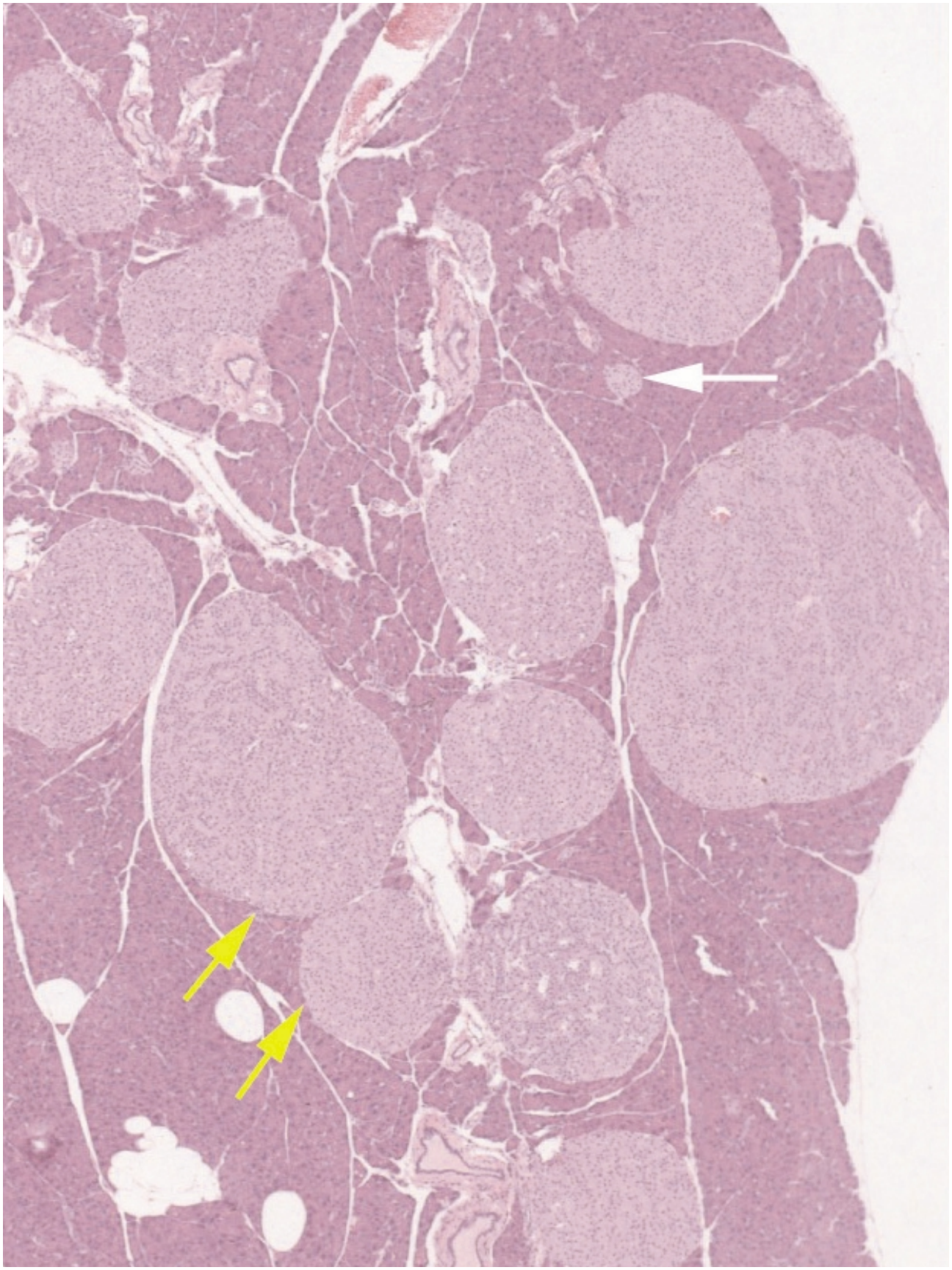

This photograph shows a stained tissue section from a mouse pancreas. Clusters of pancreatic cells called "islets" (pale purple) produce the hormone insulin, which is crucial for proper glucose metabolism. A normal insulin-producing islet is indicated by the white arrow. A mutation in one of the mouse's genes, similar to one found in the human disease multiple endocrine neoplasia, caused uncontrolled growth in a large number of the islets (yellow arrows), resulting in pancreatic islet tumors that over-produce insulin. Just as too little insulin can lead to diabetes, too much insulin can cause dangerous drops in blood sugar levels (hypoglycemia). Photo credit: Dr. Judy S. Crabtree, National Human Genome Research Institute, National Institutes of Health.

Diabetes, Endocrinology and Metabolic Diseases

Diabetes is one of the leading causes of disability and death in the U.S. It affects an estimated 16 million Americans, about one-third of whom do not even know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors play a role. Although there are several interventions currently available to help reduce the burden of this disease, there are no methods to cure it. The disease is marked by deficiencies in the body's ability to produce and properly use insulin—a hormone that is essential for the conversion of food-derived glucose into energy necessary for daily life. As a result, glucose becomes elevated in the blood, with detrimental effects on both small and large blood vessels. The most common forms of the disease are type 1 diabetes, in which insulin-producing capacity is totally destroyed, and type 2 diabetes, in which the body is resistant to insulin, even though some amount of insulin may be produced. Both forms of diabetes can lead to serious and costly complications, including kidney failure, blindness, amputations, heart disease and stroke. According to the American Diabetes Association, diabetes and its complications cost nearly $100 billion annually.

Type 1 diabetes most often occurs in children, but can appear at any age. Formerly known as insulin-dependent or juvenile-onset diabetes, it accounts for 5 to 10 percent of all diabetes in the U.S. It occurs equally among males and females, but is more common in Caucasians than in non-Caucasians. Type 1 diabetes develops when the body's system for fighting infection—the immune system—turns against itself in a disease process termed "autoimmunity." The immune system destroys clusters of cells in the pancreas called islets, which contain the body's insulin-producing beta cells. Once these cells are destroyed, type 1 diabetes patients require either lifelong insulin injections, often multiple times throughout the day, or infusion of insulin via a pump to control their blood glucose levels. Insulin therapy, however, is not a cure, nor can it always prevent the long-term complications of the disease.

