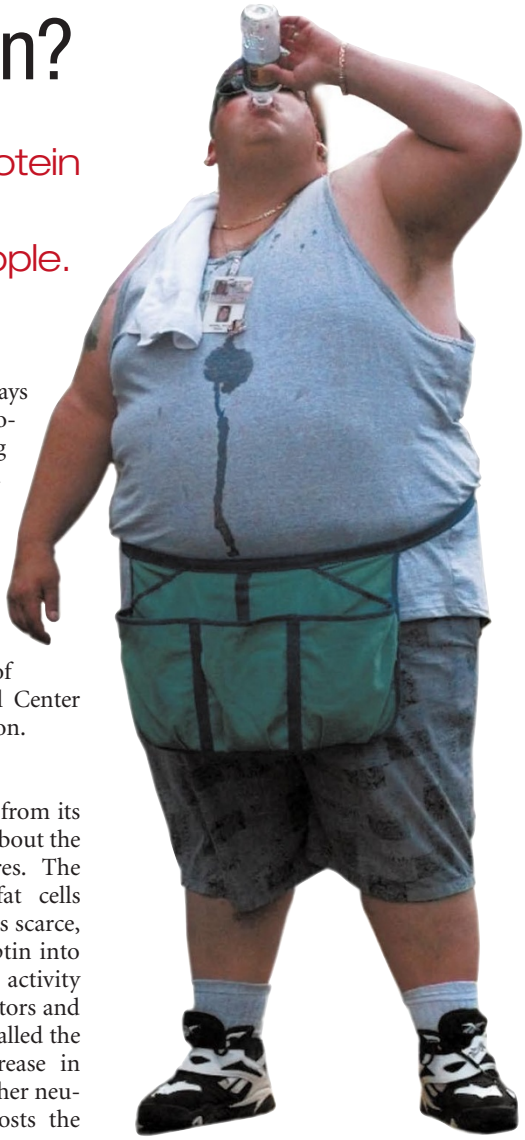
Whatever happened to leptin?

Just five years ago, it seemed that a single protein might reverse the rising tide of obesity. What worked for mice has not yet translated to people. But watch this space, says Marina Chicurel.



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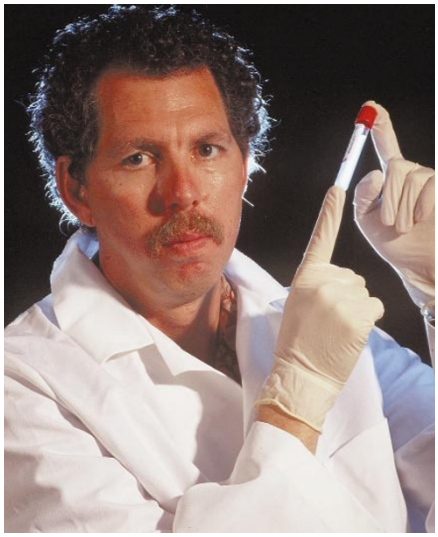
By any reckoning, US$20 million is a lot to spend on a protein. But in May 1995, Amgen of Thousand Oaks, California, paid just that for the commercial rights to leptin, a hormone that made fat mice slim. Identified only six months earlier, leptin could be injected into grotesquely obese, leptin-deficient mice where it curbed their voracious appetites and boosted their metabolic rates. Within a month, and with no apparent side-effects, these mice lost almost half of their excess weight.

Leptin was hailed as the wonder drug to defeat a burgeoning epidemic of obesity. But five years down the line, this simple script has had to be rewritten. First, data from studies on rodents and humans hinted at leptin's therapeutic shortcomings. Then, last October, came the news that the protein had performed poorly in its first clinical trial[1].

Although Amgen may not have its hands on a pharmaceutical blockbuster, obesity researchers are enthused by the wealth of advances that have followed leptin's discovery. And some of these advances may, in the long term, fulfil the therapeutic hopes once pinned on leptin. "It transformed the world of obesity from a backwater of totally ignored, rather dodgy research into one of the primary areas of biomedical research," says Stephen O'Rahilly of the University of Cambridge.



