Articles in PresS. Am J Physiol Endocrinol Metab (October 28, 2008). doi:10.1152/ajpendo.90684.2008

ANTIMÜLLERIAN HORMONE LEVELS REFLECT THE SEVERITY OF PCOS, BUT ARE NEGATIVELY INFLUENCED BY OBESITY: RELATIONSHIP WITH INCREASED LUTENEIZING HORMONE LEVELS

Athanasia Piouka [1], Dimitrios Farmakiotis [1], Ilias Katsikis [1], Djuro Macut [2], Spiros Gerou [3], Dimitrios Panidis [1]

[1] Division of Endocrinology and Human Reproduction, 2nd Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece

[2] Institute of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Centre of Serbia, Belgrade, Serbia and Montenegro

[3] "Analysis" Laboratories, Thessaloniki, Greece

Corresponding Author

Dimitrios Panidis, MD, PhD, Professor of Endocrinology

119 Mitropoleos Str, 54622, Thessaloniki, Greece

Tel no: +30 2310 992915

e-mail: panidisd@med.auth.gr

ABSTRACT

The objective of the study was the comparison of AMH levels among obese or overweight and normal-weight women with the four different PCOS phenotypes and healthy controls. AMH levels were evaluated in four age- and BMI-matched groups of 25 normal-weight and 25 obese or overweight women each, belonging to the four main subsets of the syndrome, resulting from combinations of the three diagnostic criteria (Group 1: oligo-amenorrhoea (ANOV), hyperandrogenemia (HA) and polycystic ovaries on ultrasonographic evaluation (PCO), Group 2: ANOV and HA, Group 3: HA and PCO, Group 4: ANOV and PCO), and in 50 (25 obese or overweight and 25 normal-weight) age- and BMI-matched healthy controls. Age, BMI, W, FSH, LH, prolactin, testosterone, Δ_4A, DHEA-S, 17α-OH-progesterone, fasting insulin, glucose, AMH, FAI and HoMA-IR were analysed. AMH levels were significantly higher in PCOS groups 1 and 2, compared to groups 3 and 4 and the control group and higher in PCOS groups 3 and 4, compared to the control group. AMH levels were significantly increased in normal-weight, compared to obese and overweight women. AMH concentrations were independently predicted, in order of significance, by LH and testosterone levels, BMI (negatively) and the total number of follicles 2-9 mm in diameter. The differences in circulating AMH levels between the main phenotypic groups of PCOS women seem to reflect the severity of the syndrome, but are negatively affected by obesity. Increased LH levels might be the most significant independent link between PCOS-associated disorders of ovulation and the observed increase in circulating AMH concentration.

Key words: antimüllerian hormone (AMH), polycystic ovary syndrome (PCOS), luteneising hormone (LH)

INTRODUCTION

Diagnostic criteria for polycystic ovary syndrome (PCOS), as suggested in the Rotterdam conference sponsored by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) in 2003, include: (i) oligo- and/or anovulation (ANOV), (ii) hyperandrogenemia and/or hyperandrogenism (clinical signs of high androgen levels) (HA), and (iii) polycystic ovaries (PCO). Diagnosis presupposes the presence of at least two of the three features, following the exclusion of other androgen excess disorders (1,2). Therefore, four different clinical phenotypes of PCOS arise, according to the combination of its main three manifestations (Table 1).

Despite the recommended criteria, there is still controversy among the experts about the importance of each feature and the severity of the reproductive and metabolic dysfunction every phenotype implies (3,4). Thus, the phenotypic group with all three criteria is termed ‘severe’ (3), while, notably, the presence of both ANOV and HA, independent of PCO, was required for the diagnosis of the syndrome, before 2003 (5).

Obesity is present in varying degrees (30% to 70%) in women with PCOS (6) and is usually of the central type (7). Central obesity, being a prominent feature of the so-called metabolic syndrome, is directly linked to increased peripheral insulin resistance (IR) (8). Furthermore, PCOS itself has been shown to confer a risk for IR, beyond that caused by obesity alone (9).

Oligo/anovulation in PCOS is, apparently, due to a 2-fold disorder of follicular development: first, early follicular growth is excessive; second, the selection of one follicle from the increased pool and its further maturation to a dominant follicle does not occur (follicular arrest) (10). Antimüllerian hormone (AMH) is a member of the transforming growth factor-β (TGF-β) superfamily of glycoproteins, that has been found to play an important role in both of the above processes by 1. inhibiting the initial recruitment of primordial follicles (11,12) and 2. promoting follicular arrest (11,13). Indeed, AMH levels have been found increased in both serum (14-16) and follicular fluid (17) of women with PCOS, while its expression is, paradoxically, reduced during the initial stage of follicular recruitment, in anovulatory women with PCO (18). The relationship between AMH and obesity or IR in PCOS has not been studied, so far.