Type 2 diabetes is the most common form of the disease. Once known as non-insulin-dependent or adult-onset diabetes, it affects about 90 to 95 percent of people with diabetes. Type 2 diabetes is more common in older people, especially older women who are overweight. Obesity is a major risk factor for this form of diabetes (see advances in obesity research on page 39). It also occurs more frequently among minority groups, including African Americans, Hispanic Americans, Native Americans, and Native Hawaiians. Recently, largely because of an increased incidence of childhood obesity, a disturbing increase of type 2 diabetes has been reported in children, particularly minority children. In patients with type 2 diabetes, cells in muscle, fat and liver tissue do not respond effectively to insulin. Gradually, the pancreas secretes less and less insulin in response to meals, and the timing of insulin secretion becomes abnormal. As clinically recognizable diabetes develops, production of insulin continues to decline. To control glucose levels, treatment approaches include diet, exercise and medications; some patients also need to take insulin. Type 2 diabetes is now approaching epidemic proportions in the U.S. The Centers for Disease Control and Prevention estimates that, if current trends continue, the number of people with diagnosed diabetes in the U.S. is expected to increase by 165 percent by the year 2050. Left untreated, the alarming increase of type 2 diabetes in children could cause these projections to burgeon even further.

Cell Signals and the Development of Type 2 Diabetes: Insights from Animal Models

As demonstrated by several recent advances, mouse models of diabetes have been pivotal in increasing our understanding of the pathways involved in the control of insulin secretion and the maintenance of normal, stable glucose levels in the blood. Such mouse models can help show us why the muscle, liver and fat cells of many individuals, especially those who are overweight or obese, lose the ability to respond to insulin effectively—thereby causing them to develop "insulin resistance." When this happens, the pancreatic beta cells must produce ever-increasing amounts of insulin to main-

tain normal blood sugar levels. For reasons that are poorly understood, the pancreas is unable to keep up with this increased demand in some individuals, and they progress from insulin resistance to the development of full-blown "type 2" diabetes.