REMI BANALI/LIAISON AGENCY

Weight watcher: Friedman's discovery kick-started a vibrant field of research.

Leptin is significant because it plays an important role in energy homeostasis — the physiological balancing act that regulates our food intake and our storage and expenditure of energy. It also seems to affect other physiological systems, including carbohydrate metabolism, reproduction and bone formation. "As a hormone, leptin is probably as important as insulin," says Joel Elmquist of the Beth Israel Deaconess Medical Center and Harvard Medical School in Boston.

Exercising control

Leptin's fundamental power stems from its ability to keep the brain informed about the status of the body's energy stores. The amount of leptin secreted by fat cells depends on their size. When food is scarce, the cells shrink and secrete less leptin into the bloodstream. This reduces the activity of nerve cells that have leptin receptors and are found in a region of the brain called the hypothalamus. In turn, this decrease in neural activity has two effects on other neurons in the hypothalamus: it boosts the release of neuropeptide Y, a neurotransmitter that increases appetite, and it suppresses α-melanocyte-stimulating hormone (α-MSH), which blocks feelings of hunger. Through these pathways, and perhaps through direct effects on organs such as the pancreas and liver, leptin ensures energy homeostasis.

But the balance sometimes breaks down, probably because leptin and its associated neural circuits evolved to maintain adequate fat reserves, rather than to avoid excess fat storage — our distant ancestors routinely had to cope with food scarcity.

This view of leptin is different from the protein's initial characterization as an 'anti-obesity' hormone. But given the circumstances of leptin's discovery, it is easy to see why the early view prevailed. Since 1950, researchers had realized that the bizarre plight of the *ob/ob* mouse was the result of a genetic abnormality. This inbred strain eats almost continuously, weighs three-times as much as normal mice, and suffers from diabetes. But it was not until 1994 that Jeffrey Friedman and his colleagues at the Howard Hughes Medical Institute at Rockefeller University in New York sequenced the *ob* gene, identified its human counterpart, *Ob*, and characterized the protein they produce[2,3]. The team called the protein leptin, from the Greek word *leptos*, or thin.

Despite its disappointing performance so far, leptin is still the focus of interest from drug developers.

Within months, several teams, including Friedman's, had injected leptin into *ob/ob* mice and transformed them into healthy animals with normal body weights[3–5]. Leptin specifically reduced fat mass, sparing other tissues. "Never had there been any clear circulating factor that was involved in body weight regulation," says Steven Heymsfield

of St Luke's-Roosevelt Hospital in New York, who has collaborated with Amgen on human trials of leptin. "So that was a major, major breakthrough."

Predictions of potency

Friedman believes it was the powerful images of the transformed obese mice that ignited enthusiasm for leptin. Given the extreme competitiveness of the pharmaceutical industry, Amgen was quick to buy the rights from Rockefeller University.

Almost immediately, however, studies on rodents and people began to reveal that there was much more to leptin than first met the eye. The hormone's precise physiological role seems to vary from species to species. O'Rahilly's group in Cambridge, for instance, identified two obese, leptin-deficient children — the human equivalent of *ob/ob* mice[6]. But, unlike the mice, the twins did not suffer from other conditions such as hypothermia and severe diabetes. Nevertheless, when O'Rahilly's group injected one child with leptin, she lost weight rapidly[7].

Researchers also soon realized that most obese people are not deficient in leptin. In fact, the majority have higher than normal levels of the hormone[8,9]. Many obese people seem to be, at least to some extent, resistant to leptin. In addition, leptin levels vary greatly between people with identical percentages of body fat, indicating that a variety of factors, genetic and environmental, might contribute to people's sensitivity to the hormone[8,10].

Despite this evidence, Amgen remained hopeful. After all, diabetics with high circulating levels of insulin can sometimes benefit from receiving even more of the hormone. But the limits to leptin's therapeutic potential became clear last year with the publication of results from an Amgen trial in which 73 obese volunteers injected themselves with leptin or a placebo[1].

