Leptin in Humans: Current Progress and Future Directions

Obesity is the result of a disorder in the body energy balance that occurs when energy intake chronically exceeds energy expenditure. This excess in energy intake is stored in the adipocyte. As the prevalence of obesity is increasing at a rapid pace in the US and other industrialized nations *[1]*, a more thorough understanding of the regulation of energy balance is needed to facilitate intervention in the progression of this disorder. The recently discovered hormone leptin contributes to the regulation of energy balance by informing the brain of the amount of adipose tissue in the body. The brain may then make the appropriate adjustments in either energy intake or expenditure. In this issue of *Clinical Chemistry*, Ma et al. describe their development of an RIA to measure leptin in serum or plasma of humans *[2]*.

Leptin is the protein product of the *ob* gene. Using positional cloning techniques, Friedman and coworkers identified the *ob* gene in mice *[3]*. The *ob* gene encodes an mRNA of ~4.5 kb that is expressed solely in the adipocyte. Leptin is a highly hydrophilic 167-amino-acid protein with an N-terminal secretory signal sequence. Mutations in the *ob* gene that block the synthesis of leptin have been identified in two strains of obese mice. In the SM/Ckc-+$^{Dac}ob^{2J}/ob^{2J}$ strain, no *ob* gene mRNA is detectable in the adipose tissue because of a polymorphism in the promoter region of the gene, which is hypothesized to interfere with transcription. C57BL/6J *ob/ob* mice have a single-base mutation in codon 105, which results in the replacement of arginine by a premature stop codon. Both strains of mice therefore lack leptin. No mutations in the *ob* gene have been detected in any other obese animal models studied *[4–6]*. Administration of recombinant leptin to hormone-deficient *ob/ob* mice results in weight loss through reduced food intake and increased energy expenditure *[7–11]*. Leptin also causes weight loss in normal lean and diet-induced obese mice *[9]*. This protein therefore appears to be directly involved in the regulation of total body energy balance.

In humans, the *ob* gene is expressed exclusively in adipose tissue and codes for a protein that is 84% homologous to the mouse protein *[12, 13]*. No deleterious mutations *[12]*, and only one single-base polymorphism *[14]*, have been detected in the human *ob* gene. Greater expression of the *ob* gene in obese than in normal-weight individuals has also been observed *[12, 15–17]*. *Ob* gene expression in humans is highly correlated with both percentage body fat and body mass index (BMI) *[12, 17]*. These observations support the hypothesis that mutations in the *ob* gene are not the primary cause of obesity in humans.

Leptin has recently been measured in human blood by immunoprecipitation assay *[18]* and RIA *[17]*. The RIA detected about fourfold more leptin in the serum of obese individuals (n = 139) than in the normal-weight subjects (n = 136) *[17]*. Serum leptin was most highly correlated ($r = 0.85$; $P < 0.001$) with percentage body fat in 179 subjects. Serum leptin concentrations were also correlated with BMI ($r = 0.66$; $P < 0.001$), fasting serum insulin concentration ($r = 0.57$; $P < 0.001$), and age ($r = 0.26$; $P < 0.001$) when tested as independent variables *[17]*. However, modeling of the data suggests that these factors do not have a major effect on serum leptin concentration independent of body fat *[17]*. Using the immunoprecipitation assay, Maffei et al. *[18]* found a similar correlation of plasma leptin with body fat in 46 individuals ($r = 0.86$; $P < 0.001$) and BMI ($r = 0.506$; $P < 0.001$) in 87 subjects. Increases of leptin concentrations in obese humans correlate with the increase of *ob* mRNA in the adipose tissue. Taken together, these observations suggest that most obese humans are insensitive to their endogenous leptin production.

Using their new RIA to measure human leptin, Ma et al. *[2]* report observations similar to those of the two groups discussed above. The amount of leptin they detected in normal-weight and obese humans is not different from that previously reported. Furthermore, the correlation of plasma leptin with BMI ($r = 0.721$; $P < 0.001$) they derived is similar to that of the other two research groups. Ma et al. *[2]*, however, are the first to rigorously describe the stability of leptin in various environments that could be encountered in either the clinical or research setting. In serum, the hormone appears to be stable at room temperature for at least 1 week and at least 2 months in the refrigerator. Also, the RIA is not affected by hemolysis of the sample or high concentrations of triglyceride, nor do repeated (5×) freeze/thaw operations significantly alter the amount of leptin detected.

With respect to the effect of gender on plasma leptin concentration as described by Ma et al., some clarification is necessary. These investigators correctly detect in their samples and point out that, when leptin values for men and women of equivalent BMI are compared, women have more leptin. This observation was also previously made by both Maffei et al. *[18]* and Considine et al. *[17]*. However, for any given BMI, women have more adipose tissue mass than do men *[19]*. Because leptin is most significantly correlated with percentage body fat, the gender effect discussed by Ma et al. is most likely due to gender differences in the amount of body fat. No effect from gender was detected by direct comparison of women and men with equivalent body fat *[17, 18]*.

As leptin has only recently been discovered, a substantial amount of investigation into its actions in humans remains to be done. Are there disease states such as anorexia nervosa or bulemia that could be explained by inappropriate concentrations of leptin? How do leptin amounts in children compare with those in adults? Do leptin concentrations change to account for pertubations in energy balance? Although both previous research groups demonstrated that circulating leptin is reduced by weight loss in humans *[17, 18]*, this effect may result from the combination of a reduction in fat mass and caloric restriction. Further, does the composition of the caloric intake (high or low fat) alter leptin concentrations? At the other end of the signal pathway, how do changes in leptin regulate energy expenditure? In mice, administration of recombinant leptin caused weight loss by decreasing food intake and increasing energy expenditure *[7–11]*. The mechanisms that regulate leptin production in the adipose tissue, and how the hormone quantitatively reflects the amount of adipose tissue, also require further investigation. In addition, the mechanisms responsible for clearance of the hormone from the circulation have not yet been determined. Finally, the issue of leptin resistance in humans will have to be addressed by careful analysis of the leptin receptor and its effector systems. A reliable RIA such as described by Ma et al., will greatly facilitate future investigations of leptin in humans. A better understanding of the role of leptin and energy balance in humans will provide insight into the mechanisms that lead to the development of obesity.

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