Based on the above evidence, the present study was designed in order to: (i) explore the hypothesis that the severity of PCOS is reflected upon AMH levels, and, (ii) assess the possible correlations between AMH, obesity and the hormonal or metabolic parameters of the syndrome. To our knowledge, this is the first study to classify the PCOS population in four equally-sized groups, matched for BMI and age, according to their PCOS phenotype.

SUBJECTS AND METHODS

Subjects

Two-hundred (200) women with PCOS [100 normal-weight (BMI: 20-24.99 kg/m^2) and 100 obese and overweight (BMI>25 kg/m^2)] and fifty (50: 25 normal-weight and 25 obese and overweight) healthy women with normal ovulating cycles (28±2 days, blood progesterone levels >10 ng/mL in two consecutive cycles), no signs of hyperandrogenism and normal sonographic appearance of the ovaries (controls) were recruited. All women with PCOS were outpatients at the Gynecological Endocrinology Infirmary of the Second and Fourth Departments of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece. Healthy women of the control group were volunteers.

Diagnosis of PCOS was based on the revised criteria of Rotterdam (2003) (1,2) (see “study protocol”). Women of the control group were healthy volunteers. None of the women studied had galactorrhea, nor any endocrine or systemic disease that could possibly affect their reproductive physiology. A Synacthen test was performed using tetracosactide (Synacthène 0.25 mg/1 ml; Novartis Pharma S.A., Rueil-Malmaison, France) on each woman with a basal 17-OH-progesterone plasma level greater than 1.5 ng/ml to exclude congenital adrenal hyperplasia.

No woman reported use of any medication that could interfere with the normal function of the hypothalamic–pituitary–gonadal axis during the last semester. Informed consent was obtained from all women and the study was

approved by the Institutional Review Board; the study met the requirements of the 1975 Helsinki guidelines.

Study protocol

In all women, weight, height, and waist circumferences (W) were measured. Body weight was measured using analogue scales and in light clothing; height was measured bare-foot using a stadiometre. Body mass index (BMI, kg/m^2) was calculated by dividing weight by height squared (kg per square metre) to assess obesity. Waist circumference (W) was obtained as the smallest circumference at the level of the umbilicus.

Baseline blood samples were collected between days 3 and 7 of the menstrual cycle in the control group and after a spontaneous bleeding episode in the PCOS group, following an overnight fast. The circulating levels of follicle stimulating hormone (FSH), luteneizing hormone (LH), prolactin (PRL), total testosterone (T), Δ_4-androstenedione (Δ_4A), dehydroepiandrosterone-sulfate (DHEA-S), 17-OH-progesterone (17-OH-P), sex-hormone-binding globulin (SHBG), glucose (Glu), insulin (Ins) and antimüllerian hormone (AMH) were measured.

Women with PCOS were classified into the following four groups, according to their clinical, biochemical and sonographic characteristics (Table 1):

1. Group 1 included fifty (50: 25 normal-weight and 25 obese and overweight) women presenting with oligo- or amenorrhoea (<8 spontaneous hemorrhagic episodes/year), hyperandrogenemia (early

follicular phase T > 60 ng/dl, corresponding to the mean + 2 SD of 100 controls, measured in our lab) and polycystic ovaries by sonographic evaluation (≥12 small follicles in at least one ovary and/or ovarian volume >10 cm^3) ("severe" PCOS).

2. Group 2 included fifty (50: 25 normal-weight and 25 obese and overweight) women presenting with oligo- or amenorrhoea and hyperandrogenemia without polycystic ovaries.
3. Group 3 included fifty (50: 25 normal-weight and 25 obese and overweight) women presenting with hyperandrogenemia and polycystic ovaries, without oligo- or amenorrhoea ("ovulatory" PCOS).
4. Group 4 included fifty (50: 25 normal-weight and 25 obese and overweight) women presenting with oligo- or amenorrhoea and polycystic ovaries, without hyperandrogenemia ("mild" PCOS).

All women were selected from a large cohort of outpatients at our Department (4), in order for the above groups to be matched for age and BMI (Tables 2 and 3).

Assay methods and calculations

Plasma glucose, insulin, LH, FSH, PRL, androgen and 17-OH-progesterone concentrations were measured as previously described (4).

AMH concentrations were measured with an enzymatically amplified two-side immunoassay [DSL-10-14400 Active Mllerian Inhibiting Substance / AMH enzyme-linked immunosorbent (ELISA) kit, DSL laboratories, Webster, TX]. The

theoretical sensitivity of the method is 0.006 ng/ml, the intra-assay coefficient of variation for high values 3.3% and the inter-assay coefficient of variation for high values 6.7%.

The Free Androgen Index (FAI) was calculated as T (nmol/L)×100/SHBG (nmol/L) (19). The Homeostasis Model Assessment for Insulin Resistance Index (HoMA-IR) was calculated according to the formula: fasting insulin (IU/mL) × fasting glucose (mmol/L)/22.5 (20).

