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Increased Serum Leptin Indicates Leptin Resistance in Obesity

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Featured Article: Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292–5.[2]

This study was done at a very exciting time in the field of obesity research. Jeffrey Friedman's group had published the landmark report in December 1994 that described the cloning of the *Lep*[3] (leptin; also known as *ob*) gene and the missense mutation that caused obesity in *ob/ob* mice *(1)*. The discovery of the hormone leptin in that study by Zhang et al. provided support for the lipostasis theory of body weight regulation, which postulated that adipose tissue released a signal that communicated to the brain the size of the energy stores in the body. Thus, there was great interest in knowing whether defects in leptin synthesis or secretion could cause obesity in humans, as was found in the *ob/ob* mouse.

At the time that the discovery of leptin was reported, I was working in Dr. Jose Caro's laboratory and investigating differences in the proliferation and differentiation of preadipocytes from lean and obese humans. Shortly after the publication of the mouse *Lep* sequence, we identified the human nucleotide sequence and found that *LEP* (leptin) expression was greater in adipocytes from obese individuals *(2)*. We shared the *LEP* sequence with our scientific colleagues at Eli Lilly Research Laboratories, where, under the direction of Mark Heiman, the protein chemists quickly synthesized recombinant full-length human leptin protein and immunized rabbits for antibody development. Generating human leptin antibodies and establishing the RIA quickly were very important, because we knew that other groups would be working on similar assays *(3)*.

While antibodies were in development, we expanded our investigation of *LEP* gene expression in human adipocytes. Using collagenase digestion to obtain adipocytes, we isolated total RNA and performed semiquantitative PCR with a ^{32}P-radiolabeled downstream primer. We stopped the PCR reaction at various cycle numbers to establish the linearity of amplification, resolved the radiolabeled PCR product on an agarose gel, excised the band from the gel, and measured the radioactivity. Although this cumbersome technique worked well at the time, it is much easier today to use real-time PCR to measure *LEP* mRNA in isolated adipocytes! Importantly, we were able to demonstrate that *LEP* gene expression was greater in adipocytes from obese individuals than in lean individuals, suggesting that leptin secretion should also be greater in obese humans.

When antibodies to leptin became available, Madhur Sinha and scientists at Eli Lilly collaborated to radiolabel recombinant leptin and test antibody dilutions in their development of the RIA. Once the parameters of the assay were worked out and were reproducible, we quantified leptin in serum samples from 136 lean and 139 obese individuals across a wide range of body mass indexes. It was immediately apparent from this experiment that the adipocyte protein leptin was present in blood in proportion to the amount of body fat. Thus, we concluded that in contrast to the *ob/ob* mouse model, obesity in humans was not the product of defects in leptin synthesis or secretion. We were also able to show that serum leptin concentrations decreased in individuals who had lost weight on a low-calorie diet, providing further evidence that serum leptin was a dynamic measure of body fat stores.

The frequent citation of this study in the literature is due more to the findings than to the techniques used. Although a major advantage of our RIA at the time was its use of full-length recombinant leptin, the assay used standard techniques. Eventually, many RIAs for leptin would be developed, including one from Linco Research, which we would use for many additional studies of leptin in humans. More importantly, this report introduced the concept of "leptin resistance"—that obese individuals were insensitive to their endogenous leptin production and thus continued to eat despite the presence of adequate energy stores in the body. Leptin resistance in human obesity would eventually prove to be

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[2] This article has been cited more than 3150 times since publication.
[3] Genes: *Lep*, leptin (*Mus musculus*); *LEP*, leptin (*Homo sapiens*).

a substantial barrier to the development of leptin as an effective pharmacologic intervention for weight loss *(4)*.

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