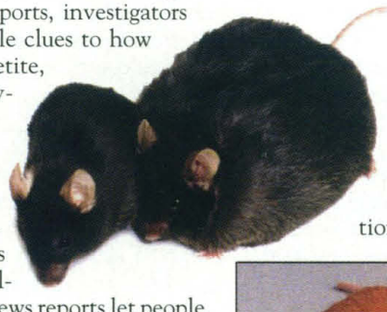
Obesity Sheds Its Secrets

While the hormone leptin has dominated obesity research, new findings suggest it has help in regulating body weight—results that may also provide potential avenues for obesity therapies

Every New Year, people make the same resolution. Glutted by holiday food and drink, they vow to shed their extra pounds by embarking on some sort of monastic weight-loss venture. And every year, most dieters fail. Figures from the National Institutes of Health show that half of the people living in the United States are overweight and one-third are clinically obese, weighing at least 20% more than they should—a problem that is both financially and medically costly. Not only do dieters spend $30 billion annually on their weight-loss endeavors, but those who remain obese are at high risk for diabetes and cardiovascular diseases, including hypertension, all of which can lead to premature death.

But in universities and biotech labs, researchers are on the trail of a different and perhaps more effective kind of weight-loss remedy—one aimed directly at the mechanisms by which the body controls its weight. In a flood of recent reports, investigators have announced telltale clues to how the body regulates appetite, metabolism, and activity. The first in the chain of findings—the discovery of the fat-busting hormone leptin 2 years ago—raised extravagant hopes that have yet to be realized. "Unfortunately, news reports let people believe that they could sit on the couch, eat whatever they want, inject themselves with leptin, and look like Cindy Crawford," recalls Art Campfield, who heads up the leptin project at drug industry giant Hoffmann-La Roche in Nutley, New Jersey.



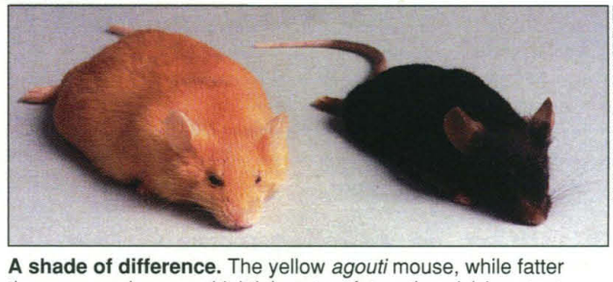
J. C. POWELL/HOFFMANN-LA ROCHE

While findings since then suggest that human obesity might not be treated so simply with leptin itself, in the past year, researchers have learned much more about how and where the hormone works in the body—information that may help them design drugs that mimic leptin's action. And the field continues to expand faster than a frustrated dieter's waistline as researchers have identified several additional molecules involved in weight control, which appear to link up with leptin in a complicated web of biochemical and brain-signaling circuits. Among these are neuropeptide Y—which jacks up appetite and slows metabolism when a person's fat stores seem to be decreasing—and a family of peptides called the melanocortins, whose receptors appear to have the opposite effect—damping down appetite in response to increased fat stores.

Defects in some of these molecules or their targets might help explain human obesity, because most overweight people have high levels of leptin itself. But even if they don't, their discovery might aid in the design of new drugs that can trim body fat. Indeed, researchers are already working on drugs that block a brain receptor of the melanocortins. "Each experiment seems to open up more and more opportunities," says Campfield. "There is so much work to do, and it will be going on like this for a long time."

A hormonal "lipostat"

Leptin still remains at the center of it all, however. Originally identified late in 1994 after an 8-year search by Jeffrey Friedman's team at Rockefeller University in New York City, leptin is a 16-kilodalton protein produced by the *obesity* (*ob*) gene of mice. Animals with defects in both copies of this gene act as if they are in a state of perpetual starvation, unable to reproduce, stay warm, grow normally, and, of course, restrain their appetites. Thus, they get grossly fat, weighing as much as three times more than normal animals.



