CELL BIOLOGY

Uncoupling Proteins Provide New Clue to Obesity's Causes

Just about everybody who has struggled to shed and keep off pounds has envied those lucky few who apparently can eat whatever they want and never change their dress size. Metabolism—the way we break down food and use it for energy—may make at least part of the difference. Some people simply have lower metabolic rates, and thus a greater tendency to gain weight, than others. Now, researchers may be getting a handle on what accounts for those differences.

Just over a year ago, they identified what appear to be the first human "uncoupling proteins" (UCPs). Originally discovered decades ago in the special brown fat cells that animals such as bears burn up while hibernating, UCPs are so called because they dissociate the reactions that break down food from those that produce the body's chemical energy. In effect, they punch holes in the energy-production pipeline, raising the body's resting metabolic rate.

Because the lost chemical energy is dissipated as heat, UCPs help hibernators and other cold-adapted animals maintain their core body temperatures in frigid weather. But people don't have brown fat, except in small amounts when they are newborns, and researchers did not think that the proteins had much effect on human metabolism. The new work now challenges that assumption, because its shows that other human tissues, including ordinary fat and muscle, make proteins very similar to the animal UCPs.

There's no proof yet that these human UCP relatives uncouple metabolism and energy production. But researchers are scrambling to pin down their role, because if human UCPs do have the predicted function, their discovery could help provide a better understanding of obesity as well as improved treatments for the condition. "The field is burgeoning," says Mary Ellen Harper, a metabolic physiologist at the University of Ottawa in Canada. She and others suggest that variations in UCP production or activity may be what cause some people to have lower or higher metabolic rates—and thus greater or lesser tendencies to get fat—than others.

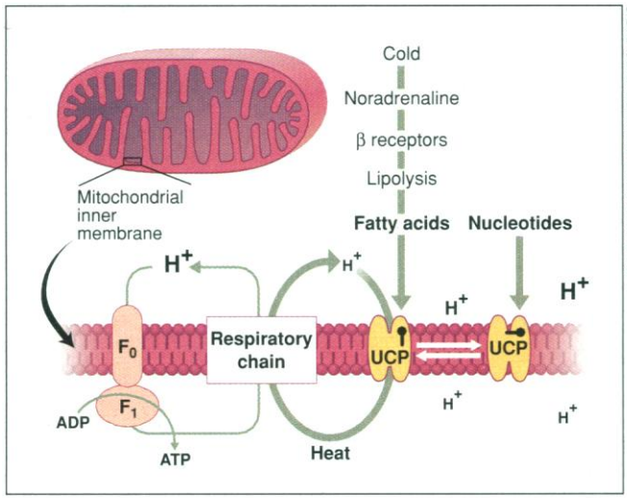
But even if that's not the case, UCPs might be good targets for obesity therapy, especially as they appear to act mainly in fat and muscle, whereas many other weight-regulatory molecules seem to work mainly in the brain (see p. 1378). By hiking up UCP activity, "you could boost your metabolic rate and you wouldn't act on the central nervous system," with the potential that has for causing side effects, says physiologist Eric Ravussin at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in Phoenix. "It's like jogging without jogging."

The first uncoupling protein (UCP1) was discovered independently in the mid-1970s by biochemist David Nicholls at the University of Dundee in the U.K. and Daniel Ricquier at the National Center for Scientific Research (CNRS) in Paris. At the time, researchers already knew that hibernating animals, and also cold-adapted rodents, use special fat cells, the brown adipocytes, to produce body heat. To try to find out more about how these cells work, Ricquier kept lab rats in either cold or warm temperatures and then looked for differences in the proteins made by the brown fat cells. Sure enough, he found that the fat cells of the chilly rats churn out a 32-kilodalton protein that is not made by the cozier animals.

At about the same time, Nicholls and his team identified the mitochondria, the tiny kidney-shaped organelles that serve as the cells' powerhouses, as the source of heat released by brown fat. The mitochondria use the energy contained in dietary sugars, fats, and other nutrients to drive the synthesis of the high-energy compound adenosine triphosphate (ATP). This process depends on an electrochemical gradient set up across the inner of the two mitochondrial membranes when protons (positively charged hydrogen ions) are pumped out of the interior chamber of the mitochondrion.