Of the 47 patients who completed the study — many withdrew because of problems with the injections — the eight receiving the highest dose lost an average of 7.1 kg, while the twelve taking a placebo lost 1.3 kg. The effects varied widely among patients — from a loss of about 15 kg to a gain of 5 kg within the group treated with the highest dose. These doses also induced skin irritation and swelling at the injection site. "The data reported were fairly moderate," concedes Carl LeBel, associate director of product development at Amgen.

But these setbacks are not the end of the road for leptin. The protein's discovery has provided countless new leads for drug development. Within the past five years, researchers have identified more than a dozen key components in the neurocircuitry underlying appetite control. They have also begun to tease out the intracellular signalling pathways activated by leptin.

Several companies are exploring the therapeutic possibilities of molecules that act at later stages in the process, after leptin has done its work. Millennium Pharmaceuticals in Cambridge, Massachusetts, for example, is working with drugs giant Hoffmann-La Roche to seek ways of heightening the effects of the appetite-suppressing α-MSH. This neurotransmitter acts on melanocortin-4 (MC-4) receptors, found on cells in the hypothalamus that are connected to leptin-responsive neurons. Mice lacking MC-4 receptors or pro-opiomelanocortin, the precursor of α-MSH, are obese[11]. Data from two unpublished studies, including one from O'Rahilly's group, suggest that up to five per cent of obese children carry mutations in the melanocortin system, making these mutations the most common genetic defects to be associated with human obesity to date.

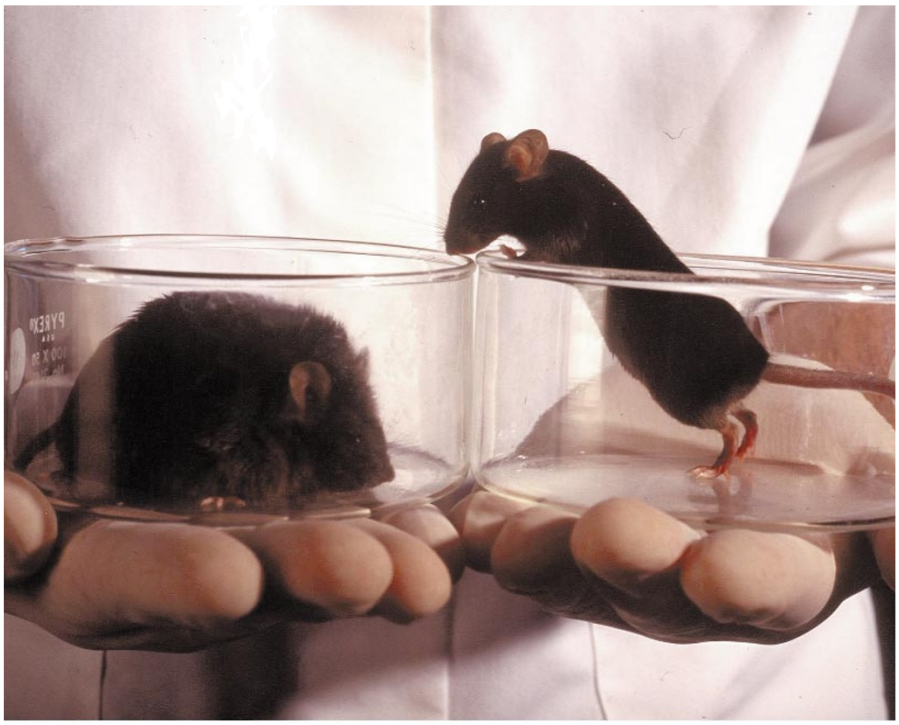
But boosting a protein's function is difficult, says Eleftheria Maratos-Flier of the Joslin Diabetes Center and Harvard Medical School. So other companies are focusing on targets that have weight-reducing effects when their function is blocked. Some researchers are trying to block melanin-concentrating hormone (MCH). Although there is disagreement about MCH's exact position in the circuitry controlled by leptin, mice that lack the hormone are much leaner than normal[12], and if the hormone is injected into rats' brains, the animals immediately start eating excessively[13].

