships of RLP-C and LDL-TG with *APOE* ε2/2 may be explained in part by the low affinity of apoE2 for the LDL (apoB/E) receptor, potentially leading to delayed clearance of VLDL and chylomicron remnants (48). The slower removal of remnant particles may lead to increased RLP-C levels in the circulation, while the reduced uptake of RLP-C via the LDL receptor may simultaneously up-regulate cellular LDL receptors, leading to increased removal of LDL and thus lower LDL-TG in these subjects. We propose a model in which defective TGRL catabolism, with subsequent increased TGRL remnants, in the presence of delayed LDL catabolism leads to increased LDL-TG levels through interaction with cholesteryl ester transfer protein. Although RLP-C and LDL-TG may both be considered markers of remnant lipoprotein metabolism (**Central Illustration**), our data suggest that LDL-TG may be a more important marker of atherogenic altered remnant/LDL metabolism not detected by a routine lipid profile. Indeed, although most circulating TGs are in chylomicron and VLDL remnants, the relatively short half-life of these particles compared with that of LDL may render remnant particles (or measures of their lipid content, such as cholesterol or TGs) less useful as cardiovascular risk markers. Alternatively, LDL-TG may represent a lipoprotein subfraction with specific proatherogenic properties. Therapies that lower LDL-C by enhanced LDL receptor–mediated clearance (e.g., statins, ezetimibe, proprotein convertase subtilisin/kexin type 9 inhibitors) would also be expected to lower LDL-TG levels. An alternative approach suggested by the genetic observations is to use therapies that inhibit apoCIII or activate LPL to clear TGRLs more rapidly, which would also be expected to lower LDL-TG levels. Future studies are needed to determine whether the relationship between LDL-TG and cardiovascular outcomes is causal, and if so, which therapies may be most effective.

**STUDY STRENGTHS AND LIMITATIONS.** Strengths of the present study include a large, well-characterized, biracial population followed for up to 16 years in a study designed to examine CVD incidence and risk factors, and the use of a homogenous assay to measure RLP-C and LDL-TG directly. A limitation is measurement at only 1 time point using frozen plasma samples. Also, despite adjustments, residual confounding is possible, and the relationships are, at best, associations.

## CONCLUSIONS

Although elevated TGs were associated with increased RLP-C and LDL-TG, only LDL-TG predicted CVD risk in models adjusted for traditional risk factors. *APOE* variants were associated with RLP-C and LDL-TG, but subjects with ε2/2 had decreased LDL-TG and increased RLP-C. Further research is needed to determine whether LDL-TG plays a causal role in CVD and may be a target for therapy.

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## PERSPECTIVES

**COMPETENCY IN MEDICAL KNOWLEDGE:** Although both RLP-C and LDL-TG levels correlate with TG levels and incident cardiovascular events, after adjusting for traditional risk factors, only LDL-TG predicts incident CHD and ischemic stroke. Hence, for risk assessment in a primary prevention setting, measurement of LDL-TG provides additional information beyond traditional risk factors and lipid levels.

**TRANSLATIONAL OUTLOOK:** Prospective clinical trials should examine whether pharmacotherapies that reduce LDL-TG reduce ischemic events.

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**KEY WORDS** coronary heart disease, remnant lipoproteins, risk, stroke, triglyceride-rich lipoproteins

**APPENDIX** For supplemental methods and tables, please see the online version of this paper.

# Adverse Effects of Statin Therapy: Perception versus the Evidence

## Focus on glucose homeostasis, cognitive, renal and hepatic function, haemorrhagic stroke and cataract

A Consensus Statement from the

European Atherosclerosis Society Consensus Panel

EAS

# Rationale

# Why We Need This Statement

- Statins are recommended by guidelines as first-line treatments for reducing LDL-C, a key driver of atherosclerotic cardiovascular disease (ASCVD), as discussed in a previous EAS Consensus Statement.
- RCTs also show that statins are safe and generally well tolerated. However, most trials are:

  - Relatively short in duration

  - Tend to enrol a more homogeneous population than seen in routine practice.

Catapano AL et al. Atherosclerosis 2016;253:281-344; Piepoli MF et al. Eur Heart J 2016;37:2315-2381; Ference BA et al. Eur Heart J 2017;38:2459-2472 ; Collins R et al. Lancet 2016;388:2532-2561

# Why We Need This Statement

- Perception by the general public of the long-term side effects of statins has also been influenced by media reportage.
- BUT are these side effects real – or a nocebo effect?

- Note: a nocebo effect is caused by negative expectations about the effects of treatment, due to information provided by clinicians and/or the media about possible side effects,. This can lead to higher reporting rates than would otherwise be expected.

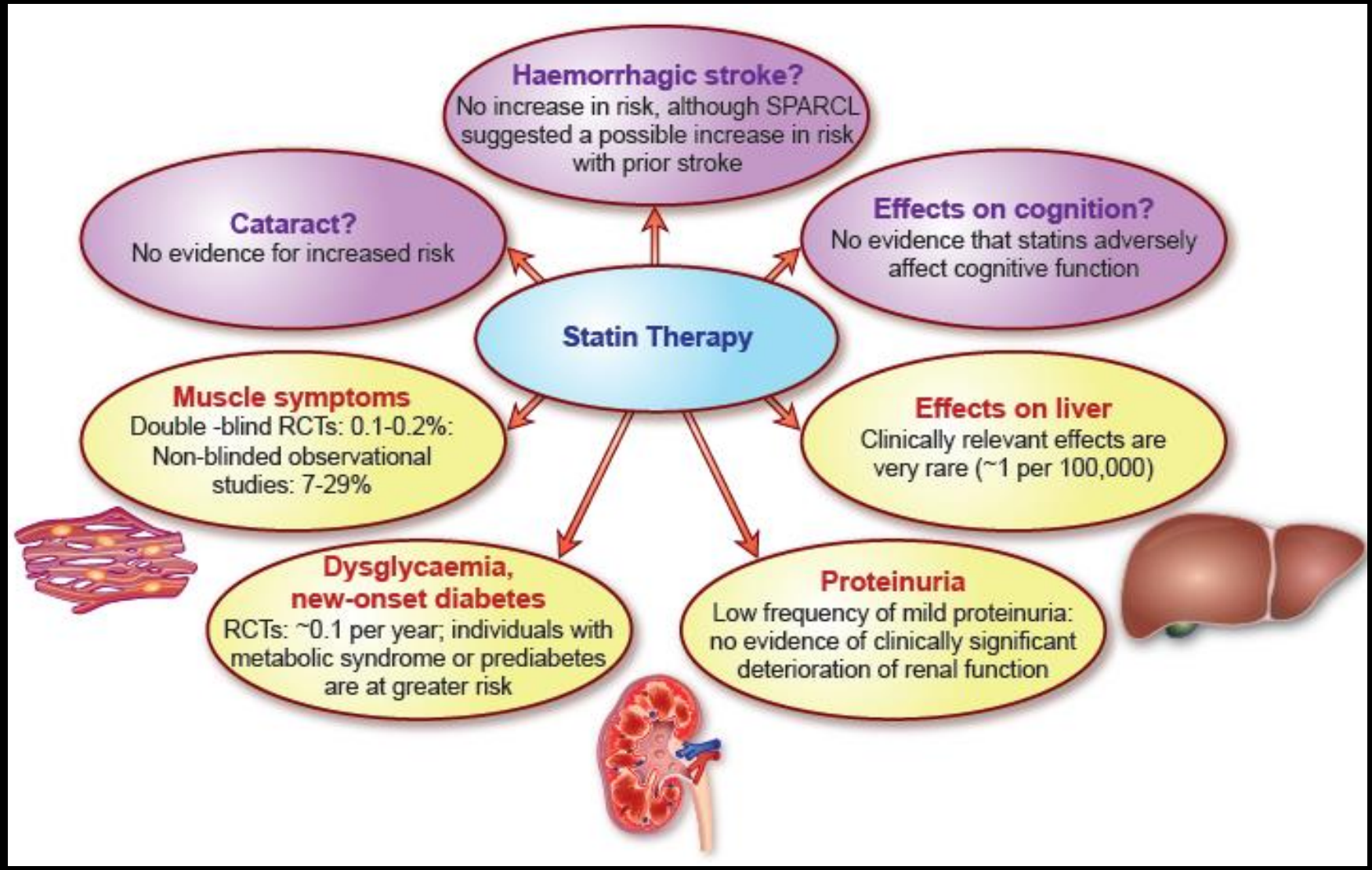
Nielsen SF, Nordestgaard BG. Eur Heart J 2016;37:908–916; Matthews A et al; BMJ 2016;353:i3283.; Tobert JA, Newman CB. J Clin Lipidol 2016;10:739-747

# Why We Need This Statement

- This EAS Consensus Panel critically appraised the evidence for possible unintended effects of long-term statin therapy.
- This Statement accurately assessed the incidence of these effects so as to place perceptions of these side effects in their correct perspective.

Mach F et al. Eur Heart J 2018;

# What are the Persistent Questions about Long-term Statin Safety?




Mach F et al. Eur Heart J 2018

# The Evidence Reviewed:

- Review and analysis of literature search 200-2017
- Key questions :

- What are the effects of statin treatment on glucose homeostasis?
- What is the benefit vs. risk of statin therapy for new-onset diabetes mellitus, especially in patients with features of the metabolic syndrome?
- What are the effects of statin treatment – and very low LDL-C levels – on cognitive function?
- Does statin treatment affect renal or hepatic function?
- Does statin treatment affect the risk for haemorrhagic stroke or cataract?

# Statins and Glucose Homeostasis

# Statins and Glucose Homeostasis: Risk Vs. Benefit

| Evidence | Risk of new-onset diabetes (per 1000 patients per year of exposure) | Benefit |
|---|---|---|
| Sattar (2010)<br>13 RCTs in 91,140 patients without diabetes at baseline | 1 new case | Prevents 5 new CVD events |
| Preiss (2011)<br>5 RCTs in 32,752 patients without diabetes at baseline, intensive statin therapy | 1 new case | Prevents 3.5 new CVD events |
| Cederberg (2015)<br>8,749 men (2,142 on a statin) with features of metabolic syndrome | 10 new cases | - |

Sattar N et al. Lancet 2010;375:735-42; Preiss D et al. JAMA 2011;305:2556-64; Cederberg H et al. Diabetologia 2015;58:1109-17

# Statins and Glucose Homeostasis

What is the mechanism(s) for the increased risk of new-onset diabetes on statin treatment?

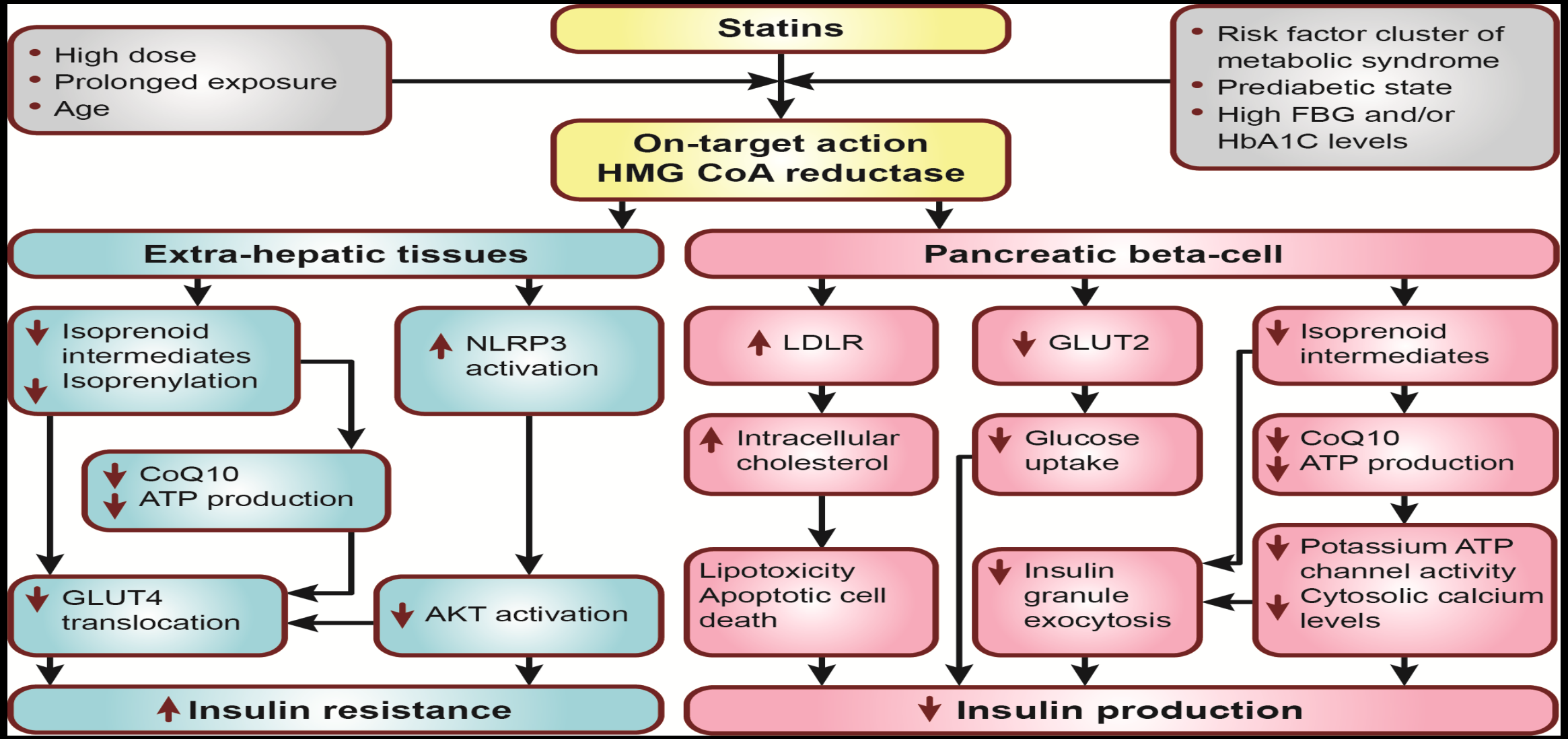
On-target effect? *HMGCR* gene variants associated with low LDL-C are also associated with higher blood glucose, insulin levels, body weight, waist circumference and BMI

Class effect mediated by LDL? *PCSK9* and *NPC1L1* gene variants associated with low LDL-C were also associated with increased risk of diabetes ONLY in subjects with impaired glucose tolerance

Off-target effects???

Swerdlow DI et al. Lancet 2015;385:351-361; Speliotes EK et al Nat Genet 2010;42: 937–948.; Scott RA et al. Nat Genet 2012;44:991-1005; Ference BA et al. N Engl J Med 2016;375:2144-2153; Schmidt AF et al. Lancet Diabetes Endocrinol 2017;5:97-105.; Lotta LA et al. JAMA 2016;316:1383-1391.

# What is the Mechanism(s) of the Diabetogenicity of Statins?

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# Statins and Glucose Homeostasis

**Take Home Messages:**

- Statins are associated with a modest risk of new-onset diabetes, about 1 new case per 1000 patients per year of exposure **BUT** also prevent 5 new cardiovascular events
- This risk may be higher in patients with features of the metabolic syndrome BUT needs to considered in context of the background conversion rate without statin treatment
- In most studies, new-onset diabetes was defined as an HbA1c >6.5 without symptoms BUT what is the relevance of this to long-term morbidity and mortality?

Mach F et al. Eur Heart J 2018;

# Statins and Cognitive Function

# Statins and Cognitive Function: What is the Evidence?

| Evidence | Conclusion |
|---|---|
| FDA (2012); Data surveillance review | Labelling amendment to include cognitive side effects such as memory loss and confusion |
| Song (2013); 8 cohort studies, n=57,020 and 2,851 cases of dementia | Statin use was associated with a lower risk of dementia |
| Richardson (2013); 3 RCTs, 16 cohort, 4 case-control and 4 cross-sectional studies | No adverse effect of statins on cognition; rates of cognitive-related AEs similar to other cardiovascular medications |
| McGuinness (2014); Cochrane review 4 RCTs, 1154 with probable or possible dementia | Statin therapy does not delay deterioration of cognitive function in patients with dementia |
| Ott (2015) ; 25 RCTs (23 with cognitive testing), n=46,836 | Statin therapy is not associated with cognitive impairment |

www.fda.gov/drugs/drugsafety; Song Y et al. Ger Gerontol Int 2013;13:817-24; Richardson K et al. Ann Intern Med 2013;159:688-97; McGuinness B et al. Cochrane systematic reviews 2014;7:CD007514; Ott BR et al. J Gen Intern Med 2015;30:348-58.

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# Statins and Cognitive Function: IMPROVE-IT

Table 2. Safety Outcomes of Evolocumab Treatment vs Placebo Stratified by Baseline LDL-C Levels[a]

| Outcome | Baseline LDL-C Level | | | |
|---|---|---|---|---|
| | <70 mg/dL (n = 2033)[b] | | ≥70 mg/dL (n = 25 491) | |
| | Evolocumab (n = 1030) | Placebo (n = 1003) | Evolocumab (n = 12 739) | Placebo (n = 12 752) |
| Serious adverse event | 268 (26.0) | 274 (27.3) | 3142 (24.7) | 3130 (24.5) |
| Adverse event related to study drug and leading to therapy discontinuation | 19 (1.8) | 19 (1.9) | 207 (1.6) | 182 (1.4) |
| Injection site reaction | 30 (2.9)[c] | 16 (1.6) | 266 (2.1)[c] | 203 (1.6) |
| Muscle-related event | 49 (4.8) | 60 (6.0) | 633 (5.0) | 596 (4.7) |
| Cataract | 19 (1.8) | 16 (1.6) | 209 (1.6) | 226 (1.8) |
| New-onset diabetes (CEC adjudicated)[d] | 45/509 (8.8) | 53/475 (11.2) | 632/7828 (8.1) | 591/7864 (7.5) |
| Neurocognitive event | 17 (1.7) | 12 (1.2) | 200 (1.6) | 190 (1.5) |
| AST or ALT level >3 times normal[e] | 27 (2.7) | 23 (2.3) | 213 (1.7) | 219 (1.7) |
| Creatine kinase level >5 times normal | 9 (0.9) | 9 (0.9) | 86 (0.7) | 90 (0.7) |

… the incidence of neurocognitive adverse events did not increase at very low LDL-C levels (<0.78 mmol/L or <30 mg/dl)….

Giugliano RP et al. JAMA Cardiol 2017;2:547-55

EAS

# Statins and Cognitive Function: FOURIER

| | LDL-cholesterol concentration at 4 weeks | | | | | $p_{trend}$ |
|---|---|---|---|---|---|---|
| | <0·5 mmol/L (n=2669) | 0·5 to <1·3 mmol/L (n=8003) | 1·3 to <1·8 mmol/L (n=3444) | 1·8 to <2·6 mmol/L (n=7471) | ≥2·6 mmol/L (n=4395) | |
| Serious adverse events | 614 (23%) | 1948 (24%) | 838 (24%) | 1684 (23%) | 1022 (23%) | 0·13 |
| Adjusted OR (95% CI) | 0·97 (0·86–1·10) | 1·01 (0·92–1·11) | 1·01 (0·90–1·13) | 0·93 (0·84–1·02) | 1 (ref) | 0·30 |
| Adverse events* leading to discontinuation of study drug | 98 (4%) | 295 (4%) | 124 (4%) | 234 (3%) | 149 (3%) | 0·11 |
| Adjusted OR (95% CI) | 1·08 (0·82–1·43) | 1·07 (0·86–1·33) | 1·07 (0·83–1·39) | 0·91 (0·73–1·14) | 1 (ref) | 0·13 |
| AST or ALT elevation (>3 times ULN) | 41 (2%) | 120 (1%) | 76 (2%) | 119 (2%) | 83 (2%) | 0·19 |
| Adjusted OR (95% CI) | 0·96 (0·64–1·43) | 0·87 (0·64–1·17) | 1·25 (0·90–1·74) | 0·91 (0·68–1·24) | 1 (ref) | 0·64 |
| Creatine kinase elevation (>5 times ULN) | 18 (1%) | 55 (1%) | 19 (1%) | 58 (1%) | 26 (1%) | 0·99 |
| Adjusted OR (95% CI) | 1·02 (0·53–1·96) | 1·07 (0·65–1·77) | 0·88 (0·47–1·65) | 1·23 (0·75–2·02) | 1 (ref) | 0·72 |
| Neurocognitive events | 49 (2%) | 122 (2%) | 51 (1%) | 100 (1%) | 52 (1%) | 0·019 |
| Adjusted OR (95% CI) | 1·28 (0·84–1·96) | 1·10 (0·78–1·55) | 1·10 (0·73–1·65) | 0·97 (0·68–1·39) | 1 (ref) | 0·15 |

… No increase in the incidence of neurocognitive adverse events at LDL-C levels <0.50 mmol/L or <20 mg/dl….

