Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans

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To elucidate whether the role of leptin in regulating neuroendocrine and immune function during short-term starvation in healthy humans is permissive, i.e., occurs only when circulating leptin levels are below a critical threshold level, we studied seven normal-weight women during a normoleptinemic-fed state and two states of relative hypoleptinemia induced by 72-h fasting during which we administered either placebo or recombinant methionyl human leptin (r-metHuLeptin) in replacement doses. Fasting for 72 h decreased leptin levels by ≈80% from a midphysiologic (14.7 ± 2.6 ng/ml) to a low-physiologic (2.8 ± 0.3 ng/ml) level. Administration of r-metHuLeptin during fasting fully restored leptin to physiologic levels (28.8 ± 2.0 ng/ml) and reversed the fasting-associated decrease in overnight luteinizing hormone pulse frequency but had no effect on fasting-induced changes in thyroid-stimulating hormone pulsatility, thyroid and IGF-1 hormone levels, hypothalamic–pituitary–adrenal and renin–aldosterone activity. FSH and sex steroid levels were not altered. Short-term reduction of leptin levels decreased the number of circulating cells of the adaptive immune response, but r-metHuLeptin did not have major effects on their number or *in vitro* function. Thus, changes of leptin levels within the physiologic range have no major physiologic effects in leptin-replete humans. Studies involving more severe and/or chronic leptin deficiency are needed to precisely define the lower limit of normal leptin levels for each of leptin's physiologic targets.

fasting | reproductive

Deficiency of the adipocyte-secreted hormone leptin (1) is associated with distinct abnormalities in energy-demanding processes such as neuroendocrine and immune function. Leptin-deficient *ob*/*ob* mice and humans with congenital complete leptin deficiency have abnormal neuroendocrine function, including hypogonadotropic hypogonadism, hypothalamic hypothyroidism, and/or growth-hormone-axis abnormalities (2–6) and impaired cell-mediated immunity (4, 7), which are improved with leptin replacement (4, 8). Similarly, starvation-induced decline of circulating leptin to very low levels in normal mice (9) and lean men (10) causes comparable neuroendocrine (9, 10) and immune defects (11, 12) that are significantly blunted or reversed with exogenous leptin.

We have shown that an 80% decline of leptin levels from ≈2 to 0.3 ng/ml in men mediates the fasting-induced suppression of gonadotropin and thyroid-stimulating hormone (TSH) pulsatility as well as sex steroid, insulin-like growth factor-1 (IGF-1), and thyroid hormone levels (10). Importantly, although observational studies have proposed that leptin regulates the hypothalamic–pituitary–gonadal axis only when serum leptin levels fall below a "threshold" of ≈2 ng/ml (13), the role of decreasing leptin levels to approximately, but not below, this threshold in leptin-replete humans with higher baseline leptin levels (e.g., normal-weight women) has not yet been directly studied.

To elucidate whether such a threshold exists, below which leptin has a "permissive" effect to regulate neuroendocrine and immune function [including peripheral blood mononuclear cell (PBMC) subpopulations, T cell proliferation, and cytokine production], we assessed pituitary hormone pulsatility and hormone levels of several neuroendocrine axes and markers of immune function in normal-weight women during a normoleptinemic-fed condition and two hypoleptinemic 72-h fasting states, with administration of either placebo (to achieve a low leptin level close to the proposed threshold) or recombinant methionyl human leptin (r-metHuLeptin) (to replace leptin to physiologic levels). To further investigate the question of a threshold leptin level in regulating immune function, we studied the effect of a range of leptin levels on T cell proliferation *in vitro*.

Results

Seventy-Two-Hour Fasting Suppresses Serum Leptin Levels out of Proportion to Changes in Body Weight and Fat Mass, and r-metHuLeptin Replacement Restores Leptin Levels Without Affecting Metabolic Parameters. In the baseline fed state, body weight increased slightly without significant changes in percent or total fat mass or fat-free mass, whereas serum leptin levels and insulin levels increased, and free fatty acid (FFA) levels decreased (Table 1). Complete fasting for 72 h significantly decreased serum leptin levels to ≈20% of baseline, out of proportion to the slight decreases in body weight and fat mass (Table 1). Leptin pulsatility on the third day of fasting was markedly suppressed (Fig. 1*a*) with loss of normal diurnal variation and decreased 24-h mean levels (20.5 ± 1.6 to 2.8 ± 0.2 ng/ml), peak height (23.8 ± 2.1 to 3.4 ± 0.3 ng/ml), valley mean (20.2 ± 1.6 to 7.8 ± 0.2 ng/ml), integrated area (29,286 ± 2,354 to 3,997 ± 277), and pulse mass (2.6 ± 0.8 to 0.5 ± 0.1) (all $P < 0.05$ vs. fed) but not pulse frequency or interpulse interval. r-metHuLeptin during fasting fully corrected the fasting-induced suppression of leptin to levels that were higher than baseline but within the physiological range for women (24-h mean: 42.4 ± 4.0 ng/ml vs. trough level on day 4 at 8 a.m. in Table 1) (Fig. 1*a*). Similar decreases in body weight and fat mass were observed as during fasting alone, with a slightly greater decrease in fat-free mass with r-metHuLeptin (Table 1). Resting metabolic rate was not affected by fasting or r-metHuLeptin, and r-metHuLeptin did not alter fasting-induced changes in insulin, respiratory quotient, or FFA (Table 1).

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Abbreviations: ACTH, adrenocorticotropic hormone; FFA, free fatty acid; FSH, follicle-stimulating hormone; T3, triiodothyronine; IGF-BP, IGF-binding protein; LH, luteinizing hormone; PBMC, peripheral blood mononuclear cell; PRA, plasma renin activity; TSH, thyroid-stimulating hormone.

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