

Human leptin characterization

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SIR — Injections of leptin, the hormone encoded by the mouse *ob* gene, reduce body weight in wild type and *ob* mice¹⁻⁴. However, the weight-reducing effects of recombinant leptin expressed from bacteria in wild-type mice require relatively high doses of leptin¹⁻³. The basis for the high dosage requirement of recombinant leptin could be partly pharmacokinetic but could also be the result of post-translational modifications of the native protein.

To address this question we looked for differences in the molecular mass between recombinant and endogenous human lep-

teins from SDS-PAGE for mass spectrometric analysis. The method makes use of reversible, negative staining protocols (for example copper⁵ or zinc⁶ staining) and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)⁷. We could readily see bands with as little as 3 pmol (50 ng) loading of refolded bacterial recombinant leptin on copper-stained (Bio-Rad) SDS gels measured by mass spectrometry. We excised protein bands from the gel, destained them, and extracted the protein into a MALDI matrix solution.⁸ Full details of this new protein extraction procedure will be given else-

The presence of an amino-terminal start methionine in the recombinant leptin accounts for the mass difference observed between the endogenous and recombinant proteins.

We undertook further comparison of the proteins by performing in-gel trypsin and cyanogen bromide digests followed by extraction and MALDI-MS analysis. Except for the amino-terminal methionine on the recombinant protein, the fragmentation patterns of the recombinant and endogenous proteins are virtually identical. In addition, molecular mass measurements of the peptides generated after digestion indicate the presence of a carboxy-terminal disulphide bond in both the endogenous and refolded recombinant proteins (data not shown).

The results show that immunopurified leptin from human serum has the wild-type sequence with the removal of the predicted amino-terminal signal peptide and with the presence of a carboxy-terminal intramolecular disulphide bond. There is no evidence of other post-translational modifications. Further studies are required to determine the basis for the high dosage requirements of leptin to induce weight loss.

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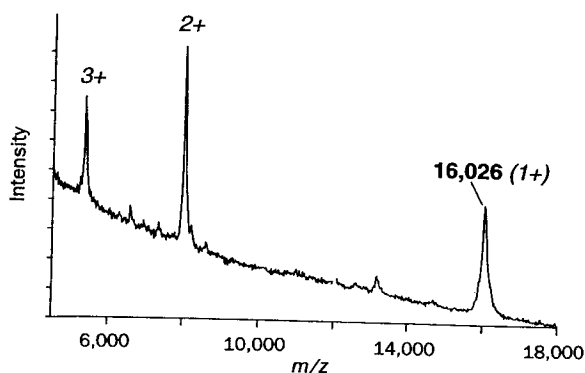
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Mass spectrum of endogenous human leptin. The MALDI mass spectrum is dominated by a single component (leptin) with a measured molecular mass of $16,026 \pm 9$. The three main peaks represent singly, doubly and triply protonated forms of the protein arising from the MALDI mass spectrometric process⁷. *m*, mass; *z*, charge.

tin. We isolated endogenous leptin using two stages of immunoaffinity chromatography from 50 ml serum pooled from three moderately obese human female donors (leptin concentration 54 ng ml^{-1}). We eluted the protein from a monoclonal antibody-affinity resin and subjected it to SDS-PAGE. The principal band, well separated from contaminating and irrelevant proteins, showed the same mobility as recombinant human leptin (apparent relative molecular mass 16,000); we estimated it to contain more than 250 ng protein.

Although the endogenous and recombinant human leptins have the same mobility after immunoblotting, SDS-PAGE has insufficient accuracy to exclude the possibility of subtle differences (for example, post-translational modifications). To measure accurately the molecular mass of the native protein, we developed a method to elute whole pro-

teins (and are available directly from S.L.C and B.T.C.).

Using this technique, we find the molecular mass of endogenous human leptin to be $16,026 \pm 9$ (see figure), a value identical, within experimental error, to the molecular mass calculated from the *ob* cDNA sequence minus the putative signal sequence, 16,024 (ref. 9). In a parallel experiment, recombinant leptin extracted from the gel gives a mass of $16,178 \pm 10$.

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