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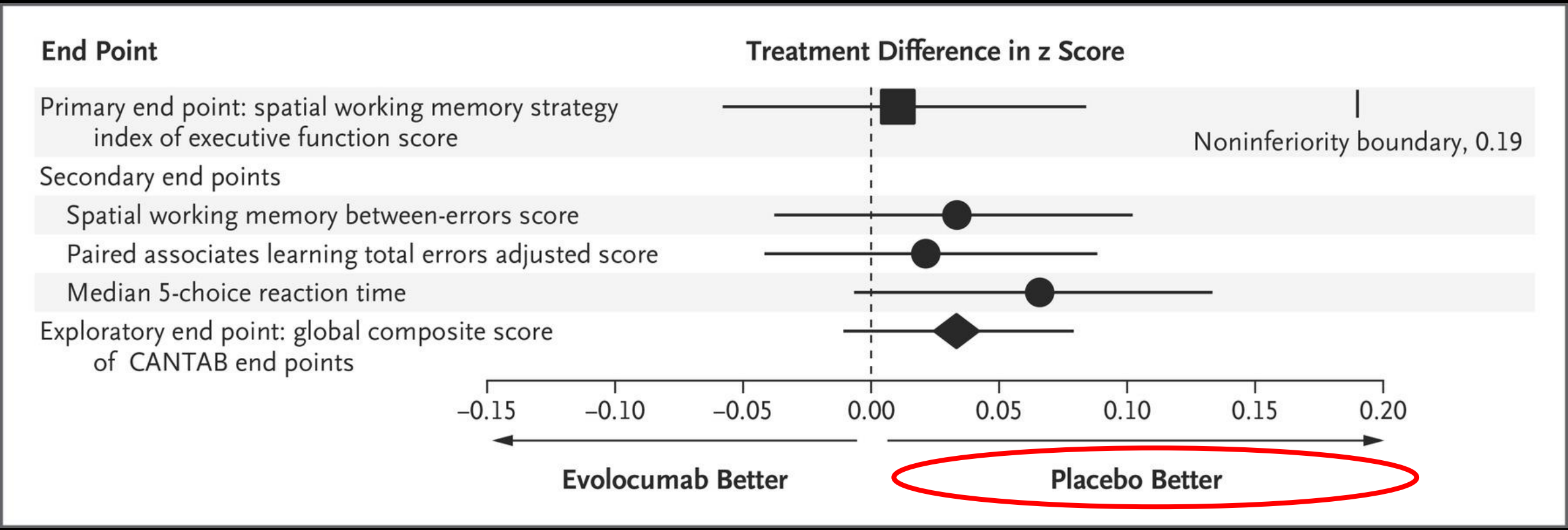
Giugliano RP et al. Lancet 2017;390:1962-71

# Statins and Cognitive Function: EBBINGHAUS




…Low LDL-C levels were not associated with adverse effects on cognitive function as assessed prospectively over 19 months…

Giugliano RP et al. N Engl J Med 2017;377:633-43.

EAS

# Statins and Cognitive Function

**Take Home Messages:**

- Statin treatment does not adversely affect cognitive function.
- At very low LDL-C levels attained with the combination of statin plus ezetimibe or a PCSK9 inhibitor, there was no signal for any adverse effect on cognitive function.
- Mendelian randomization analyses support the finding that low LDL-C levels, due to PCSK9 and HMGCR variants mimicking PCSK9 inhibitors and statins, had no causal effect on the risk of Alzheimer's disease, vascular dementia, any dementia, or Parkinson's disease

Mach F et al. Eur Heart J 2018;

# Statins and Renal Function

# Statins: Effects on Major CVD events stratified by renal function

Statin therapy reduces CVD events in patients with CKD, especially those with mild kidney disease

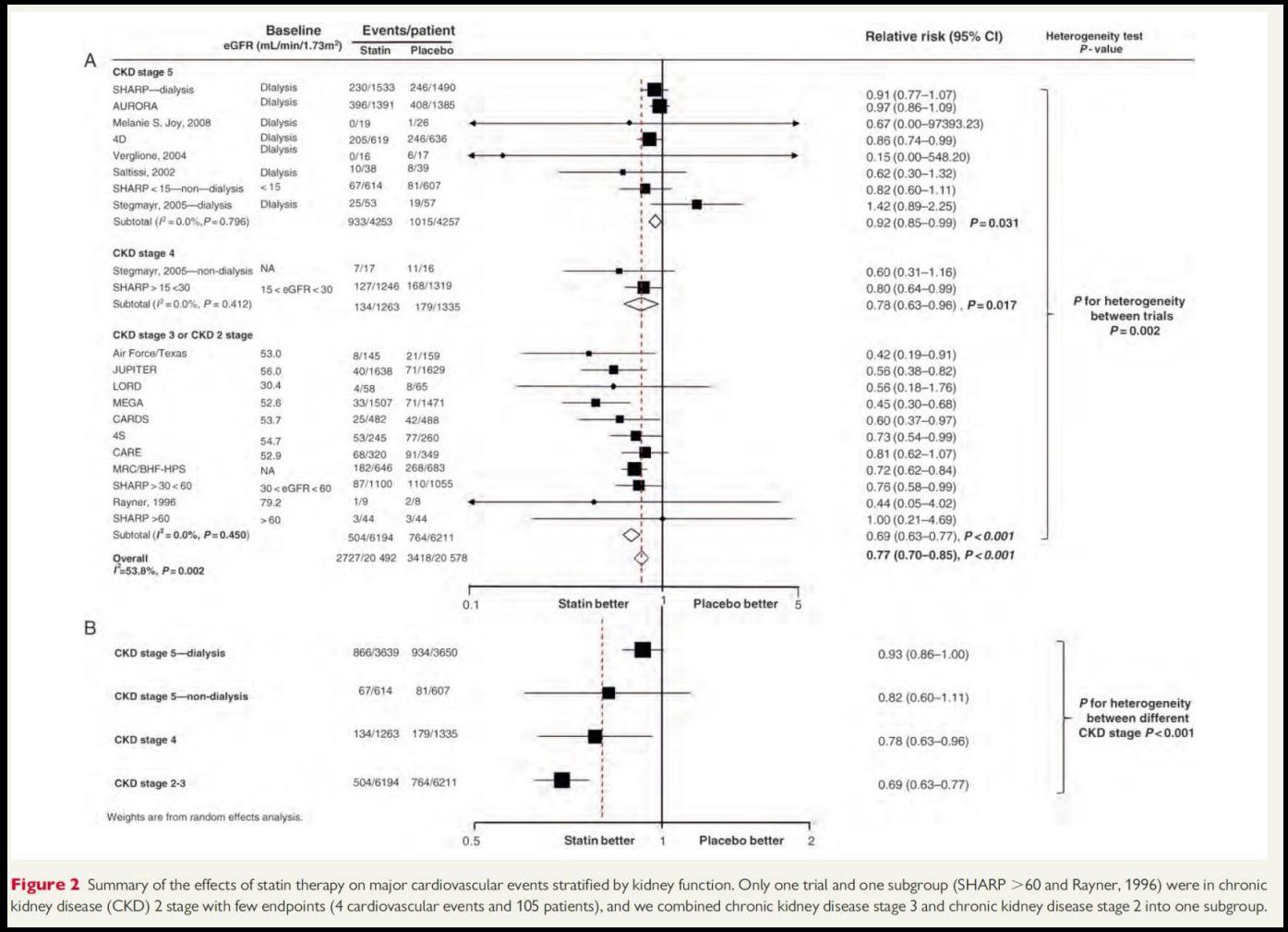



**Figure 2** Summary of the effects of statin therapy on major cardiovascular events stratified by kidney function. Only one trial and one subgroup (SHARP >60 and Rayner, 1996) were in chronic kidney disease (CKD) 2 stage with few endpoints (4 cardiovascular events and 105 patients), and we combined chronic kidney disease stage 3 and chronic kidney disease stage 2 into one subgroup.

Hou W et al. Eur Heart J 2013;34:1807-1817

# Renal Clearance of Statins

- Most statins are metabolized by the liver and the renal clearance is minimal
- Hydrophilic statins, pravastatin and rosuvastatin, have significant clearance by the kidneys
- With the exception of patients on dialysis or with end-stage renal disease, no dose adjustment is recommended except for pravstatin and rosuvastatin

Mach F et al. Eur Heart J 2018;

# Statins and Renal Function

| Benefit | ? Risk |
| --- | --- |
| • Statin treatment reduces CVD events by 20% in patients with CKD<br>• No benefit in dialysis patients | • Mild proteinuria, especially with intensive statin therapy BUT not associated with deterioration in function |
| | • Meta-analyses in CKD patients showed no increase in progression of CKD or acute renal events on statin therapy |

Baigent C et al. Lancet 2011;377:2181-92; Palmer SC et al. Cochrane Database Syst Rev 2014:CD007784; Sanguankeo A et al. PLoS One 2015;10:e0132970; Zhang Z et al. Pharmacol Res 2016;105:74-83

# Statins and Renal Function

## Take Home Messages:

- Statin treatment is not associated with clinically significant deterioration of renal function
- Dose reduction based on eGFR may be prudent in patients with severe kidney dysfunction who are receiving intensive statin regimens
- A protective effect of statins on the kidney cannot be excluded but further study is merited

Mach F et al. Eur Heart J 2018;

# Statins and Hepatic Function

# Background to Drug-Related Hepatotoxicity

- Drug-related hepatotoxicity is relatively uncommon
- True incidence is difficult to determine; spontaneously reported rates are likely to be an underestimate
- Difficult to detect in clinical trials
- Need to distinguish between injury and function
- Adults and females more sensitive
- Genetic susceptibility may be relevant

Mach F et al. Eur Heart J 2018;

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# Statin-induced Elevation in Liver Enzymes

| Evidence | Conclusion |
| --- | --- |
| Retrospective pooled analysis of 49 trials (n=14,236)<br>• 0.1%, 0.6%, and 0.2% of patients on atorvastatin 10 mg, 80 mg, or placebo had clinically relevant ALT elevation (≥3 x ULN on 2 occasions) | **Clinically relevant transaminase elevation with statin therapy is rare** |
| Network meta-analysis of 135 RCTs (n=246,955)<br>• Low frequency of clinically significant transaminase elevation with statin therapy<br>• Higher doses of statins were associated with higher likelihood of transaminase elevation | |

Naci H et al. Circ Cardiovasc Qual Outcomes 2013;6:390-9; Newman C et al. Am J Cardiol 2006;97:61–7.

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# Statin-Induced Liver Injury

| Evidence | Conclusion |
| --- | --- |
| UK General Practice Database (1997-2006)<br>• Statin-induced liver injury is rare but higher with atorvastatin than simvastatin (0.09% versus 0.06%, hazard ratio 1.9, 95% CI 1.4-2.6, p<0.001)<br>• Reporting rates were higher at higher doses | **Statin-induced liver injury is very rare** |
| FDA Adverse Drug Event Reporting System database<br>• Reporting rates for severe statin-induced liver injury were very low (≤2 per million patient-years) | |
| Swedish Adverse Drug Reactions Advisory Committee (1998-2010)<br>• Statin-induced liver injury reported for 1.2 per 100,000<br>• Re-exposure to statin can produce the same response | |

http://www.fda.gov/; Björnsson E et al. J Hepatol 2012;56:374-80; Clarke AT et al. PLoS One 2016;11:e0151587

# Statins and Hepatic Function

**Take home messages:**

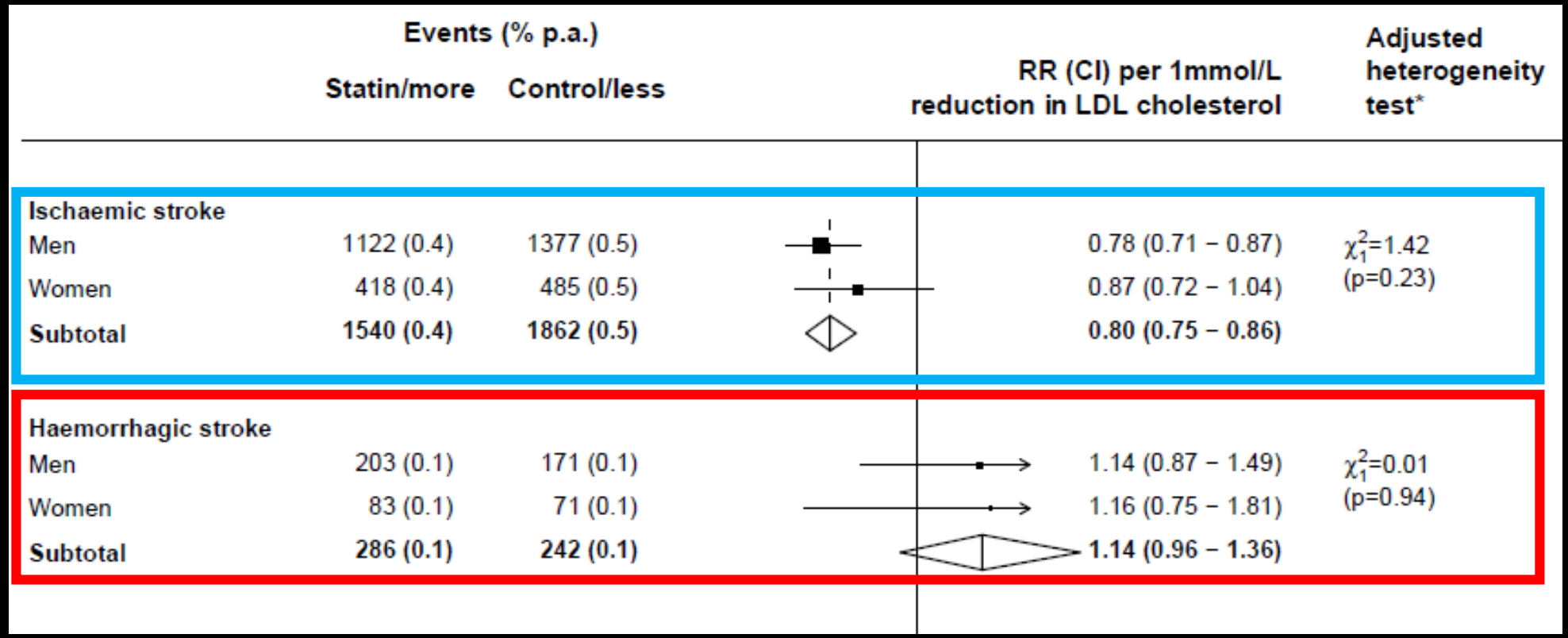
- Mild ALT elevation in isolation in asymptomatic statin users is not clinically relevant. In patients with mild ALT elevation due to steatosis or non-alcoholic fatty liver disease, statin therapy does not worsen liver disease.
- Clinically apparent liver injury with statin therapy is very rare and likely to be a class effect of statins.
- Routine periodic monitoring of liver enzymes is not justified
- Liver enzymes should be measured in the rare patient who develops symptoms suggestive of hepatotoxicity.

Mach F et al. Eur Heart J 2018;

# Statins and Risk for Haemorrhagic Stroke

# Statins and Risk for Haemorrhagic Stroke

- CTT meta-analysis of statin trials: 14% increase in haemorrhagic stroke/ mmol/L LDL-C reduction; BUT this was driven by the SPARCL data
- The risk for haemorrhagic stroke is outweighed by reduction in the risk of ischaemic stroke



| | Events (% p.a.) | | RR (CI) per 1mmol/L reduction in LDL cholesterol | Adjusted heterogeneity test* |
|---|---|---|---|---|
| | Statin/more | Control/less | | |
| **Ischaemic stroke** | | | | |
| Men | 1122 (0.4) | 1377 (0.5) | 0.78 (0.71 – 0.87) | $\chi^2_1$=1.42 (p=0.23) |
| Women | 418 (0.4) | 485 (0.5) | 0.87 (0.72 – 1.04) | |
| **Subtotal** | **1540 (0.4)** | **1862 (0.5)** | **0.80 (0.75 – 0.86)** | |
| **Haemorrhagic stroke** | | | | |
| Men | 203 (0.1) | 171 (0.1) | 1.14 (0.87 – 1.49) | $\chi^2_1$=0.01 (p=0.94) |
| Women | 83 (0.1) | 71 (0.1) | 1.16 (0.75 – 1.81) | |
| **Subtotal** | **286 (0.1)** | **242 (0.1)** | **1.14 (0.96 – 1.36)** | |

Collins R et al. Lancet 2016;388:2532-2561

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# Statins and Risk for Haemorrhagic Stroke

Collaborative meta-analysis (n=248,391)

- No increase in risk of intracerebral haemorrhage in
  - RCTs (RR 1.10, 0.86-1.41),
  - Cohort studies (RR 0.94, CI 0.81-1.10)
  - Case-control studies (RR 0.60, 0.41-0.88)

Hackam DG et al. Circulation 2011;124:2233-2242

EAS

# Statins and Risk for Haemorrhagic Stroke

**Take Home Messages**:

- Statin treatment reduces the risk of first or subsequent ischaemic strokes by 15-35% per mmol/L reduction in LDL-C.
- While SPARCL suggested a small increase in haemorrhagic stroke in subjects with prior stoke, this possible increased risk associated with LDL-C reduction has not been confirmed by analysis of a substantive evidence base of RCTs, cohort studies and case-control studies.
- No alteration in the statin regimen in patients with a history of cerebrovascular disease is indicated

Mach F et al. Eur Heart J 2018;

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# Statins and Risk for Cataract

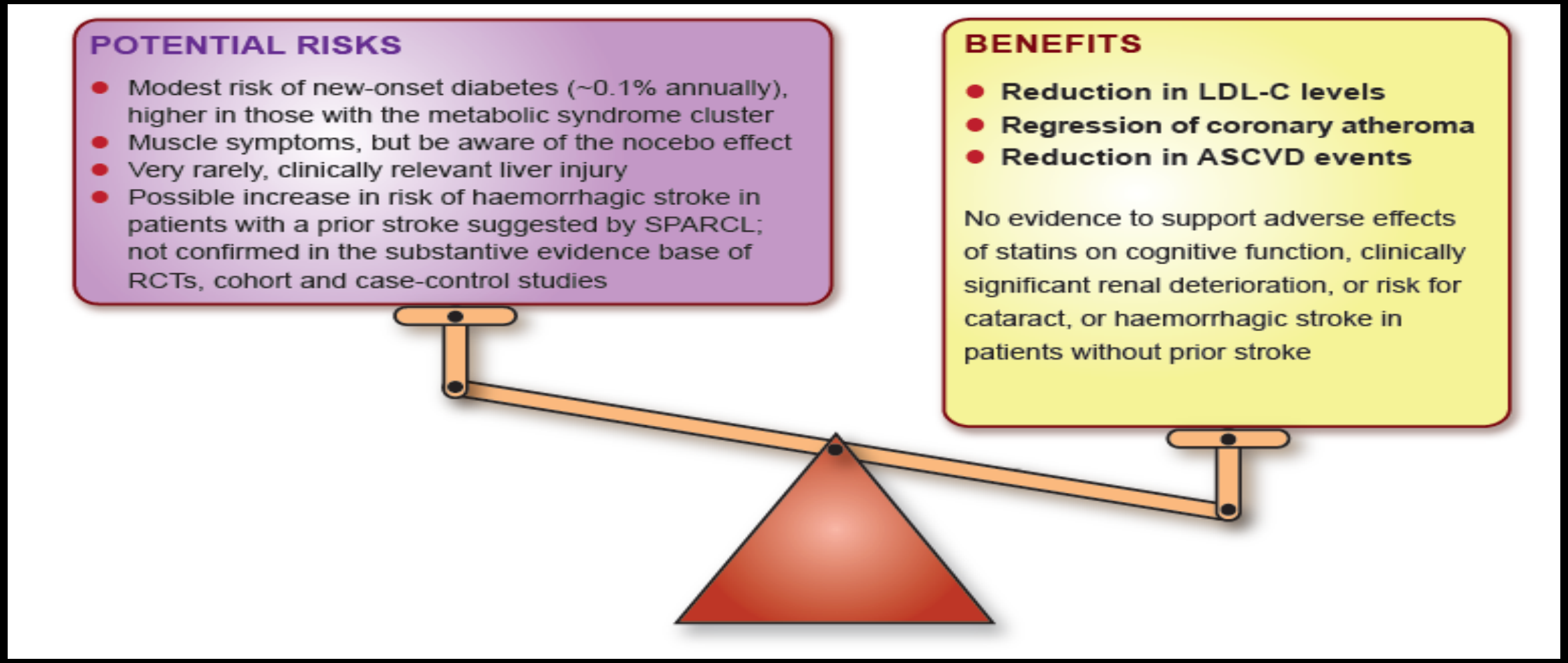
# Statins and Risk for Cataract

**Take Home Messages:**

- Statin treatment is not associated with cataract development.
- No change in cardiovascular prevention strategies are indicated, even in patients with cataracts.

Mach F et al. Eur Heart J 2018;

# Statins: Highly Favourable Benefit vs. Risk Ratio




***..' the Panel emphasizes that the established cardiovascular benefits of statin therapy far outweigh the risk of any such adverse effects '***

Mach F et al. Eur Heart J 2018;

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EAS

# European Atherosclerosis Society Consensus Panel:

François Mach, Kausik K. Ray, Olov Wiklund, Alberto Corsini,
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Lawrence A. Leiter, Winfried März, Børge G. Nordestgaard, Frederick J. Raal,
Michael Roden, Raul D. Santos, Evan A. Stein, Erik S. Stroes, Paul D. Thompson,
Lale Tokgözoğlu Georgirene D. Vladutiu, Baris Gencer, Jane K. Stock,
Henry N. Ginsberg, M. John Chapman

***European Heart Journal* 2018;**

Circulation

ORIGINAL RESEARCH ARTICLE

# Benefit of Adding Ezetimibe to Statin Therapy on Cardiovascular Outcomes and Safety in Patients With Versus Without Diabetes Mellitus

## Results From IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial)

Editorial, see p 1583

Robert P. Giugliano, MD, SM
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Jennifer A. White, MS
Erin A. Bohula, MD, DPhil
Eugene Braunwald, MD
On behalf of the IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial) Investigators

**BACKGROUND:** Ezetimibe, when added to simvastatin, reduces cardiovascular events after acute coronary syndrome. We explored outcomes stratified by diabetes mellitus (DM).

**METHODS:** In IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial), 18 144 patients after acute coronary syndrome with low-density lipoprotein cholesterol 50 to 125 mg/dL were randomized to 40 mg ezetimibe/simvastatin (E/S) or 40 mg placebo/simvastatin. The primary composite end point was cardiovascular death, major coronary events, and stroke. DM was a prespecified subgroup.

