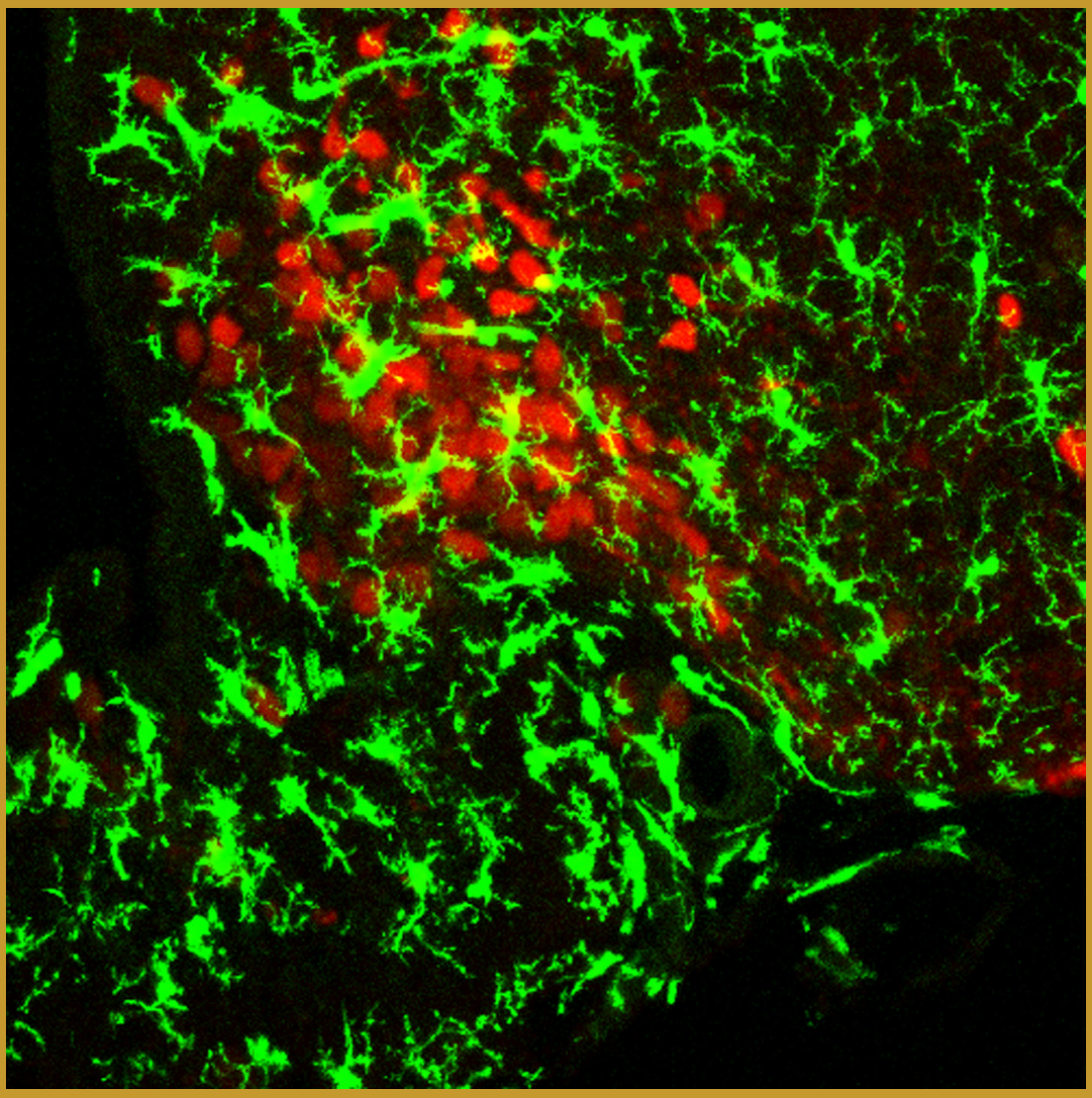

The brain plays a crucial role in feeding behavior and the regulation of body weight, and brain cells have long been the target of drugs to treat obesity. Recent research in mice, however, has shown that immune cells that reside in the brain can trigger inflammation in response to a high-fat diet, leading to weight gain. This figure shows immune cells (green) interacting with specific brain cells (red) that are critical in the regulation of food intake and body weight. As described in this chapter, this recent discovery could lead to new targets for obesity treatment that could avoid side effects of current drugs in use.

Image courtesy of Martin Valdearcos, Ph.D., laboratory of Suneil Koliwad, M.D., Ph.D.

Obesity

Obesity has risen to epidemic levels in the United States. Individuals who have obesity may suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. Obesity is a strong risk factor for type 2 diabetes, fatty liver disease, and many other diseases and disorders within the NIDDK's mission. Nearly 40 percent of U.S. adults are considered to have obesity based on body mass index (BMI), a measure of weight relative to height.[1] More than 18 percent of children and adolescents also have obesity, and thus are at increased risk for developing serious diseases both during their youth and later in adulthood.[1,2] Obesity disproportionately affects people from certain racial and ethnic groups and those who are socioeconomically disadvantaged.

The high prevalence of obesity in the United States is thought to result from the interaction of genetic susceptibility with behaviors and factors in the environment that promote increased caloric intake and sedentary lifestyles. Diet, activity, and aspects of our environment may also modify biologic factors in ways that promote obesity. Research is providing the foundation for actions to address this major public health problem by illuminating the causes and consequences of obesity, evaluating potential prevention and treatment strategies, and providing an evidence base to inform policy decisions.

The NIDDK supports a multi-dimensional research portfolio on obesity, spanning basic, clinical, and translational research. NIDDK-funded studies investigate a variety of approaches for preventing and treating obesity. These span behavioral and environmental interventions in families and in health care and other settings, using a variety of approaches and technologies; surgical interventions; and combinations of strategies. In parallel, Institute-supported investigations into the biologic processes associated with body weight have continued to spark new ideas for intervention approaches. To help bring research results to those affected by obesity and their families, health professionals, and the general public, the Institute sponsors health information programs.[3]

The NIDDK also continues to play a leading role in the NIH Obesity Research Task Force. The NIDDK Director co-chairs the Task Force along with the Directors of the National Heart, Lung, and Blood Institute and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. The Task Force includes representatives from these and numerous other NIH Institutes, Centers, and Offices.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter.