JUNE SHIIGI/VOLLUM INSTITUTE

A shade of difference. The yellow *agouti* mouse, while fatter than a normal mouse *(right)*, is not as fat as the *ob/ob* mutant, shown at upper left with its normal counterpart.

Friedman's group and others traced the source of leptin to the body fat itself. The investigators found that fat cells called adipocytes manufacture leptin and spew it into the bloodstream, so that leptin levels in the bloodstream normally increase as the fat deposits grow. "When you get fat, mainly what happens is the adipocytes enlarge and store more lipids," says Jeffrey Flier, a leptin researcher at Beth Israel Deaconess Medical Center and Harvard Medical School in Boston. "The amount of leptin is usually in proportion to how big the adipocytes get."

These findings led to the idea that leptin functions as a lipostat: When fat stores rise, adipocytes produce leptin, and the hormone in turn tells the brain that it's time to stop eating and increase activity levels. Conversely, when fat stores decline, leptin concentrations go down, too, and that signals the brain to try to counteract the weight loss with increased feeding and lowered activity.

Subsequent work on the receptor that receives leptin's signal in the brain supports this view of how the molecule works. A year after leptin's discovery, Louis Tartaglia's team at Millennium Pharmaceuticals in Cambridge, Massachusetts, and their colleagues at Hoffman-La Roche found the receptor and cloned its gene in mice and humans. Shortly thereafter, three groups of investigators—Tartaglia's, Friedman's, and a third headed by Rudolph Liebel, also at Rockefeller—independently traced the obesity seen in another strain of mice, the so-called *db* (for *diabetic*) mouse, to mutations in the leptin-receptor gene (*Science*, 16 February 1996, pp. 913 and 994). Investigators have since mapped leptin-receptor mutations in two other grossly fat rodents—the *corpulent* rat and the fatty Zucker rat. All three types of animals presumably become fat because they can't respond to the hormone.

In addition, the leptin receptor's location in the brain is consistent with the idea that the hormone plays a role in weight control. Denis Baskin at the Veterans Administration Puget Sound Health Care System in Seattle and others have found that the leptin-receptor gene is expressed in four main regions of the hypothalamus, including two—the arcuate nucleus and the paraventricular nucleus—that had previously been implicated as hot spots for the regulation of feeding and metabolism.

But despite all the evidence implicating leptin and its receptor in weight control, mutations in their genes do not seem to be at fault in human obesity. "So far, no one has

Leptin's Other Hormonal Links

During the 2 years since its discovery, the protein hormone leptin has paraded through headlines because of its possible role in keeping fat levels in check (see main text). But that may not be its main function, says endocrinologist Jeffrey Flier of Harvard Medical School: "Throughout evolution, starvation—not obesity—has always been a problem." And recent work by Flier's team and others indicates that leptin, working through glands around the body, including the adrenals, the thyroid, and even the ovaries and testes, helps the body deal with the stresses of starvation.

Starving animals commonly experience a host of endocrine changes. To help conserve energy, for example, they lose their sex drives as production of the sex hormones by the ovaries and testes plummet. At the same time, starving animals turn down production of the thyroid hormones that stimulate cells' metabolic activities and turn up the production of adrenal stress hormones, such as cortisol, which perform a number of functions, such as maintaining blood pressure, that help the body adapt to starvation.

These changes, Flier and his colleagues found, may be triggered by a drop in leptin, which normally occurs when fat stores decline during starvation. By giving leptin to starving animals, the researchers were able to reverse the reproductive, metabolic, and stress responses. "The three classical endocrine changes that you see with starvation are apparently caused to a very large degree, if not entirely, by the fact that with starvation, leptin levels go down," Flier concludes.

In addition to helping explain the loss of sex drive in starving animals, the leptin work is also shedding light on normal sexual development. Flier's team and that of Farid Chehab at the University of California, San Francisco, have evidence that the hormone appears to signal, and perhaps orchestrate, the onset of puberty (*Science*, 29 November 1996, p. 1466; and 3 January, p. 88). Both teams found, for example, that giving the hormone to normal female mice could speed up the attainment of sexual maturity.