**RESULTS:** The 4933 (27%) patients with DM were more often older and female, had had a prior myocardial infarction and revascularization, and presented more frequently with non-ST segment elevation acute coronary syndrome compared with patients without DM (each $P<0.001$). The median admission low-density lipoprotein cholesterol was lower among patients with DM (89 versus 97 mg/dL, $P<0.001$). E/S achieved a significantly lower median time-weighted average low-density lipoprotein cholesterol compared with placebo/simvastatin, irrespective of DM (DM: 49 versus 67 mg/dL; no DM: 55 versus 71 mg/dL; both $P<0.001$). In patients with DM, E/S reduced the 7-year Kaplan–Meier primary end point event rate by 5.5% absolute (hazard ratio, 0.85; 95% confidence interval, 0.78–0.94); in patients without DM, the absolute difference was 0.7% (hazard ratio, 0.98; 95% confidence interval, 0.91–1.04; $P_{int}=0.02$). The largest relative reductions in patients with DM were in myocardial infarction (24%) and ischemic stroke (39%). No differences in safety outcomes by treatment were present regardless of DM. When stratified further by age, patients ≥75 years of age had a 20% relative reduction in the primary end point regardless of DM ($P_{int}=0.91$), whereas patients <75 years of age with DM had greater benefit than those without ($P_{int}=0.011$). When stratified by the TIMI (Thrombolysis in Myocardial Infarction) Risk Score for Secondary Prevention, all patients with DM demonstrated benefit with E/S regardless of risk. In contrast, among patients without DM, those with a high risk score experienced a significant (18%) relative reduction in the composite of cardiovascular death, myocardial infarction, and ischemic stroke with E/S compared with placebo/simvastatin, whereas patients without DM at low or moderate risk demonstrated no benefit with the addition of ezetimibe to simvastatin ($P_{int}=0.034$).

**CONCLUSIONS:** In IMPROVE-IT, the benefit of adding ezetimibe to statin was enhanced in patients with DM and in high-risk patients without DM.

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## Clinical Perspective

### What Is New?

- In IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial), patients with recent acute coronary syndrome were randomized to ezetimibe versus placebo on top of background simvastatin. We found that patients with diabetes mellitus derived significantly greater relative and absolute benefit with the addition of ezetimibe relative to patients without diabetes mellitus.
- This enhanced benefit was driven by reductions of acute ischemic events, including myocardial infarction and ischemic stroke in patients with diabetes mellitus, whereas patients without diabetes mellitus >75 years of age or with a high risk score also significantly benefited from the addition of ezetimibe to simvastatin.
- The benefits of ezetimibe were achieved without an increase in safety events compared with placebo.

### What Are the Clinical Implications?

- In patients admitted with an acute coronary syndrome and low-density lipoprotein cholesterol ≥50 mg/dL, healthcare providers should consider adding ezetimibe to statin to reduce the risk of cardiovascular events.
- Two patient subgroups likely to achieve greater benefits with the addition of ezetimibe include patients with diabetes and patients without diabetes who have a high risk score.
- These findings support the 2017 American Association of Clinical Endocrinologists and American College of Endocrinology Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease treatment goal of a low-density lipoprotein cholesterol <55 mg/dL in patients with extreme risk, including patients with diabetes mellitus with established clinical cardiovascular disease.

The number of individuals with diabetes mellitus (DM) has more than doubled in the last 3 decades,[1] affecting 9% of all adults worldwide in 2014.[2] Because patients with DM are at increased risk of developing coronary artery disease[3] and have poorer outcomes after acute coronary syndromes (ACS),[4] more effective treatments to prevent ischemic cardiovascular events in patients with DM are highly desirable. Statins, lifestyle modifications, and other interventions to reduce coronary artery disease risk factors, such as antihypertensive medications, are recommended for all patients with DM.[5] However, despite the recognition of this multifaceted approach, patients with DM who have experienced an acute coronary event remain at increased risk for subsequent coronary events, stroke, and vascular death.[6]

Ezetimibe is a nonstatin that inhibits absorption of cholesterol from the small intestine, reducing low-density lipoprotein cholesterol (LDL-C) by 23% to 24% when added to a statin.[7] In patients with DM, ezetimibe lowers LDL-C and reduces levels of other atherogenic particles, such as remnant-like particle cholesterol, small dense LDL-C, malondialdehyde-modified LDL, apoliprotein B-48, and ratios of total cholesterol/high-density lipoprotein cholesterol and apolipoprotein B/apolipoprotein A-I.[8,9] Although statins have been shown to improve cardiovascular outcomes in patients with DM with[6,10,11] and without prior clinically recognized coronary artery disease,[6,12] guidelines for the management of patients with DM published in 2015[12a] note that there has been insufficient evidence to support the addition of nonstatin therapies (ie, ezetimibe, niacin, fenofibrate, bile acid sequestrants) to further reduce cardiovascular risk in patients with DM.

As previously reported,[13] the combination of ezetimibe and simvastatin (E/S) reduced the median time-weighted average LDL-C by 16 mg/dL compared with placebo and simvastatin (P/S), with a significant 2.0% absolute reduction (6.4% relative reduction, $P$=0.016) in the primary composite end point (cardiovascular death, major coronary event, or stroke) after a median of 6 years in patients admitted with ACS. Of 19 subgroup analyses prespecified in the statistical analysis plan, 2 treatment-subgroup interactions (baseline diabetes mellitus status and age dichotomized at 75 years) had a nominally significant $P_{int}$ <0.05 for the primary end point. Here we present an analysis of the efficacy and safety of E/S versus P/S in patients enrolled in IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial) stratified by the presence of DM at randomization.

## METHODS

The authors support the spirit and intent of sharing of clinical trial data. We encourage interested parties to contact the corresponding author directly for further discussions. The IMPROVE-IT protocol[14] and the main results[13] have been described previously. The ethics committee at each participating center approved the protocol and amendments, and all subjects provided informed consent. DM at hospital admission for the qualifying ACS event was determined by the investigators based on a history of DM (regardless of duration), treatment with an antidiabetic agent, or a fasting blood sugar >126 mg/dL. A sensitivity analysis was performed to also include patients identified from review of the trial database who had a fasting glucose >126 mg/dL, a nonfasting glucose >200 mg/dL, or a hemoglobin A1c ≥6.5% on the first sample obtained after randomization.

Baseline characteristics, medications, and laboratory test results were compared in patients with and without DM. Lipid levels (total cholesterol, LDL-C, HDL-C, triglycerides) were measured locally on admission with the qualifying ACS event (defined as ≤24 hours after presentation, or if unavailable, a

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value from the prior 6 months was used provided the patient had been clinically stable with no changes in lipid-lowering therapy). Lipid levels and high-sensitivity C-reactive protein (hsCRP) were measured at a core laboratory at randomization, after randomization at 1, 4, and 8 months, and annually thereafter. A combined analysis of LDL-C and hsCRP at 30 days was conducted with the prespecified dual target achievement defined as <70 mg/dL for LDL-C and <2.0 mg/dL for hsCRP.[15]

The primary efficacy end point was a composite of cardiovascular death, major coronary event (eg, myocardial infarction [MI], unstable angina requiring hospital admission, coronary revascularization occurring ≥30 days after randomization), or stroke, and it was reported as a Kaplan–Meier event rate at 7 years. Other efficacy end points and safety outcomes of special interest were as described in the main trial.[13] Efficacy end points and muscle-related adverse events were adjudicated by an independent clinical end point committee that was unaware of treatment assignment. Because patients ≥75 years of age derived particular benefit with E/S compared with P/S,[13] an analysis stratified by age and diabetes mellitus status was also performed. In addition, analyses were conducted in patients stratified by the TIMI (Thrombolysis in Myocardial Infarction) Risk Score for Secondary Prevention, a simple 9-point risk stratification tool previously developed in a large population with atherothrombosis[16] to predict cardiovascular events that was subsequently validated in the IMPROVE-IT population.[17] Because patients treated with insulin represent an especially high-risk subgroup with more advanced DM, outcomes by treatment group, among patients with DM stratified by use of insulin, were also conducted.

## Statistical Analysis

The primary analyses were performed using the intention-to-treat principle, including all patients randomized, and counting first events between randomization and the final visit or last patient contact. A sensitivity analysis was conducted in the on-treatment population (including all patients who took ≥1 dose of the study drug), censoring events that occurred >30 days after the last dose of study drug. Continuous variables were reported as mean values±standard deviation or median values with 25th and 75th percentiles depending on their distribution and compared using Wilcoxon rank-sum test statistics. Categorical variables were compared using the $\chi^2$ test. A $P$ value <0.05 was considered to represent nominal statistical significance. Adjustments for multiple testing were not performed for the analyses because all comparisons, other than the prespecified analysis of the primary end point stratified by the presence of diabetes mellitus at baseline, were considered exploratory. Cox proportional hazard models were developed to assess the time to the first clinical end point. Models were stratified by protocol-specified stratification factors to evaluate the presence of an interaction between diabetic mellitus status and randomized treatment. $P$ values for subgroup x treatment interactions were calculated using Cox proportional hazard or logistic regression models as appropriate, with a $P_{int}$ <0.05 indicative of a significant interaction. $P$ values for comparisons of 2 groups on dichotomous/categorical responses controlled by a covariate were calculated using the Cochran–Mantel–Haenszel test or logistic regression (for binary outcomes). All analyses were performed using SAS (version 9.3).

# RESULTS

## Baseline Characteristics

The investigators identified DM in 4933 (27%) patients randomized (Table 1). On average, patients with DM were 2 years older; more likely to be female and have had a prior MI or coronary artery bypass grafting; and less likely to present with an ST-elevation MI ($P$<0.001 for each compared with patients without DM). Patients with DM were more likely to have been treated with guideline-supported therapies (aspirin, β-blockers, statins, angiotensin-converting-enzyme inhibitors/angiotensin receptor blockers) before the qualifying event. Before admission, statins were prescribed more frequently in patients with DM than those without. Three-quarters of patients with DM were being treated with an antidiabetic medication before admission, mostly commonly metformin (46%), sulfonylureas (25%), or insulin (21%), and 27% were treated with >1 antidiabetic agent. There were no differences in baseline characteristics, treatments, or laboratory values at admission between randomized treatment groups among patients with or without DM (Table I in the online-only Data Supplement). Patient adherence to the study drug was 2% to 3% higher among patients without DM (Table II in the online-only Data Supplement).

## Laboratory Data at Admission

The median LDL-C at admission was lower in patients with DM (89 mg/dL) compared with those without DM (97 mg/dL, $P$<0.001; Table 1, Figures I and II in the online-only Data Supplement). Patients with DM had lower median HDL-C and higher median triglycerides compared with patients without DM (both $P$<0.001).

## Changes in Lipids

In patients with DM, the median decline in LDL-C from admission to 1 year was 40 mg/dL (to a median achieved level of 46 mg/dL) with E/S compared with a median decrease of 22 mg/dL (to a median achieved level of 65 mg/dL) with P/S, resulting in a median difference in LDL-C reduction between treatments in the first year of 18 mg/dL ($P$<0.001; Figure II in the online-only Data Supplement). In patients without DM, the median LDL-C values at 1 year decreased by 44 and 27 mg/dL, and achieved median LDL-C values at 1 year were 51 and 68 mg/dL, with E/S and P/S, respectively ($P$<0.001 for both comparisons by treatment). The resultant median difference in LDL-C reduction from admission to

**Table 1. Baseline Characteristics**

| Demographics | Diabetes Absent 13 202 (72.6) | Diabetes Present 4933 (27.4) | *P* Value |
|---|---|---|---|
| Mean age (SD), y | 63.7 (9.9) | 65.3 (9.2) | <0.001 |
| Female | 2905 (22.8) | 1407 (28.5) | <0.001 |
| White | 11 359 (86.0) | 3837 (77.8) | <0.001 |
| Median weight, kg (IQR) | 80.0 (70.0, 90.7) | 84.8 (74.0, 98.0) | <0.001 |
| Median body mass index, kg/m$^2$ (IQR) | 27.0 (24.5, 30.1) | 29.2 (26.1, 33.0) | <0.001 |
| Medical history | | | |
| Hyperlipidemia | 9504 (72.0) | 3647 (73.9) | <0.001 |
| Hypertension | 7266 (55.0) | 3871 (78.5) | <0.001 |
| Current smoking | 4784 (36.2) | 1194 (24.2) | <0.001 |
| Myocardial infarction | 2541 (19.3) | 1265 (25.7) | <0.001 |
| Percutaneous coronary intervention | 2360 (17.9) | 1202 (24.4) | <0.001 |
| Coronary artery bypass grafting | 998 (7.6) | 686 (13.9) | <0.001 |
| Congestive heart failure | 410 (3.1) | 380 (7.7) | <0.001 |
| Peripheral arterial disease | 617 (4.7) | 388 (7.9) | <0.001 |
| Medications before admission | | | |
| Aspirin | 5011 (38.0) | 2643 (53.6) | <0.001 |
| β-Blocker | 4115 (31.2) | 2181 (44.2) | <0.001 |
| Statin | 3934 (29.8) | 2313 (46.9) | <0.001 |
| Angiotensin-converting enzyme inhibitor or angiotensin receptor blocker | 4470 (33.9) | 2946 (59.8) | <0.001 |
| Medications at randomization | | | |
| Aspirin | 12 827 (97.2) | 4765 (96.6) | 0.003 |
| β-Blocker | 11 517 (87.3) | 4274 (86.6) | 0.034 |
| Angiotensin-converting enzyme inhibitor or angiotensin receptor blocker | 9589 (72.6) | 4111 (83.3) | <0.001 |
| At index event | | | |
| ST-segment elevation myocardial infarction | 4177 (31.6) | 1013 (20.5) | <0.001 |
| Diagnostic angiography | 11 788 (89.3) | 4136 (83.9) | <0.001 |
| Percutaneous coronary intervention | 9499 (72.0) | 3207 (65.0) | <0.001 |
| Laboratory values at admission (median and IQR) | | | |
| Low-density lipoprotein cholesterol, mg/dL | 97 (81, 112) | 89 (74, 103) | <0.001 |
| Prior statin use | 81 (70, 93) | 78 (66, 89) | <0.001 |
| No prior statin use | 105 (91, 116) | 100 (84, 113) | <0.001 |
| High-density lipoprotein cholesterol, mg/L | 41 (34, 50) | 38 (31, 46) | <0.001 |
| Triglycerides, mg/L | 115 (81, 164) | 137 (96, 193) | <0.001 |
| Creatine clearance, ml/min | 84 (66, 106) | 86 (64, 111) | 0.027 |
| Laboratory values at randomization (median and IQR) | | | |
| Low-density lipoprotein cholesterol, mg/dL | 81 (67, 97) | 75 (61, 91) | <0.001 |
| Statin during admission | 78 (65, 93) | 73 (59, 87) | <0.001 |
| No statin during admission | 93 (76, 110) | 89 (71, 106) | <0.001 |
| C-reactive protein,* mg/L | 9.5 (3.9, 26.5) | 9.7 (4.0, 26.6) | 0.740 |

Data shown are n (%) unless otherwise indicated. IQR indicates interquartile range.
*C-reactive protein was not routinely collected at admission; values closest to randomization are shown.

year 1 in patients without DM between treatments of 17 mg/dL was similar to that observed in patients with DM (18 mg/dL, $P_{int}$ =0.58). The reduction in LDL-C with E/S compared with P/S persisted throughout follow-up (Figure I in the online-only Data Supplement), although the difference between treatment groups in the time-

weighted reduction in LDL-C after admission was 3 mg/dL greater in patients with DM (17 mg/dL) compared with patients without DM (14 mg/dL, $P_{int}$=0.03).

Patients treated with E/S compared with P/S also achieved greater reductions in total cholesterol, triglycerides, and non-HDL-C during the trial among patients with and without DM. The median time-weighted average reduction in total cholesterol with E/S versus P/S was greater in patients with DM (19 mg/dL) than in patients without DM (16 mg/dL; $P_{int}$ =0.022), whereas reductions with the addition of ezetimibe in triglycerides (DM: 11 mg/dL; no DM: 8 mg/dL; $P_{int}$ =0.58) and non-HDL-C (DM: 19 mg/dL; no DM: 17 mg/dL; $P_{int}$ =0.10) were similar regardless of diabetic mellitus status.

## Reduction in hsCRP at 1 Month

The median hsCRP levels at randomization were 9.7 and 9.5 mg/L among patients with versus without DM, respectively (*P*=0.74). E/S compared with P/S reduced hsCRP to a similar degree in patients with DM (−0.3 mg/dL) compared with patients without DM (−0.2 mg/dL, $P_{int}$ =0.93).

## Achievement of Prespecified Dual Targets of LDL-C and hsCRP

The prespecified dual targets of LDL-C <70 mg/dL and hsCRP <2.0 mg/L were achieved more frequently with E/S than with P/S, both among patients with DM (46% versus 30%, *P*<0.001) and in those without DM (52% versus 31%, *P*<0.001). There was evidence of statistical heterogeneity indicating an even greater likelihood of achieving the dual targets with E/S in patients without DM (Cochran–Mantel–Haenszel, *P*<0.001, with *P*=0.02 for treatment arm difference in odds ratios).

## Clinical Efficacy

Efficacy outcomes occurred more frequently in patients with DM compared without those without DM. For the primary composite end point, the Kaplan–Meier event rates at 7 years in patients with DM were 40.0% versus 45.5% in patients treated with E/S versus P/S (hazard ratio [HR], 0.85; 95% confidence interval [CI], 0.78–0.94), and the corresponding rates in patients without DM were 30.2 versus 30.8% (HR, 0.98; 95% CI, 0.91–1.04; Figures 1 and 2, Table III in the online-only Data Supplement). This difference in treatment benefit with the ad-

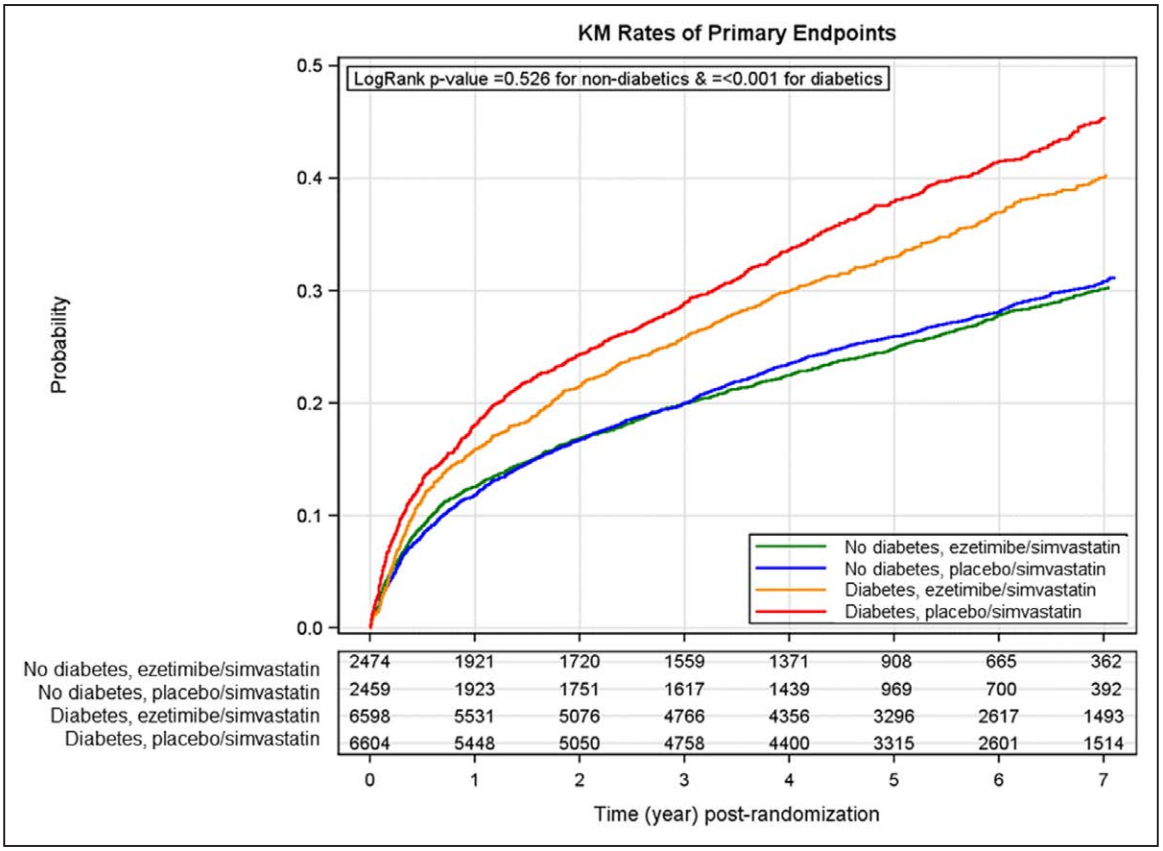


**Figure 1. Kaplan–Meier curves for the primary efficacy end point.**
Cumulative event rates for the primary composite end point of cardiovascular death, major coronary event (eg, nonfatal myocardial infarction, unstable angina requiring hospitalization, or coronary revascularization occurring ≥30 days after randomization), or nonfatal stroke in the intention-to-treat population during the overall study period (ie, from randomization to the first occurrence of a primary end point event or last contact with the patient).

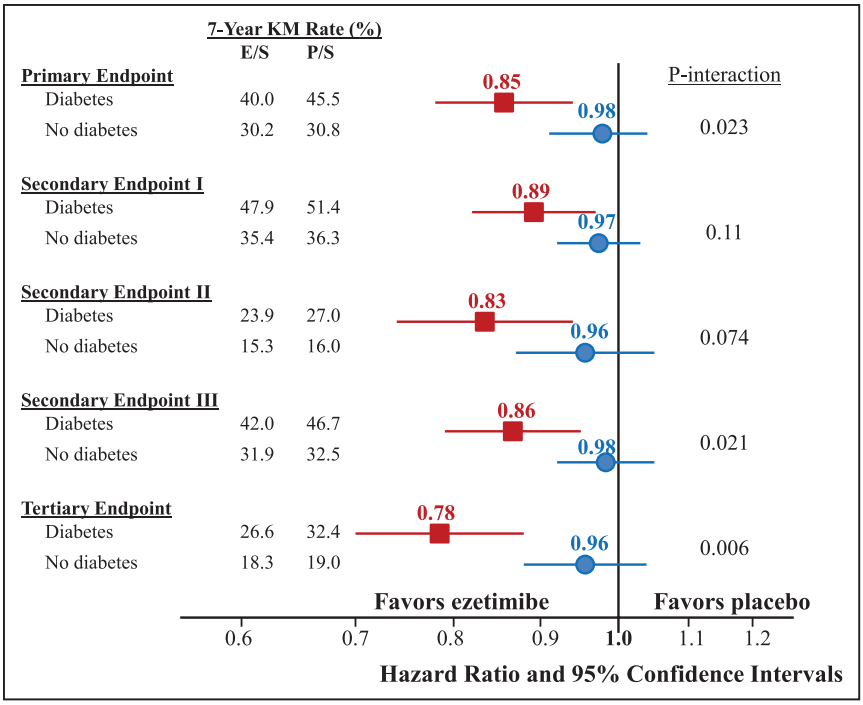


**Figure 2. Composite efficacy outcomes stratified by treatment and diabetes mellitus status.** Hazard ratios and 95% confidence intervals are shown for the comparison of ezetimibe/simvastatin (E/S) versus placebo/simvastatin (P/S) in patients with (red) and without (blue) diabetes mellitus. See text and the footnotes to Table 2 for definitions of the composite endpoints.

dition of ezetimibe in patients with DM versus without DM was significant (*P* value of 0.023 for interaction). Among patients with DM, combination E/S prevented 1 event for every 18 (95% CI, 12.0–42.0) patients treated on average for 6 years compared with P/S.