AN ENZYME'S ROLE IN A COMPLEX GENETIC OBESITY SYNDROME

An Enzyme Deficiency Contributes to Disease Symptoms in Prader-Willi Syndrome: Researchers have discovered a critical role for the enzyme prohormone convertase 1 (PC1) in the complex genetic disorder, Prader-Willi Syndrome (PWS). PWS is caused when a part of the genome is missing, resulting in several genes not passing down from a father to a child, leading to many detrimental effects on the infant's body that persist throughout adulthood. Beginning in childhood, affected individuals are often short in stature and develop insatiable appetites, which leads to chronic overeating, obesity, and an increased risk for diabetes and other disorders. Physical symptoms arise from poor regulation of various hormones, including insulin; growth hormone (GH); possibly the appetite-regulating hormone, ghrelin; and others.

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1 *Hales CM et al. CDC. National Center for Health Statistics Data Brief No. 288, 2017.*

2 *For children and adolescents, obesity refers to a BMI at or greater than the 95th percentile on growth charts (which are based on previous national surveys).*

3 *NIDDK Weight-control Information Network, www.win.niddk.nih.gov*

Most PWS instances are due to a large genetic deletion on chromosome 15. However, researchers identified five PWS patients with a smaller deletion, defining a critical region sufficient to cause the major PWS-associated traits. This region contains three genes, including one known as *SNORD116*. While none of the existing PWS mouse models develop obesity, mice lacking a paternal copy of *Snord116* (referred to as *Snord116*$^{p-/m+}$ mice) develop many of the other clinical features exhibited in their human counterparts, including overeating, decreased body length, and hormone impairments.

It is well-established that a part of the brain called the hypothalamus plays a crucial role in regulating appetite through production of and interactions with various hormones. To study how the PWS genetic deletions affect the brain, investigators generated brain cells (neurons) in the laboratory from another type of cell that, unlike neurons, can be obtained from patient volunteers. Using a technique developed by other researchers, they first "reprogrammed" samples of patients' skin cells to an early stage, stem cell-like (or pluripotent) state in the laboratory and then had them differentiate into (become) neurons. Because the induced pluripotent stem cell-derived (iPSC-derived) neurons contain the PWS patients' genetic material, scientists could study the effects of the gene deletion encompassing *SNORD116*. In addition, they studied male *Snord116*$^{p-/m+}$ mice. Analysis of human iPSC-derived neurons revealed that the gene *PCSK1*, which codes for the PC1 enzyme, had reduced activity, suggesting the possibility of PC1's involvement in the development of PWS. Furthermore, mice lacking paternal *Snord116* had decreased PC1 levels compared to normal mice.

PC1 processes prohormones (precursors to hormones) including proinsulin, pro-GH-releasing hormone, and proghrelin, into their bioactive forms—insulin, GH, and ghrelin, respectively. To determine if the hormonal impairments observed in PWS are a consequence of impaired prohormone processing by deficient PC1, researchers measured hormone levels *in vivo* in *Snord116*$^{p-/m+}$ mice and human PWS patients. Compared to normal mice, *Snord116*$^{p-/m+}$ mice exhibited increased levels of proinsulin, pro-GH-releasing hormone, and proghrelin, indicating an inability of PC1 to properly process the prohormones. The ratio of proinsulin to insulin in the blood of PWS patients was elevated, but not to the extent as that of a patient completely lacking PC1. These data suggest impaired PC1 activity due to paternal deletion of *SNORD116* drives the hormonal features of PWS.

This research highlights the effectiveness of a combined approach using human cells and blood samples along with mouse models to study a complex genetic disorder. While the findings contribute to a growing body of knowledge investigating how the loss of a gene alters hormone levels and function in PWS, more research is necessary to determine if other mechanisms are involved.

Burnett LC, LeDuc CA, Sulsona CR,...Leibel RL. Deficiency in prohormone convertase PC1 impairs prohormone processing in Prader-Willi syndrome. J Clin Invest 127: 293-305, doi:10.1172/JCI88648, 2017.

NEW INSIGHTS INTO FAT CELL DEVELOPMENT

A Strategy for Synthesizing Potential New Drugs and Research Tools Yields Insights into Fat Cell Development: With a creative strategy for generating new knowledge that may lead to future drug development, researchers developed a set of small chemicals that bind many different proteins in cells and can be used to learn the proteins' functions. Using this approach, they discovered a protein important in fat cell development.

Different types of proteins perform key tasks throughout the body, and many drugs are chemicals that target specific proteins, either blocking or increasing their functions for therapeutic effects. Chemicals that bind to proteins can also be used to gain new understanding of what the respective proteins do. However, only a fraction of human proteins are targets of current drugs. Thus, scientists devised a way to target many previously untargeted proteins with small chemicals. To get a sense of how many proteins could be targeted this way, they developed a collection of small chemicals of varying structure (which they also called "fragments"), each with a special tag, and mixed these with human cells grown in the lab. Using the tags, they retrieved the chemical-protein

pairs that formed in the cells. In their initial screen, they identified thousands of interactions between the chemicals and different types of proteins; each chemical could bind multiple proteins. A search of a large database revealed that most of these proteins were not targets of existing drugs. These results showed that a large variety of proteins—more than previously thought possible—could be bound by small chemicals. Further experiments with a few of the chemicals showed that these inhibited the functions of the bound proteins. The researchers also found that, by modifying a chemical's structure, they could strengthen its interaction with a particular protein of interest, an approach similar to the methods scientists use to fine-tune the structure of a potential new drug.

The researchers then explored whether their small, tagged chemicals could be used to discover proteins important for biological processes. As a test case, they chose the process of fat cell development. They screened for chemicals that could prompt precursor (immature) cells from mice to develop into fully mature fat cells. They found one chemical that could do this; identified the protein it bound, PGRMC2; and showed that the chemical stimulated the protein's activity. Through these and additional experiments, they discovered that PGRMC2 plays a role in fat cell development.

This research demonstrates that small, tagged chemicals can be used to target a wide variety of proteins in human cells. Future studies may advance understanding of the proteins they bind, and these chemicals could potentially serve as starting points for new drug development.

