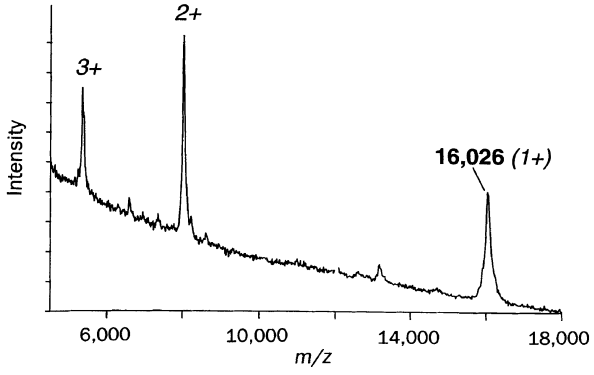
Human leptin characterization

SIR — Injections of leptin, the hormone encoded by the mouse *ob* gene, reduce body weight in wild type and *ob* mice[1–4]. However, the weight-reducing effects of recombinant leptin expressed from bacteria in wild-type mice require relatively high doses of leptin[1–3]. 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 leptin. 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 proteins from SDS-PAGE for mass spectrometric analysis. The method makes use of reversible, negative staining protocols (for example copper[5] or zinc[6] 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 elsewhere (and are available directly from S.L.C and B.T.C.).



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 ± 9. The three main peaks represent singly, doubly and triply protonated forms of the protein arising from the MALDI mass spectrometric process[7]. *m*, mass; *z*, charge.

Using this technique, we find the molecular mass of endogenous human leptin to be 16,026 ± 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 ± 10. 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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tRNA-dependent asparagine formation

SIR — It is commonly assumed that there are 20 aminoacyl-transfer (t)RNA synthetases, which form the different aminoacyl-tRNAs required for protein biosynthesis, in every organism. Direct acylation of tRNA with the amide amino acid, asparagine, is catalysed by asparaginyl-tRNA synthetase (AsnRS) in all organisms studied biochemically to date[1]. Genomic analysis of eubacteria[2,3] and a eukaryote[4] also predict both cytoplasmic and organellar AsnRS activity. In Gram-negative eubacteria and in the cytoplasm of eukaryotic cells, glutaminyl-tRNA synthetase acylates $tRNA^{Gln}$ directly with glutamine providing Gln-$tRNA^{Gln}$. However, in Gram-positive eubacteria, archaebacteria and organelles Gln-$tRNA^{Gln}$ formation is achieved by transamidation of misacylated Glu-$tRNA^{Gln}$ (refs 5–7). We now show that transamidation, and not direct acylation, provides Asn-$tRNA^{Asn}$ for protein biosynthesis in the halophilic archaebacterium *Haloferax volcanii*.

We investigated transamidation of mischarged tRNAs in *H. volcanii* as neither $tRNA^{Asn}$ nor $tRNA^{Gln}$ isolated from this archaeon could be aminoacylated *in vitro*[8]. *H. volcanii* total tRNA is considerably less well charged by Gln than by Glu in the presence of a dialysed S100 extract (data not shown). Comparable results were previously observed in *Lactobacillus bulgaricus*[9], and are consistent with the existence of a tRNA-dependent transamidation pathway for the formation of Gln-$tRNA^{Gln}$. We also find that Asn is a signif-