Peering into the neurons that sport leptin receptors, other groups are looking for intracellular drug targets. Louis Tartaglia at Millennium believes the most promising approach is boosting the responsiveness of the signalling pathway activated by leptin. One component of this pathway, called suppressor of cytokine signalling-3 (SOCS-3), was recently identified in Jeffrey Flier's lab at Beth Israel Deaconess and Harvard[14]. Leptin seems to boost production of SOCS-3, which in turn seems to stifle further leptin signalling. It is possible, therefore, that the resistance of obese people to leptin is the result of SOCS-3 overactivity.

Copycat molecules

Dissection of leptin's signalling path has also helped reveal the weight-regulating properties of another molecule — ciliary neurotrophic factor (CNTF). This is one of a range of nerve growth factors being explored as treatments for neurodegenerative diseases. One trial using CNTF to treat amyotrophic lateral sclerosis revealed its leptin-like behaviour in humans. The patients did not show much neurological improvement, but they lost lots of weight[15].

The cell-surface receptor for CNTF closely resembles the leptin receptor, it activates similar intracellular signalling pathways, and it is found in related areas of the hypothalamus. Extending work on mice, in which Ralph Laufer's team at IRBM, the Institute for Research in Molecular Biology in Rome, showed that CNTF made obese mice lose fat[16], George Yancopoulos and colleagues at Regen-



REMI BANALI/LIAISON AGENCY

Before and after: dramatic images such as this one fired pharmaceutical enthusiasm for leptin.

nature insight Obesity
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eron Pharmaceuticals in Tarrytown, New York, started experimenting with axokine, a patented derivative of CNTF. When they made normal rodents obese by putting them on a high-fat diet, the animals became resistant to leptin. But when the researchers injected these same animals with axokine, the rodents shed 30 per cent of their body weight and maintained their moderate eating habits, even several days after their last injection.

Sights set on success

Last September, Regeneron completed a phase I clinical trial of axokine. Although at high doses the drug seemed to cause cold sores and nausea, patients tolerated low doses well. The results have yet to be published, but most patients lost significant amounts of weight, reducing their energy intake by about 500 calories a day. Yancopoulos calls axokine "the most promising weight loss agent ever described", but others are more cautious. Tartaglia at Millennium, for example, worries that tinkering with a neuronal growth factor could have delayed effects that are difficult to predict.

Despite its disappointing performance so far, leptin itself is still the focus of interest from drug developers. Many scientists, including those from Amgen, think leptin might help people maintain weight loss. For most people, the hardest part of dieting is not shedding weight, but keeping it off — they tend to return to their previous weight. The drop in the level of circulating leptin is probably partly to blame for this because the ensuing shrinkage of fat cells sounds a 'starvation alarm' that boosts the appetite. Leptin injections could help overcome this by fooling the brain into thinking that its energy stores are not at risk.

Amgen is also designing second-generation versions of leptin. The goal is to make leptin mimics that are more soluble — to increase the dose that can be injected into patients — and more stable, to last longer in the bloodstream. Other researchers are designing small molecules with leptin-like activity that can easily cross the blood–brain barrier. This could be important, because the ratio of leptin in cerebrospinal fluid to that in blood is four times lower in obese people than in those of normal weight[17,18].

The success or failure of these strategies, however, depends on how leptin sensitivity varies from person to person. Because of their resistance to leptin, flushing the cerebrospinal fluid of obese people with leptin-like molecules might make very little difference. So Amgen is searching for biomarkers that correlate with leptin sensitivity. This may allow leptin to be prescribed selectively to those who are likely to respond more strongly to the hormone.

Leptin's reach may also extend to the treatment of other, related diseases. Roger Unger at the University of Texas Southwestern Medical Center in Dallas believes that obesity-induced diabetes develops when pancreatic β-cells die after accumulating excessive fat in their cytoplasm. By stimulating receptors on the surface of β-cells, Unger thinks leptin can stop this from happening. But other researchers caution that leptin's interaction with insulin remains mysterious and controversial.