The HRs comparing E/S with P/S for the 3 secondary efficacy composite end points and the tertiary composite end point are shown in Figure 2 and Table III in the online-only Data Supplement. For 2 of these composite end points (secondary composite III: cardiovascular death, MI, unstable angina, all revascularization on/after 30 days, stroke; tertiary composite of CHD death, unstable angina, MI, and ischemic stroke), the $P_{int}$ values were significant (0.021 and 0.006, respectively). For the 2 other secondary composite end points, the HRs comparing E/S with P/S were numerically lower in patients with DM, although the $P_{int}$ values were not significant (0.11 and 0.074, respectively).

The results for other end points are shown in Table IV in the online-only Data Supplement. Patients with DM exhibited significantly lower HRs with E/S versus P/S for the end points of MI (HR, 0.76; 95% CI, 0.66–0.88), ischemic stroke (HR, 0.61; 95% CI, 0.46–0.82), and the composite of cardiovascular death, MI, or stroke (HR, 0.80; 95% CI, 0.71–0.90) compared with patients without DM (interaction *P* values of 0.028, 0.031, and 0.016, respectively). Urgent revascularization was significantly and similarly reduced in patients with DM (HR, 0.76; 95% CI, 0.62–0.93) and without DM (HR, 0.84; 95% CI, 0.73–0.97; $P_{int}$ =0.40). Mortality end points and hospitalization for unstable angina were not reduced with E/S versus P/S in patients with or without DM.

## Efficacy Outcomes Stratified by Age and DM Status

Among patients ≥75 years of age, E/S when compared with P/S significantly reduced the primary end point to a similar degree in patients with DM (HR, 0.80) and without DM (HR, 0.79; $P_{int}$ =0.91; Table 2, Figure III in the online-only Data Supplement). The high event rates in elderly patients at 7 years (Figure 3) resulted in numbers needed to treat of 10 (95% CI, 5.0–73.0) in patients with DM and 12 (95% CI, 7.0–28.0) in patients without DM.

In contrast, among patients <75 years of age, there was evidence of a significant treatment–DM subgroup interaction. In these patients with DM, E/S significantly reduced the primary end point compared with P/S (HR, 0.87; 95% CI, 0.78–0.96; *P*=0.008; numbers needed to treat, 21 [95% CI, 12.0–73.0]), whereas in patients <75 years of age without DM, there was no difference between treatments (HR, 1.02; 95% CI, 0.95–1.10; $P_{int}$= 0.01). Likewise, there was evidence of similar interactions in patients <75 years of age for several secondary end points (Table 2, Figure III in the online-only Data Supplement), whereby the treatment benefit with E/S was greater among such patients with DM than in such patients without DM.

## Risk Stratification and Outcomes in Patients With and Without DM

When patients were stratified by the TIMI Risk Score for Secondary Prevention,[16] more patients with DM versus no DM were classified as high risk (≥3 risk indicators: 55% versus 13%), whereas far fewer patients with DM

**Table 2. Outcomes, by Age and Diabetes Mellitus Status**

| End Points | Age, y | History of Diabetes Mellitus | Placebo/Simvastatin | | Ezetimibe/Simvastatin | | Hazard Ratio (95% CI) | *P* Value | Interaction *P* Value* |
|---|---|---|---|---|---|---|---|---|---|
| | | | n (%) | KM Event (%) at 7 y | n (%) | KM Event (%) at 7 y | | | |
| Primary end points | <75 | No | 1429 (25.6) | 28.82 | 1460 (25.9) | 29.44 | 1.02 (0.95–1.10) | 0.522 | 0.011 |
| | | Yes | 749 (36.4) | 42.90 | 658 (32.0) | 38.17 | 0.87 (0.78–0.96) | 0.008 | |
| | ≥75 | No | 363 (36.0) | 42.94 | 288 (29.8) | 34.46 | 0.79 (0.68–0.92) | 0.003 | 0.913 |
| | | Yes | 200 (47.8) | 59.94 | 166 (41.3) | 49.86 | 0.80 (0.65–0.99) | 0.039 | |
| Secondary end points I | <75 | No | 1666 (29.8) | 33.27 | 1664 (29.5) | 33.12 | 1.00 (0.94–1.07) | 0.963 | 0.069 |
| | | Yes | 852 (41.4) | 47.81 | 777 (37.8) | 44.53 | 0.90 (0.81–0.99) | 0.026 | |
| | ≥75 | No | 478 (47.4) | 53.02 | 420 (43.4) | 48.75 | 0.88 (0.77–1.00) | 0.049 | 0.900 |
| | | Yes | 249 (59.6) | 69.96 | 228 (56.7) | 63.96 | 0.86 (0.72–1.03) | 0.105 | |
| Secondary end points II | <75 | No | 672 (12.0) | 14.09 | 684 (12.1) | 14.26 | 1.02 (0.92–1.14) | 0.707 | 0.042 |
| | | Yes | 414 (20.1) | 24.17 | 355 (17.3) | 21.95 | 0.85 (0.74–0.98) | 0.023 | |
| | ≥75 | No | 223 (22.1) | 27.45 | 174 (18.0) | 21.82 | 0.79 (0.65–0.96) | 0.019 | 0.967 |
| | | Yes | 138 (33.0) | 42.39 | 109 (27.1) | 34.36 | 0.78 (0.61–1.01) | 0.059 | |
| Secondary end points III | <75 | No | 1513 (27.1) | 30.51 | 1546 (27.4) | 31.13 | 1.03 (0.96–1.10) | 0.489 | 0.012 |
| | | Yes | 778 (37.8) | 44.16 | 688 (33.4) | 39.88 | 0.87 (0.79–0.97) | 0.009 | |
| | ≥75 | No | 371 (36.8) | 44.08 | 306 (31.6) | 36.18 | 0.82 (0.71–0.96) | 0.012 | 0.993 |
| | | Yes | 206 (49.3) | 61.02 | 176 (43.8) | 53.52 | 0.83 (0.68–1.02) | 0.070 | |
| Tertiary end points | <75 | No | 802 (14.3) | 16.83 | 820 (14.5) | 17.18 | 1.02 (0.93–1.13) | 0.639 | 0.003 |
| | | Yes | 492 (23.9) | 28.98 | 397 (19.3) | 24.15 | 0.79 (0.69–0.90) | 0.001 | |
| | ≥75 | No | 268 (26.6) | 31.63 | 205 (21.2) | 25.39 | 0.77 (0.64–0.92) | 0.004 | 0.856 |
| | | Yes | 168 (40.2) | 50.97 | 129 (32.1) | 39.93 | 0.75 (0.60–0.95) | 0.016 | |

CI indicates confidence interval; and KM, Kaplan-Meier. Primary end points: cardiovascular death, nonfatal myocardial infarction, unstable angina, coronary revascularization ≥30 days after randomization, or nonfatal stroke.

Secondary end points I: all death, nonfatal myocardial infarction, unstable angina, coronary revascularization ≥30 days after randomization, or nonfatal stroke.

Secondary end points II: coronary heart disease death, nonfatal myocardial infarction, or urgent coronary revascularization ≥30 days after randomization.

Secondary end points III: cardiovascular death, nonfatal myocardial infarction, unstable angina, all arterial revascularization (coronary and noncoronary) ≥30 days after randomization, or nonfatal stroke.

Tertiary end points: coronary heart disease death, unstable angina requiring hospitalization, nonfatal myocardial infarction, and nonfatal ischemic stroke.

*Interaction effect of treatment arm and history of diabetes mellitus using Cox proportional hazards regression modeling.

were classified as low risk (0–1 risk indicators: 9% versus 59%, *P*<0.001 for both; Figure IV in the online-only Data Supplement). In patients with DM, the benefit of E/S over P/S in reducing the composite of cardiovascular death, MI, and ischemic stroke was consistent across the risk strata ($P_{int}$ =0.59; Figure 4A). In contrast, in patients without DM, there was a significant effect modification by the risk score ($P_{int}$ =0.034), with patients without DM at high risk experiencing a significant 18% reduction with E/S compared with P/S, whereas patients without DM with moderate and low risk did not demonstrate a significant difference between treatments (Figure 4B).

## Safety Outcomes

Overall, patients with and without DM had similar rates of transaminase elevation and cancer. However, patients with DM were more likely to experience gall bladder- and muscle-related adverse events than those without DM (Table 3). Rates of prespecified safety events of special interest were similar between E/S and P/S, irrespective of DM status, with the possible exception of hemorrhagic stroke. In patients with DM, the rates of hemorrhagic stroke were 0.9% with E/S versus 0.4% with P/S (*P*=0.023). However, the treatment-subgroup interaction *P* value was not statistically significant (*P*=0.092).

## Sensitivity Analyses

In the first sensitivity analysis using a definition of existing DM that incorporated glucose values at randomization, the 5284 patients meeting this broader definition of DM who were randomized to E/S versus P/S had a greater reduction of the primary composite end point (HR, 0.84) compared with those without DM (HR, 0.99; $P_{int}$ =0.006). There were similar significant interactions for the 3 secondary and 1 tertiary composite end points, demonstrating consistently greater benefit of E/S among patients with this alternative definition of existing DM (Table V in the online-only Data Supplement). There were no differences in the safety out-

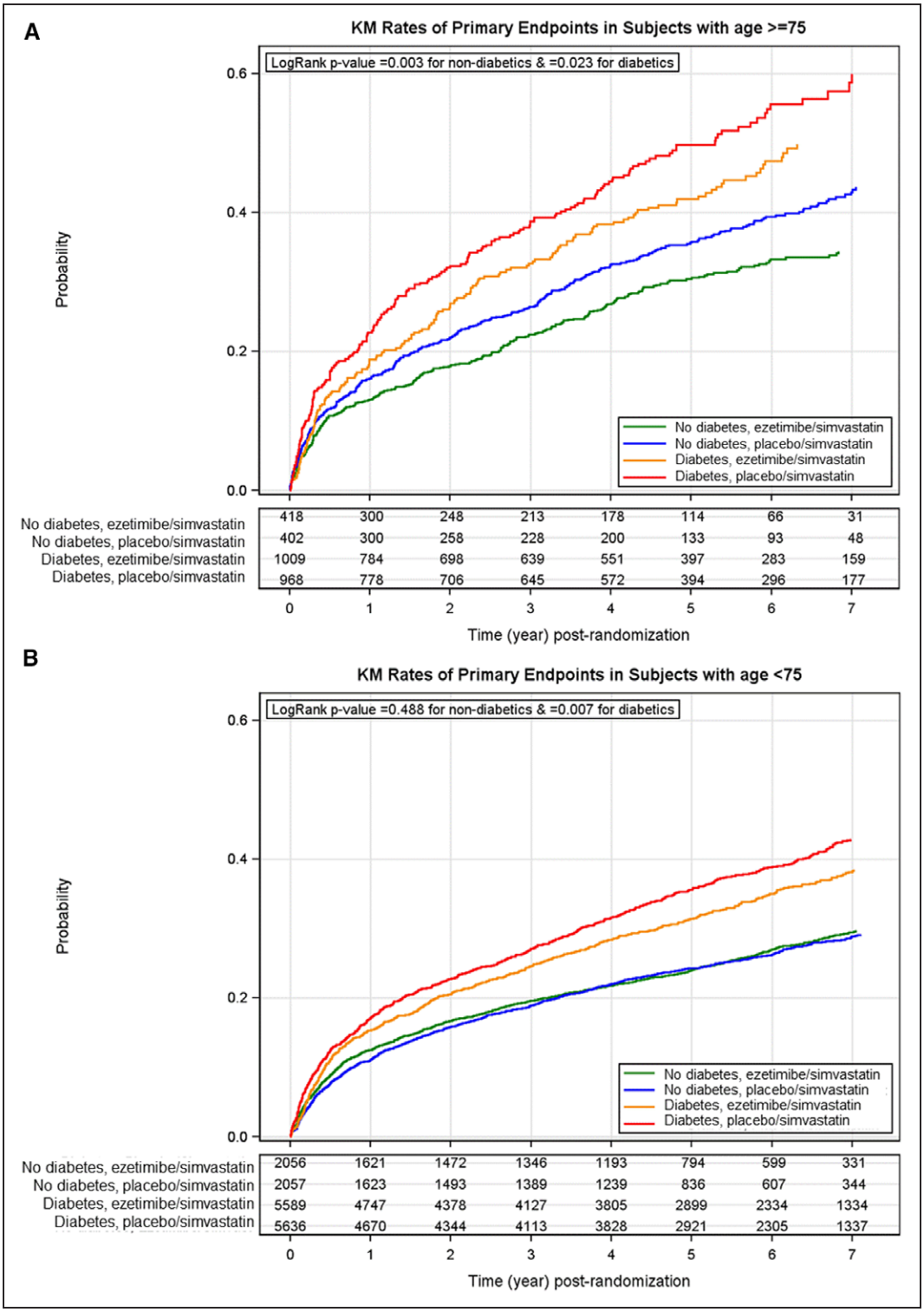


**Figure 3. Kaplan–Meier curves for the primary efficacy end point stratified by age and diabetes mellitus status.** **A,** Cumulative event rates for the primary composite end point in patients ≥75 years of age stratified by diabetes mellitus status. **B,** Similar curves for patients <75 years of age.

comes of special interest when patients were stratified by this definition of DM.

In the second sensitivity analysis of primary composite end points conducted in 17 706 patients while on treatment (Table VI in the online-only Data Supplement), a qualitatively similar pattern of greater relative benefit was seen with E/S versus P/S among patients with DM (HR, 0.85) compared with those without DM (HR, 0.96), although the *P* interaction was of borderline significance (0.067). The pattern of greater relative ef-

**Table 3. Safety Outcomes**

| | Placebo/ Simvastatin | Ezetimibe/ Simvastatin | *P* Value | *P* Interaction |
|---|---|---|---|---|
| Alanine aminotransferase or aspartate aminotransferase >3× upper limit of normal (N=432) | | | | 0.36 |
| DM absent | 150 (2.3) | 153 (2.3) | 0.91 | |
| DM present | 58 (2.3) | 71 (2.9) | 0.25 | |
| Cholecystectomy (N=267) | | | | 0.94 |
| DM absent | 90 (1.4) | 89 (1.3) | 0.94 | |
| DM present | 44 (1.8) | 44 (1.8) | >0.99 | |
| Gall bladder adverse event (N=603) | | | | 0.76 |
| DM absent | 215 (3.3) | 186 (2.8) | 0.14 | |
| DM present | 106 (4.3) | 96 (3.9) | 0.52 | |
| Rhabdomyolysis (N=31) | | | | 0.69 |
| DM absent | 7 (0.1) | 6 (0.1) | 0.79 | |
| DM present | 11 (0.4) | 7 (0.3) | 0.48 | |
| Rhabdomyolysis, myopathy, or elevated creatine phosphokinase >5× upper limit of normal (N=111) | | | | |
| DM absent | 38 (0.6) | 37 (0.6) | 0.91 | 0.64 |
| DM present | 20 (0.8) | 16 (0.7) | 0.62 | |
| Hemorrhagic stroke (N=102) | | | | 0.092 |
| DM absent | 33 (0.5) | 36 (0.5) | 0.81 | |
| DM present | 10 (0.4) | 23 (0.9) | 0.023 | |
| Cancer (N=1480) | | | | 0.96 |
| DM absent | 543 (8.2) | 551 (8.3) | 0.83 | |
| DM present | 189 (7.6) | 197 (8.0) | 0.63 | |

DM indicates diabetes mellitus.

ficacy with E/S in patients with DM was directionally consistent in other prespecified composite efficacy end points in the on-treatment analysis (HRs ranging from 0.76–0.86 in patients with DM versus 0.94–0.96 in patients without DM; Table VI in the online-only Data Supplement), with statistically significant subgroup-treatment interactions observed for 2 of these 4 additional composite efficacy end points.

## DISCUSSION

In this prespecified subgroup analysis of IMPROVE-IT, patients with DM derived significantly greater relative and absolute benefit from E/S compared with P/S in patients after ACS with LDL-C 50 to 125 mg/dL relative to patients without DM. This enhanced benefit was driven by reductions of acute ischemic events, including MI and ischemic stroke.

It would be incorrect to conclude that patients without DM experienced no benefit with the addition of ezetimibe. Although the benefit of adding ezetimibe to simvastatin in patients without DM was modest overall, among patients without DM who were at high risk for cardiovascular events, either on the basis of advanced age or an elevated risk score, significant reductions in cardiovascular events were observed with E/S compared with P/S. Patients without DM who were <75 years of age or with a low risk score did not exhibit any added benefit with ezetimibe. The safety profile of E/S was similar to that of P/S in both patients with and without DM.

Because this is the only large cardiovascular outcomes study comparing ezetimibe with placebo on the background of a statin, a comparison of the current results to other similarly designed outcomes studies is not possible. It is notable that the only large placebo-controlled trial of a statin conducted solely in patients with DM[12] was stopped early because of overwhelming efficacy, with a 37% (17%–52%) reduction in major cardiovascular events, and was conducted in a primary prevention population, whereas IMPROVE-IT enrolled patients in ≤10 days of ACS. Moreover, 2 meta-analyses of cholesterol-lowering therapy (predominantly statins) did not show a differential benefit of lipid-lowering therapy between patients with no DM, type I DM, or type II DM.[6,18]

The explanation for the findings that patients with DM benefited more than patients without DM is not clear. It is notable that there was a greater incremental reduction in the median time-averaged LDL-C (by 3 mg/dL) in patients with DM with E/S versus P/S, but there

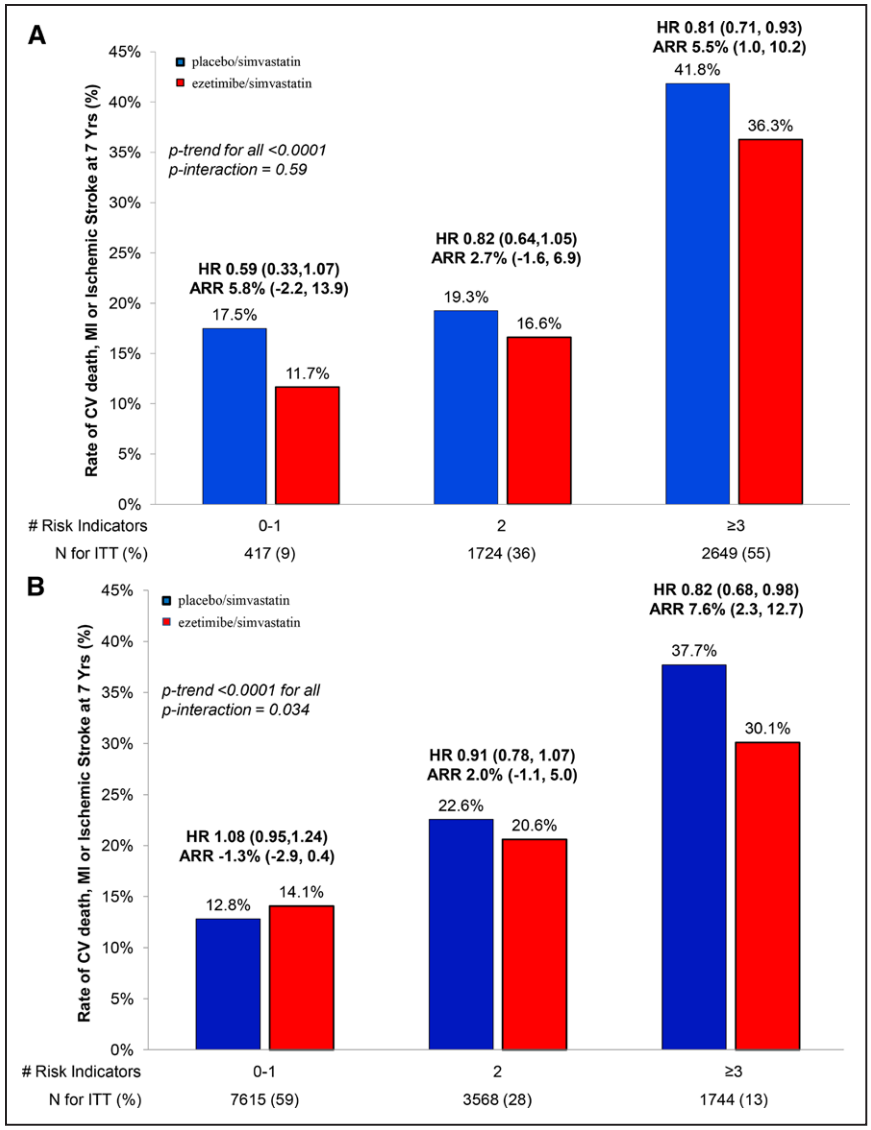


**Figure 4. Efficacy of ezetimibe stratified by diabetes mellitus status and TIMI Risk Score for Secondary Prevention.**
**A** and **B,** Cumulative event rates of the composite of cardiovascular death, myocardial infarction, or ischemic stroke in patients at low (0–1 risk indicators), intermediate (2 risk indicators), and high (≥3 risk indicators) risk are shown for placebo/ezetimibe (blue) and ezetimibe/simvastatin (red) in patients with diabetes mellitus (**A**) and without (**B**) diabetes mellitus.

were no similar incremental benefits in triglycerides, HDL-C, or hsCRP, and this difference in LDL-C reduction appears to be too modest to be the sole reason. Furthermore, the odds of achieving the dual targets of LDL-C <70 mg/dL and hsCRP <2 mg/dL were greater with E/S compared with P/S among patients without DM than in patients with DM. The effect of ezetimibe on other atherogenic lipid particles in patients with DM[8,9] or the favorable effects of ezetimibe on glucose metabolism, including reductions in fasting plasma glucose, insulin levels, and insulin resistance,[8] may have also contributed to the enhanced benefit of E/S in patients with DM in IMPROVE-IT.