The researchers haven't yet worked out exactly how low leptin levels bring about starvation-induced hormonal changes. Flier's work suggests that they may lead to increased activity of an important regulator of stress-hormone production. He has found that normal leptin levels damp down production of a brain peptide called corticotropin-releasing hormone that works through the pituitary gland to boost production of the adrenal steroids. Stephen Woods and Michael Schwartz at the University of Washington, Seattle, have contrary results, however, although both Flier and Schwartz agree that more time and research will probably reconcile the disparity. "Either one of us might be wrong, and I don't feel dogmatic on this," Schwartz says.

Even if these connections between leptin and other hormonal systems evolved to cope with starvation rather than obesity, they may help guide obesity therapies. For example, Woods's team has evidence from studies on rats that adrenal steroids blunt leptin's activity in starving animals. Because, for reasons not yet understood, "obese people often have high levels of steroids," Woods says, this may help explain why they are resistant to their high blood leptin levels. It also suggests that drugs that block the steroids' effects might help in weight control.

This tangle of effects on hormones and neuropeptides hints that leptin's true role may be less of an obesity meter and more of a starvation shield, says Flier. "What we are saying is that there is this huge biology of leptin that may not intrinsically have anything to do with obesity or the prevention of obesity," says Flier. "Even apart from starving or overfeeding, leptin is an important way for there to be communication between the periphery and the brain." –T.G.

identified a human being with a mutated Ob receptor or mutated *ob* gene," Campfield says. And while a study of Pima Indians reported in the February issue of *Nature Medicine* by a team led by Eric Ravussin of the National Institute of Diabetes and Digestive and Kidney Diseases suggests that low leptin levels may have contributed to weight gains by some members of this population, other investigators, including Jose Caro, then at Thomas Jefferson University in Philadelphia, have generally found that overweight people have high leptin levels in their bloodstream, not the low levels seen in the *ob* mice. The large fat stores in the people seem to be pumping out the hormone normally, which implies that leptin itself won't work as an obesity cure. Instead, something must be going wrong in obese people's response to the hormone, although the difficulty does not seem to be in their leptin receptors.

One possibility is that leptin has problems making its way from the bloodstream into the brain. Michael Schwartz at the University of Washington Medical Center in Seattle and Caro found, for example, that the spinal fluid of obese people contains only slightly more leptin than that of their thinner counterparts, despite the fact that overweight individuals had, on average, a fivefold higher concentration of the hormone in their bloodstreams.

Alternatively, the brain may lose its ability to respond to whatever leptin does get in. "Somewhere between the circulation and the brain, there is decreased sensitivity," says Campfield. "We're trying to identify where the weak places are." One place to look is among the other molecules that interact with leptin to control weight, such as the potent appetite-stimulating neurotransmitter called neuropeptide Y (NPY).

Possible leptin partners

Normally, NPY levels are held in check by leptin. Production of the appetite stimulator skyrockets, for example, in leptin-lacking *ob/ob* mice. And in October 1995, Thomas Stephens and his colleagues at Eli Lilly Co. in Indianapolis reported that giving the animals leptin suppresses NPY production, a finding confirmed a few months later by Schwartz's group. These results suggest, Schwartz says, "that if [the animals] don't have any leptin, the NPY system is completely unrestrained," and that in turn leads to their obesity. Baskin's mapping of the brain locations of the leptin receptors fit neatly with this idea, because one of those locations, the arcuate nucleus, is a major site of NPY production.

More recently, Richard Palmiter's group at the University of Washington provided more direct evidence that leptin works through NPY to regulate appetite. Early in 1996, the researchers had made a puzzling observation when they created a strain of mice in which the NPY gene was inactivated. Rather than being thin, as expected, these animals appeared normal in body weight, fat levels, and food intake.