Ultrasound evaluation

Transvaginal ultrasound scans of the ovaries were performed by an experienced sonographer (I.K.) in women who participated in the study. Ovarian volume was calculated by the formula: $V= (\pi/6) \times D_{length} \times D_{width} \times D_{thickness}$ (D: dimension).The presence of polycystic ovaries was diagnosed by the presence of 12 or more follicles in each ovary, measuring 2–9 mm in diameter, and/or increased ovarian volume (> 10 cm^3).

Statistical analysis

Normality of distribution was assessed with the one-sample Kolmogorov-Smirnoff test and values that were not normally distributed were log-transformed. In order to avoid type I error, comparisons of means were performed with General Linear Model (GLM)- based two-way ANalysis Of VAriance (ANOVA) with post-hoc analysis for pairwise comparisons after Bonferroni adjustment.

Correlations were assessed with calculation of the Spearman coefficient. Independent relationships were assessed by means of multiple regression analysis, after log-transformation. Two-tailed statistical significance was set at 5%. Statistical analyses were performed with SPSS software v. 15.0 (SPSS inc, Chicago, Illinois). The graph was designed with MedCalc software (version 8.0.0.1, www.medcalc.be).

RESULTS

Effects of PCOS

The women of the five groups did not differ significantly in age, BMI, W, and PRL levels. FSH levels were borderline lower in women with PCOS (F=2.5, p=0.04), specifically those of group 4, compared to controls (p=0.03). LH levels were higher in women with PCOS (F=5.5, p<0.001); in pairwise comparisons, women of group 1 had significantly higher LH levels, compared to women of group 3 (p=0.002) and controls (p=0.001) (Tables 2 and 3).

By definition, T (F=68.2), Δ_4A (F=35.9) and DHEA-S (F=13.0) levels, as well as FAI values (F=52.0) were significantly higher in women with HA (PCOS groups 1-3), compared to those without (PCOS 4 and controls) (p<0.001 in all comparisons), whereas no difference was observed between the former three groups. No difference in T and DHEA-S levels was observed between the latter two groups (4 and controls), whereas Δ_4A levels and FAI values were higher in women of group 4, compared to controls (p=0.003 and 0.028, respectively). 17-OH-progesterone levels were higher in all women with PCOS, compared to controls (F=6.7, p<0.001; p=0.005 for group 1, 0.009 for group 2, <0.001 for group 3 and 0.02 for group 4), but no significant differences were observed between the four groups of women with the syndrome (Tables 2 and 3).

The total number of follicles 2-9 mm on ultrasonography (F=57.60), as well as the mean ovarian volume (F=8.24) were by definition higher in women with PCO (PCOS groups 1,3 and 4), compared to those without (group 2 and

controls, p<0.001 in all comparisons). No difference was observed between the former three or the latter two groups. (Tables 2 and 3).

SHBG levels were significantly higher in controls, compared to all HA groups of women with PCOS (1-3, F=9.4, p<0.001 in all comparisons) and in women of group 4 (non-HA PCOS), compared to group 2 (p=0.021) (Tables 2 and 3). No statistical significance was observed between women of the five groups in indices of IR (fasting insulin levels and HoMA-IR levels), although a trend for higher HoMA-IR values was observed in women with PCOS (Tables 2 and 3, F=2.0, p=0.093).

The concentrations of AMH (F=40.4, p<0.001) were significantly higher in groups 1 and 2, compared to all other groups of women with PCOS and the control group (p<0.001 in all comparisons, except for group 2 vs. 3 and 4, p=0.002 and 0.001, respectively). AMH levels were significantly higher in women with PCOS of groups 3 and 4, compared to the control group (p=0.008 and 0.025, respectively) (Figure 1, Tables 2 and 3).

Effects of obesity

Obese and overweight women had significantly higher levels of fasting insulin (F=86.3), HoMA-IR (F=91.1) and FAI (40.1) values, compared to normal-weight women (Tables 2 and 3, p<0.001 in all comparisons). LH, SHBG and AMH levels were significantly lower in obese and overweight women, compared to normal-weight women (F=7.9, p=0.005; F=44.8, p<0.001; F=64.7, p<0.001,

respectively). No significant interactions were observed (Tables 2 and 3, Figure 1).

AMH determinants

AMH levels were negatively correlated with age (r=-0.215, p=0.001), BMI (r=-0.310, p<0.001), W (r=-0.263, p<0.001), and positively with LH (r=+0.374, p<0.001), T (r=+0.444, p<0.001), Δ_4A (r=+0.398, p<0.001), 17-OH-progesterone (r=0.186, p-=0.003), FAI values (r=+0.219, p=0.001), the total number of small follicles (r=0.263, p<0.001) and the mean ovarian volume (r=0.178, p=0.007).