Mechanisms of Insulin Resistance: Genetically engineered mice have been used by NIDDK-funded scientists to help unravel the mechanisms underlying the insulin resistance seen in type 2 diabetes, and its association with obesity. The mice were engineered to have mutations that would eliminate, or "knock out," the function of a protein, GLUT4, that transports glucose into cells. Recent technology enabled the scientists to remove GLUT4 only from fat cells, rendering them unresponsive to insulin-induced glucose uptake, but leaving the insulin signaling apparatus intact in other tissues such as muscle and liver. Surprisingly, the lack of GLUT4 in fat cells not only made these cells insulin-resistant, but also made the muscle and liver insulin-resistant. This finding suggests that fat cells normally secrete a factor that travels in the blood to the muscles and liver and that the absence of GLUT4 changes the amount of this factor circulating in the blood. After further experiments, researchers concluded that fat cells must use messengers to "talk" to liver and muscle cells. These results suggest that there may be a number of as yet undiscovered signaling molecules involved in insulin-induced glucose uptake that could prove to be useful drug targets in the treatment of type 2 diabetes and obesity.

Control of Glucose Production: In related research in mice, another team of investigators made a significant discovery about the cell-signaling machinery that controls glucose production by the liver. They found that low blood glucose signals tell liver cells to turn on the production of a known protein, PGC-1, which—in turn—interacts with other molecules to activate a series of genes required to produce glucose.

Normally, the body maintains exquisite control over levels of glucose in the blood. During periods of fasting, the liver manufactures glucose for cells, especially brain cells, to use as an energy source. After a meal, insulin signals the liver to turn down its glucose production. This process maintains blood glucose levels within a very narrow range. When insulin production is abnormally reduced or nearly abolished, as in type 2 or type 1 diabetes, respectively, the liver does not receive the signal to reduce glucose production. Instead, the liver continues to pump glucose into the bloodstream, and high blood glucose levels can lead to devastating complications.

In their studies, the NIDDK-funded researchers found that, whereas PGC-1 is present in the liver cells of normal mice only when glucose production is needed, diabetic mice appear to make PGC-1 continuously. Thus, PGC-1 functions as an "on-off switch" for glucose production by the liver. These results are the first demonstration that PGC-1 is important for glucose production by the liver, and have major implications for the treatment of diabetes. For example, the anti-diabetes drug, metformin, shuts down glucose production in the liver, but exactly how it does this is unknown. Future research based on the findings about PGC-1 could lead to insights into the mechanism of action of metformin and other anti-diabetes drugs, and may also aid in the development of new and more effective drugs for use in the treatment of type 2 diabetes.

Effects of the Appetite-Controlling Hormone Leptin: In yet another study of diabetes in mice, NIDDK scientists, in collaboration with scientists at Kyoto University in Japan, investigated the effects of increasing the levels of leptin, an appetite-controlling hormone. The mice had a severe deficiency of fat cells, which can cause a form of diabetes known as lipoatrophic diabetes. Patients with lipoatrophic diabetes lack body fat, have severe insulin resistance and elevated blood lipid levels, and eat excessively. One explanation for this is that leptin is normally produced by fat cells. When glucose metabolism changes in response to a meal, fat cells release more leptin, which then travels to the appetite control center in the brain and tells it to "stop eating!" Plasma leptin concentrations are markedly reduced both in patients with lipoatrophic diabetes and in rodent models of the disease. Earlier research had indicated that leptin, in addition to regulating appetite, can also act as an anti-diabetic hormone by increasing both glucose metabolism and insulin sensitivity. By genetically engineering mice to produce extra leptin in their livers, or by administering laboratory-made leptin to the animals, the scientists were able to improve the diabetic symptoms of the mice. Based on these findings, leptin may be a potential long-term therapeutic agent for the treatment of lipoatrophic diabetes.