Parker CG, Galmozzi A, Wang Y,...Cravatt BF. Ligand and target discovery by fragment-based screening in human cells. Cell 168: 527-541, doi: 10.1016/j.cell.2016.12.029, 2017.

Large-scale Human Genetics Study of Fat Depots Identifies Gene Regions Associated with Depot- and Gender-specific Fat Cell Development: In the largest-scale human genetics study of its kind, researchers have identified seven new gene regions associated with individual variability in body fat traits. In addition, genetic analysis in a mouse model suggests an important physiological role for two of these genes in fat cell development.

Differences in fat tissue (adipose tissue) distribution affect disease risk, including diabetes and heart disease. Mammals store different kinds of adipose tissue in various repositories, or depots, in the body. Subcutaneous adipose tissue (SAT) is found just beneath the skin, while visceral adipose tissue (VAT) surrounds abdominal organs, and pericardial adipose tissue (PAT) surrounds the heart. Research has shown that various traits, such as the volume, density, and relative distribution of adipose tissue have an associated genetic component and are important predictors of disease risk. To gain new insights into the genetic contribution to adipose tissue traits, investigators searched the genomes of 18,332 ethnically diverse men and women to identify small, common variants in their DNA. Then, using non-invasive imaging techniques, they analyzed adipose tissue traits of study participants; each trait was examined in all or a large number (several thousand) of the participants. They found that these traits were associated with common genetic variants in seven gene regions. Of these, three gene regions were associated with SAT and VAT volume, two were associated with PAT volume, one with SAT density, and one with the relative distribution between VAT and SAT depots. In some cases, associations were gender specific. Further evaluation found that genetic variations associated with PAT volume and a higher amount of VAT relative to SAT were also significantly associated with different metabolic conditions, including type 2 diabetes and levels of cholesterol and circulating fat in both men and women.

To examine the functional significance of these findings, they turned to an animal model. They measured activity of four analogous mouse genes in different fat depots in male mice and found variable activity for two genes: the *Ube2e2* gene was more highly activated in VAT than in SAT or PAT during fat cell development. The *Atxn1* gene was more active in the SAT of mice with diet-induced obesity than in lean mice. These data suggested a potential regulatory role for the genes in adipose tissue development. To explore this possibility, the team isolated early-stage cells from SAT and VAT depots of mice and allowed them to differentiate into (become) fat cells in the laboratory. Both *Atxn1* and *Ube2e2* showed evidence of dynamic regulation of activity at different timepoints. When they used a molecular technique to silence the activity of either of these genes in the cells, they observed impaired fat cell formation in SAT, whereas only *Ube2e2* disruption impaired adipose tissue development in VAT.

Through a combined approach of a large-scale human genetics study coupled with experiments in a mouse model, this research provides new insight into the genetics of body fat distribution and supports physiological roles for specific genes in fat cell development. Future studies are necessary to determine the mechanism by which *ATXN1* and *UBE2E2* affect adipose tissue formation in humans and if they influence the development of cardiometabolic disease.

Chu AY, Deng X, Fisher VA,...Fox CS. Multiethnic genome-wide meta-analysis of ectopic fat depots identifies loci associated with adipocyte development and differentiation. Nat Genet 49: 125-130, doi: 10.1038/ng.3738, 2017.

REGULATING METABOLISM VIA SIGNALS FROM FAT, LIVER, AND BONE

An Appetite-suppressing Hormone in Bones: Researchers discovered, in a study in mice, that bones secrete an appetite-suppressing hormone called lipocalin 2 (LCN2). This hormone also regulates blood glucose (sugar) levels.

Building on previous findings that hinted at a possible role for bones in regulating appetite, the scientists searched for hormones produced in bone cells of mice and identified LCN2. This hormone had earlier been found to be made in fat cells and was associated with body weight. However, as shown in this study, compared to fat cells, bones produce much larger quantities of LCN2. To gain insight into the function of LCN2, the researchers explored what happens in its absence. They genetically engineered a group of male mice to be deficient specifically in bone-derived LCN2; these mice could still produce LCN2 in their fat cells. Without LCN2 from bones, these mice ate more than normal mice, gained more weight, and accumulated more body fat. The absence of bone-derived LCN2 also impaired blood glucose regulation. Thus, they concluded that LCN2 from bones normally dampens appetite and regulates blood glucose.

To confirm these results, the research team next studied the effects of excess LCN2. When they injected LCN2 into normal mice, the extra hormone reduced food intake, body fat, and weight. The researchers then found that levels of LCN2 naturally fluctuate with fasting and feeding. Delving deeper into how LCN2 affects appetite, the researchers discovered, in experiments in male and female mice, that LCN2 can travel to the brain, where it partners with another molecule, MC4R, to suppress appetite. An additional study revealed clues that LCN2 might work similarly in humans. Examining men with type 2 diabetes, the researchers found that higher levels of LCN2 in the blood correlated with lower body weights and better blood glucose levels.

This study highlights the importance of a bone-produced hormone in regulating appetite and blood glucose. Other research teams had previously identified LCN2 as playing a role in blood glucose control and other biologic processes, but had not detected its production in bones, and, curiously, had not observed any effects on appetite in mice. Further investigation may yield additional insights into the complexity of this hormone's activities. If LCN2 suppresses appetite and improves blood glucose levels in people, it could potentially be targeted in the development of new therapies for type 2 diabetes and obesity.

Mosialou I, Shikhel S, Liu JM...Kousteni S. MC4R-dependent suppression of appetite by bone-derived lipocalin 2. Nature 543: 385-390, doi: 10.1038/nature21697, 2017.

Signals from Body Fat to Other Organs—Small Packages with Big Metabolic Impact: Researchers discovered a way that fat tissue regulates metabolism—it produces a variety of tiny but powerful molecules, called miRNAs (or microRNAs), and sends these out through the bloodstream to control biological processes elsewhere in the body. Previous studies had shown that fat tissue also releases hormones with far-ranging effects. The new discoveries thus add miRNAs to the metabolic toolbox of fat.