Using multiple regression analysis on all of the above parameters, four models predicted serum AMH concentrations: In model 1, LH concentration was the only significant determinant. In model 2, T was added, in model 3 BMI and in model 4, LH and T levels, BMI and the total no of follicles 2-9 mm were the significant independent determinants (Table 4).

DISCUSSION

The present study confirmed previous results (14-16) on increased serum AMH levels in women with the polycystic ovary syndrome, compared to healthy controls (Tables 2 and 3, Figure 1). Ovulatory disorders in women with the syndrome are caused by: a) an increased early follicular growth, resulting in a larger than normal reserve of selectable follicles and b) defective selection of one follicle from this increased pool, leading to follicular arrest (10). Since AMH has been shown to inhibit the initial follicle recruitment (12), but also cause follicular arrest (13), and AMH levels are increased in the syndrome, it has been proposed that its involvement in PCOS-associated oligo-anovulation lies only in the second mechanism (10). Nevertheless, more recent data support an additional PCOS-associated defect in the early production of AMH by the granulosa cells (GC) of growing follicles, indicating a possible involvement of decreased AMH in the disordered early follicle development observed in anovulatory women with the syndrome (18).

It has also been reported that the excess in AMH production by polycystic ovaries is a result of the increased small follicle number per se (10). However, based on the present results, the increased number of follicles 2-9 mm in diameter, demonstrated as PCO sonographic morphology, is not the only determinant of serum AMH. Indeed, AMH levels were higher in anovulatory and hyperandrogenic women with NIH-defined “classical” PCOS, without PCO morphology (group 2), compared to both ovulatory women with PCO and HA (group 3) and anovulatory women with PCO but normal androgen levels (group

4) (Tables 2 and 3, Figure 1). Therefore, AMH levels reflect the severity of PCOS, traditionally defined by its two cardinal elements, ie. oligo-anovulation and hyperandrogenemia (5).

Moreover, the total number of 2-9 mm-sized follicles, although an independent determinant, contributed only an additional 5.3% to the variance of AMH levels, as opposed to 18% by LH levels alone and an additional 9.5% by circulating testosterone (Table 4). Notably, cells from normal ovaries have been found to produce very little response to LH, whereas this gonadotropin stimulated AMH production 4-fold in cells from PCOs (17). Furthermore, a significant and independent positive association between LH and AMH levels has been recently observed in women with clomiphene-resistant PCOS, undergoing low-dose FSH treatment of anovulation (21). These findings support our own observation that serum AMH concentration was positively influenced by LH levels (Table 4). After all, LH levels were markedly high in women with “severe” PCOS (group 1), who also had the highest AMH concentrations (Figure 1, Tables 2 and 3).

The strong independent positive effect of LH on GC production of AMH was also demonstrated in the consistently higher AMH levels in normal-weight women, which have been shown to have higher LH levels, compared to obese and overweight women, both in the present (Tables 2 and 3) and previous (16,22-24) studies. Although in a previous report on adolescent girls with the syndrome (16), no significant association between AMH levels and BMI was observed, our results are in agreement with another recent study in the adult

population, where AMH levels were by 65% lower in obese women, compared to normal-weight women of late reproductive age (25).

To sum up, a strong positive association between LH and AMH levels was demonstrated in the present study by: 1. The strong independent correlation between the two hormones, explaining the most significant proportion of AMH variance (Table 4). 2. The significantly higher LH concentrations in women with “severe” PCOS (group 1) (Figure 1, Tables 2 and 3), along with the highest levels of serum AMH. 3. Most important, the substantially lower AMH levels in obese and overweight, compared to normal-weight women, which could not possibly be accounted for by any of the other PCOS-associated hormonal or metabolic defects (Figure 1, Tables 2 and 3).

It should be noted that an earlier LH receptor gain in anovulatory patients with PCOS has been proposed (10,26,27). Therefore, premature LH action on the GC of anovulatory women with the syndrome might offer an additional contribution to the follicular arrest, being the link between PCOS-associated disorders of ovulation and the observed increase in GC production of AMH. Enhanced and premature LH action could also compensate, during mid-to-late follicular development, for the aforementioned initial defect of AMH production by primary and transitional follicles, in anovulatory women with PCOS (18).

The intra-ovarian hyperandrogenism may be the main culprit for the follicular arrest. Indeed, it seems to lead to follicle excess, increasing the AMH intra-ovarian level, which then could exert an inhibiting effect on the selection process (10). This notion is supported by our own observation that serum T

levels were significantly and independently associated with circulating AMH (Table 4). Nevertheless, ovulatory hyperandrogenic women with PCOS (group 3, classified as PCOS, based on the revised criteria) had significantly lower AMH levels, compared to anovulatory ones (groups 1 and 2, “classical” PCOS), whereas no difference was observed between groups 3 and 4, despite the presence of HA in the former (Figure 1, Tables 2 and 3). Moreover, in multiple regression analysis, several other significant determinants were identified (Table 4). Therefore, we postulate that AMH levels reflect a dynamic interaction between LH secretion and influence, androgens and endogenous disorders of follicular maturation. It should be noted that circulating T might not accurately reflect intra-ovarian androgen concentration and action.