But results reported later in the year by Palmiter and his colleagues help resolve that puzzle (*Science*, 6 December 1996, p. 1704). When the investigators mated the normal-weight NPY knockout mice with the obese, leptin-lacking *ob/ob* mutants, they found, says Schwartz, that "a deficiency in NPY attenuated the defect in the *ob/ob* mouse." The appetites, general metabolism, and fat levels of the double-mutant offspring were midway between those of normal and *ob/ob* mice. This result indicates that part—but not all—of the weight gain of the *ob* mouse results from the increased NPY production induced

by the animal's low leptin levels.

"I think it makes a strong argument that excessive NPY is at least a component of the obesity syndrome that results when you don't have leptin," says Schwartz. And because the NPY defect didn't completely counteract the leptin defect in the double-mutant offspring, the finding also suggests that NPY isn't the only obesity regulator that responds to leptin, a circumstance that might explain why normal mice do just fine without NPY. "Obviously," says Schwartz, "other things in the brain are also sensitive to leptin."

Two papers published just a month ago provide some indication of what those other things might be. Roger Cone and his colleagues at Oregon Health Sciences University in Portland and Dennis Huszar's team at Millennium and Hoffmann-La Roche simultaneously unearthed another obesity signal. This one comes from a group of molecules that normally regulate hair color—the melanocortins and their receptors.

The *agouti* connection

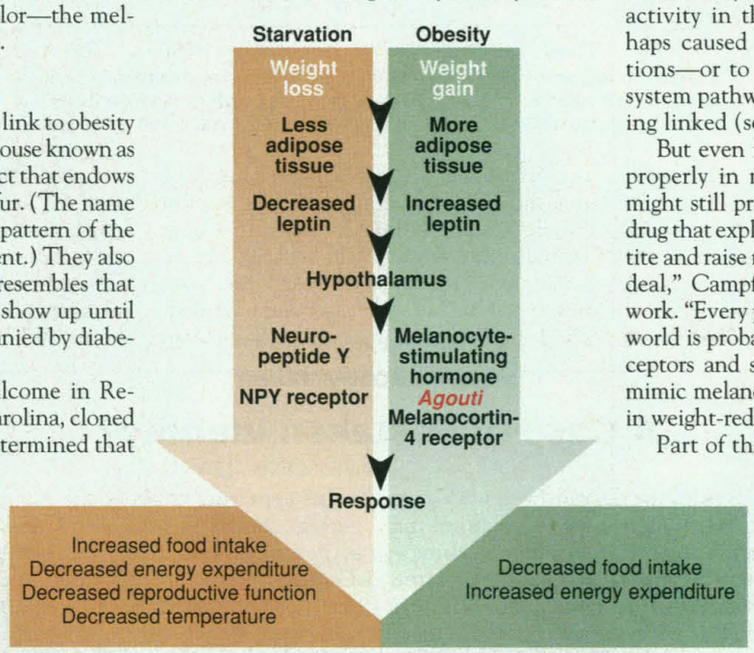
The story of the melanocortins' link to obesity actually began with a mutant mouse known as *agouti*, which has a genetic defect that endows the animals with bright yellow fur. (The name *agouti* comes from the banded pattern of the fur of the South American rodent.) They also have an obesity problem that resembles that of many humans, as it doesn't show up until late in life and is often accompanied by diabetes, particularly in males.

Researchers at Glaxo Wellcome in Research Triangle Park, North Carolina, cloned the *agouti* gene in 1992 and determined that its product is a 131-amino-acid protein produced by hair follicles. At the time, however, the Glaxo team, led by biochemist Bill Wilkison, couldn't figure out what a protein normally made by hair follicles had to do with obesity, although they did note that in the *agouti* mutants the gene is "on" all the time in every tissue in the body. It wasn't until after Cone's group cloned the first melanocortin receptor later that year that researchers had even an inkling of how *agouti* could affect body weight.