The researchers investigated miRNAs from fat tissue because past studies had illuminated their importance in cells throughout the body; and altered levels of miRNAs had been observed in obesity, diabetes, and another condition, lipodystrophy, associated with abnormalities in body fat. To gain new insights, the researchers began by comparing normal male mice to male mice that were deficient in miRNA production in fat tissue (but not elsewhere), to see whether this deficiency affected miRNAs in the blood. In the bloodstream, miRNAs often travel in small packages called exosomes, and the researchers found that levels of hundreds of different types of miRNAs were reduced in exosomes from the mice deficient in

fat-derived miRNAs. These mice also had metabolic problems, including impaired blood glucose (sugar) regulation. When the researchers transplanted fat tissue, particularly the type known as brown fat tissue, from normal mice into the miRNA-deficient mice, they found partial restoration of miRNAs in the blood and improvement in glucose levels. These results demonstrated that normal fat tissue is a key source of circulating miRNAs, with effects on health. Other experiments also showed that fat deploys miRNAs to control metabolism from afar. For these experiments, the researchers drew upon knowledge that miRNAs block the flow of information encoded by various genes, so that the information cannot be used to make proteins. They found that miRNA-containing exosomes from mouse fat tissue travel to the liver and regulate a gene there, reducing levels of the protein it encodes.

The researchers also studied miRNAs in blood samples from human volunteers, including healthy individuals and people with lipodystrophy, a condition marked by loss of body fat where it should be, accumulation of fat where it can be toxic, and increased risk for diabetes. The levels of many miRNAs were lower in people with lipodystrophy than in healthy people, a sign that normal fat tissue is a source of circulating miRNAs in humans.

This study highlights a role for miRNAs in the regulation of metabolism by body fat. With further research, scientists may be able to develop interventions that target fat-derived miRNAs to improve health.

Thomou T, Mori MA, Dreyfuss JM,...Kahn CR. Adipose-derived circulating miRNAs regulate gene expression in other tissues. Nature 542: 450-455, doi :10.1038/nature21365, 2017.

THE BRAIN'S REGULATION OF APPETITE AND OBESITY

Brain Immune Cells Control Food Intake and Obesity: Researchers have discovered that a type of brain immune cell, called microglia, controls food intake and obesity in mice, providing a novel therapeutic target to combat obesity and its associated health consequences. The research focused on the hypothalamus, a part of the brain governing hunger and other physiologic functions, and was conducted primarily in male mice. Scientists built on previous research showing the association between obesity and the accumulation of microglia in the hypothalamus of people and mice. It was not known, however, whether microglia-induced inflammation played a role in regulating food intake or rather was a consequence of excess food intake. To examine this question, researchers used a drug called PLX5622 to deplete microglia. They found that the drug had no effect on mice eating a standard, low-fat diet. They then compared two groups of mice that were on a high-fat diet. Although both groups gained more weight than mice fed the standard diet, drug-treated mice eating a high-fat diet ate less, gained less weight, and had lower body fat compared to untreated mice also eating a high-fat diet. These and other results suggested that microglia play a role in regulating food intake. To confirm this finding, the researchers performed the opposite experiment: using genetic techniques, they generated mice in which they could activate microglia and induce microglial inflammation. They found that mice with activated microglia were vulnerable to weight gain even on the standard diet, experiencing a rapid, 4-fold increase in the amount of weight gained compared to control mice on the same diet. Contributing to this weight gain, the experimental mice ate more food and had lower energy expenditure (calorie burning) compared to control mice, further suggesting that microglia directly regulate metabolism. Other experiments showed that, when exposed to a high-fat diet, microglia signal to immune cells found in the bone marrow and recruit them to the hypothalamus to participate in the inflammatory response. These results suggest that microglia are key regulators of food intake, calorie burning, and obesity in mice.

If found to play a similar role in women and men, microglia represent novel therapeutic targets for obesity and its associated health consequences. Further research into the detailed mechanisms by which these cells exert their effects—such as understanding how a high-fat diet promotes microglial inflammation in mice—could shed light on other targets and potential therapies.

Valdearcos M, Douglass JD, Robblee MM,...Koliwad SK. Microglial inflammatory signaling orchestrates the hypothalamic immune response to dietary excess and mediates obesity susceptibility. Cell Metab 26: 185-197, doi: 10.1016/j.cmet.2017.05.015, 2017.

Brain Control of Obesity-related Behaviors: Two studies in mice have demonstrated new links between nerve cell activity in specific brain regions and both physical activity and the drive to eat, further defining the critical roles of hormonal signaling in obesity-related behaviors.

The first study focused on understanding the association between obesity and physical inactivity. Although it is well known that physical activity benefits overall health, it is not known why people with obesity have reduced physical activity levels. In other words, does obesity cause physical inactivity, or does physical inactivity cause obesity? To begin to address this question, researchers fed male mice either a standard, low-fat diet or a high-fat diet for 18 weeks. As expected, mice on the high-fat diet gained more weight and were less physically active than the animals eating the standard diet. Next, the researchers sought to identify the mechanisms underlying the observed physical inactivity in obese mice and focused on studying dopamine signaling. The chemical dopamine is produced by neurons (nerve cells) and binds to protein "receptors" found on the surface of nerve and other cells to exert its effect of transmitting signals. Experiments showed that obese mice had deficits in the function of dopamine D2-type receptors (D2R) in the brain's basal ganglia, meaning that the animals had less active dopamine signaling. Experimentally restoring signaling in obese mice increased their physical activity, whereas lean mice genetically altered to lack D2R in a subset of nerve cells in the basal ganglia were not as physically active as control animals. When the researchers further studied those genetically altered mice, they uncovered a surprising finding: the animals did not gain more weight on a high-fat diet compared to control mice eating the same diet. That is, even though the experimental mice were less active, they did not gain more weight than control mice with intact dopamine signaling. This result suggests that inactivity is more a consequence than a cause of obesity in mice.