Insulin resistance (IR) has been proposed as a “second hit” that non-specifically contributes to PCOS-associated follicular arrest (9,10,28). In the present study, the well-known (7,8,29) association between IR and obesity was confirmed; however, no independent significant association with PCOS was found, although a trend for higher HoMA-IR values in women with PCOS, especially its “severe” form was observed (Tables 2 and 3). It should be noted, though, that these negative results may have well been limited by the wide spread of fasting indices of IR values, which, in small samples, have been shown to be relatively inaccurate, compared to the “golden standard”, ie the euglycemic hyperinsulinemic clamp (30). Nevertheless, no correlation between AMH and indices of IR was observed whatsoever and we presume that a direct relationship between the two is quite unlikely.

In conclusion, AMH levels reflect the severity of PCOS and are significantly increased in its “classical” phenotypic forms, based on the 1990 criteria, as opposed to its recently introduced subtypes. Moreover, AMH levels are negatively affected by increased adiposity in both women with the syndrome and normal controls. Opposite to previous suggestions, increased AMH levels in women with PCOS do not seem to be merely the result of increased small follicle number per se, neither increased androgen levels alone, since, in the present study, LH levels have emerged as the most important independent determinant of AMH. It should be emphasised, though, that a causal relationship is at present difficult to establish and more properly designed studies are definitely needed.

REFERENCES

1. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19: 41-47, 2004.
2. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Fertil Steril 81: 19-25, 2004.
3. Norman RJ, DeWailly D, Legro SR, Hickey TE. Polycystic ovary syndrome. Lancet 370: 685-697, 2007.
4. Diamanti-Kandarakis E, Panidis D. Unravelling the phenotyping map of polycystic ovary syndrome (PCOS): a prospective study of 634 women with PCOS. Clin Endocrinol (Oxf.) 67: 735-742, 2007.
5. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Polycystic ovary syndrome. Current issues in endocrinology and metabolism, edited by Dunaif A, Givens JR, Haseltine FP, Merriam GE, eds. Hershman SM. Blackwell Scientific Publications, Boston, 1992.

6. Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, O'Keefe M, Ghazzi MN; PCOS/Troglitazone Study Group. Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab 86: 1626-1632. 2001.

7. Legato MJ. Gender-specific aspects of obesity. Int J Fertil Womens Med 42: 184-197, 1997.

8. Bjontorp P. Abdominal obesity and the metabolic syndrome. Ann Med 24: 465-468, 1992.

9. Dunaif A, Futterweit W, Segal KR, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in the polycystic ovary syndrome. Diabetes 38: 1165-1174, 1989.

10. Jonard S, DeWailly D. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. Hum Reprod Update 10: 107-117, 2004.

11. La Marca A, Volpe A. Anti-Müllerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool? Clin Endocrinol (Oxf) 64: 603-610, 2006.

12. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, Uilenbroek JT, Grootegoed JA, Themmen AP. Anti-Müllerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology 143: 1076-1084, 2002.

13. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Kumar TR, Matzuk MM, Rose UM, de Jong FH, Uilenbroek JT, Grootegoed JA, Themmen AP. Anti-Müllerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology 142: 4891-4899, 2001.

14. Pigny P, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, DeWailly D. Elevated serum level of antimüllerian hormone in patients with polycystic ovary syndrome: Relationship to the ovarian follicle excess and the follicular arrest. J Clin Endocrinol Metab 88: 5957-5962, 2003.

15. Pigny P, Jonard S, Robert Y, DeWailly D. Serum antimüllerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab 91: 941-945, 2006.

16. Siow Y, Kives S, Hertweck P, Perlman S, Fallat ME. Serum müllerian inhibiting substance levels in adolescent girls with normal menstrual cycles or with polycystic ovary syndrome. Fertil Steril 84: 938-944, 2005.

17. Pellat L, Hanna L, Brincat M, Galea R, Brain H, Whitehead S, Mason H. Granulosa cell production of antimüllerian hormone is increased in polycystic ovaries. J Clin Endocrinol Metab 92: 240-245, 2007.

18. Stubbs A, Hardy K, Da Silva-Buttkus P, Stark J, Webber LJ, Flanagan AM, Themmen APN, Visser JA, Groome NP, Franks S. Antimüllerian hormone protein expression is reduced during the initial stages of follicle development in human polycystic ovaries. J Clin Endocrinol Metab 90: 5536-5543, 2005.

19. Morley JE, Patrick P, Perry HM III. Evaluation of assays available to measure free testosterone. Metabolism 5: 554-559, 2002.

20. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and b-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 12-19, 1985.

