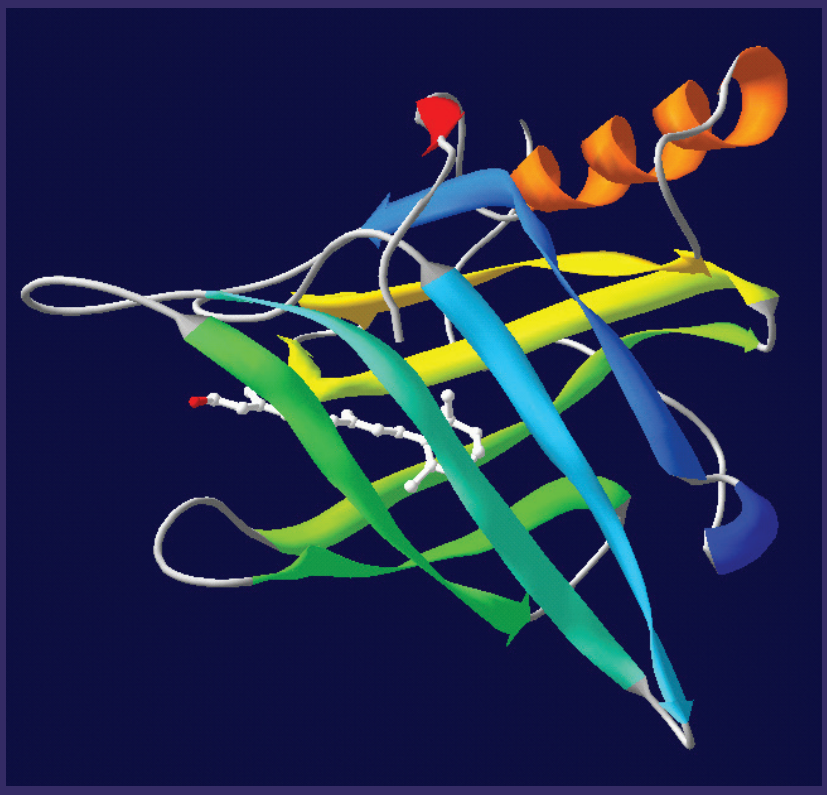

Scientists supported by the NIDDK are elucidating how specific molecules in the body contribute to obesity and its comorbid conditions, such as type 2 diabetes. One molecule of interest is a protein called retinol binding protein 4 (RBP4), produced by fat cells. A retinol binding protein is shown here as a ribbon diagram of the structure, in complex with retinol (vitamin A) shown in white and red in the center. Knowledge of a protein's structure can provide key insights into its function, and in some cases may suggest avenues for medical intervention. Other investigations into the role of RBP4 in obesity and insulin resistance are chronicled in this chapter, as well as in the chapter on "Diabetes, Endocrinology, and Metabolic Diseases."

Image created using DeepView/Swiss-PdbViewer 3.7, using data from Cowan SW, Newcomer ME, and Jones TA. Crystallographic refinement of human serum retinol binding protein at 2Å resolution, Proteins 8: 44-61, 1990.

Obesity

Obesity has risen to epidemic levels in the U.S. Obese individuals suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. A strong risk factor for type 2 diabetes, obesity is also associated with other health conditions within the NIDDK's mission, including, for example, urinary incontinence, gallbladder disease, and the fatty liver disease non-alcoholic steatohepatitis.

Approximately 32 percent of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1,2] Furthermore, while obesity and overweight have risen in the population in general, the greatest increases observed over approximately the past two decades have been in the prevalence of extreme obesity; those who are severely obese are most at risk for serious health problems.[3] Levels of childhood overweight have also escalated in the past several decades; approximately 17 percent of children and teens ages 2 through 19 are now overweight.[2,4] The levels of pediatric overweight have ominous implications for the development of serious diseases both during youth and later in adulthood. Overweight and obesity also disproportionately affect racial and ethnic minority populations, and those of lower socioeconomic status.