A second study examined hunger and the drive to eat in relation to other competing behaviors in mice. Previous research has primarily studied feeding behavior in isolation. However, in nature, mice must navigate feeding while engaging in other behaviors, such as avoiding predators. The scientists hypothesized that hunger could suppress other behaviors in favor of eating. To test this, they used three different groups of male mice: (1) fed (sated) mice, (2) hungry (fasted) mice, and (3) fed mice with experimentally-activated AgRP neurons found in the arcuate nucleus (ARC) of the brain's hypothalamus (or fed-ARC^{AgRP}-activated mice). It is known that ARC^{AgRP} neurons play an important role in feeding behavior, as experimentally activating them promotes feeding even when mice are not hungry. Confirming prior results, the researchers first demonstrated that activating ARC^{AgRP} neurons in fed mice promoted feeding, and thus their fed ARC^{AgRP}-activated mice represented a model of hunger. The researchers then conducted several behavioral tests using the three different groups of mice to see where hunger/feeding would rank related to engaging in other behaviors. It turns out that, when food is present, hunger wins. For example, the scientists found that when housed in a large open-field apparatus, hungry mice and fed ARC^{AgRP}-activated mice were more likely to spend time in the center—a location that is anxiety-provoking in mice—compared to fed mice when food was placed there. Similarly, when placed in a two-chamber apparatus with a chemical produced by a predator, foxes, in one chamber, the hungry mice and fed ARC^{AgRP}-activated mice were more likely to venture over to the "unsafe" side if that side also had food. By contrast, the fed mice tended to stay on the safe side, which had no food but also lacked the fear-inducing chemical. Other experiments showed that hunger won over other behaviors, such as drinking water and promoting social interactions. The scientists suggest that it is possible that ARC^{AgRP} neurons produce signals to suppress these other behaviors in favor of feeding.

These studies shed important new light on the complex neurological signaling pathways that regulate obesity-related behaviors such as physical inactivity and the drive to eat. Future research could help determine whether the same results are observed in women and men, as well as delve deeper into the underlying molecular mechanisms toward finding safe strategies to modify behavior to prevent or treat obesity.

Friend DM, Devarakonda K, O'Neal TJ,...Kravitz AV. Basal ganglia dysfunction contributes to physical inactivity in obesity. Cell Metab 25: 312-321, doi: 10.1016/j.cmet.2016.12.001, 2017.

Burnett CJ, Li C, Webber E,...Krashes MJ. Hunger-driven motivational state competition. Neuron 92: 187-201, doi: 10.1016/j.neuron.2016.08.032, 2016.

Identification of Brain Cells That Drive Binge Eating and Weight Gain: Researchers have identified a group of brain cells (referred to here as ZI-GABA cells) that, upon activation, induces rapid binge eating and weight gain in mice. Previous studies have shown that humans who receive brain stimulation of the region including these cells, as a form of therapy to treat neurological disorders, can develop compulsive eating habits. However, it was not clear why this treatment would elicit such a response.

To determine the role ZI-GABA cells play in eating behavior and body weight regulation, investigators used a technique that allowed them to use a light source to selectively control brain cells in mice that had been genetically modified to have light-responsive proteins on the surface of these cells. When they activated the ZI-GABA cells in these mice with laser light, the mice rapidly consumed large quantities of food compared to mice without light-activated proteins. Because previous observations have shown that ZI-GABA cells project (extend long fibers) into an area of the brain that may help regulate feeding, they investigated whether this area is a critical target for ZI-GABA cell control of food intake. Upon light stimulation, they found that ZI-GABA cells sent signals to cells in this target area that reduced their activity, subsequently inducing food foraging behavior, and dramatically increasing food intake. To assess whether activation of the ZI-GABA pathway leads to weight gain, they selectively light-stimulated the cells several times per day over the course of two weeks. Repeated activation led to increased feeding and weight gain in mice, both of which were significantly reduced when the light stimulus was removed. Consistent with the notion that mice resume normal feeding behavior and body weight in the absence of ZI-GABA activation, the researchers then selectively deleted ZI-GABA cells from a group of mice and found that these mice reduced their food intake and gained less weight.

Finally, the researchers explored the idea that, because ZI-GABA cells project an inhibitory signal to their target cells, in turn increasing feeding behavior, the target cells have an opposite effect on food intake. When they selectively activated the target cells, bypassing ZI-GABA, mice significantly reduced their food intake. Moreover, when they selectively eliminated the target cells, mice substantially increased feeding and body weight.

Taken together, these data suggest that activation of a robust inhibitory pathway involving ZI-GABA brain cells is capable of evoking rapid binge eating episodes. With further research, knowledge gleaned from these and future results could lead to the development of novel treatment strategies for binge eating disorders in humans.

Zhang X and van den Pol AN. Rapid binge-like eating and body weight gain driven by zona incerta GABA neuron activation. Science 356: 853-859, doi: 10.1126/science.aam7100, 2017.

Explaining the Link Between an Antipsychotic Medication and Excessive Weight Gain: Researchers have identified a receptor (cell-surface protein) in the mouse brain responsible for the metabolic syndrome caused by an antipsychotic medication, as well as a therapy that may prevent this side effect. Olanzapine is part of a drug family called the "atypical antipsychotics" (AATPs). AATPs can be effective treatments for disorders such as schizophrenia, bipolar disorder, and depression. However, AATPs can also cause side effects like food cravings and binge eating that can lead to obesity and type 2 diabetes within months of starting treatment. AATPs have been known to interact with multiple receptors in the brain, including HTR2C, which helps regulate food intake, body weight, and glucose metabolism. Blocking HTR2C signaling in mice was known to cause overeating and obesity, which resembled side effects induced by AATPs. Moreover, many AATPs, including olanzapine, interfere with HTR2C function, which led researchers to suggest that it was olanzapine's block of HTR2C function that was causing the metabolic syndrome.

To test this hypothesis, researchers fed mice the drug to reproduce the olanzapine blood concentrations seen during human olanzapine therapy. This mouse model was then used to investigate how olanzapine causes weight gain. Female mice fed olanzapine ate more and moved less, leading to excessive weight gain and other indications of metabolic syndrome similar to that seen in humans. Overeating was less prominent in male mice fed olanzapine than in female mice, and the male mice gained less weight, though the reason for the difference between the males' and females' responses was unclear. This result, compared with that of other experiments, suggested that, at least in mice, the main contributor to olanzapine-induced weight gain was overeating.