Additional possible explanations for the enhanced benefit in patients with DM include inhibition by ezetimibe of the heighted levels of platelet aggregation and activation,[19] a reduction in campesterol cholesterol ratio, which has been linked to regression of atherosclerotic plaques,[20] or other pleiotropic effects of ezetimibe to reduce oxidative stress/inflammation,[21,22] smooth muscle proliferation,[23] and plaque instability.[24,25] Greater platelet inhibition has been associated with additional incremental treatment benefit in patients with DM with several[26–28] but not all[29] potent platelet inhibitors, whereas it is less clear whether the other nonlipid effects of ezetimibe would be of greater benefit in patients with DM.

The enhanced benefit of E/S in patients with DM is consistent with the findings reported in other high-risk subgroups in IMPROVE-IT, including patients >75 years of age,[30] with prior coronary artery bypass grafting,[31] and with prior stroke.[32] Indeed, each of these high-risk features contributes to the TIMI Risk Score for Secondary Prevention, and all were associated with increased benefit of E/S in IMPROVE-IT.[17] Thus, these observations in patients with DM are consistent with the hypothesis that patients at highest risk for cardiovascular events have the most to benefit from ezetimibe. This finding may reflect a greater proportion of modifiable events with aggressive lipid-lowering in high- compared with low-risk patients.

Several limitations of this analysis deserve consideration. Although this study was an analysis of a prespecified subgroup involving 4933 patients from a large clinical trial, it has limited statistical power and was not adjusted for multiple comparisons. Hence, we cannot exclude a chance finding. Patients enrolled in clinical trials often differ in baseline characteristics and have fewer comorbidities than patients treated in clinical practice, thus limiting the generalizability of the findings. Investigator-determined assessment of the presence or absence of DM at randomization was used without a systematic collection of hemoglobin A1c levels, which may have resulted in some misclassification. However, this would be expected to bias toward a null finding. In addition, 2 sensitivity analyses were performed and were consistent with the main analysis.

## CONCLUSIONS

In the IMPROVE-IT of 18 144 patients with ACS and LDL-C 50 to 125 mg/dL, the benefit of adding ezetimibe to statin appeared to be enhanced among patients with DM, with no adverse effect in safety. These findings support the use of intensive, combination lipid-lowering therapy in patients with DM to optimize cardiovascular outcomes, as recommended by the American Association of Clinical Endocrinologists and the American College of Endocrinology.[33]

## ARTICLE INFORMATION

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# Benefit of Adding Ezetimibe to Statin Therapy on Cardiovascular Outcomes and Safety in Patients With Versus Without Diabetes Mellitus: Results From IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial)

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On behalf of the IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial) Investigators

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# SUPPLEMENTARY MATERIAL

## Supplementary Appendix: Table of Contents

**Table S1: Baseline Characteristics by Diabetes Status and Treatment Group**

| | **Diabetes Absent** 13,202 (72.6%) | | | **Diabetes Present** 4933 (27.4%) | | |
|---|---|---|---|---|---|---|
| | **Simvastatin** N=6598 | **Ezetimibe/Simva** N=6604 | P | **Simvastatin** N=2474 | **Ezetimibe/Simva** N=2459 | P |
| **Demographics** | | | | | | |
| Mean Age (SD) | 63.7 (9.9) | 63.7 (9.9) | 0.76 | 65.3 (9.3) | 65.3 (9.1) | 0.71 |
| Female | 1473 (22.3) | 1532 (23.2) | 0.23 | 716 (28.9) | 691 (28.1) | 0.51 |
| White | 5682 (86.1) | 5677 (86.0) | 0.80 | 1938 (78.3) | 1899 (77.2) | 0.35 |
| Median Weight, kg [IQR] | 80.0 [70.0, 91.0] | 80.0 [70.0, 90.3] | 0.50 | 84.5 [74.0, 97.7] | 85.0 [74.0, 98.0] | 0.62 |
| Median BMI [IQR] | 27.0 [24.5, 30.1] | 27.1 [24.6, 30.0] | 0.76 | 29.2 [26.2, 33.1] | 29.2 [26.0, 32.9] | 0.81 |
| **Medical history** | | | | | | |
| Hyperlipidemia | 4742 (71.9) | 4762 (72.1) | 0.76 | 1845 (74.6) | 1802 (73.3) | 0.30 |
| Hypertension | 3631 (55.0) | 3635 (55.0) | 0.99 | 1926 (77.8) | 1945 (79.1) | 0.29 |
| Current smoking | 2437 (36.9) | 2347 (35.6) | 0.099 | 598 (24.2) | 596 (24.2) | 0.96 |
| Myocardial infarction | 1243 (18.9) | 1298 (19.7) | 0.24 | 638 (25.8) | 627 (25.6) | 0.85 |
| PCI | 1180 (17.9) | 1180 (17.9) | 0.97 | 616 (24.9) | 586 (23.9) | 0.39 |
| CABG | 489 (7.4) | 509 (7.7) | 0.52 | 353 (14.3) | 333 (13.5) | 0.46 |
| Congestive heart failure | 192 (2.9) | 218 (3.3) | 0.20 | 179 (7.2) | 201 (8.2) | 0.22 |
| Peripheral arterial disease | 329 (5.0) | 288 (4.4) | 0.088 | 189 (7.6) | 199 (8.1) | 0.55 |
| **Medications prior to admission** | | | | | | |
| Aspirin | 2522 (38.3) | 2489 (37.7) | 0.52 | 1333 (53.9) | 1310 (53.3) | 0.68 |
| Beta-blocker | 2041 (31.0) | 2074 (31.4) | 0.56 | 1102 (44.6) | 1079 (43.9) | 0.63 |
| Statin | 1939 (29.4) | 1995 (30.2) | 0.31 | 1172 (47.4) | 1140 (46.4) | 0.47 |
| ACE-I or ARB | 2202 (33.4) | 2268 (34.4) | 0.24 | 1474 (59.6) | 1472 (59.9) | 0.85 |
| **Medications at randomization** | | | | | | |
| Aspirin | 6412 (97.2) | 6415 (97.2) | 0.68 | 2382 (96.3) | 2383 (96.9) | 0.24 |
| Beta-blocker | 5746 (87.1) | 5771 (87.4) | 0.44 | 2133 (86.2) | 2141 (87.1) | 0.52 |
| ACE-I or ARB | 4834 (73.3) | 4755 (72.0) | 0.10 | 2044 (82.6) | 2067 (84.1) | 0.18 |

| | **Diabetes Absent** 13,202 (72.6%) | | | **Diabetes Present** 4933 (27.4%) | | |
|---|---|---|---|---|---|---|
| | **Simvastatin** N=6598 | **Ezetimibe/Simva** N=6604 | P | **Simvastatin** N=6598 | **Ezetimibe/Simva** N=6604 | P |
| **At index event** | | | | | | |
| ST-segment elevation MI | 2089 (31.7) | 2088 (31.6) | 0.95 | 517 (20.9) | 496 (20.2) | 0.53 |
| Diagnostic angiography | 5581 (89.1) | 5907 (89.5) | 0.51 | 2055 (83.1) | 2081 (84.6) | 0.15 |
| PCI | 4739 (71.8) | 4760 (72.1) | 0.74 | 1582 (64.0) | 1625 (66.1) | 0.12 |
| **Laboratory Values at Qualifying Event** | | | | | | |
| LDL-C (mg/dL) | 97 [81, 112] | 97 [81, 112] | 0.55 | 88 [73, 103] | 89 [74, 103] | 0.37 |
| Prior statin use | 81 [69, 93] | 81 [70, 92] | 0.82 | 78 [66, 89] | 78 [67, 89] | 0.47 |
| No prior statin use | 105 [91, 116] | 105 [91, 116] | 0.79 | 101 [84, 113] | 100 [85, 113] | 0.74 |
| HDL-C (mg/dL) | 41 [34, 50] | 41 [34, 50] | 0.71 | 38 [31, 46] | 38 [31, 46] | 0.15 |
| Triglycerides (mg/dL) | 115 [81, 163] | 115 [81, 164] | 0.96 | 138 [96, 192] | 135 [95, 193] | 0.87 |
| C-reactive protein (mg/L) | 5.0 [2.0, 15.0] | 5.0 [2.0, 17.7] | 0.68 | 6.0 [2.3, 21.0] | 5.4 [2.0, 21.0] | 0.47 |
| **Laboratory Values at Randomization** | | | | | | |
| LDL-C (mg/dL) | 81 [67, 98] | 81 [66, 96] | 0.20 | 75 [60, 92] | 75 [61, 91] | 0.92 |
| Statin use during admit | 78 [65, 93] | 78 [64, 92] | 0.46 | 73 [59, 88] | 72 [59, 87] | 0.42 |
| No statin use during admit | 94 [76, 111] | 92 [76, 109] | 0.15 | 87 [69, 104] | 90 [73, 108] | 0.024 |
| C-reactive protein* (mg/L) | 9.6 [4.0, 26.7] | 9.5 [3.8, 26.2] | 0.60 | 9.4 [4.0, 25.4] | 9.9 [4.0, 27.5] | 0.13 |

*Values shown are those closest to randomization

ACE-I = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, BMI = body mass index, HDL-C = high-density lipoprotein cholesterol, IQR = interquartile range, Kg = kilograms, LDL-C = low-density lipoprotein cholesterol, M = meter, MI = myocardial infarction, SD = standard deviation

**Table S2 – Patient adherence to study drug among those at risk for the primary endpoint, stratified by diabetes status**

| Year End | No Diabetes Mellitus (N, %) | Diabetes Mellitus (n, %) |
|---|---|---|
| 1 | 9161 (83.7) | 3118 (81.6) |
| 2 | 7839 (77.7) | 2597 (75.3) |
| 3 | 6924 (72.9) | 2246 (71.1) |
| 4 | 6037 (69.0) | 1891 (67.6) |
| 5 | 4364 (66.2) | 1208 (64.7) |
| 6 | 3340 (64.2) | 827 (60.9) |
| 7 | 1842 (61.4) | 444 (59.0) |

## Table S3 – Efficacy Outcomes

| | | SIMVA/alone | | SIMVA/EZE | | | | |
|---|---|---|---|---|---|---|---|---|
| Endpoints[1] | History of diabetes | n (%) | KM event(%) at 7 yrs | n (%) | KM event(%) at 7 yrs | Hazard Ratio (95%CI) | p-value | Interaction p-value[2] |
| **Primary Endpoints** | **Non-diabetics** | 1792 (27.2) | 30.84 | 1748 (26.5) | 30.16 | 0.98 (0.91, 1.04) | 0.471 | 0.023 |
| | **Diabetics** | 949 (38.4) | 45.50 | 824 (33.5) | 40.04 | 0.85 (0.78, 0.94) | 0.001 | |
| **Secondary Endpoints I** | **Non-diabetics** | 2144 (32.5) | 36.26 | 2084 (31.6) | 35.40 | 0.97 (0.92, 1.03) | 0.378 | 0.109 |
| | **Diabetics** | 1101 (44.5) | 51.42 | 1005 (40.9) | 47.86 | 0.89 (0.82, 0.97) | 0.009 | |
| **Secondary Endpoints II** | **Non-diabetics** | 895 (13.6) | 15.98 | 858 (13.0) | 15.31 | 0.96 (0.87, 1.05) | 0.396 | 0.074 |
| | **Diabetics** | 552 (22.3) | 26.98 | 464 (18.9) | 23.89 | 0.83 (0.74, 0.94) | 0.004 | |
| **Secondary Endpoints III** | **Non-diabetics** | 1884 (28.6) | 32.45 | 1852 (28.0) | 31.86 | 0.98 (0.92, 1.05) | 0.640 | 0.021 |
| | **Diabetics** | 984 (39.8) | 46.72 | 864 (35.1) | 42.04 | 0.86 (0.79, 0.95) | 0.002 | |
| **Tertiary Endpoints** | **Non-diabetics** | 1070 (16.2) | 18.97 | 1025 (15.5) | 18.33 | 0.96 (0.88, 1.04) | 0.322 | 0.006 |
| | **Diabetics** | 660 (26.7) | 32.39 | 526 (21.4) | 26.63 | 0.78 (0.70, 0.88) | 0.000 | |

[1] Primary endpoints: CV death, non-fatal MI, Unstable angina, Coronary revascularization (PCI or CABG) at least 30 days post-randomization, or non-fatal stroke.
[1] Secondary endpoints I: All death, non-fatal MI, Unstable angina, Coronary revascularization (PCI or CABG) at least 30 days post-randomization, or non-fatal stroke.
[1] Secondary endpoints II: CHD death, non-fatal MI, Urgent coronary revascularization (PCI or CABG) at least 30 days post-randomization.
[1] Secondary endpoints III: CV death, non-fatal MI, Unstable angina, All revascularization (both coronary and non-coronary) at least 30 days post-randomization, or non-fatal stroke.
[1] Tertiary Endpoints: composite of CHD death, UA required hospitalization, MI, and Ischemic Stroke.
[2] Interaction effect of treatment arm and history of diabetes using Cox PH regression modeling.

## Table S4 – Other Efficacy Endpoints by Treatment Group and Diabetes Status

| | | SIMVA/alone | | | SIMVA/EZE | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | History of diabetes | n (%) | | KM event(%) at 7 yrs | n (%) | | KM event(%) at 7 yrs | p-value | Hazard Ratio (95%CI) | | Interaction p-value[1] |
| **CV Death** | **Non-diabetics** | 302 | (4.6) | 5.29 | 312 | (4.7) | 5.28 | 0.696 | 1.03 (0.88, 1.21) | | 0.570 |
| | **Diabetics** | 235 | (9.5) | 11.15 | 225 | (9.2) | 11.68 | 0.687 | 0.96 (0.80, 1.16) | | |
| **MI** | **Non-diabetics** | 706 | (10.7) | 12.73 | 660 | (10.0) | 11.99 | 0.211 | 0.93 (0.84, 1.04) | | 0.028 |
| | **Diabetics** | 412 | (16.7) | 20.81 | 317 | (12.9) | 16.41 | 0.000 | 0.76 (0.66, 0.88) | | |
| **Hosp. for Unstable Angina** | **Non-diabetics** | 94 | (1.4) | 1.64 | 100 | (1.5) | 1.80 | 0.658 | 1.07 (0.80, 1.41) | | 0.941 |
| | **Diabetics** | 54 | (2.2) | 2.74 | 56 | (2.3) | 2.81 | 0.821 | 1.04 (0.72, 1.52) | | |
| **CHD Death** | **Non-diabetics** | 247 | (3.7) | 4.32 | 248 | (3.8) | 4.23 | 0.973 | 1.00 (0.84, 1.20) | | 0.450 |
| | **Diabetics** | 213 | (8.6) | 10.07 | 192 | (7.8) | 10.07 | 0.327 | 0.91 (0.75, 1.10) | | |
| **Stroke** | **Non-diabetics** | 216 | (3.3) | 3.99 | 201 | (3.0) | 3.79 | 0.472 | 0.93 (0.77, 1.13) | | 0.151 |
| | **Diabetics** | 129 | (5.2) | 7.14 | 95 | (3.9) | 5.25 | 0.020 | 0.73 (0.56, 0.95) | | |
| **Ischemic Stroke** | **Non-diabetics** | 180 | (2.7) | 3.35 | 164 | (2.5) | 3.24 | 0.399 | 0.91 (0.74, 1.13) | | 0.031 |
| | **Diabetics** | 117 | (4.7) | 6.48 | 72 | (2.9) | 3.94 | 0.001 | 0.61 (0.46, 0.82) | | |
| **Any Death** | **Non-diabetics** | 759 | (11.5) | 12.93 | 746 | (11.3) | 12.57 | 0.740 | 0.98 (0.89, 1.09) | | 0.842 |
| | **Diabetics** | 471 | (19.0) | 21.79 | 469 | (19.1) | 23.46 | 1.000 | 1.00 (0.88, 1.14) | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CVDeath/MI/Stroke** | **Non-diabetics** | 1060 | (16.1) | 17.99 | 1019 | (15.4) | 17.16 | 0.310 | 0.96 | (0.88, | 1.04) | 0.016 |
| | **Diabetics** | 643 | (26.0) | 29.88 | 525 | (21.4) | 25.31 | 0.000 | 0.80 | (0.71, | 0.90) | |
| **PCI/CABG 30days-post** | **Non-diabetics** | 1224 | (18.6) | 21.45 | 1173 | (17.8) | 20.59 | 0.326 | 0.96 | (0.89, | 1.04) | 0.514 |
| | **Diabetics** | 569 | (23.0) | 29.07 | 517 | (21.0) | 25.38 | 0.148 | 0.92 | (0.81, | 1.03) | |
| **Urgent PCI/CABG 30days-post** | **Non-diabetics** | 409 | (6.2) | 7.50 | 346 | (5.2) | 6.44 | 0.020 | 0.84 | (0.73, | 0.97) | 0.395 |
| | **Diabetics** | 217 | (8.8) | 11.84 | 164 | (6.7) | 8.63 | 0.007 | 0.76 | (0.62, | 0.93) | |

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[1] Interaction p-value between treatment arm and diabetes status.

**Table S5 – Sensitivity Analysis of Efficacy Endpoints Using a Broader Definition* of Pre-existing Diabetes Mellitus**

| | | SIMVA/alone | | SIMVA/EZE | | | | |
|---|---|---|---|---|---|---|---|---|
| Endpoints | Diabetes status | n (%) | KM event(%) at 7 yrs | n (%) | KM event(%) at 7 yrs | Hazard Ratio (95%CI) | p-value | Interaction p-value* |
| **Primary Endpoints** | **Non-diabetics** | 1660 (27.0) | 30.65 | 1633 (26.7) | 30.27 | 0.99 (0.92, 1.06) | 0.778 | 0.006 |
| | **Diabetics** | 985 (37.5) | 44.07 | 855 (32.2) | 38.50 | 0.84 (0.77, 0.93) | 0.000 | |
| **Secondary Endpoints I** | **Non-diabetics** | 1989 (32.4) | 36.10 | 1945 (31.8) | 35.46 | 0.98 (0.92, 1.05) | 0.610 | 0.036 |
| | **Diabetics** | 1148 (43.7) | 49.96 | 1044 (39.3) | 46.34 | 0.88 (0.81, 0.96) | 0.003 | |
| **Secondary Endpoints II** | **Non-diabetics** | 822 (13.4) | 15.72 | 812 (13.3) | 15.61 | 0.99 (0.90, 1.10) | 0.911 | 0.005 |
| | **Diabetics** | 581 (22.1) | 26.61 | 472 (17.8) | 22.29 | 0.79 (0.70, 0.90) | 0.000 | |
| **Secondary Endpoints III** | **Non-diabetics** | 1746 (28.4) | 32.20 | 1729 (28.3) | 31.96 | 1.00 (0.93, 1.07) | 0.959 | 0.006 |
| | **Diabetics** | 1022 (38.9) | 45.40 | 898 (33.8) | 40.55 | 0.85 (0.78, 0.93) | 0.001 | |
| **Tertiary Endpoints** | **Non-diabetics** | 992 (16.2) | 18.81 | 967 (15.8) | 18.56 | 0.98 (0.90, 1.07) | 0.648 | <0.001 |
| | **Diabetics** | 690 (26.2) | 31.84 | 540 (20.3) | 25.28 | 0.76 (0.68, 0.85) | 0.000 | |

*Diabetes mellitus identified by the investigator at time of admission, or first glucose after randomization $\geq$ 126 mg/dL (fasting) or 200 mg/dL (non-fasting).

Primary endpoints: Cardiovascular (CV) death, non-fatal myocardial infarction (MI), unstable angina (UA), coronary revascularization $\geq$30 days post-randomization, or stroke.
Secondary endpoints I: All death, MI, UA, coronary revascularization $\geq$30 days post-randomization, or stroke.
Secondary endpoints II: Coronary heart disease (CHD) death, MI, urgent coronary revascularization $\geq$30 days post-randomization.