These advances highlight the enormous contributions of mouse genetic models to our understanding of diseases such as diabetes. Discovering or defining the role of different signals in type 2 diabetes through such animal research is pivotal to understanding disease development and progression in humans. Through the creative use of mouse genetic models, researchers will continue to make discoveries about the signaling pathways involved in type 2 diabetes, enabling them to better target future research endeavors to yield improved therapies for this devastating disease.

Abel ED, Peroni O, Kim JK, Kim Y-B, Boss O, Hadro E, Minnemann T, Shulman GI, and Kahn BB. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 409(6821):729-733, 2001.

Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB III, Kaestner KH, Bartolomei MS, Shulman GI, and Birnbaum MJ. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKBß). *Science* 292(5522):1728-1731, 2001.

Ebihara K, Ogawa Y, Masuzaki H, Shintani M, Miyanaga F, Aizawa-Abe M, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Gavrilova O, Reitman ML, and Nakao K. Transgenic overexpression of leptin rescues insulin resistance and diabetes in a mouse model of lipoatrophic diabetes. *Diabetes* 50(6):1440-1448, 2001.

Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman BM, Montminy M. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* 413(6852):179-183, 2001.

Kim JK, Zisman A, Fillmore JJ, Peroni OD, Kotani K, Perret P, Zong H, Dong J, Kahn CR, Kahn BB, and Shulman GI. Glucose toxicity and the development of diabetes in mice with muscle-specific inactivation of GLUT4. *J Clin Invest* 108(1):153-160, 2001.

Tansey JT, Sztalryd C, Gruia-Gray J, Roush DL, Zee JV, Gavrilova O, Reitman ML, Deng C-X, Li C, Kimmel AR, and Londos C. Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc Natl Acad Sci USA* 98(11):6494-6499, 2001.

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, and Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7(8):941-946, 2001.

Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM: Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 413(6852):131-138, 2001.

Stress Hormone May Determine Fat Deposition that Promotes Diabetes

Obesity is the consequence of greater energy intake as food than energy output as metabolism and exercise; the excess energy is stored as fat. However, the best predictor of obesity-associated diseases such as diabetes is not total body fat, but the amount of visceral fat—fat built up from deep within the abdominal area. One therapeutic approach to the complications of obesity could be to control where fat is deposited in the body. Recent research studies have indicated that the stress hormone cortisol may play a key role in determining where fat is deposited. Normally present in low amounts, active cortisol can be regenerated inside cells from an inactive precursor molecule by an enzyme, 11ß hydroxysteroid dehydrogenase type 1 (11ß HSED-1). In obese humans, one site of abnormally high activity of this enzyme is in fat cells. NIDDK-supported researchers recently investigated the significance of this enzyme activity in fat cells using a mouse model. They found that mice genetically engineered to make more 11ß HSED-1 in just their fat cells ate more than normal mice. Moreover, all of their fat cells, but especially their visceral fat cells, became bigger than fat cells in normal mice, resulting in visceral obesity. As the mice grew older, they also developed metabolic complications similar to those observed in obese persons, including insulin resistance and hyperlipidemia (increased lipids in the blood). There is evidence that one drug already in use to treat type 2 diabetes reduces visceral fat by repressing the activity of 11ß HSED-1 in fat cells. Strategies that repress activity of this enzyme, and hence reduce cortisol production, may therefore become an effective treatment for visceral obesity and its complications.

Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS. A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294(5549):2166-2170, 2001.

Preventing Type 2 Diabetes and Its Complications

A person with diabetes and no known cardiovascular problems has the same risk of having a heart attack as a person who does not have diabetes but who has

already had a heart attack. What can individuals do to prevent the development of type 2 diabetes, its strong association with cardiovascular disease, and its many other devastating complications? Recent advances emerging from clinical trials are providing useful answers.