The increased prevalence of obesity in the U.S. is thought to result from the interaction of genetic susceptibility with behavior and factors in the environment that promote increased caloric intake and sedentary lifestyles. Thus, the NIDDK has been supporting a multidimensional research portfolio on obesity ranging from basic studies to large clinical trials. This research includes, for example, investigations to elucidate the hormones and signaling pathways that influence appetite and energy expenditure; exploration of genetic factors that predispose individuals to obesity; studies of nutrition; research encompassing physical activity; and studies aimed toward obesity prevention through the development and testing of modifications of environmental factors in schools, the home, and other settings.

The NIDDK additionally supports research on eating disorders that are associated with obesity in some people.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter. To help bring the results of research to the public and health care providers, the NIDDK also sponsors education and information programs. Given the importance of the obesity epidemic as a public health problem, and its relevance to the mission of the NIDDK, the Institute has played a leading role in the NIH Obesity Research Task Force. Established by the NIH Director and co-chaired by the Acting Director of the NIDDK and the Director of the National Heart, Lung, and Blood Institute, the Task Force also includes representatives from numerous other NIH Institutes, Centers, and Offices. With extensive input from external scientists and the public, the Task Force developed the *Strategic Plan for NIH Obesity Research*, published in August 2004 (*http://obesityresearch.nih.gov/About/strategic-plan.htm*). The NIH is currently supporting a spectrum of research studies consistent with the recommendations of the *Strategic Plan.*

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[1] *Statistics Related to Overweight and Obesity. http://win.niddk.nih.gov/statistics/index.htm*

[2] *Ogden et al. 2006. JAMA 295: 1549-1555.*

[3] *Flegal et al. 2002. JAMA 288: 1723-1727; Flegal and Troiano. 2000. Int. J. Obes Relat Metab Disord 24: 807-818; Freedman et al. 2002. JAMA 288: 1758-1761.*

[4] *This document uses the terms overweight and obesity interchangeably for children and adolescents because there is no generally accepted definition for obesity, as distinct from overweight, in this age group.*

PRIMARY PREVENTION OF OBESITY AND KEEPING THE WEIGHT OFF

New Strategy for Fighting Obesity: A seemingly straightforward approach to avoiding excess weight gain is to eat less and exercise more, but this is difficult to accomplish. Recent research in an animal model has suggested a novel approach to helping overweight or obese people achieve that goal, through a strategy used for centuries to prevent infectious disease: vaccination. The researchers vaccinated adult rats against ghrelin, a protein hormone that signals hunger and reduces activity level. Interestingly, the ghrelin-vaccinated rats did not eat less than controls, but they did gain much less weight, suggesting that the vaccine had the effect of accelerating metabolism. It is important to note, however, that even if the effect of ghrelin vaccination is the same in people as in rats, there may be serious long-term side effects associated with vaccinating against a protein produced by the normal human body. Whether or not vaccination against ghrelin proves viable as an obesity treatment, this research is providing valuable insights into the modes of action of this intriguing hormone. It may also suggest that inhibiting ghrelin action (perhaps by developing a drug, as a potential alternative to a vaccine) could help prevent or treat obesity.

Zorrilla EP, Iwasaki S, Moss JA, Chang J, Otsuji J, Inoue K, Meijler MM, and Janda KD: Vaccination against weight gain. Proc Natl Acad Sci USA 103: 13226-13231, 2006.