21. Jonard S, Pigny P, Reyss AC, Decanter C, Poncelet E, DeWailly D. Changes in serum AMH level during low-dose recombinant follicular stimulating hormone therapy in polycystic ovary syndrome. J Clin Endocrinol Metab 92: 4138-4143, 2007.

22. Lobo RA, Kletzky OA, Campeau JD, di Zerega GS. Elevated bioactive luteneizing hormone in women with polycystic ovary syndrome. Fertil Steril 39: 674-678, 1983.

23. Panidis D, Koliakos G, Kourtis A, Farmakiotis D, Mouslech T, Rousso D. Serum resistin levels in women with polycystic ovary syndrome. Fertil Steril; 81: 361-366, 2004.

24. Panidis D, Farmakiotis D, Rousso D, Katsikis I, Kourtis A, Diamanti-Kandarakis E. Luteinising hormone levels are markedly increased and significantly correlated with delta 4-androstenedione levels in lean women with polycystic ovary syndrome. Fertil Steril 84: 538-540, 2005.

25. Freeman EW, Gracia CR, Sammel MD, Lin H, Lim LCL, Strauss JF. Association of anti-mullerian hormone levels with obesity in late reproductive age women. Fertil Steril 87: 101-106, 2007.

26. Willis DS, Willis D, Watson H, Mason HD, Galea R, Brincat M, Franks S. Premature response to luteinizing hormone of granulosa cells from anovulatory women with polycystic ovary syndrome: relevance to mechanism of anovulation. J Clin Endocrinol Metab 83: 3984-3991, 1998.

27. Jakimiuk AJ, Weitsman SR, Navab A, Magoffin DA. Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidogenic enzyme messenger ribonucleic acids are overexpressed in thecal and granulosa cells from polycystic ovaries. J Clin Endocrinol Metab 86: 1318-1323, 2001.

28. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implication for pathogenesis. Endocr Rev 18: 774-800, 1997.

29. Panidis D, Macut D, Farmakiotis D, Rousso D, Kourtis A, Katsikis I, Spanos N, Petakov M, Bjekic J, Damjanovic S. Indices of insulin sensitivity, beta cell function and serum proinsulin levels in the polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 127: 99-105, 2006.

30. Diamanti-Kandarakis E, Kouli C, Alexandraki K, Spina G. Failure of mathematical models to accurately assess insulin resistance in lean, overweight or obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 89: 1273-1276, 2004.

Table 1. Definition of the five groups studied (n=50 in each), based on the 2003 Rotterdam criteria for the diagnosis of the Polycystic ovary syndrome (PCOS): ANOV: Anovulation; HA: Hyperandrogenemia (hirsuitism, as well as increased testosterone levels); PCO: Polycystic ovaries on transvaginal sonography. The shaded area represents groups that were also classified as PCOS, before 2003, with the 1990 (NIH) criteria (5).

GROUP		ANOV	HA	PCO	Epidemiology in Greece *	
PCOS	1	+	+	+	"Severe"	~46.4 %
	2	+	+	-	"With anovulation & Hyperandrogenemia"	~39.6 %
	3	-	+	+	"Ovulatory"	~7.2 %
	4	+	-	+	"Mild"	~6.8 %
CONTROLS		-	-	-	* Modified from Reference no 4	

Table 2. Anthropometric, hormonal and metabolic features of normal-weight women with PCOS and matched controls (mean ± S.D.)

	PCOS				Controls
	1	2	3	4	
Age (years)	24.2 ± 4.7	25.2 ± 4.4	25.8 ± 5.6	24.8 ± 6.4	27.0 ± 5.0
BMI (kg/m^2)	22.2 ± 1.8	22.2 ± 1.8	22.1 ± 1.8	22.2 ± 1.7	22.4 ± 1.5
W (cm)	74.6 ± 6.0	74.2 ± 5.9	71.3 ± 4.5	73.0 ± 6.0	73.6 ± 6.9
FSH (mIU/ml)	6.4 ± 1.5	6.2 ± 1.7	6.3 ± 2.0	5.8 ± 1.7	6.5 ± 1.8
LH (mIU/ml)	10.4 ± 7.4	8.1 ± 4.3	6.6 ± 3.3	7.6 ± 4.6	5.9 ± 2.5
PRL (ng/ml)	15.8 ± 8.9	14.5 ± 7.0	14.7 ± 6.7	13.7 ± 7.7	13.4 ± 7.1
T (ng/dl)	92.1 ± 26.1	78.9 ± 21.1	83.4 ± 22.8	47.7 ± 10.4	40.1 ± 11.52
Δ_4A (ng/ml)	3.6 ± 1.2	2.7 ± 0.9	3.0 ± 1.1	2.4 ± 0.8	1.6 ± 0.4
DHEA-S (mg/ml)	2855 ± 1167	2923 ± 1075	3160 ± 1474	1991 ± 794	1907 ± 785
17α-OH-P (ng/ml)	1.1 ± 0.6	1.2 ± 0.8	1.3 ± 0.6	1.2 ± 0.6	0.7 ± 0.4
SHBG (nmol/l)	45.0 ± 19.3	42.1 ± 15.0	46.6 ± 22.3	60.4 ± 28.2	69.8 ± 21.9
FAI	9.7 ± 9.3	7.4 ± 3.3	8.0 ± 4.6	3.5 ± 2.2	2.2 ± 1.0
Glucose (mg/dl)	94.9 ± 13.7	94.0 ± 11.7	97.6 ± 14.1	103.3 ± 10.8	93.0 ± 13.1
Insulin (μIU/ml)	8.8 ± 5.3	8.0 ± 4.4	8.1 ± 4.9	7.1 ± 3.8	6.9 ± 3.5
HoMA-IR	2.1 ± 1.6	1.8 ± 1.0	1.9 ± 1.1	1.8 ± 1.0	1.6 ± 0.9
No of follicles 2-9 mm	25.0 ± 10.6	14.2 ± 3.3	24.8 ± 7.9	30.8 ± 10.8	13.6 ± 10.8
Mean ovarian volume (cm^3)	7.9 ± 3.8	5.2 ± 1.3	7.6 ± 3.3	7.5 ± 3.9	5.3 ± 1.9
AMH (ng/ml)	9.2 ± 8.6	6.3 ± 3.2	5.1 ± 2.6	5.3 ± 1.9	3.9 ± 1.4