Impressive Clinical Trial Results Tranform Hope into Reality: Results from a major NIDDK clinical trial are providing important knowledge about diabetes prevention strategies. The Diabetes Prevention Program (DPP) demonstrated that individuals with impaired glucose tolerance and at risk of developing type 2 diabetes can prevent disease onset and improve their blood glucose levels through modest improvements in diet and exercise (see "Story of Discovery: The Ominous Link Between Obesity and Type 2 Diabetes"). Approximately 20 million Americans suffer from impaired glucose tolerance (IGT)—a condition in which blood glucose levels are higher than normal but not yet at diabetic levels. Left untreated, IGT often progresses to type 2 diabetes, and it is also associated with an increased risk of cardiovascular disease. Of the over 3,200 participants in the DPP, 45 percent were from minority populations who suffer disproportionately from type 2 diabetes—African Americans, Hispanic Americans, Asian Americans and Pacific Islanders, and American Indians. All participants were overweight, with impaired glucose tolerance, and were randomly assigned to one of the following groups: intensive lifestyle changes, treatment with the medication metformin, or a placebo control.

Lifestyle Interventions Have Enormous Benefits: The goal for the intensive lifestyle intervention group was to reduce weight by 7 percent through a low-fat diet and exercising for at least 150 minutes per week; the other two groups were also given information on diet and exercise, but there was no intensive lifestyle intervention. The study showed that the patients in the intensive lifestyle intervention group reduced their risk of developing type 2 diabetes by 58 percent. The lifestyle intervention was effective for both men and women and in all of the racial/ethnic groups. Lifestyle intervention also worked well in people over age 60, reducing the development of diabetes by 71 percent in this group. Participants randomized to treatment with metformin also reduced their risk of developing type 2 diabetes, but by 31 percent. Metformin was most effective in younger and heavier study participants.

Translating Message of Diabetes Prevention Program: This landmark study clearly demonstrated that, with instruction and encouragement, patients at high-risk for type 2 diabetes could be successful in improving their diet and activity levels and that these relatively modest changes had a major impact in reducing the onset of diabetes. Because of the strikingly positive results of the DPP, the NIDDK ended the study earlier than planned in order to disseminate its important prevention message as rapidly as possible to the public and to health practitioners. To this end, the NIDDK is expanding the National Diabetes Education Program (see sidebar: "Getting the Message Out: The National Diabetes Education Program"), which it supports in collaboration with the Centers for Disease Control and Prevention and 200 participating organizations in the private sector. The NIDDK is also supporting a cost-effectiveness study to determine the most efficient ways to achieve the translation of the DPP prevention message to the public and to health practitioners.

Post-DPP Study: Long-term follow-up of the DPP cohort will be undertaken to see how long the interventions will be effective. In addition, the researchers will further analyze the data to determine whether the interventions reduced cardiovascular disease and atherosclerosis, major causes of death in people with type 2 diabetes. The DPP cohort is the largest population of individuals with impaired glucose tolerance ever to be studied. The DPP was co-sponsored by the National Institute of Child Health and Human Development, the National Institute on Aging, the National Center for Minority Health and Health Disparities, the National Center for Research Resources, the NIH's Office of Research on Women's Health, and the Office of Behavioral and Social Science Research. The Centers for Disease Control and Prevention, the American Diabetes Association, and industry provided additional support.

Cardiovascular Complications: Heart disease is two-to-four times more common in diabetics than in non-diabetic adults. Women with diabetes are particularly at risk for heart disease. A recent analysis of data from the Nurses' Health Study revealed a dramatically increased death rate from heart disease associated with type 2 diabetes in women. As noted previously, diabetes confers nearly the same risk of death from heart disease as a previous heart attack. In other studies, researchers found that exercise can markedly reduce this risk. The American Diabetes

Association, the National Diabetes Education Program, and the National Cholesterol Education Program now recommend aggressive management of cardiovascular risk factors in diabetic patients to control cholesterol and high blood pressure and to address such other risks as smoking and obesity. These new epidemiological findings provide important insights into the prevention of cardiovascular complications of type 2 diabetes.