Secondary endpoints III: CV death, MI, UA, all revascularization (both coronary and non-coronary) ≥30 days post-randomization, or stroke.
Tertiary Endpoints: CHD death, UA, MI, and ischemic stroke.
*Interaction effect of treatment arm and history of diabetes using Cox proportional hazards regression modeling

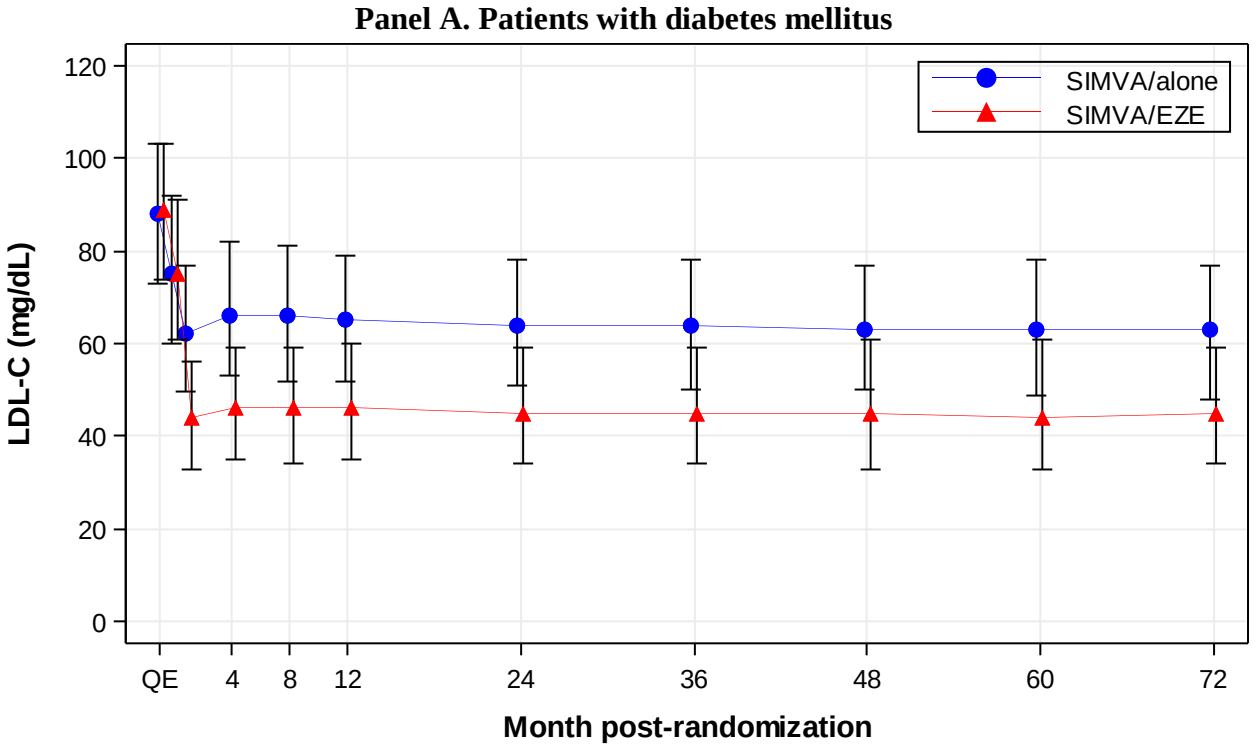
## Table S6 – On-treatment Sensitivity Analysis of Efficacy Endpoints

| Within 30 days of stopping study drug in subjects who were on treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | SIMVA/alone | | SIMVA/EZE | | | | |
| Endpoints | History of diabetes | n (%) | KM event(%) at 7 yrs | n (%) | KM event(%) at 7 yrs | Hazard Ratio (95%CI) | p-value | Interaction p-value* |
| **Primary Endpoints** | **Non-diabetics** | 1383 (21.5) | 29.33 | 1332 (20.6) | 27.64 | 0.96 (0.89, 1.03) | 0.281 | 0.067 |
| | **Diabetics** | 696 (28.9) | 41.27 | 600 (25.1) | 36.32 | 0.85 (0.76, 0.95) | 0.003 | |
| **Secondary Endpoints I** | **Non-diabetics** | 1445 (22.4) | 30.65 | 1380 (21.3) | 28.74 | 0.95 (0.88, 1.02) | 0.182 | 0.144 |
| | **Diabetics** | 728 (30.2) | 43.16 | 640 (26.8) | 39.27 | 0.86 (0.78, 0.96) | 0.007 | |
| **Secondary Endpoints II** | **Non-diabetics** | 625 (9.7) | 14.07 | 595 (9.2) | 12.94 | 0.95 (0.85, 1.06) | 0.341 | 0.042 |
| | **Diabetics** | 368 (15.3) | 22.59 | 291 (12.2) | 18.90 | 0.78 (0.67, 0.91) | 0.001 | |
| **Secondary Endpoints III** | **Non-diabetics** | 1461 (22.7) | 31.07 | 1411 (21.8) | 29.21 | 0.96 (0.89, 1.04) | 0.311 | 0.069 |
| | **Diabetics** | 728 (30.2) | 42.50 | 636 (26.6) | 38.70 | 0.86 (0.77, 0.95) | 0.004 | |
| **Tertiary Endpoints** | **Non-diabetics** | 770 (11.9) | 17.28 | 727 (11.2) | 15.97 | 0.94 (0.85, 1.04) | 0.219 | 0.015 |
| | **Diabetics** | 445 (18.5) | 27.40 | 345 (14.5) | 22.46 | 0.76 (0.66, 0.87) | 0.000 | |

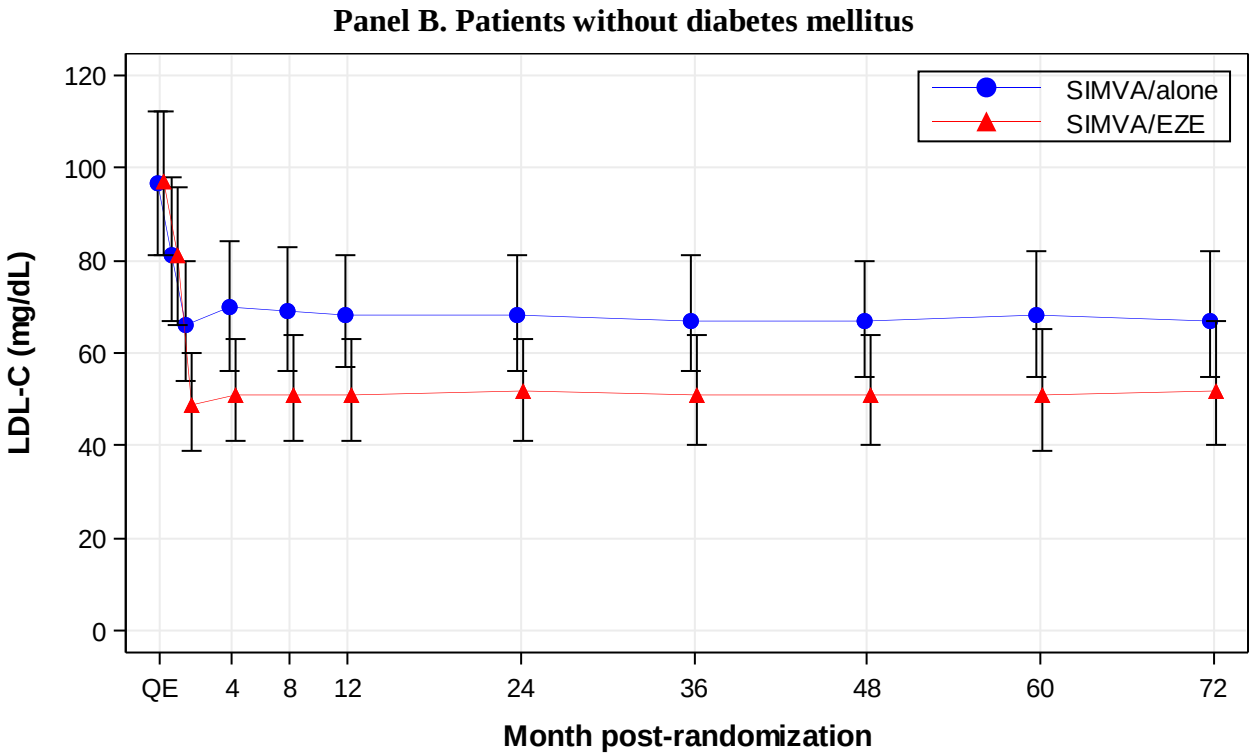
See footnote to Table S3 for explanation of endpoints.

*Interaction effect of treatment arm and history of diabetes using Cox proportional hazards regression modeling

**Figure S1**



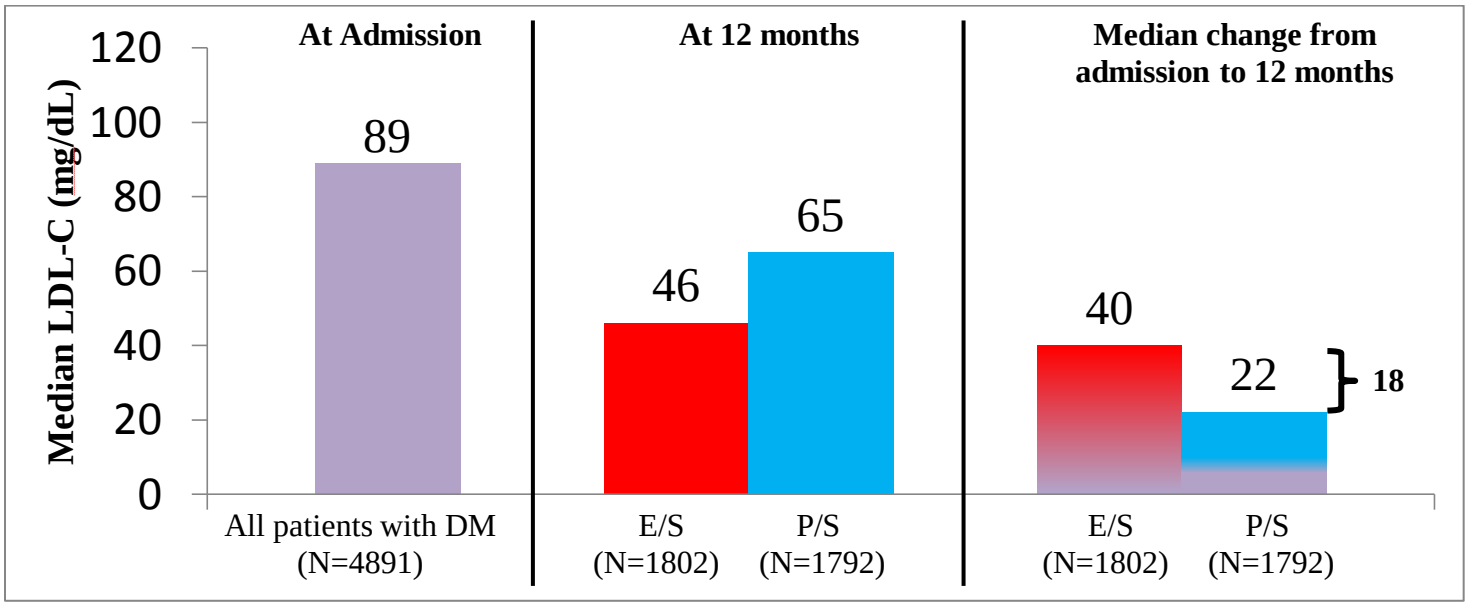

**LDL-C over time.** In Panel A, the median LDL-C (dark circle) and interquartile range (bars) are shown for patients with diabetes from prior to the time of the qualifying event (QE) though 72 months for placebo/simvastatin (solid line) and ezetimibe/simvastatin (dashed line). Panel B shows similar data among patients without diabetes.




**LDL-C over time.** In Panel A, the median LDL-C (dark circle) and interquartile range (bars) are shown for patients with diabetes from prior to the time of the qualifying event (QE) though 72 months for placebo/simvastatin (solid line) and ezetimibe/simvastatin (dashed line). Panel B shows similar data among patients without diabetes.

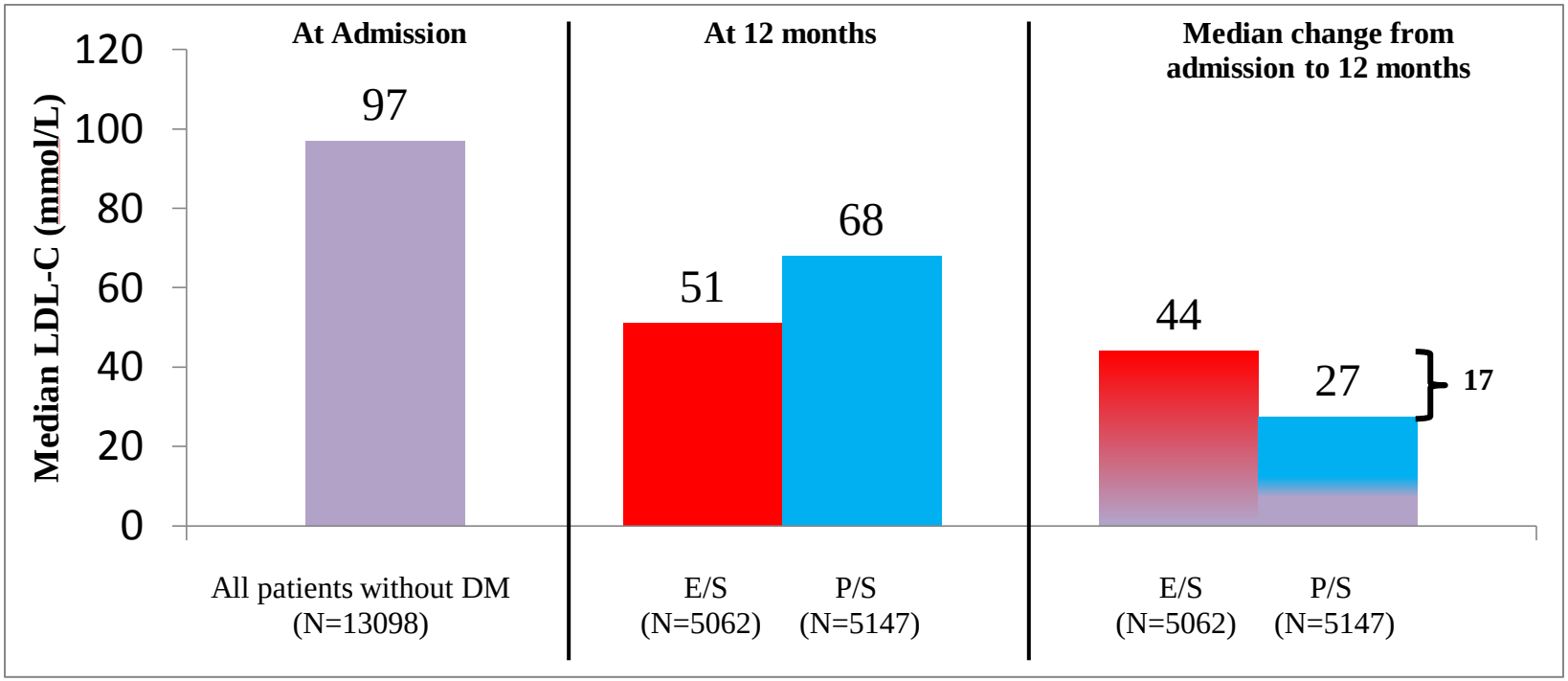
**Figure S2 – Change in median LDL-C between admission and 12 months by diabetic status and treatment group**

**A. Median LDL-C at admission and 12 months in patients with diabetes mellitus**




**Change in median LDL-C between admission and 12 months by diabetic status and treatment group.** Results in patients with diabetes are shown in Panel A and results in patients without diabetes are shown in Panel B. There was no significant effect modification by diabetes status on the treatment difference in LDL-C reduction from admission to 12 months ($P_{interaction}$ 0.12).

**B. Median LDL-C at admission and 12 months in patients without diabetes mellitus**




**Change in median LDL-C between admission and 12 months by diabetic status and treatment group.** Results in patients with diabetes are shown in Panel A and results in patients without diabetes are shown in Panel B. There was no significant effect modification by diabetes status on the treatment difference in LDL-C reduction from admission to 12 months ($P_{interaction}$ 0.12).

**Figure S3. Efficacy composite endpoints by treatment group stratified by age and diabetes status**

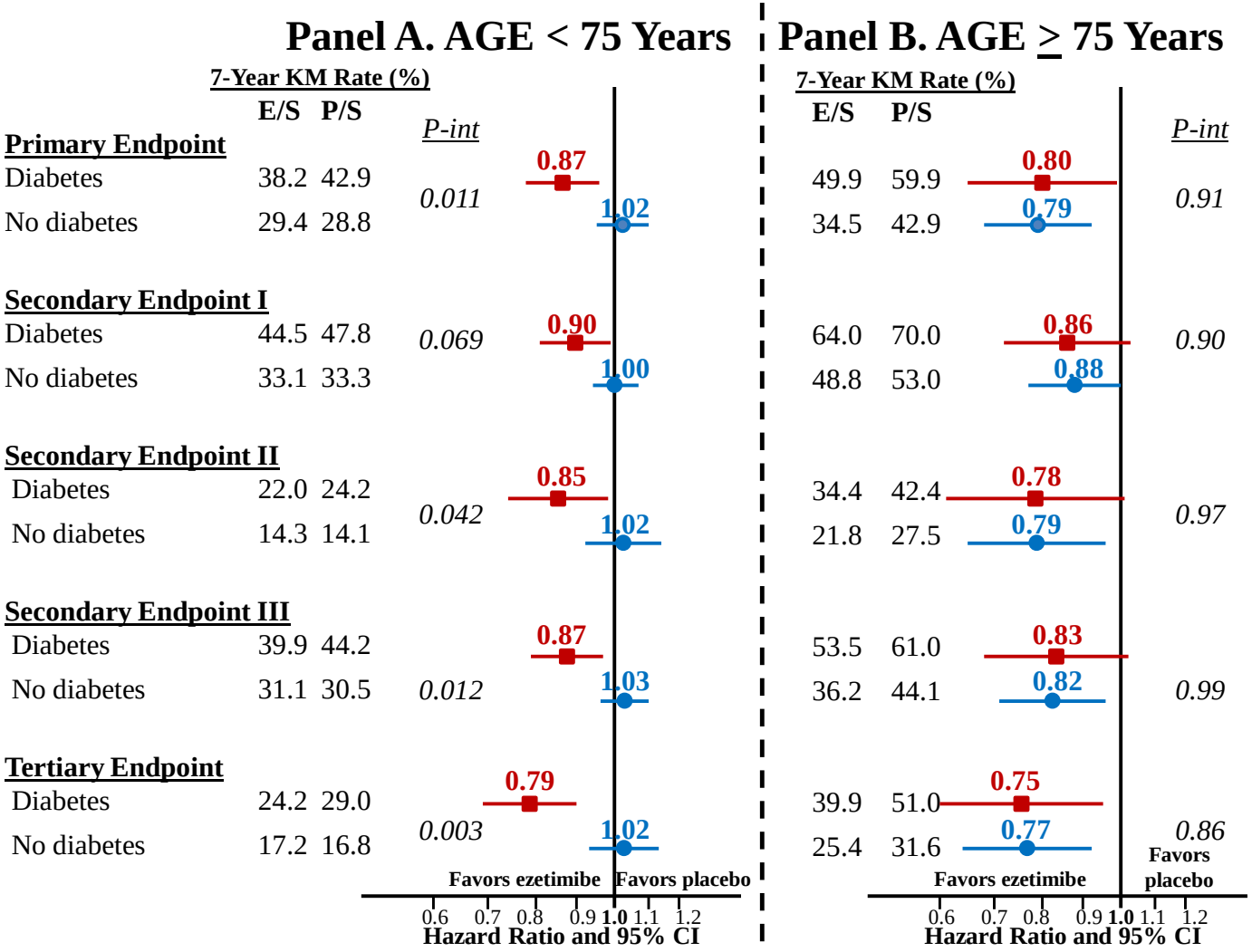
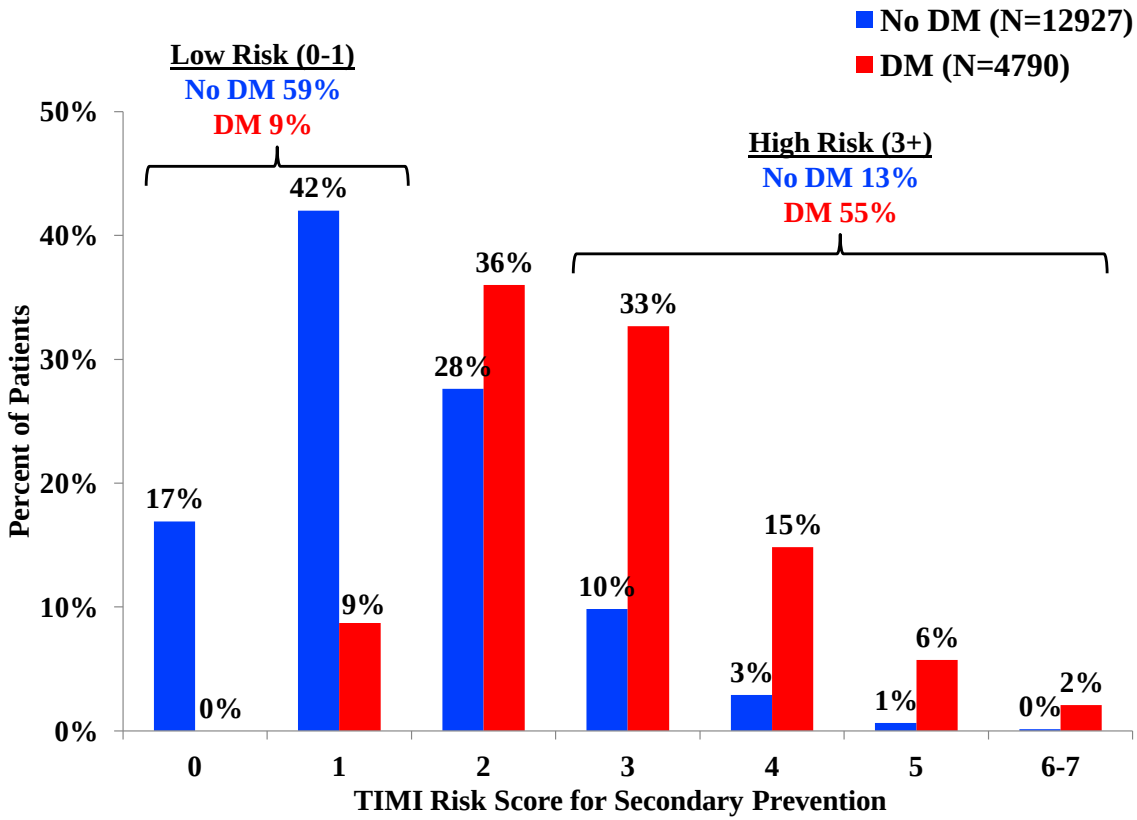


**Figure S4. Risk distribution of patients with vs without diabetes mellitus**




**Risk distribution of patients with vs without diabetes mellitus.** Patients with diabetes mellitus (red) were at higher risk (mean score 2.8) compared to patients without diabetes mellitus (blue, mean score 1.4), as categorized by the TIMI Risk Score for Secondary Prevention.[1, 2] Note: The score could not be calculated in 427 patients (2.4%) due to missing data.