Self-Regulation Program for Maintenance of Weight Loss: Anyone who has struggled to lose weight knows how disheartening it is to see the weight come back. A recent study has found that a face-to-face intervention incorporating daily self-weighing can help people maintain a desired weight once they have reached it. In a clinical trial, the Study to Prevent Regain (STOP Regain), investigators recruited people who had previously managed to lose at least 10 percent of their body weight, an amount that would have important health benefits. Participants were then assigned to one of three groups to assess ways of maintaining this weight loss over 18 months. One group received a face-to-face intervention; another received a similar intervention delivered through the Internet; and the third group, the control, received a quarterly newsletter. Both interventions emphasized self-regulation of body weight and daily self-weighing. Participants in each of these groups were also given a scale, and were asked to report their weight on a weekly basis. Those who had maintained weight loss ("green zone") were given positive reinforcement. Those who had regained weight ("yellow zone" or "red zone" depending upon amount regained) were encouraged to lose that weight by adjusting their eating and physical activity through the use of problem-solving skills, a tool kit provided in the study, or weight-loss counseling. This approach helped participants to catch gains in weight early and gave them a plan to help lose the weight, before the weight regain escalated. As compared to the control group, both the Internet and the face-to-face interventions reduced the risk of regaining five pounds or more, but the face-to-face intervention was particularly beneficial in decreasing the amount of weight regained. Furthermore, those who weighed themselves daily also had better weight maintenance. This study thus provides encouraging results for weight loss maintenance.

Wing RR, Tate DF, Gorin AA, Raynor HA, and Fava JL: A self-regulation program for maintenance of weight loss. N Engl J Med 355: 1563-1571, 2006.

MOLECULAR CAUSES OF OBESITY

New Insights into Molecular Signals that Influence Hunger: New research indicates that signaling by a protein known as mTOR may regulate food intake. Hunger is the evolutionarily developed message the body uses to influence feeding behavior when it needs fuel. Hunger is controlled by an array of hormones and metabolites through complex mechanisms that are gradually coming into focus through research. The mTOR protein is proving to have a central role in some of the pathways that sense nutrients and respond to metabolic hormones. Researchers found that in rats, in a part of the brain called the hypothalamus, this protein is highly active when the animals are well fed, but much less active

when they are fasting. They also found that the protein's activity is increased by experimental conditions designed to simulate certain changes in nutrient and hormone levels that can result from feeding. Further, they showed that specific inhibition of this protein's activity in this part of the brain induced rats to eat, while its stimulation reduced feeding and led to weight loss, even in rats that had been fasting. This improved understanding of the molecular control of feeding behavior could help scientists design improved approaches to treating or preventing obesity and its many associated health problems.

Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, Woods SC, and Seeley RJ: Hypothalamic mTOR signaling regulates food intake. Science 312: 927-930, 2006.

Molecular Mediators of Eating Behavior: Scientists are elucidating the signaling molecules in the brain that regulate eating behavior. The hope is that a better understanding of the elements that control food intake may lead to new ways to combat obesity, a complex condition arising from a combination of genetic predisposition and environmental and behavioral factors. Recent studies have uncovered some of the key molecules involved in transmitting signals from the appetite-regulating neurotransmitter serotonin. The investigators used an array of mouse models and a number of chemical agents to identify the specific subtype of serotonin receptor that mediates the decrease in food intake observed following exposure to this neurotransmitter. In further studies, the researchers found that this decrease in food intake was not seen in animals in which signaling was disrupted from another molecule—the melanocortin 4 receptor. These experiments identified this molecule as a key downstream mediator of serotonin-regulated feeding behavior. As shown by these experiments, the serotonin-melanocortin 4 receptor signaling pathway is an important component in maintaining energy balance. As serotonin analogs have been widely developed as weight-loss agents, this research may identify targets for novel future approaches to modifying food intake and controlling weight.

Heisler LK, Jobst EE, Sutton GM, Zhou L, Borok E, Thornton-Jones Z, Liu HY, Zigman JM, Balthasar N, Kishi T, Lee CE, Aschkenasi CJ, Zhang CY, Yu J, Boss O, Mountjoy KG, Clifton PG, Lowell BB, Friedman JM, Horvath T, Butler AA, Elmquist JK, and Cowley MA: Serotonin reciprocally regulates melanocortin neurons to modulate food intake. Neuron 51: 239-249, 2006.