The researchers performed further experiments with genetically modified mice to identify factors responsible for olanzapine-induced metabolic syndrome. Mice who were fed olanzapine but also lacked the HTR2C protein did not overeat, develop altered glucose metabolism or insulin levels, or gain weight, indicating that HTR2C is important for these side effects. The researchers then asked: if olanzapine's metabolic side effects are mediated by its blockage of HTR2C function, then could the side effects be alleviated by activating HTR2C? To test this, they fed mice olanzapine and treated the mice with an FDA-approved weight-loss drug called lorcaserin, which activates HTR2C. Lorcaserin blocked olanzapine's overeating, weight gain, and metabolic syndrome side effects.

Since other existing anti-obesity and anti-diabetic medications have limited effectiveness against AATP-induced metabolic syndrome, this work offers hope that HTR2C activators like lorcaserin may be useful tools to prevent these side effects.

Lord CC, Wyler SC, Wan R,...Elmquist JK. The atypical antipsychotic olanzapine causes weight gain by targeting serotonin receptor 2C. J Clin Invest 127: 3402-3406, doi: 10.1172/JCI93362, 2017.

HOW APPETITE COUNTERS WEIGHT LOSS

Weight Loss Leads to Strong Increase in Appetite—The Body's Internal Feedback Control of Calories: Studying people in a clinical trial of a type 2 diabetes drug that causes weight loss, researchers discovered that as people lost weight, their appetite increased proportionately, leading to increased calorie consumption and a leveling off of weight loss. The findings provide the first measurement in people of how strongly appetite counters weight loss, as part of the body's feedback control system regulating weight.

For this study, the researchers sought further insight into why it is so difficult to maintain a lower body weight after loss of excess weight. To investigate whether the body may have internal, biological controls that compensate for weight loss by boosting appetite, they developed a strategy for measuring real-world calorie consumption over the long term in response to weight loss. They examined body weight data from a year-long clinical trial of canagliflozin, a type 2 diabetes drug that substantially increases the amount of glucose (sugar) that is excreted in the urine. This drug causes a gradual decrease in weight, averaging about eight pounds. In the clinical trial, participants were randomly assigned to either the drug or a placebo, without knowing which they received. The participants were not directly aware of the loss of calories in the excreted glucose, and they were not on a restricted diet or in an exercise program. Thus, because the participants were not making intentional behavior changes, the researchers could study what the body is inherently wired to do.

Analyzing data from 153 men and women who received the drug and 89 who received a placebo, the research team calculated calorie intake using a previously validated mathematical model. They compared the measured body weight changes with the weight changes expected based on the loss of glucose calories, and realized that calorie intake increased. For every pound of lost weight, the people treated with canagliflozin consumed about 50 calories per day more than they had been eating before the trial. The increased appetite and calorie intake led to a slowing of weight loss after about 6 months. These changes were not seen in people who got a placebo.

The researchers then analyzed data from a separate trial of a lifestyle weight-loss program for overweight and obese individuals. Based on the weight-loss and regain data, the researchers calculated the compensatory changes in appetite. Although the participants did not maintain their initial diet through the trial, they still consumed fewer calories than expected based on appetite changes—a sign that they persisted in their efforts despite an increased appetite.

These findings underscore the challenges faced by those seeking obesity treatment. Past research had shown that, after weight loss, the body also slows its rate of burning of calories, yet the change in appetite shown in this study is even stronger. Weight loss strategies thus need to overcome the body's multiple systems designed to regain that weight.

Polidori D, Sanghvi A, Seeley RJ, and Hall KD. How strongly does appetite counter weight loss? Quantification of the feedback control of human energy intake. Obesity 24: 2289-2295, doi: 10.1002/oby.21653, 2016.

HEAT PRODUCTION, BROWN FAT, AND OBESITY

Finding Factors Important to Stoking Brown Fat: An international research team including NIDDK-supported scientists has newly identified a molecular factor key to the activation of energy-burning brown fat, furthering possibilities for targeting brown fat in treatment of obesity.

Unlike white adipose tissue (fat), which stores energy derived from the food we eat and expands in volume when calorie consumption exceeds bodily energy expenditure (calorie-burning), brown fat "burns" energy, releasing it as heat. Because active brown fat has been found in adult humans, its characteristics and development are being studied intensively to determine if it might be or harbor a treatment target for obesity and metabolic diseases. Through experiments in laboratory-grown mouse cell lines, male mice, and human tissue samples, researchers sought in this study to identify key molecular factors, called transcription regulators, controlling activation and maintenance of patterns of gene expression—turning genes "off" or "on"—specific to brown fat. Using a technique that enabled them to assess thousands of active chromosomal regions in cells simultaneously, they detected in mouse brown fat a DNA sequence motif associated with binding by a family of transcription regulators called nuclear factor I. Further experiments revealed that one member of this family, nuclear factor I A (NFIA), was highly enriched in mouse brown fat compared to other tissues, such as white fat and muscle. Brown fat cells share a common progenitor with skeletal muscle cells. Thus, to confirm a role for NFIA in defining brown fat, the scientists introduced NFIA into laboratory-grown mouse muscle precursor cells. These cells developed characteristics of fat cells and their gene expression was modified, with brown fat genes "turned on" and muscle genes "turned off." Conversely, when NFIA was inhibited in laboratory-grown mouse brown fat cells, expression of brown fat-specific genes decreased significantly, indicating that NFIA is needed for both activation and maintenance of genes determining brown fat identity. Examining the underlying mechanism involved, the researchers found evidence in cell-based experiments that NFIA facilitates binding of PPARγ—the master transcriptional regulator governing fat cell development—to regions of the genome associated with brown fat-specific genes. This co-localization resulted in significantly increased expression of the genes examined.

Moving from cells to whole organisms, experiments in mouse models appeared to confirm that NFIA is important to brown fat development and suppresses muscle cell development. The researchers then analyzed samples of human brown fat from volunteers. They found that samples from patients with a tumor causing activation of brown fat surrounding the kidneys showed higher expression of the genes encoding NFIA, as well as other brown fat-specific genes, than similar samples obtained from patients with other tumors. Analyses of brown fat cells and white fat cells cultured from tissue samples from shoulder and belly areas, respectively, of other volunteers, also showed higher NFIA expression in the brown fat cells. Taken together, the study results suggest that NFIA is a key factor in brown fat activation and development in mice and may act similarly in humans. Future studies may reveal ways to manipulate NFIA and its ability to "reprogram" cells to become brown fat as part of therapeutic strategies for obesity.