Cone's group found that the agouti protein binds to a melanocortin receptor (MCR-1) located on skin cells called melanocytes. By doing so, it gets in the way of a melanocortin known as α melanocyte-stimulating hormone (α-MSH), which binds to the receptor and signals the melanocytes to produce black pigment. Normally, that inhibition comes on briefly to cause yellow banding in the mouse's fur. But because *agouti* is constantly turned on in the mutant mice, the MCR-1 receptor is always blocked and the animals are yellow instead of black. And Cone speculated that additional MCRs may be located in the brain, where they would have a different function: regulating weight gain and metabolism. "It's typical that hormone systems serve multiple physiological roles," says Cone. "For example, NPY is also in the [brain] cortex affecting learning and other functions clearly unrelated to feeding."

Cone's team went on to confirm his speculation by showing that two MCRs—MCR-3 and MCR-4—are produced in the arcuate nucleus of the hypothalamus, a prime target of leptin action as well as a seat of NPY production. And in 1994, he and his graduate student Dongsi Lu reported that *agouti* can work on those brain receptors.

That work led to Cone's most recent paper, published in *Nature* on 9 January. When Cone and his colleagues injected synthetic peptides mimicking melanocortins into the brains of both normal and *ob* mutant mice, they found that the molecules bind to MCR-4 and suppress feeding, even when the mice are given extra NPY to boost their appetites. At the other side of the spectrum, molecules that mimic the agouti protein and block the receptor can bolster the animals' appetites during the night or after a prior fast.



Weight watchers. Decreased leptin levels work through neuropeptide Y to combat starvation, while high leptin levels may work through the melanocortin path to help counteract overweight. The agouti protein can lead to obesity by blocking the melanocortin-4 receptor.

Further evidence that the MCR-4 receptor is important in regulating weight comes from Huszar's team, working in collaboration with Campfield and others. In results that appeared in the 10 January issue of *Cell*, they found that mice in which MCR-4 had been knocked out mimicked the weight gain and other symptoms of the mutant *agouti* mice. "What is remarkable about this is that we got a phenotype that virtually mirrors the *agouti* effect," says Huszar. "The *agouti* mouse itself is one of those happy accidents that points to an interesting pathway." Cone agrees: "This finding all of a sudden puts melanocortin-producing neurons in the center of weight homeostasis and metabolism. That was never known before."

Still to be established is what ties, if any, MSH and its receptor have to leptin, although Cone's team and others have preliminary evidence suggesting that MCR-4 knockouts and *ob/ob* mice both have high NPY expression in a region of the hypothalamus called the dorsal medial nucleus, indicating that all three are connected. Human obesity might then be due to either overactivity of the NPY pathway or too little activity in the melanocortin branch, perhaps caused by as yet unidentified mutations—or to problems in other hormonal-system pathways to which leptin is now being linked (see sidebar).

But even if all these systems are working properly in most overweight people, they might still provide a target for a weight-loss drug that exploits the pathways to lower appetite and raise metabolism. "I think this is a big deal," Campfield says of the melanocortin work. "Every pharmaceutical company in the world is probably now getting the [MCR] receptors and screening them" for drugs that mimic melanocortins' effects for possible use in weight-reducing therapies.

Part of the appeal of the MCRs is their nature. Unlike leptin receptors, MCRs are so-called G protein–coupled receptors, which traditionally are the easiest targets to block with small-molecule drugs. "If you had your choice, G-coupled [receptors] are the best possible target," Campfield says. And drugs that bind and stimulate MCR-4 could be developed even before the details of the receptor's mechanism are fully worked out.

There are likely to be plenty of other opportunities for biotech and basic science lurking in the tangle of pathways investigators have uncovered. "There may be multiple limbs, and the limbs themselves are likely to branch and interconnect," says leptin discoverer Friedman. Since his discovery set it all in motion, he says, "it's been a great deal of fun to see it happen."

–Trisha Gura

Trisha Gura is a free-lance writer based in Cleveland.