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Insulin Resistance in Obesity and Type 2 Diabetes: From Clinical Observation, to Laboratory Mice and DNA, and Back to the Patient's Bedside

Dr. Barbara Kahn

Dr. Barbara Kahn is the George R. Minot Professor of Medicine at Harvard Medical School and Chief of the Endocrinology, Diabetes, and Metabolism Division at the Beth Israel Deaconess Medical Center in Boston. She received her M.D. from Stanford University, and completed an internship, residency, and clinical fellowship in general internal medicine at the University of California, Davis. Additionally, she completed an endocrinology fellowship at the NIDDK in Bethesda, Maryland. Dr. Kahn has received numerous honors and awards, including election to the Institute of Medicine of the National Academies. Among the major research areas pursued by her research group are the molecular underpinnings of obesity, type 2 diabetes, and insulin resistance, a condition that precedes and characterizes type 2 diabetes. Dr. Kahn was invited to present her research at the NIDDK's National Advisory Council meeting in May 2006. This summary is intended to capture highlights of Dr. Kahn's talk, focusing on her exciting research on obesity, diabetes, and the RBP4 protein.

CLUES FROM CLINICAL OBSERVATIONS: THE FAR-REACHING EFFECTS OF FAT TISSUE

Dr. Kahn presented a compelling research story of "translating" biomedical research between the laboratory "bench" and the patient's "bedside." The discoveries began with an intriguing clinical observation in patients with type 2 diabetes that sparked research with animal models and DNA technology, and finally culminated in new insights from studies of patients, with implications for novel approaches to patient care.

The prevalence of type 2 diabetes in both adults and children has risen dramatically in the U.S., as has the prevalence of obesity, a strong risk factor for type 2 diabetes. The consequences of diabetes are severe, including increased risk for heart attack and stroke and reduced life expectancy, among other adverse effects on health. One avenue of scientific research to address these public health problems is the illumination, at the molecular level, of the biological defects that lead to type 2 diabetes.

Normally, Dr. Kahn explained, the body is in a state of "metabolic harmony." The pancreas, liver, muscle, fat tissue, and brain work together to make sure that fuel, in the form of glucose (sugar) or fats, is available when and where it is needed in the body, and kept at healthy levels. The body obtains glucose from food and can also synthesize it in the liver. The hormone insulin, produced by the pancreas, directs fat and muscle cells to take up glucose from the blood and can also signal the liver to dampen glucose production when necessary. In type 2 diabetes, this metabolic harmony is destroyed. The uptake of glucose from blood into the tissues is impaired because the tissues do not respond properly to insulin. This condition is called "insulin resistance." The liver also becomes resistant to insulin signaling in type 2 diabetes. Blood glucose levels thus rise too

high. In people who do not yet have type 2 diabetes, a state of insulin resistance is an ominous sign of high risk for diabetes onset.

Clinical investigators had previously observed that, in type 2 diabetes patients, most of the impairment in insulin-mediated glucose uptake is a result of defects in glucose uptake by muscle. To understand this defect, Dr. Kahn and others examined people and animals with obesity and type 2 diabetes. They assessed levels of a protein, called GLUT4, which transports glucose into cells. Surprisingly, the levels of this glucose transporter in muscle were not changed as a result of obesity or diabetes—but its levels were reduced in fat cells. This finding sparked additional research.

LABORATORY DISCOVERY: OBESITY, TYPE 2 DIABETES, AND THE RBP4 PROTEIN

To further explore how a defect in fat tissue could affect muscle and liver function, Dr. Kahn and the members of her research group developed a mouse model. They genetically engineered mice so they would lack the GLUT4 glucose transporter in their fat tissue, but not in other tissues. As would be expected, without this glucose transporter, the mice had reduced transport of glucose into fat and their fat tissue was insulin resistant. However, the muscle and liver of these mice also became insulin resistant, and the mice were at increased risk for type 2 diabetes. Something, then, must be sent out by fat cells to contribute to insulin resistance in tissues elsewhere in the body.