Table 3. Anthropometric, hormonal and metabolic features of obese and overweight women with PCOS and matched controls (mean ± S.D.)

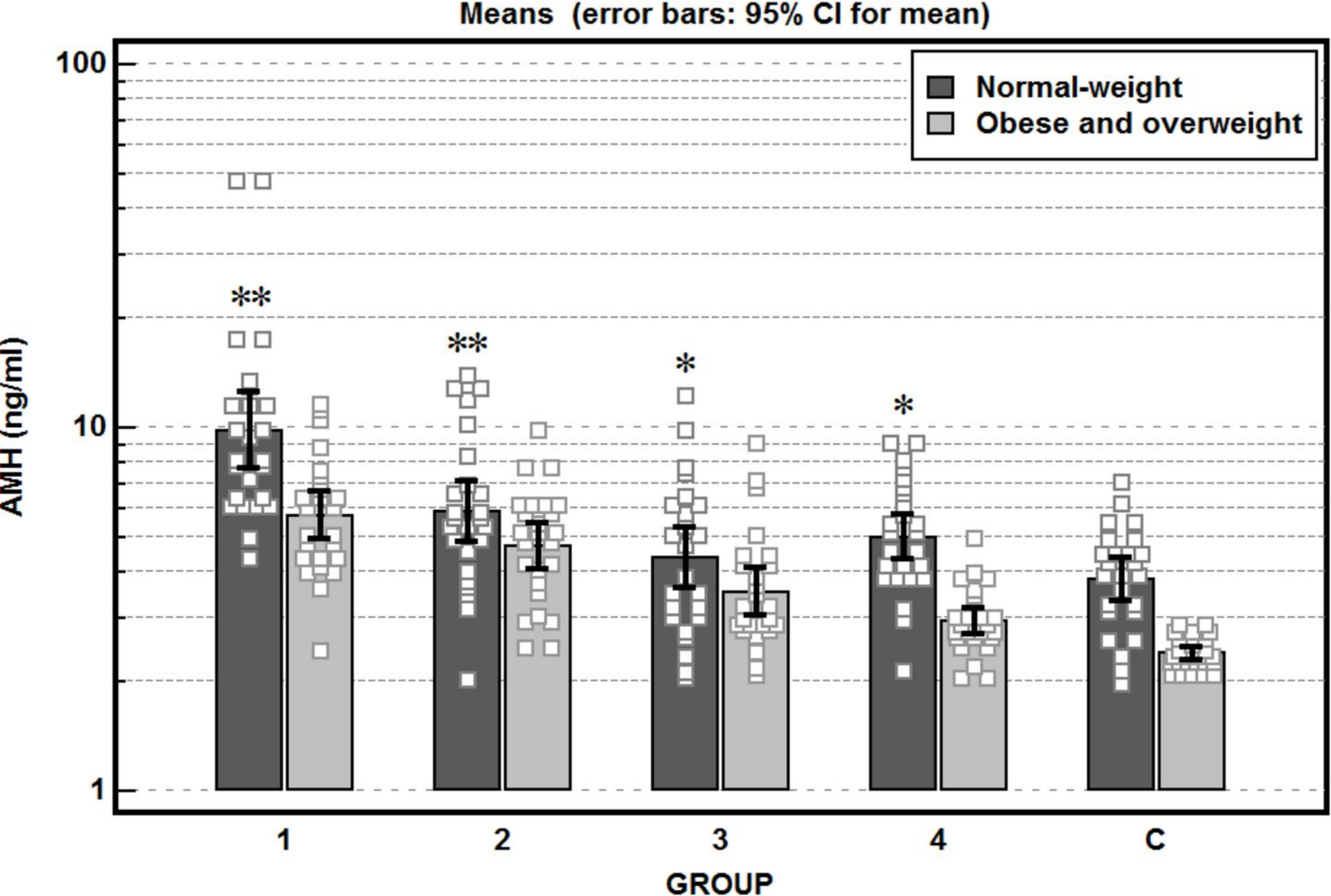
	PCOS				Controls
	1	2	3	4	
Age (years)	25.4 ± 4.7	24.8 ± 5.4	27.3 ± 6.5	23.9 ± 5.7	26.6 ± 4.3
BMI (kg/m^2)	30.7 ± 2.9	30.3 ± 3.6	30.9 ± 3.9	31.1 ± 4.8	30.4 ± 4.6
W (cm)	93.3 ± 8.1	91.9 ± 7.6	89.4 ± 10.3	93.6 ± 11.9	90.6 ± 10.3
FSH (mIU/ml)	5.3 ± 1.4	5.9 ± 1.2	5.7 ± 1.6	5.2 ± 1.9	6.6 ± 2.7
LH (mIU/ml)	8.5 ± 3.9	7.4 ± 5.0	4.9 ± 3.5	5.6 ± 3.0	5.3 ± 2.2
PRL (ng/ml)	14.9 ± 6.0	14.4 ± 7.0	13.9 ± 6.2	14.5 ± 12.3	14.1 ± 5.7
T (ng/dl)	83.4 ± 22.3	97.2 ± 31.7	82.5 ± 23.5	44.8 ± 10.3	43.5 ± 8.9
Δ_4A (ng/ml)	2.8 ± 0.8	3.1 ± 1.1	2.9 ± 0.8	1.9 ± 0.7	1.8 ± 0.5
DHEA-S (mg/ml)	3562 ± 1551	4130 ± 1668	3216 ± 1264	2247 ± 1100	2321 ± 921
17α-OH-P (ng/ml)	1.1 ± 0.4	1.2 ± 0.5	1.5 ± 0.5	1.2 ± 0.8	0.9 ± 0.3
SHBG (nmol/l)	33.3 ± 17.2	28.1 ± 10.7	36.1 ± 16.9	35.4 ± 16.3	46.1 ± 32.2
FAI	10.2 ± 3.9	14.5 ± 9.6	9.4 ± 4.8	5.4 ± 2.8	4.3 ± 2.3
Glucose (mg/dl)	104.1 ± 14.1	101.9 ± 10.5	99.5 ± 12.6	105.8 ± 13.4	99.7 ± 13.8
Insulin (μIU/ml)	17.9 ± 9.5	14.9 ± 6.7	11.2 ± 7.9	17.7 ± 11.6	13.8 ± 8.8
HoMA-IR	4.6 ± 2.5	3.8 ± 1.7	2.8 ± 2.0	4.6 ± 2.9	3.4 ± 2.3