In a large study of risk factors for type 2 diabetes in women, researchers found that a healthy diet and lifestyle dramatically reduce the risk of type 2 diabetes. The researchers assessed weight, dietary, and lifestyle factors in nearly 85,000 women from the Nurses' Health Study. From these data, the scientists determined how combinations of risk factors were associated with a diagnosis of diabetes during the study, which lasted for 16 years. Excess body fat was the single most important risk factor for type 2 diabetes. Lifestyle factors were also associated with diabetes, including lack of exercise, poor diet, and smoking. While limited alcohol consumption correlated with decreased risk, the investigators cautioned against alcohol overuse. A healthy body weight, diet, and lifestyle also reduced the risk in women with a family history of diabetes. Encouragingly, even in overweight and obese women, a reduced risk of diabetes was achieved with exercise, a healthy diet, and abstinence from smoking.

These data complement the critically important prevention message from the recently-completed Diabetes Prevention Program (DPP) clinical trial. Both studies underscore the benefits that can be achieved from a healthy diet and exercise in preventing or reducing the risk of type 2 diabetes.

Diabetes in Pregnancy: Babies born to mothers with diabetes have an increased risk of becoming diabetic and obese themselves. However, it is not clear whether this is solely due to the genes inherited by the children, or whether the diabetic condition of the mother also plays a role. In a new NIDDK-supported study, researchers looked at families in which one child was born before and another after their mother's diagnosis with diabetes. The children born after their mothers had developed diabetes were more likely to be diabetic and obese themselves. Thus, the diabetic condition of the mother during pregnancy appears to affect a child's risk of diabetes and obesity. Since type 2 diabetes is increasingly occurring in younger women, the results of this study are particularly important, suggesting that prevention of diabetes in women of child-bearing age improves not only their health, but also the health of their offspring.

In addition to NIDDK's research efforts focused on women with type 2 diabetes, the NIDDK is also pursuing prevention and treatment of type 2 diabetes in children and adolescents. Recent epidemiologic data reveal an increasing number of cases of type 2 diabetes in the pediatric population, especially among adolescents and in certain minority populations. To address this alarming finding, the NIDDK is currently seeking research partners for the development of two separate clinical trials for the prevention and treatment of type 2 diabetes in children.

Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, Roumain J, Bennett PH, and Knowler WC. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity. *Diabetes* 49(12):2208-2211, 2000.

Hu FB, Stampfer MJ, Solomon C, Liu S, Colditz GA, Speizer FE, Willett WC, and Manson JE. Physical activity and risk for cardiovascular events in diabetic women. *Ann Int Med* 134(2):96-105, 2001.

Hu FB, Stampfer MJ, Solomon CG, Liu S, Willett WC, Speizer FE, Nathan DM, and Manson JE. The impact of diabetes mellitus on mortality from all causes and coronary heart disease in women. *Arch Int Med* 161(15):1717-1723, 2001.

Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* 345(11):790-797, 2001.

Improved Long-Term Survival for Patients with Type 1 Diabetes

Type 1 diabetes affects nearly every organ system in the body causing serious complications and significantly reducing life expectancy. Indeed, before the introduction of insulin as a treatment in the 1920s, the onset of type 1 diabetes—usually in childhood—meant almost certain death. A later study of Americans who were diagnosed with type 1 diabetes between 1950 and 1981 found that these patients had mortality rates that were up to seven times higher than the general population. Since that time, great strides have been made in the medical care of diabetes such as the introduction of self-monitoring of blood glucose, the measurement of hemoglobin A1c, and better blood pressure management. However, little information has been available on trends in mortality from diabetes for patients diagnosed in more recent years.

Recently, researchers have examined the mortality rate within the Allegheny County (Pennsylvania) Registry of patients who were diagnosed with type 1 diabetes before their 18th birthdays between 1965 and 1979. Patients in this registry have been living with type 1 diabetes for an average of more than 25 years. By dividing the patients into three groups based on when they were diagnosed—those diagnosed between 1965 and 1969, between 1970 and 1974, and between 1975 and 1979—the researchers found clear evidence that survival rates for these type 1 diabetes patients had improved over time. For example, death rates between 10 and 20 years after diagnosis declined from 8.4 percent in the earliest group of patients to 3.5 percent in the latest. Importantly, both male and female patients showed equal improvements in survival. African Americans and Caucasians also both demonstrated improved outcomes, though mortality rates remained significantly higher in African American patients.