While Amgen's multimillion-dollar bet on leptin doesn't look like yielding an immediate bonanza for the company, obesity researchers agree that the protein has kick-started an exciting field — both for basic research and for drug development. "Whatever has happened to leptin as an immediate therapeutic tool, as a discovery that sparked an area of biomedical research, it's phenomenal," says O'Rahilly. ■

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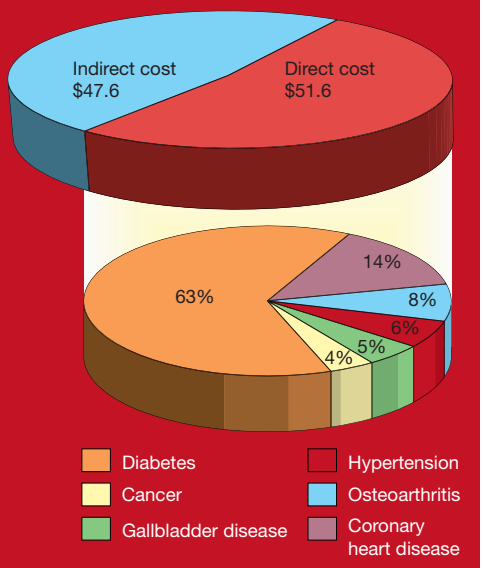
ERIC SANDER/LIAISON AGENCY

Diet plan: Amgen still believes leptin is the key to the lucrative obesity market.

Counting the cost of obesity

For the first company to bring a potent weight-loss drug to the market, the rewards could be huge. Across the industrialized world, the prevalence of obesity is rising. Last month, the Worldwatch Institute in Washington DC reported that 1.1 billion people worldwide are now suffering from the health consequences of overconsumption — equal to the number plagued by hunger. In the United States alone, where more than one in three adults is classified as obese, the condition is estimated to cause 300,000 premature deaths each year. And, as these figures reveal, the associated burden of disease causes a significant annual dent in the US economy — both from the direct cost of treating conditions triggered by obesity, and from lost economic productivity.



Source: Wolf, A. M. & Colditz, G. A., Obes. Res. 6, 97-106 (1998)

1. Heymsfield, S. B. *et al. J. Am. Med. Assoc.* **282,** 1568–1575 (1999).
2. Zhang, Y. *et al. Nature* **372,** 425–432 (1994).
3. Halaas, J. L. *et al. Science* **269,** 543–546 (1995).
4. Pelleymounter, M. A. *et al. Science* **269,** 540–543 (1995).
5. Weigle, D. S. *et al. J. Clin. Invest.* **96,** 2065–2070 (1995).
6. Montague, C. T. *et al. Nature* **387,** 903–908 (1997).
7. Farooqi, I. S. *et al. N. Engl. J. Med.* **341,** 879–884 (1999).
8. Maffei, M. *et al. Nature Med.* **1,** 1155–1161 (1995).
9. Considine, R. V. *et al. N. Engl. J. Med.* **334,** 292–295 (1996).
10. Lonnqvist, F., Arner, P., Nordfors, L. & Schalling, M. *Nature Med.* **1,** 950–953 (1995).
11. Huszar, D., *et al. Cell* **88,** 131–141 (1997).
12. Shimada, M., Tritos, N. A., Lowell, B. B., Flier, J. S. & Maratos-Flier, E. *Nature* **396,** 670–674 (1998).
13. Qu, D. *et al. Nature* **380,** 243–247 (1996).
14. Bjorbaek, C., Elmquist, J. K., Frantz, J. D., Shoelson, S. E. & Flier, J. S. *Mol. Cell.* **1,** 619–625 (1998).
15. ALS CNTF Treatment Study Group *Neurology* **46,** 1244–1249 (1996).
16. Gloaguen, I. *et al. Proc. Natl Acad. Sci. USA* **94,** 6456–6461 (1997).
17. Caro, J. *et al. Lancet* **348,** 159–161 (1996).
18. Schwartz, M. W., Peskind, E., Raskind, M., Boyko, E. J. & Porte, D. Jr *Nature Med.* **2,** 589–591 (1996).