1. Bohula EA, Bonaca MP, Braunwald E, Aylward PE, Corbalan R, De Ferrari GM, et al. Atherothrombotic risk stratification and the efficacy and safety of vorapaxar in patients with stable ischemic heart disease and previous myocardial infarction. *Circulation*. 2016;134:304-313
2. Bohula EA, Morrow DA, Giugliano RP, Blazing MA, He P, Park JG, et al. Atherothrombotic risk stratification and ezetimibe for secondary prevention. *Journal of the American College of Cardiology*. 2017;69:911-921

JAMA Cardiology | Original Investigation

# Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease Risks

## Meta-analysis of 10 Trials Involving 77 917 Individuals

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**IMPORTANCE** Current guidelines advocate the use of marine-derived omega-3 fatty acids supplements for the prevention of coronary heart disease and major vascular events in people with prior coronary heart disease, but large trials of omega-3 fatty acids have produced conflicting results.

**OBJECTIVE** To conduct a meta-analysis of all large trials assessing the associations of omega-3 fatty acid supplements with the risk of fatal and nonfatal coronary heart disease and major vascular events in the full study population and prespecified subgroups.

**DATA SOURCES AND STUDY SELECTION** This meta-analysis included randomized trials that involved at least 500 participants and a treatment duration of at least 1 year and that assessed associations of omega-3 fatty acids with the risk of vascular events.

**DATA EXTRACTION AND SYNTHESIS** Aggregated study-level data were obtained from 10 large randomized clinical trials. Rate ratios for each trial were synthesized using observed minus expected statistics and variances. Summary rate ratios were estimated by a fixed-effects meta-analysis using 95% confidence intervals for major diseases and 99% confidence intervals for all subgroups.

**MAIN OUTCOMES AND MEASURES** The main outcomes included fatal coronary heart disease, nonfatal myocardial infarction, stroke, major vascular events, and all-cause mortality, as well as major vascular events in study population subgroups.

**RESULTS** Of the 77 917 high-risk individuals participating in the 10 trials, 47 803 (61.4%) were men, and the mean age at entry was 64.0 years; the trials lasted a mean of 4.4 years. The associations of treatment with outcomes were assessed on 6273 coronary heart disease events (2695 coronary heart disease deaths and 2276 nonfatal myocardial infarctions) and 12 001 major vascular events. Randomization to omega-3 fatty acid supplementation (eicosapentaenoic acid dose range, 226-1800 mg/d) had no significant associations with coronary heart disease death (rate ratio [RR], 0.93; 99% CI, 0.83-1.03; $P = .05$), nonfatal myocardial infarction (RR, 0.97; 99% CI, 0.87-1.08; $P = .43$) or any coronary heart disease events (RR, 0.96; 95% CI, 0.90-1.01; $P = .12$). Neither did randomization to omega-3 fatty acid supplementation have any significant associations with major vascular events (RR, 0.97; 95% CI, 0.93-1.01; $P = .10$), overall or in any subgroups, including subgroups composed of persons with prior coronary heart disease, diabetes, lipid levels greater than a given cutoff level, or statin use.

**CONCLUSIONS AND RELEVANCE** This meta-analysis demonstrated that omega-3 fatty acids had no significant association with fatal or nonfatal coronary heart disease or any major vascular events. It provides no support for current recommendations for the use of such supplements in people with a history of coronary heart disease.

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Supplemental content

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Observational studies in Western and Asian populations have reported that regular consumption of fish once or twice a week is associated with lower risks of death from coronary heart disease (CHD).[1,2] These observations, together with the lower rates of CHD in populations that consumed large amount of foods rich in very-long-chain polyunsaturated fatty acids containing omega-3 fatty acids have prompted interest in assessing whether consumption of marine-derived very-long-chain omega-3 fatty acids (abbreviated "omega-3 FA" in this article) may be protective for CHD.[3] These marine-derived omega-3 FAs include eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) found in fish and other seafood, but not alpha-linolenic acid, which is plant-derived.

The initial Diet and Reinfarction Trial-1 study[4] examined the associations of consumption of oily fish twice or more per week with CHD risk in men who had had a myocardial infarction and reported that fish consumption was associated with a significant reduction in fatal CHD and all-cause mortality but had no association with nonfatal myocardial infarction (MI) recurrence.[4] However, the subsequent Diet and Reinfarction Trial-2 study in men with angina reported that consumption of fish or fish oil supplements increased the risk of CHD death.[5] Subsequently, several large trials have reported conflicting results of the associations of supplementation with omega-3 FA supplements vs placebo or untreated controls on fatal and nonfatal vascular events.[6-16]

Ten large randomized trials[6-15] have been conducted comparing the associations of treatment with omega-3 FA supplementation vs placebo or no treatment for at least 12 months in populations with prior CHD, stroke, or high risk of cardiovascular disease (CVD). These trials have reported conflicting results for the associations of treatment with fatal CHD, nonfatal CHD, or other subtypes of CVD. The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione trial,[6] an open-label trial involving 11 323 recent survivors of MI, reported that patients who received supplementation with omega-3 FAs experienced a 10% reduced risk of major cardiovascular events compared with untreated controls. The Japan EPA Lipid Intervention Study (JELIS) trial, an open-label trial involving 18 645 participants with total cholesterol of 243.24 mg/dL (to convert to mmol/L, multiply by 0.0259) or greater, of whom only 20% with prior CHD, also reported[14] that supplementation with fish oil was associated with a 19% reduction in major CHD events (95% CI, 5%-31%). None of the other large placebo-controlled trials reported any significant association with CHD or mortality. Hence, it is unclear whether the discrepant results reflect different associations of omega-3 FAs with CHD subtypes, different outcomes in primary vs secondary prevention of CHD, increasing use of statins with better control of lipid levels, or an artifact of chance or bias in open-label trials. Previous meta-analyses of these trials of omega-3 FA supplements[16-18] appeared to suggest a significant beneficial association of omega-3 FAs with fatal CHD but not nonfatal CHD. However, these meta-analyses were constrained because they included trials of dietary advice to eat fish[17] or excluded trials that did not include a placebo control.[18]

The Omega-3 Treatment Trialists' Collaboration was established to conduct a collaborative meta-analysis based on aggregated study-level data obtained from the principal investigators of all large randomized clinical trials of omega-3 FA supplements for the prevention of cardiovascular disease, using a prespecified protocol and analysis plan. The aims of this meta-analysis were to assess the associations of supplementation with omega-3 FAs on (1) fatal CHD, nonfatal MI, stroke, major vascular events, and all-cause mortality and (2) major vascular events in prespecified subgroups.

### Key Points

**Question** Does supplementation with marine-derived omega-3 fatty acids have any associations with reductions in fatal or nonfatal coronary heart disease in people at high risk of cardiovascular disease?

**Findings** This meta-analysis of 10 trials involving 77 917 participants demonstrated that supplementation with marine-derived omega-3 fatty acids for a mean of 4.4 years had no significant association with reductions in fatal or nonfatal coronary heart disease or any major vascular events.

**Meaning** The results provide no support for current recommendations to use omega-3 fatty acid supplements for the prevention of fatal coronary heart disease or any cardiovascular disease in people who have or at high risk of developing cardiovascular disease.

## Methods

We performed a systematic search of randomized clinical trials in PubMed and Medline data sets, supplemented by manual hand-searching of reference lists from individual trials, review articles, or previous meta-analyses of omega-3 FAs and CVD (eFigure 1 in the Supplement). Search terms included "omega-3 FA," "omega-3 polyunsaturated fat," "fish oils," and "ω-3 FA" and "cardiovascular disease" or "coronary heart disease" or "stroke" (eFigure 1 in the Supplement). The prespecified eligibility criteria were randomized clinical trials of marine-derived very-long-chain omega-3 FA supplements vs placebo or open-label control, with a sample size of at least 500 participants and a scheduled duration of treatment of at least 1 year. All eligible trials required use of supplements, but no minimum daily dose of EPA or DHA was specified. The prespecified end points included nonfatal MI; death caused by CHD; ischemic, hemorrhagic, and unclassified stroke; coronary or noncoronary arterial revascularization events; major vascular events (a composite of first occurrence of nonfatal MI or death caused by CHD; nonfatal or fatal stroke; or any revascularization procedure); and all-cause mortality. Deaths caused by CHD included sudden cardiac deaths, deaths due to ventricular arrhythmias, and heart failure in patients with CHD, MI, or deaths occurring after coronary revascularization or heart transplant.

All included trials were also assessed for risk of bias. Individual trials had approval from their respective institutional review boards, and all participants provided written informed consent. No additional ethical approval was required for this meta-analysis.

Table. Characteristics of Included Trials

| Study (Year) | Patients, No. | Dose of EPA/ DHA (mg/d) | Male, No. (%) | Mean Trial Duration, y | Mean (SD) Age, y | No. (%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Prior CHD | Prior Stroke | Prior Diabetes | Statin Use |
| DOIT (2010) | 563 | 1150/800 | 563 (100) | 3 | 70 (3) | 133 (23.6) | 37 (6.6) | 46 (8.2) | NA |
| AREDS-2 (2014) | 4203 | 650/350 | 1816 (43.2) | 4.5 | 74 (NA) | 405 (9.7) | 211 (5.0) | 546 (13.0) | 1866 (44.4) |
| SU.FOL.OM3 (2010) | 2501 | 400/200 | 1987 (79.4) | 4.7 | 61 (NA) | 1863 (74.5) | 638 (25.5) | 440 (17.9) | 2079 (83.1) |
| JELIS (2007)[a,b] | 18 645 | 1800/NA | 5859 (31.4) | 4.6 | 61 (8) | NA | NA | 3040 (16.3) | 18 645 (100.0) |
| Alpha Omega (2010) | 4837 | 226/150 | 3783 (78.2) | 3.3 | 69 (6) | 4837 (100.0) | 345 (7.2) | 1014 (21.0) | 4122 (85.2) |
| OMEGA (2010) | 3818 | 460/380 | 2841 (74.4) | 1 | 64 (NA) | 796 (22.5) | 192 (5.5) | 948 (27.0) | 3566 (94.2) |
| R&P (2013) | 12 505 | 500/500 | 7687 (61.5) | 5 | 64 (NA) | Not stated (30) | 594 (4.8) | 7494 (59.9) | 12 505 (100.0) |
| GISSI-HF (2008) | 6975 | 850/950 | 5459 (78.3) | 3.9 | 67 (11) | 3614 (51.8) | 346 (5.0) | 1974 (28.3) | NA |
| ORIGIN (2012) | 12 536 | 465/375 | 8150 (65.0) | 6.2 | 64 (8) | 8094 (64.6) | 10 877 (86.8) | 11 081 (88.4) | 6739 (53.8) |
| GISSI-P[b] (1999) | 11 334 | 850/1700 | 9658 (85.2) | 3.5 | 59 (11) | 11 334 (100.0) | NA | 2139 (18.9) | NA |
| Total | 77 917 | NA | 47 803 (61.4) | 4.4 | 64 | 31 076/46 767 (66.4) | 13 240/47 938 (27.6) | 28 722 (36.9) | 49 522 (83.4) |

Abbreviations: AREDS-2, Age-Related Eye Disease Study 2; DOIT, Diet and Omega-3 Intervention Trial; GISSI-HF, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Heart Failure; GISSI-P, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Prevenzione; JELIS, Japan Eicosapentaenoic Acid (EPA) Lipid Intervention Study; NA, not available; OMEGA, Effect of Omega 3-Fatty Acids on the Reduction of Sudden Cardiac Death After Myocardial Infarction; ORIGIN, Outcome Reduction With Initial Glargine Intervention; SU.FOL.OM3, Supplémentation en Folates et Omega-3; R&P, Risk and Prevention Study.

[a] All trials used eicosapentaenoic acid and docosahexanoic acid supplements, with the exception of the JELIS trial (eicosapentaenoic acid only).

[b] All trials were blind, placebo-controlled randomized clinical trials with the exception of JELIS and GISSI-P, which were open-label without placebo.

A protocol outlining the eligibility criteria, prespecified analyses, and plans for publication together with standardized data request forms were sent to the principal investigators of all eligible trials. The study used the PRISMA guidelines for the conduct of meta-analysis of randomized trials.[19] Aggregated study-level (tabular) data were successfully obtained from 9 of the 10 trials (**Table**; eTable in the Supplement).[6-13,15] The JELIS trial[14] declined to participate in this collaboration, but the published results of the trial were sufficiently detailed to allow its inclusion in this study. Any discrepancies between data supplied and the published reports were clarified by contacting trial investigators.

## Statistical Analysis

The association of treatment with outcomes in each trial was analyzed separately, and summary statistics were calculated for each trial. For each trial, we calculated the observed minus expected statistic (O−E) and its variance (V) from the number of patients who developed the relevant end point and the total number of patients in each treatment group, using standard formulas for 2 × 2 contingency tables. One O−E value from each trial was summed to produce a grand total (G), with variance (V) equal to the sum of their separate variances. The value exp(G/V) is Peto 1-step estimate of the rate ratio (RR), and its continuity-corrected 95% confidence interval is given by $\exp(G/V \pm [0.5/V + (1.96/\sqrt{V})])$.[20] Rate ratios are given with 95% CI for the overall results for major diseases and with 99% CI (which is calculated by replacing 1.96 in the formula above by 2.58) for the results of individual trials or subgroups of trials or subgroups of such major diseases. Heterogeneity between the different subgroups is assessed by first calculating $S-(G^2/V)$, where S is the sum of $(O-E)^2/V$ for each trial (or subgrouping), and then testing this statistic against a $\chi^2$ distribution with the degrees of freedom equal to 1 fewer than the number of subgroups. The meta-analysis was repeated after excluding the JELIS trial,[14] since it tested EPA alone rather than the combination of EPA and DHA used in all other trials.[6-13,15]

Additional analyses of the primary outcomes assessed the associations of treatment with major vascular events in predefined subgroups, including age, sex, prior CHD, prior stroke, prior diabetes, blood lipids (total cholesterol, triglyceride, high-density lipoprotein, and calculated or measured low-density lipoprotein), prior use of statins, and trial design (open-label or blinded). In interpreting subgroup results, the chief emphasis was placed on the overall results, unless there was strong evidence of heterogeneity ($P < .001$). Sensitivity analyses compared the results of the Peto method with log-rank method in the 1 trial that had also provided individual participant data on all events.

# Results

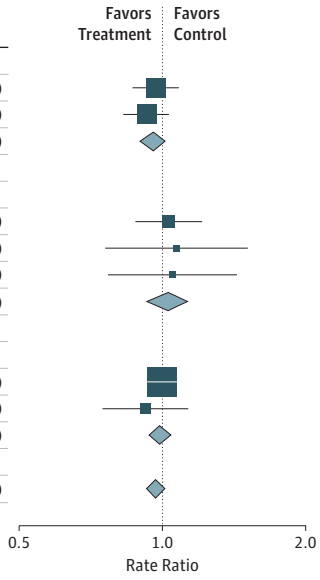
## Characteristics of Individual Trials

Study level data were obtained on a total of the 10 trials[6-15] that met the inclusion criteria. A total of 77 917 participants were involved, and trials ranged in size from 563 to 18 645 participants (Table; eTable in the Supplement). Of the 10 trials, 8 had a double-blind design and used a placebo control, and 2 trials had an open-label design.[6,14] The risk of bias of the included trials was low, with exception of the 2 trials that did not use a placebo-treated control group[6,14] (eFigure 2 in the Supplement).

Combinations of polyunsaturated fatty acid ethyl esters of EPA and DHA were used in all but 1 trial,[14] which tested daily

Figure 1. Associations of Omega-3 Fatty Acids With Major Vascular Events

| Source | No. of Events (%) Treatment | Control | Rate Ratios (CI) |
|---|---|---|---|
| Coronary heart disease | | | |
| Nonfatal myocardial infarction | 1121 (2.9) | 1155 (3.0) | 0.97 (0.87-1.08) |
| Coronary heart disease death | 1301 (3.3) | 1394 (3.6) | 0.93 (0.83-1.03) |
| Any | 3085 (7.9) | 3188 (8.2) | 0.96 (0.90-1.01) |
| | | | *P* = .12 |
| Stroke | | | |
| Ischemic | 574 (1.9) | 554 (1.8) | 1.03 (0.88-1.21) |
| Hemorrhagic | 117 (0.4) | 109 (0.4) | 1.07 (0.76-1.51) |
| Unclassified/other | 142 (0.4) | 135 (0.3) | 1.05 (0.77-1.43) |
| Any | 870 (2.2) | 843 (2.2) | 1.03 (0.93-1.13) |
| | | | *P* = .60 |
| Revascularization | | | |
| Coronary | 3044 (9.3) | 3040 (9.3) | 1.00 (0.93-1.07) |
| Noncoronary | 305 (2.7) | 330 (2.9) | 0.92 (0.75-1.13) |
| Any | 3290 (10.0) | 3313 (10.2) | 0.99 (0.94-1.04) |
| | | | *P* = .60 |
| Any major vascular event | 5930 (15.2) | 6071 (15.6) | 0.97 (0.93-1.01) |
| | | | *P* = .10 |




The number of events by allocated treatment are presented for individual trials and subgroups of trials; participants can contribute only once to subtotals and totals of major vascular events. Rate ratios for individual trials or subgroups of trials are indicated by squares and 99% CIs by horizontal lines. Overall totals and their 95% confidence intervals are represented by diamonds. The size of the squares and the diamonds are proportional to the statistical information conveyed.

dose of 1800 mg EPA alone. The daily doses of EPA varied from 226 to 1800 mg/day, and DHA varied from 0 to 1700 mg/day. The mean duration of treatment in individual trials varied from 1.0 year to 6.2 years (weighted mean, 4.4 years).

Of the 77 917 participants, 47 803 (61.4%) were men, and the mean age at entry was 64 years. After accounting for missing data, about two-thirds of participants had a prior history of CHD (31 076/46 767; 66.4%), 13 240 of 47 938 (28%) had prior stroke, and 28 722 of the total 77 917 participants (37%) had prior diabetes. Among the 77 917 participants, there were a total of 12 001 major vascular events (15.4% of 77 917 participants), including 2276 incidents of nonfatal MI (2.9%), 2695 CHD deaths (3.5%), 1713 strokes (2.2%), and 6603 revascularization events (8.5%) during the study duration (eTable in the Supplement). Data were available on the association of treatment by prior use of statin therapy in 7 trials involving 49 522 participants.[8,10-12,14,15]

## Associations of Omega-3 Fatty Acid Use With CHD and Major Vascular Events

**Figure 1** shows that randomization to receive omega-3 FA supplementation had no significant association with the rate ratios (RRs) for any CHD event (RR, 0.96; 95% CI, 0.90-1.01; *P* = .12) and no significant association with RRs in subgroups of CHD events, including CHD death (RR, 0.93; 99% CI, 0.83-1.03; *P* = .05) and nonfatal myocardial infarction (RR, 0.97; 99% CI, 0.87-1.08; *P* = .40). Likewise, randomization of patients to an omega-3 FA supplementation regimen had no associations with the RRs for major vascular events (RR, 0.97; 95% CI, 0.93-1.01; *P* = .10), stroke (RR, 1.03; 95% CI, 0.93-1.13; *P* = .56), or revascularization events (RR, 0.99; 95% CI, 0.94-1.04; *P* = .61). This meta-analysis also showed no significant heterogeneity between the results of individual trials for nonfatal MI, CHD death, any CHD events, or all major vascular events (**Figure 2**). The association of omega-3 FA supplementation with major vascular events were unaltered after excluding the JELIS trial[14] (odds ratio [OR], 0.98; 95% CI, 0.94-1.02; *P* = .30) (eFigure 3 in the Supplement). Additional sensitivity analyses in 1 trial[12] that compared the results of the Peto method (O−E statistic) with the log-rank method demonstrated that analysis of individual participant and study-level data yielded identical results for association of omega-3 FA supplementation with major vascular events (eFigure 4 in the Supplement).

## Associations of Omega-3 Fatty Acid Use With Major Vascular Events in Prespecified Subgroups

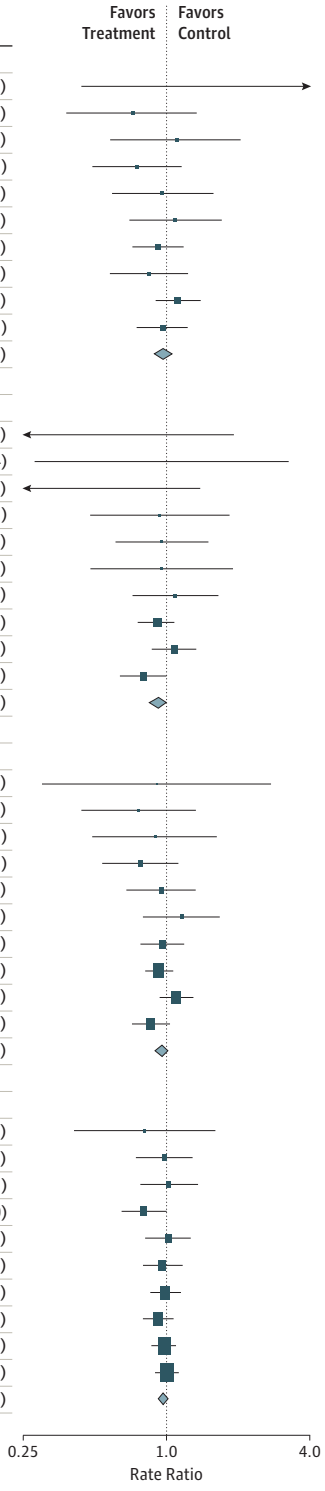
**Figure 3** shows that after adjustment for multiple testing, randomization of patients to study arms involving supplementation by omega-3 FAs had no significant association with major vascular events in any of the prespecified subgroups, including those defined by sex, history of CHD, history of diabetes, pretreatment levels of total cholesterol, high-density lipoprotein levels, low-density lipoprotein levels, triglyceride levels, or prior use of statin therapy. However, there was some evidence of heterogeneity in the associations of omega-3 FAs with major vascular events by age (unadjusted *P* = .02) and by history of stroke (*P* = .06), respectively. While it was not possible to assess the associations of treatment with race, the results were unaltered after exclusion of the JELIS trial,[14] which was conducted in a Japanese population only (eFigure 3 in the Supplement).