The encouraging results from this NIDDK-supported study suggest that research has led to improvements in medical care for type 1 diabetes, which have had a measurable impact on the survival of patients diagnosed in recent years. Continued follow-up of these type 1 diabetes patients will be necessary to document whether survival trends continue to improve over time. It will also identify reversible causes of mortality related to type 1 diabetes, suggest strategies to further increase survival, and help to find and address the causes of racial disparities in survival of patients with this disease.

Nishimura R, LaPorte RE, Dorman JS, Tajima N, Becker D, and Orchard TJ. Mortality trends in type 1 diabetes. *Diabetes Care* 24(5):823-828, 2001.

Beta Cells That Resist Destruction by the Immune System—Implications for Type 1 Diabetes

Great strides are being made in the search for techniques to help the insulin-producing pancreatic beta cells ward off attack and destruction by the body's immune system, and thus prevent the onset of type 1 diabetes in susceptible individuals. Beta cells appear to be particularly vulnerable to such autoimmune attack, as their ability to tolerate damage seems to be lower than that of other cell types in the body.

To gain insight into the role of the immune system in the onset of type 1 diabetes, NIDDK-supported researchers studied two genetically related strains of mice: the non-obese diabetic (NOD) mouse and the ALR/Lt mouse. Though these mouse strains share a common origin, NOD mice are highly susceptible to type 1 diabetes, while ALR/Lt mice are resistant to it. Thus, studies that compare these two mouse models may provide clues about the genetic signals that contribute to susceptibility to type 1 diabetes.

To better understand the reasons for ALR/Lt resistance to type 1 diabetes, the research team removed pancreatic islets, clusters of cells in the pancreas that include beta cells, from ALR/Lt mice. They then exposed these cells to chemicals that are known to promote inflammation and destroy the insulin-producing capacity of beta cells in NOD mice. Interestingly, ALR/Lt beta cells, unlike those from NOD mice, were able to maintain their ability to release insulin in the presence of these chemicals. Furthermore, ALR/Lt beta cells resisted destruction by the same type of T cells that kill NOD beta cells. To see if ALR/Lt beta cells could avoid destruction by the immune system in an animal, the researchers next exposed NOD and ALR/Lt mice to radiation that destroyed the animals' bone marrow, the part of the body that produces the cells of the immune system. Both sets of animals were then given replacement bone marrow from other NOD mice that had not been treated with radiation. Within 18 weeks after treatment with new bone marrow, all of the irradiated NOD mice had become diabetic. In contrast, no ALR/Lt mouse developed diabetes during this time, indicating that their beta cells were not destroyed by immune cells from the transplanted NOD bone marrow.

These experiments show that, far from being passive victims of the immune system, beta cells, such as those in ALR/Lt mice, can effectively fight off destruction by both chemical and biological agents. Importantly, when ALR/Lt mice are mated with NOD mice, their offspring are also resistant to type 1 diabetes. This observation strongly suggests that resistance of ALR/Lt mice to diabetes is a dominant genetic characteristic. Though the gene (or genes) that confers resistance is not yet known, identifying it could have important clinical implications for preventing type 1 diabetes in at-risk individuals.

Mathews CE, Graser RT, Savinov A, Serreze DV, and Leiter, EH. Unusual resistance of ALR/Lt mouse ß cells to autoimmune destruction: Role for ß cell-expressed resistance determinants. *Proc Natl Acad Sci USA* 98(1):235-240, 2001.