No of follicles 2-9 mm	28.6 ± 9.5	15.1 ± 3.6	27.4 ± 6.5	23.8 ± 7.7	13.9 ± 3.9
Mean ovarian volume (cm^3)	8.4 ± 3.9	5.6 ± 1.8	7.9 ± 3.6	8.5 ± 3.5	5.4 ± 1.7
AMH (ng/ml)	6.2 ± 2.1	4.9 ± 2.0	3.8 ± 1.6	3.0 ± 0.6	2.4 ± 0.3

Table 4. Regression equations of the variables that influenced plasma AMH concentrations in the women of the present study.

Independent variables	Regression Statistics			R^2
	B	SE	p	
Model 1				0.180
Constant	+0.366	0.042	0.000	
LH	+0.345	0.051	0.000	
Model 2				0.275
Constant	–0.258	0.125	0.040	
LH	+0.279	0.049	0.000	
T levels	+0.377	0.072	0.000	
Model 3				0.351
Constant	+0.751	0.235	0.002	
LH	+0.239	0.047	0.000	
T levels	+0.388	0.068	0.000	
BMI	–0.705	0.142	0.000	
Model 4				0.404
Constant	+0.478	0.235	0.043	
LH	+0.225	0.046	0.000	
T levels	+0.360	0.066	0.000	
BMI	–0.708	0.137	0.000	
Total no of follicles 2-9 mm	+0.260	0.161	0.000	

LEGENDS OF FIGURES

Figure 1. Serum AMH levels in the four basic phenotypes of normal-weight vs. obese and overweight women with polycystic ovary syndrome (PCOS) and healthy controls – scatterplot and mean ± S.D on a logarithmic scale.


Means (error bars: 95% CI for mean)
Normal-weight
Obese and overweight
AMH (ng/ml)
100
10
1
**
**
*
*
1
2
3
4
C
GROUP