Hiraike Y, Waki H, Yu J,...Kadowaki T. NFIA co-localizes with PPARγ and transcriptionally controls the brown fat gene program. Nat Cell Biol 19: 1081-1092, doi: 10.1038/ncb3590, 2017.

Insights into How the Body Adjusts Its Thermostat To Regulate the Balance Between Food Intake and Calorie Burning: Researchers identified two factors that contribute to the critical balance of food intake and energy expenditure (calorie burning), in studies in mice. Mammals have precise and linked regulatory mechanisms to maintain body temperature and stabilize body weight. Food is converted to chemical energy, which the body uses, stores as fat, or converts to and dissipates as heat to adapt to various conditions (a process referred to as "adaptive thermogenesis"). The body can adjust its calorie burning, to some extent, based on the amount of food eaten. Decreased food intake leads to reduced energy expenditure, which limits weight loss. Conversely, increased food intake stimulates calorie-burning brown and beige fat cells to increase energy expenditure through the generation of heat (a form of adaptive thermogenesis referred to as "diet-induced thermogenesis"), limiting weight gain. When this regulation is disrupted, excess food intake and/or reduced energy expenditure can eventually

lead to an increase in storage of the excess calories as fat, resulting in obesity. Scientists hope that, by understanding the body's regulatory mechanisms, they can develop strategies to stimulate increased energy expenditure and to help combat the growing prevalence of obesity. Toward that goal, two studies from the same research group provided important insights into these regulatory mechanisms.

Previously the investigators demonstrated that a small molecule known as creatine enhanced energy expenditure. To explore the role of creatine specifically in adipose (fat) tissue, the researchers genetically engineered mice to lack a gene critical to the synthesis of creatine in fat cells and characterized the male mice. When these mice were transferred to a cold environment, the researchers observed that the mice lacking creatine in fat had reduced body temperatures, indicating that creatine plays a role in the production of heat to maintain body temperature. When the mice were fed a diet that can induce obesity, the researchers found that the mice lacking creatine in fat more rapidly developed obesity, compared to mice with creatine, and exhibited mild metabolic dysfunction, such as impaired glucose tolerance.

Importantly, both types of mice were eating the same amount of food and exhibiting similar levels of physical activity, so the scientists measured the resting energy expenditure in the mice to see if that would explain the acceleration of obesity and metabolic dysfunction. They found that, on the obesity-inducing diet, mice with creatine in their fat increased their energy expenditure and metabolic rate concomitantly to balance the extra calories. Mice lacking creatine in their fat, however, did not increase their energy expenditure and metabolic rate to the same extent; their diet-induced thermogenesis was suppressed. To determine whether this was specifically due to the loss of creatine, the scientists fed a creatine-supplemented diet to mice lacking creatine in their fat and observed that these mice showed an increase in metabolic rate, "rescuing" the loss of diet-induced thermogenesis. These results indicated that creatine in fat promotes diet-induced thermogenesis and combats obesity.

Equally important as the processes that promote thermogenesis are the processes that put a "brake" on thermogenesis, protecting animals from wasting energy by counterbalancing thermogenic processes. In a second study, the researchers identified a protein in fat, named KCNK3, that appears to act as this brake. Male and female mice genetically engineered to lack KCNK3 specifically in fat cells had increased body temperatures when placed in a cold environment compared to mice with KCNK3 in fat, indicating increased adaptive thermogenesis. When fed a diet that induces obesity, the genetically engineered mice gained significantly less weight than mice with KCNK3 in their fat cells and demonstrated increased energy expenditure. These mice also showed metabolic benefits, including improved glucose tolerance. Furthermore, the researchers demonstrated how KCNK3 activity leads to these outcomes: it increases the amount of potassium flowing out of a brown or beige fat cell, which limits the amount of calcium that can enter the cell; this process subsequently suppresses thermogenesis.

These two studies provide important details toward understanding the balance of energy intake and expenditure in mammals, and identify two new potential therapeutic targets in brown and beige fat for treatment of obesity and type 2 diabetes. As both these studies were conducted in mice, additional research will be necessary to determine the roles that creatine and KCNK3 play in human energy metabolism, and if creatine supplementation and/or KCNK3 antagonists can promote energy expenditure in humans.

Kazak L, Chouchani ET, Lu GZ,...Spiegelman BM. Genetic depletion of adipocyte creatine metabolism inhibits diet-induced thermogenesis and drives obesity. Cell Metab 26: 660-671, doi: 10.1016/j.cmet.2017.08.009, 2017.

Chen Y, Zeng X, Huang X, Serag S, Woolf CJ, Spiegelman BM. Crosstalk between KCNK3-mediated ion current and adrenergic signaling regulates adipose thermogenesis and obesity. Cell 171: 836-848, doi: 10.1016/j.cell.2017.09.015, 2017.

CLINICAL RESEARCH ON WEIGHT-LOSS INTERVENTIONS

Gastric Bypass Surgery Provides Long-term Health Benefits: In one of the first of its kind, a long-term, observational study of outcomes from gastric bypass, a form of bariatric surgery, has shown durability of weight loss and effective remission and prevention of type 2 diabetes in U.S. adults for more than a decade. More than 800 participants with severe obesity

who sought to undergo "Roux-en-Y" gastric bypass at a bariatric surgical center were studied. Of the participants who sought bariatric surgery, half of them proceeded with the operation, and half initially did not, although some of those individuals had surgery at a later time. The researchers also recruited a group of more than 300 people with severe obesity who were not seeking surgery, as another control group for the study. The study included women and men; a majority of the participants were women. The investigators conducted clinical examinations of participants upon enrollment, and 2, 6, and 12 years later to assess weight and to determine the presence of type 2 diabetes, high blood pressure, and a disorder marked by abnormal amounts of fat in the bloodstream called "dyslipidemia." Remarkably, most of the participants took part in follow-up exams at the 12-year mark. Of these, 388 people had sought and undergone bariatric surgery. Their health outcomes were compared with data from participants who did not have surgery, including 217 individuals who originally sought but did not undergo surgery, and 262 who had not sought surgery.