By injecting a radioactive compound into fat cells and then measuring its concentration on either side of the mitochondrial membrane, Nicholls and his colleagues showed that the inner membrane of brown fat mitochondria is very permeable to protons. Ultimately, the researchers traced this leak to a protein in the mitochondrial membrane that came to be known as UCP1.

By creating the leak, UCP1 reduces the number of ATPs that can be made from a given amount of food, thereby raising the body's metabolic rate and generating heat. Normally, though, the protein is kept in an inactive state by nucleotides that bind to the protein. Then, when the animal needs extra heat, it activates neurons that release the neurotransmitter norepinephrine at the surfaces of the brown fat cells, and the hormone then sets in motion a chain of events that releases the inhibition.



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Regulator. When activated by cold, uncoupling proteins (UCPs) let hydrogen ions pass through the inner mitochondrial membrane, thereby abolishing the hydrogen ion gradient needed to drive ATP synthesis. Nucleotide binding prevents the UCP from doing that.

For many years, uncoupling proteins didn't appear to play an important role in body metabolism in people. Humans have a UCP1 gene, but it's active only in their brown fat, which disappears shortly after birth. Still, measurements of the amount of oxygen that human and other animal cells consume when they metabolize food show that anywhere from 25% to 35% of that oxygen is being used to compensate for mitochondrial proton leaks. "There is a significant proportion of uncoupling going on all the time," says Harper, who performed such oxygen-consumption studies in the laboratory of Martin Brand at the University of Cambridge. What causes that uncoupling has been unclear, but the novel UCP family members may provide an explanation.

Craig Warden, a geneticist at the University of California, Davis, working with Ricquier in Paris and Sheila Collins's team at Duke University, came on the first of these early last year. While combing through genetic databases, the researchers identified sequences strongly resembling those in UCP1. After cloning the corresponding gene, they showed that the protein, UCP2, is expressed in tissues ranging from the brain to muscle and fat cells.

Warden says he was inspired to look for UCP1 relatives because he had heard Louis Tartaglia of the biotech firm Millennium Pharmaceuticals in Cambridge, Massachusetts, talking about his team's discovery of

such a protein at a meeting. The Millennium team held up on publishing their results, however, until the company filed for patents, which it has since received on the UCP2 gene and its potential use in diagnosing an individual's risk of becoming obese.

A third human UCP made its debut on the heels of UCP2's discovery. A trio of research groups—one headed by Jean-Paul Giacobino at the University of Geneva in Switzerland, another by Brad Lowell at Beth Israel Deaconess Medical Center and Harvard Medical School in Boston, and a third by Mark Reitman at the NIDDK labs in Bethesda, Maryland—independently cloned the gene for this protein, UCP3, which seems to be active mostly in muscle cells.

No one knows for sure whether UCP2 and -3 behave like UCP1, although circumstantial evidence suggests they do. For example, the family members look startlingly alike. Both the UCP2 and -3 genes are about 56% identical to the UCP1 gene. "That is pretty high homology," Lowell says, and suggests that the genes' products have similar functions.

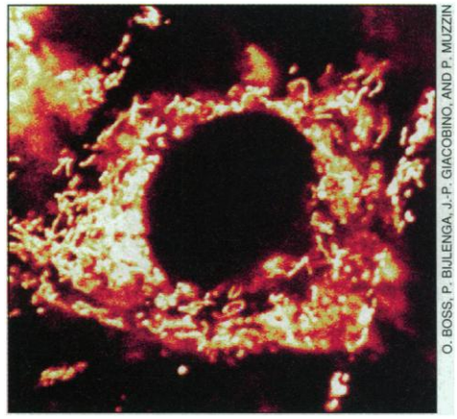
Further evidence that the new proteins uncouple oxidation and ATP synthesis comes from experiments in which the researchers engineered either yeast or muscle cells from mice to express extra copies of UCP2 and UCP3. Compared to mitochondria of normal cells, those from the UCP-loaded cells show a lower membrane potential—a sign that the proton gradient is leaking. "It's a strong indication that UCP2 and UCP3 uncouple, at least in yeast and other transfected cells," says Giacobino.

Mitochondria from UCP2-altered yeast cells also tend to use more oxygen, another sign of uncoupling. Researchers are now conducting similar experiments to measure oxygen consumption by mitochondria from the mouse cells.