## Associations of Omega-3 Fatty Acid Use With CHD Events by Study Design

**Figure 4** demonstrates that randomization of patients to receive omega-3 FAs had no significant association with their experience of nonfatal MI, CHD death, or overall CHD in trials that used either an open-label and blind design. However, there was some evidence of heterogeneity between the results of open-label trials vs blind trials for all participants with CHD (open-

Figure 2. Associations of Omega-3 Fatty Acids With Subtypes of Coronary Heart Disease and Major Vascular Events, by Trial

| Source | No. of Events (%) Treatment | Control | Rate Ratios (CI) |
|---|---|---|---|
| Nonfatal myocardial infarction | | | |
| DOIT | 8 (2.8) | 4 (1.4) | 1.97 (0.44-8.85) |
| AREDS-2 | 30 (1.4) | 40 (1.9) | 0.72 (0.38-1.33) |
| SU.FOL.OM3 | 32 (2.6) | 29 (2.3) | 1.10 (0.56-2.15) |
| JELIS | 62 (0.7) | 83 (0.9) | 0.75 (0.49-1.15) |
| Alpha Omega | 56 (2.3) | 59 (2.4) | 0.96 (0.59-1.56) |
| OMEGA | 74 (3.8) | 67 (3.6) | 1.08 (0.70-1.69) |
| R&P | 228 (3.7) | 248 (4.0) | 0.92 (0.72-1.17) |
| GISSI-HF | 92 (2.6) | 108 (1.3) | 0.84 (0.58-1.22) |
| ORIGIN | 316 (5.0) | 284 (4.5) | 1.11 (0.90-1.38) |
| GISSI-P | 223 (3.9) | 233 (4.1) | 0.96 (0.75-1.22) |
| All | 1121 (2.9) | 1155 (3.0) | 0.97 (0.89-1.05) |
| | | | *P* = .40 |
| Coronary heart disease death | | | |
| DOIT | 3 (1.1) | 8 (2.8) | 0.39 (0.08-1.90) |
| AREDS-2 | 9 (0.4) | 9 (0.4) | 0.96 (0.28-3.24) |
| SU.FOL.OM3 | 5 (0.4) | 13 (1.0) | 0.41 (0.12-1.38) |
| JELIS | 29 (0.3) | 31 (0.3) | 0.93 (0.48-1.82) |
| Alpha Omega | 67 (2.8) | 71 (2.9) | 0.95 (0.61-1.49) |
| OMEGA | 28 (1.5) | 29 (1.5) | 0.95 (0.48-1.89) |
| R&P | 82 (1.3) | 76 (1.2) | 1.08 (0.72-1.64) |
| GISSI-HF | 512 (14.7) | 555 (15.9) | 0.91 (0.76-1.07) |
| ORIGIN | 319 (5.1) | 296 (4.7) | 1.08 (0.87-1.33) |
| GISSI-P | 247 (4.4) | 306 (5.4) | 0.80 (0.64-1.00) |
| All | 1301 (3.3) | 1394 (3.6) | 0.93 (0.85-1.00) |
| | | | *P* = .05 |
| Any coronary heart disease | | | |
| DOIT | 11 (3.9) | 12 (4.3) | 0.91 (0.30-2.73) |
| AREDS-2 | 39 (1.8) | 49 (2.4) | 0.76 (0.44-1.32) |
| SU.FOL.OM3 | 37 (3.0) | 41 (3.3) | 0.90 (0.49-1.62) |
| JELIS | 88 (0.9) | 113 (1.2) | 0.78 (0.54-1.12) |
| Alpha Omega | 120 (5.0) | 128 (5.3) | 0.95 (0.68-1.32) |
| OMEGA | 112 (5.8) | 96 (5.1) | 1.16 (0.80-1.67) |
| R&P | 130 (5.0) | 324 (5.2) | 0.96 (0.78-1.18) |
| GISSI-HF | 1309 (37.5) | 1360 (39.1) | 0.93 (0.82-1.06) |
| ORIGIN | 635 (10.1) | 580 (9.3) | 1.10 (0.94-1.29) |
| GISSI-P | 424 (7.5) | 485 (8.6) | 0.86 (0.72-1.03) |
| All | 3085 (7.9) | 3188 (8.2) | 0.96 (0.90-1.01) |
| | | | *P* = .12 |
| Major vascular events | | | |
| DOIT | 29 (10.3) | 35 (12.5) | 0.81 (0.41-1.60) |
| AREDS-2 | 213 (9.9) | 208 (10.1) | 0.98 (0.75-1.28) |
| SU.FOL.OM3 | 216 (17.2) | 211 (16.9) | 1.02 (0.78-1.35) |
| JELIS | 262 (2.8) | 324 (3.5) | 0.80 (0.65-1.00) |
| Alpha Omega | 332 (13.8) | 331 (13.6) | 1.02 (0.82-1.26) |
| OMEGA | 534 (27.7) | 541 (28.6) | 0.96 (0.80-1.16) |
| R&P | 733 (11.7) | 745 (11.9) | 0.99 (0.86-1.14) |
| GISSI-HF | 783 (22.4) | 831 (23.9) | 0.92 (0.80-1.07) |
| ORIGIN | 1276 (20.3) | 1295 (20.7) | 0.98 (0.87-1.09) |
| GISSI-P | 1552 (27.4) | 1550 (27.3) | 1.00 (0.90-1.12) |
| All | 5930 (15.2) | 6071 (15.6) | 0.97 (0.93-1.01) |
| | | | *P* = .10 |

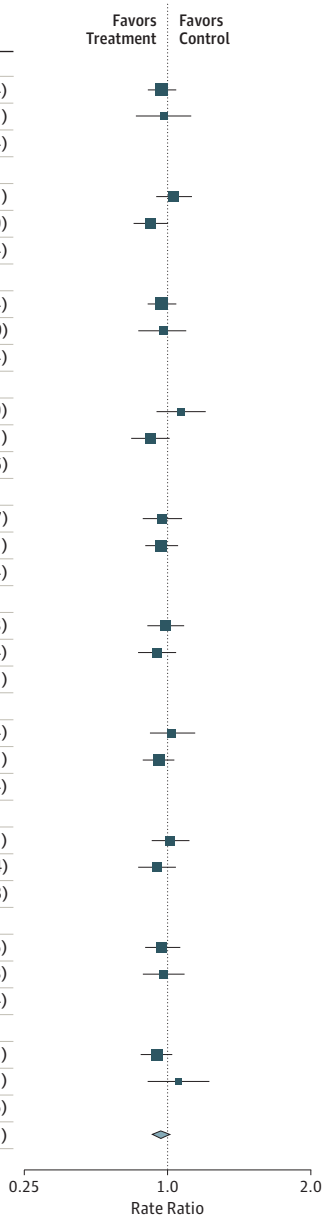



Symbols and conventions as in Figure 1. Study names are AREDS-2, Age-Related Eye Disease Study 2; DOIT, Diet and Omega-3 Intervention Trial; GISSI-HF, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Heart Failure; GISSI-P, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Prevenzione; JELIS, Japan Eicosapentaenoic Acid (EPA) Lipid Intervention Study; OMEGA, Effect of Omega 3-Fatty Acids on the Reduction of Sudden Cardiac Death After Myocardial Infarction; ORIGIN, Outcome Reduction With Initial Glargine Intervention; SU.FOL.OM3, Supplémentation en Folates et Omega-3; R&P, Risk and Prevention Study. Rate ratios for individual trials or subgroups of trials are indicated by squares and the 99% CIs by the horizontal lines. Overall totals and their 95% confidence intervals are represented by diamonds. Arrowheads indicate error bars that extend beyond the area shown. Heterogeneity between all trials ($\chi^2_9$ in all cases) for nonfatal myocardial infarction, coronary heart disease death, any coronary heart disease, and major vascular events were 10.18 (*P* = .34), 12.3 (*P* = .20), 12.92 (*P* = .17), and 7.68 (*P* = .57), respectively.

label trials: RR, 0.85; 99% CI, 0.72-0.99; *P* = .01; blinded trials: RR, 0.99; 99% CI, 0.91-1.07; *P* = .69; heterogeneity *P* = 0.03), but not for either fatal CHD or nonfatal MI, respectively. Overall, the results of this meta-analysis demonstrated no significant association of supplementation with omega-3 FAs for a mean duration of 4.4 years with the risk of fatal CHD, nonfatal MI, any CHD, or any major vascular events in the full study population and in all relevant subgroups.

Figure 3. Associations of Omega-3 Fatty Acids With Major Vascular Events, in Prespecified Subgroups

| Source | No. of Events (%) Treatment (n = 39 017) | Control (n = 38 900) | Rate Ratios (CI) |
|---|---|---|---|
| Sex | | | |
| Male | 3989 (23.2) | 4040 (23.7) | 0.97 (0.91-1.04) |
| Female | 946 (15.1) | 962 (15.5) | 0.98 (0.86-1.12) |
| Missing | 995 (6.4) | 1069 (6.9) | 0.93 (0.82-1.04) |
| Age, y | | | |
| <65 | 2528 (21.8) | 2432 (21.3) | 1.03 (0.95-1.12) |
| ≥65 | 2407 (20.5) | 2570 (22.0) | 0.92 (0.85-1.00) |
| Missing | 995 (6.4) | 1069 (6.9) | 0.93 (0.82-1.04) |
| Prior coronary heart disease | | | |
| Yes | 3499 (22.5) | 3550 (22.9) | 0.97 (0.91-1.04) |
| No | 1435 (18.5) | 1448 (19.0) | 0.98 (0.87-1.09) |
| Missing | 996 (6.4) | 1073 (6.9) | 0.92 (0.82-1.04) |
| Prior stroke | | | |
| Yes | 1332 (21.1) | 1262 (20.0) | 1.07 (0.95-1.20) |
| No | 2046 (18.3) | 2185 (19.6) | 0.92 (0.84-1.01) |
| Missing | 2552 (12.0) | 2624 (12.3) | 0.97 (0.89-1.05) |
| Prior diabetes | | | |
| Yes | 2087 (22.8) | 2111 (23.3) | 0.97 (0.89-1.07) |
| No | 2846 (20.1) | 2889 (20.5) | 0.97 (0.90-1.05) |
| Missing | 997 (6.4) | 1071 (6.9) | 0.93 (0.82-1.04) |
| Total cholesterol | | | |
| <193.1 | 2326 (21.5) | 2315 (21.5) | 0.99 (0.91-1.08) |
| ≥193.1 | 2346 (22.8) | 2441 (23.7) | 0.95 (0.87-1.04) |
| Missing | 1258 (7.1) | 1315 (7.4) | 0.94 (0.84-1.05) |
| High-density lipoprotein cholesterol | | | |
| <38.6 | 1532 (25.4) | 1512 (24.8) | 1.02 (0.92-1.14) |
| ≥38.6 | 2961 (20.7) | 3041 (21.5) | 0.96 (0.89-1.03) |
| Missing | 1437 (7.8) | 1518 (8.2) | 0.94 (0.85-1.04) |
| Low-density lipoprotein cholesterol | | | |
| <115.8 | 2096 (21.0) | 2051 (20.6) | 1.01 (0.93-1.11) |
| ≥115.8 | 2295 (23.2) | 2385 (24.2) | 0.95 (0.87-1.04) |
| Missing | 1539 (8.1) | 1635 (8.6) | 0.93 (0.84-1.03) |
| Triglycerides | | | |
| ≤150.4 | 2626 (21.3) | 2638 (21.7) | 0.97 (0.90-1.06) |
| >150.4 | 1998 (23.4) | 2061 (23.7) | 0.98 (0.89-1.08) |
| Missing | 1306 (7.3) | 1372 (7.7) | 0.94 (0.84-1.04) |
| Prior statin use | | | |
| Yes | 2824 (11.4) | 2959 (11.9) | 0.95 (0.88-1.02) |
| No | 742 (15.4) | 696 (14.9) | 1.05 (0.91-1.22) |
| Missing | 2364 (25.0) | 2416 (25.6) | 0.97 (0.89-1.06) |
| All | 5930 (15.2) | 6071 (15.6) | 0.97 (0.93-1.01) |
| | | | P = .10 |




Symbols and conventions as in Figure 1. Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were measured in mg/dL (to convert cholesterol to mmol/L, multiply by 0.0259; triglycerides, multiply by 0.0113). Heterogeneity between all trials ($\chi^2_1$ in all cases) was 0.04 ($P$ = .84) for sex, 5.59 ($P$ = .02) for age, 0.0 ($P$ = .96) for prior coronary heart disease, 7.03 ($P$ = .01) for prior stroke, 0.0 ($P$ > .99) for prior diabetes, 0.87 ($P$ = .35) for total cholesterol, 1.56 ($P$ = .21) for high-density lipoprotein cholesterol, 1.8 ($P$ = .18) for low-density lipoprotein cholesterol, 0.02 ($P$ = .89) for triglycerides, and 2.55 ($P$ = .11) for prior statin use.

### Associations of Omega-3 Fatty Acid Use With All-Cause Mortality

Randomization to omega-3 FA intervention had no significant association with RRs of all-cause mortality (RR, 0.96; 95% CI, 0.92-1.01; $P$ = .16). Further information is presented in eFigure 5 in the Supplement.

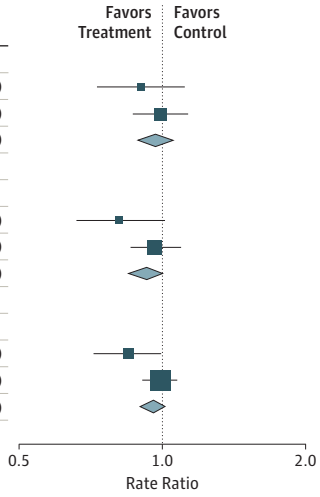
## Discussion

This meta-analysis of 10 randomized clinical trials, involving 77 917 participants, demonstrated that randomization to trial arms with omega-3 FA supplementation for a mean of 4.4 years had no significant effect on either of fatal CHD, nonfatal MI, stroke, revascularization events, or any major vascular events. Importantly, this meta-analysis also demonstrated no significant effect on major vascular events in any particular subgroups, including prior vascular disease, diabetes, lipid levels, or statin use. Likewise, the present meta-analysis showed no significant association of omega-3 FA supplementation with all-cause mortality or cancer (data not shown). Moreover, the overall results were unaltered after exclusion of the JELIS trial,[14] which tested the effects of EPA alone rather than EPA and DHA combined.

The chief strength of this study was the availability of study-level data extracted by the trial principal investigators for all prespecified outcomes in this meta-analysis (with the exception of the JELIS trial,[14] in which the published data

Figure 4. Associations of Omega-3 Fatty Acids With Fatal and Nonfatal Vascular Events, by Trial Design

| Source | No. of Events (%) Treatment | Control | Rate Ratios (CI) |
|---|---|---|---|
| Nonfatal myocardial infarction | | | |
| Open | 285 (1.9) | 316 (2.1) | 0.90 (0.73-1.11) |
| Blind | 836 (3.5) | 839 (3.5) | 0.99 (0.87-1.13) |
| All | 1121 (2.9) | 1155 (3.0) | 0.97 (0.89-1.05) |
| | | | *P* = .40 |
| Coronary heart disease death | | | |
| Open | 276 (1.8) | 337 (2.2) | 0.81 (0.66-1.01) |
| Blind | 1025 (4.3) | 1057 (4.4) | 0.96 (0.86-1.09) |
| All | 1301 (3.3) | 1394 (3.6) | 0.93 (0.85-1.00) |
| | | | *P* = .05 |
| Any coronary heart disease | | | |
| Open | 512 (3.4) | 598 (4.0) | 0.85 (0.72-0.99) |
| Blind | 2573 (10.7) | 2590 (10.8) | 0.99 (0.91-1.07) |
| All | 3085 (7.9) | 3188 (8.2) | 0.96 (0.90-1.01) |
| | | | *P* = .12 |




Symbols and conventions as in Figure 1. Heterogeneity between trial designs ($\chi^2_1$ in all cases) was 1.05 (*P* = .31) for nonfatal myocardial infarction, 3.26 (*P* = .07) for coronary heart disease death, and 4.81 (*P* = .03) for any coronary heart disease.

were used). The inclusion criteria and vascular disease outcomes differed from previous meta-analyses of the published results.[16-18] The present meta-analysis had a low risk of selection bias or confounding because it did not include trials testing the effects of dietary advice to eat fish nor trials that were either too small or insufficient in treatment duration. In contrast with previous meta-analyses, the present meta-analysis also examined effects of supplementation with omega-3 FA supplementation in prespecified subgroups of major vascular events by history of disease, history of diabetes, lipid levels, or statin use.

The reasons for the discrepant results of the previous trials of omega-3 FA supplementation on fatal and nonfatal CHD events are unclear. In contrast with the null findings for most trials, the GISSI-Prevenzione trial[6] reported a 14% reduction in major vascular events, chiefly owing to an 11% reduction in cardiac deaths. But the JELIS trial reported a 19% (95% CI, 5%-31%) reduction in major CHD events (albeit based on only 586 events), chiefly owing to a reduction in nonfatal CHD events.[14] It is unclear whether differences in inclusion criteria for prior diseases, concomitant use of statins, or other secondary prevention treatments may explain some of the conflicting results of individual trials.

For example, previous reports had suggested that the effects of omega-3 FA use may vary by patients' prior use of statin medications.[21,22] The Alpha Omega trial reported that use of low-dose omega-3 FAs reduced the risk of major vascular events in patients with prior MI who were not treated with statin medications.[22] However, the present meta-analysis demonstrated no heterogeneity in the effects of omega-3 FA supplementation on CHD death or nonfatal MI between the individual trials and reported no differences in the effects of omega-3 FAs on major vascular events by subgroups of those with or without prior cardiovascular disease or diabetes; those with lipid levels less than or greater than specified cutoff points; or those who had histories of statin therapy. The results of the present meta-analysis were also unaltered by the exclusion of the JELIS trial,[14] in which all participants were also treated with statin medications.

The present meta-analysis reported weak evidence of heterogeneity between the results of open-label vs blind trials for any CHD. This may reflect reporting bias, chance, or greater compliance in the open-label trials than in the blinded trials.

Previous meta-analyses of omega-3 FA trials,[16-18] which were limited by being incomplete, including trials of dietary advice to increase fish consumption,[16,17] or failure to distinguish the effects on a wide range of subtypes of CVD.[16-18,23,24] In contrast, the present meta-analysis demonstrated that omega-3 FA supplementation had no significant effect on fatal CHD or any other CVD subtypes. Moreover, the conclusions of the present meta-analysis are consistent with those of a 2016 report[24] for the US Agency for Healthcare Research and Quality that also involved study-level data from the same 10 large trials for prevention of major vascular events, and concluded that omega-3 FA supplementation had no association with the risk of major vascular events, all-cause mortality, sudden cardiac death, or revascularization. In contrast with this report, the present article was able to assess effects on a wide range of subtypes of CVD and on major vascular events in all relevant subgroups.[24]

## Limitations

This meta-analysis had several limitations. The protocol did not prespecify assessment of the effects of treatment by smoking status or by site-specific cancer incidence. An additional limitation of this meta-analysis involved the use of aggregated study-level data rather than individual-level data. A meta-analysis of individual participant data may have a greater chance of detecting effects of omega-3 FA supplements on subtypes of fatal CHD events (ie, sudden death or ventricular arrhythmias) in a wider range of subgroups. However, the overall null results of the present meta-analysis, which assesses effects on a wide range of prespecified CVD subtypes, provides little encouragement for such an approach. In addition, sensitivity analyses

using data from 1 trial[12] that also provided data on all individual participants indicated identical effect estimates and 99% CI for analyses using both O-E and log-rank methods.

The 95% CI in the present meta-analysis of 10 trials, involving 77 917 high-risk individuals, 12 001 major vascular events, and 6273 CHD events, cannot exclude a 7% lower risk of major vascular events and a 10% lower risk of CHD associated with omega-3 FA supplements. Several ongoing large randomized trials involving a total of 54 354 additional participants (A Study of Cardiovascular Events in Diabetes [ASCEND],[25] n = 15 480; VITamin D and OmegA-3 TriaL [VITAL],[26] n = 25 874; STatin Residual risk reduction with EpaNova in hiGh CV risk patienTs with Hypertriglyceridemia [STRENGTH],[27] n = 13 000 and Reduction of Cardiovascular Events With EPA–Intervention Trial [REDUCE-IT], n = 8000) will provide additional evidence about the associations of omega-3 FA supplementation with the risk of major vascular events, any CHD, and subtypes of fatal and nonfatal CHD. Importantly, the STRENGTH[27] and REDUCE-IT trials will test the effects on major vascular events of much higher doses of omega-3 FAs (3-4 g/d), which will lower plasma levels of triglycerides.

## Conclusions

The 2016 European Society of Cardiology and European Atherosclerosis Society guidelines for prevention of cardiovascular disease[28] indicated that it is debatable whether omega-3 FAs may exert a protective effect, and the 2016 guidelines on the management of dyslipidaemia[29] indicated that more evidence on the efficacy of omega-3 FA supplements for prevention of clinical outcomes is needed to justify their prescription. In contrast, the American Heart Association recommended[30] that the use of omega-3 FAs for prevention of CHD is probably justified in individuals with prior CHD and those with heart failure and reduced ejection fractions. However, the results of the present meta-analysis provide no support for the recommendations to use approximately 1 g/d of omega-3 FAs in individuals with a history of CHD for the prevention of fatal CHD, nonfatal MI, or any other vascular events. The results of the ongoing trials are needed to assess if higher doses of omega-3 FAs (3-4 g/d) may have significant effects on risk of major vascular events.

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