Previously, the investigators reported that participants who underwent bariatric surgery lost significantly more weight than people who did not at the 2-year mark and at 6 years after surgery. Although the participants who had surgery, on average, gained back some of the weight, at 12 years after the surgery, more than 70 percent of these participants maintained greater than 20 percent of the weight loss, and 40 percent maintained greater than 30 percent weight loss. Additionally, on average, the surgery participants' weight remained stable between the 6- and 12-year follow-up exams. Those who did not have bariatric surgery did not lose weight. Moreover, among participants who had type 2 diabetes at the study's onset, those who had surgery were significantly more likely to experience remission than those who did not have surgery, especially if the diabetes had not progressed to the point of needing medication prior to the surgery. The investigators also observed a reduction–by more than 90 percent–of new-onset type 2 diabetes at 12 years among people who had the surgery.

Finally, when the researchers assessed other obesity-related conditions in the participants, they found a significant reduction in the occurrence of both high blood pressure and dyslipidemia in people who underwent bariatric surgery compared to those who sought out the surgery but did not receive it. Although the overall effects of the surgery were of strong benefit, there were 7 suicide deaths, all in people who had surgery; this finding highlights the need for greater attention to patients' psychological health before and after surgery.

The results from this first U.S.-based, 12-year, observational study of bariatric surgery in adults indicate long-term durability of weight loss after "Roux-en-Y" gastric bypass. Moreover, the weight loss was associated with improvement and prevention of type 2 diabetes and obesity-related cardiovascular conditions.

Adams TD, Davidson LE, Litwin SE,...Hunt SC. Weight and metabolic outcomes 12 years after gastric bypass. N Engl J Med 377: 1143-1155, doi: 10.1056/NEJMoa1700459, 2017.

A Weight-loss Intervention Shows Promise for Low-income Mothers Post-childbirth: A recent study has shown that an Internet-based weight loss intervention produces greater weight loss in low-income women who have recently given birth than a standard care program alone.

Weight retention post-childbirth can increase a woman's risk of developing obesity and associated complications later in life. In addition, maintaining excess weight can compromise future pregnancies, affecting the health of both mother and child. However, few effective interventions exist for new mothers in low-income populations, who are at higher than average risk for weight retention. Researchers enrolled more than 370 new mothers, most of them Hispanic, from a federally funded nutrition assistance program in a 12-month study to assess the effects on weight of adding a primarily Internet-based weight-loss program to the standard care of the nutrition assistance program versus the standard care alone. While women in the standard care group received supplemental foods and newsletters with information about nutrition, exercise, and weight loss, women in the intervention group additionally received interactive and motivational Internet-based weight loss guidance, activity trackers, and dietary assessment tools, as well as multiple weekly text messages providing information and feedback, and monthly face-to-face group meetings with study staff; the entire program was available in English and Spanish. At the end of the trial, the researchers compared the weights of participants

in both groups and found that participants in the intervention group achieved a greater weight loss (7 pounds) than those who received standard care alone (2 pounds), indicating that the addition of the Internet-based program was effective in promoting weight loss in low-income women post-childbirth who were enrolled in the nutrition assistance program. In addition, more women in the intervention group returned to their self-reported pre-pregnancy weight, providing more evidence that the intervention is an effective weight-loss tool.

Even a modest reduction in weight can improve blood glucose (sugar) and lower the risk of developing obesity later in life. This study highlights a practical and successful method of losing weight after giving birth among women at high risk of weight retention, potentially improving their long-term health. Future studies could help determine which components of this multi-faceted intervention were most critical to promoting weight loss, what might be added to enhance weight loss, and whether a primarily Internet-based weight loss intervention such as the one tested is cost effective.

Phelan S, Hagobian T, Brannen A,...Tate DF. Effect of an Internet-based program on weight loss for low-income postpartum women: A randomized clinical trial. JAMA 317: 2381-2391, doi: 10.1001/jama.2017.7119, 2017.

Shining a Light on Obesity Genetics

It takes a lot of detective work to understand what is written in our DNA—and how changes in the genome's DNA sequence affect health. Yet, deciphering the nature of genetic variants can enhance understanding of diseases and disorders and identify molecular targets for developing novel interventions. Three leading scientists, Drs. Ruth Loos, Rudolph Leibel, and Manolis Kellis, highlighted their research on the genetics of obesity at a 2017 seminar on DNA sequence variants in a gene called *FTO* (fat mass and obesity-associated). The research was supported by several NIH Institutes, including the NIDDK, and other sources. The seminar was organized by the NIDDK as part of the NIH Obesity Research Task Force seminar series.

Dr. Loos and others discovered the obesity-associated genetic variants in *FTO*. These variants are common, and they contribute to increased body mass index (a measure of weight relative to height) in people of European, African, and Asian ancestries. The association between *FTO* variants and obesity is also stronger than that of other genetic variants. But, genetics is not always destiny. Dr. Loos and her team found that people with *FTO* variants do not necessarily develop obesity, and physical activity can attenuate the risk. How do these variants increase body weight? They may, in part, affect the *FTO* gene, which encodes a protein. Intriguingly, however, the genetic variants are not in protein-coding regions of *FTO*; rather, they reside in intervening segments of DNA.

Drs. Leibel and Kellis discovered that the variant-containing segments of *FTO* have regulatory effects: they modulate the activity of *FTO* and several other genes, or the extent to which these genes are turned on or off, in different cells. Dr. Leibel and his team found that *FTO* variants modify the activity of *FTO* and an adjacent gene, called *RPGRIP1L*, in brain cells. They also showed that lower *RPGRIP1L* activity in the brain leads to overeating and excess body fat, likely by blunting response to an appetite-reducing hormone. Dr. Kellis and his team focused on a different part of the body, early-stage fat cells, and found that an *FTO* variant boosts the activity of other genes, *IRX3* and *IRX5*, which are further from *FTO* in the genome. As a result, these cells, which could have matured into cells that burn calories ("beige" fat cells), instead turned into the more common type of fat cells, which store extra calories as body fat.

The multiple effects of *FTO* genetic variants—increasing appetite through brain cell pathways, and decreasing calorie burning in fat tissue—may help explain their strong association with obesity. These research findings not only advance our understanding of body weight, but also provide insights for genetic research in general. Shining light on different areas of the genome, and on different cells and tissues, can reveal different clues as to how genetic variants affect biological processes and lead to new ideas for prevention and treatment strategies.