While researchers wait for proof that UCP2 and -3 are bona fide uncouplers, they are also pursuing hints that variations in these genes could affect body weight. In one study of 640 French Canadians, for example, Claude Bouchard at Laval University in Quebec, working with Ricquier and Warden, showed that certain DNA sequences that flank the UCP2 gene are found primarily in people with low metabolic rates. The researchers also have evidence that those markers may be linked to body mass and percentage of fat in obese individuals within the group.

Similarly, NIDDK's Ravussin, Warden, and their colleagues found that certain changes in the UCP2 gene seem to be associated with a sluggish resting metabolic rate in a group of 76 Pima Indians and more weakly associated with obesity in a group of 1000 Pimas. Only old people showed the linkage with obesity, however. "If this variant really does something to metabolic rate, we think it may take time to show up in body weight," Ravussin suggests.

Now his team is looking at some promising variations (polymorphisms) located in the regions that help to regulate the activity of the UCP3 gene. These, too, seem to be linked with obesity or slow metabolism, although Ravussin emphasizes that these findings are preliminary.



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Heat source. As shown by the fluorescent staining, muscle cells are loaded with mitochondria, so that even a small amount of uncoupling could lead to significant energy loss as heat.

On a more cautious note, however, not all studies have supported a connection between the human UCPs and obesity. For example, Danish investigators at the Steno Diabetes Center and Hegedorn Research Institute in Copenhagen couldn't find anything unusual about the UCP2 variants carried by 35 obese or diabetic patients. In a study of 60 patients, they also failed to find any link between those conditions and UCP3 polymorphisms.

Muddying the picture still further are studies of how UCP and gene expression change when animals and people are temporarily starved. If the proteins are, in fact, calorie burners, their activity would be expected to drop when food is scarce. Instead, the studies seem to show that total fasting actually increases the activity of their UCP2 and -3 genes. "You would think that starvation would be the worst time your body would decide to increase energy expenditures," says Lowell.

Olivier Boss, working with Giacobino and Patrick Muzzin at Geneva, has results that may resolve this paradox, however. They show that although going without food altogether sparks UCP2—and more dramatically, UCP3—gene activity in various tissues of rats, restricting the animals' food by more than 60% of normal amounts actually decreases the expression of UCP1 in brown fat and UCP3 in muscles. The difference, Boss and others say, could hinge on how the body responds to a reduction in food as opposed to total cessation.

The body has to maintain its core temperature, and when totally deprived of food, it may do this by turning up its UCPs, even at the cost of burning its fat stores. In less dire circumstances this may not be necessary, however, and the body may turn down UCP activity to save energy.

One dietary study does fit the calorie-burner hypothesis: Collins, at Duke, found that a high-fat diet can turn up the activity of the UCP2 gene and protect mice against obesity. She, along with Duke's Richard Surwit and their colleagues, stumbled onto the finding when they started feeding high-fat diets to their murine subjects. Two strains, called A/J and black kalas, managed to stay svelte on such diets, but the third, known as C57-black, immediately fattened up.

When Collins compared the UCP2 genes in the strains, she found a possible explanation: differences in genes' regulatory regions. What's more, the sequence differences apparently affect gene expression. "Kala behaves like [the equally slim] A/J in that it upregulates UCP2 messenger RNA very quickly in response to a high-fat diet," says Collins. In contrast, the C57 mice did not display a comparable increase.

To get more definitive proof that UCP2 and -3 are involved in regulating basal metabolic rates, and thus weight gain or loss, researchers like Lowell and many others are working to engineer mice in which the genes encoding the proteins are either knocked out or overactive. If these manipulations have the postulated effects, then the race will be on to find drugs that can combat obesity by turning up the activity of the proteins. "If one were to identify small molecules that one would take through the oral route, then we might be able to stimulate the uncoupling proteins enough to reduce body weight," says Tartaglia at Millennium.

Still, obesity research is full of dashed hopes. Biochemist Ricquier remembers how in the 1960s physicians treated obese individuals with nonspecific uncoupling drugs. They did help burn fat by hiking resting metabolic rates. But in some cases ATP formation plunged to zero—with fatal results.

Given those failures, he says, the key now is to figure out how the uncoupling proteins work and then nudge them to work just a little harder. "If we could develop compounds that very slightly increase the level of uncoupling—by 1% or 2%," Ricquier suggests, "then we would simply increase fat oxidation and thermogenesis." And that could boost the resting metabolic rates of millions of people and whittle away their days of perpetual dieting.

–Trisha Gura

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