To discover the nature of this suspected fat-cell-produced factor, the investigators delved deeper into the molecular pathways underlying type 2 diabetes. They employed microarrays, little plates containing thousands of pieces of DNA representing the different genes in a cell. This technology enabled the scientists to examine the regulation of myriad genes of fat cells from mice that were insulin resistant as a result of the glucose transporter alteration. They homed in on a gene that was turned on at an increased level in fat cells from the insulin resistant mice. This gene encodes a protein called RBP4. The scientists then observed that RBP4 levels were also elevated in mice that were obese as a result of certain other genetic mutations, as well as in mice that became obese after eating a high-fat diet. As part of experiments to explore whether high RBP4 levels might be involved in causing insulin resistance, they injected RBP4 protein into normal mice. This subsequently caused insulin resistance.

If high RBP4 levels reflect insulin resistance, then a potential therapeutic approach for obesity or type 2 diabetes would be to try to lower RBP4 levels or block its metabolic activity. To begin exploration of this strategy, Dr. Kahn's research group turned to another aspect of RBP4: its previously-known function as a transporter for vitamin A. RBP4's name, in fact, stands for Retinol (vitamin A) Binding Protein, based on earlier research. It remains unclear how RBP4's retinol binding function might relate to diabetes. However, the knowledge of what RBP4 binds enabled Dr. Kahn and her group to obtain and test an agent that would disrupt RBP4 activity. In searching the scientific literature, they learned about a synthetic, retinol-like molecule called fenretinide that can bind to RBP4 and disrupt its function. The scientists fed mice a high-fat diet and also administered fenretinide to some of the mice. The mice that got only the high-fat chow developed elevated RBP4 levels and insulin resistance, but the mice that additionally received fen-

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retinide did not show a rise in RBP4 levels, and their insulin resistance improved. The beneficial effect on insulin resistance occurred even though the mice still gained excess weight. From these studies in mice, Dr. Kahn thought that disrupting RBP4 function could be a new strategy for preventing or treating type 2 diabetes. First, however, it was important to go back to the clinic, to investigate whether elevated RBP4 levels were associated with insulin resistance in humans.

BACK TO THE CLINIC: ADVANCING RESEARCH FROM THE LABORATORY TO PATIENTS ("BENCH TO BEDSIDE")

Dr. Kahn noted that several years ago, others had reported elevated serum RBP4 levels in people with type 2 diabetes, but the meaning of these observations was unclear. With new-found insights from mice, Dr. Kahn's research group was now poised to explore in depth the association between RBP4 levels and insulin resistance in people. In collaboration with others, her research group found that RBP4 levels, measured in blood samples, were elevated in people who were obese (based on a measure of weight relative to height), and in those who were obese and had type 2 diabetes as well. High RBP4 levels also correlated with the degree of insulin resistance in these individuals. The more obese or insulin resistant their bodies were, the higher their levels of RBP4.

Next, the researchers examined the effects of exercise training in people who had been newly-diagnosed with type 2 diabetes or abnormal glucose levels. Although the exercise affected these individuals differently, those whose body's responsiveness to insulin improved the most from the exercise also had the greatest change in their RBP4 levels. Dr. Kahn's research group then sought to determine whether RBP4 might be useful as a marker of risk for diabetes. To do this, they measured RBP4 levels and insulin resistance in people who were lean and who did not have diabetes, but who were nonetheless at risk for the disease based on family history (a close relative with type 2 diabetes). In these individuals, RBP4 levels reflected the degree of insulin resistance. Additionally, the scientists found that elevated RBP4 levels occurred together with cardiovascular risk factors that are often associated with insulin resistance: high triglyceride levels, high blood pressure, a low level of the "good" form of cholesterol, and abdominal obesity. Finally, reaching back to their earlier studies of the glucose transporter protein GLUT4 in mice, the researchers assessed whether elevated RBP4 levels correlated with changes in GLUT4 levels and insulin resistance in people, and found that this was the case.

IMPLICATIONS FOR FUTURE CARE OF TYPE 2 DIABETES PATIENTS AND THOSE AT RISK

Dr. Kahn's studies have important implications for future medical care. Her research—from the clinic, to the laboratory, and back—not only suggests that a blood test for RBP4 levels may be a convenient way to assess risk for type 2 diabetes and cardiovascular risk factors, but also illuminates a potential new strategy for combating this disease: the development of drugs that could lower RBP4 levels.