Type 1 Diabetes TrialNet

Knowledge of immune factors that contribute to the development of type 1 diabetes is now being used to identify individuals at risk for the disease and to design clinical trials aimed at preventing or delaying disease onset. A major new research program sponsored by the NIDDK, the Type 1 Diabetes TrialNet, has been initiated to develop and test strategies for prevention of type 1 diabetes. TrialNet will fund a consortium of clinical centers and core support facilities that will perform intervention studies with the purpose of preserving beta cell function from autoimmune attack. TrialNet will facilitate rapid, preliminary testing of emerging therapeutic strategies such as new ideas for immunoprevention. Those that prove most promising can then be moved quickly into larger-scale trials. TrialNet researchers will also complete the current oral insulin study of the Diabetes Prevention Trial for Type 1 Diabetes (DPT-1). The goal of the DPT-1 trial is to determine whether the use of oral insulin by non-diabetic relatives of individuals with type 1 diabetes can delay disease onset.

TrialNet will also be a valuable tool for identifying patients who may be able to help researchers find genes that predispose people to developing type 1 diabetes and diabetic complications. To further leverage the resources supported by TrialNet, biological samples and other data from participating patients may be placed in genetic repositories for use by many investigators. Thus, the NIDDK's TrialNet initiative represents a significant and promising investment in the ongoing search for methods to prevent and cure type 1 diabetes.

Prevention strategies tested in the TrialNet infrastructure will be particularly valuable to those at risk for type 1 diabetes. In this insidious disease, most symptoms do not begin until almost all of the pancreatic beta cells have been destroyed by immune system attack. Thus, by the time a person is diagnosed, damage to his or her beta cells is nearly complete. Finding ways to prevent or delay the onset of type 1 diabetes by interfering with this immune attack would be an enormous clinical achievement that would permit many people to avoid or reduce the severe health burden imposed by this disease and its complications.

Translating Diabetes Information into Intervention

In order to bring important research findings about diabetes prevention and control from "the bench to the bedside," effective strategies for translating these advances into clinical practice need to be developed continually. To this end, the NIDDK already supports a number of Diabetes Research and Training Centers (see sidebar on page 26), and has recently issued a research solicitation to promote diabetes prevention and control efforts. Through the support of both clinical and behavioral studies, this translational research program is expected to develop and test strategies for achieving objectives that have already been proven beneficial, such as control of glycemia and other risk factors for diabetic complications, and for enhancing behaviors that are expected to improve health outcomes for individuals with either type 1 or type 2 diabetes. This program will be especially supportive of interventions that focus on translating new advances into practice in under-served and minority populations.

The NIDDK has also established a program to develop diabetes-focused science education in American Indian tribal middle and high schools. The NIDDK plans to support faculty at Tribal Colleges and Universities and tribal community middle and high schools in the creation of an education program that will both increase awareness of diabetes and its risk factors and also highlight the role of science in the attainment of health and a healthy lifestyle. Through this initiative, the NIDDK hopes to increase the interest and competitiveness of American Indian students in pursuit of biomedical careers by exposing them to biomedical science through the prism of diabetes.

Inborn Errors of Metabolism

Just as insufficient amounts of an important signaling molecule, insulin, can lead to diabetes, deficiencies in metabolic enzymes can cause a number of devastating

disorders. Important research progress has been made in two inherited metabolic diseases in which inadequate levels of a needed enzyme lead to the storage of excessive amounts of normal biologic substances in cells and organs, resulting in toxicity. Through this work, the NIDDK is moving toward its long-standing goal of identifying the functional changes in the bodies of patients suffering from genetic metabolic diseases in order to develop and test possible treatments. By studying both the normal and abnormal proteins made from genes, scientists are determining their function in healthy individuals and trying to understand how faulty proteins cause disease.

The first inherited metabolic disease in which an advance has been made is Fabry disease. This is a rare disorder caused by insufficient amounts of an enzyme critical to the breakdown of fat in cells. Without sufficient enzyme, fat builds up and damages organs such as heart, kidneys, and brain. Eventually, untreated patients develop kidney disease, heart disease, and the potential for having a stroke. This year, investigators tested enzyme replacement therapy as a means for decreasing the levels of built-up fat. Two clinical trials demonstrated that patients treated with enzyme replacement therapy were able to reduce the amount of fat deposited in their heart, kidneys, and skin. The treatment also improved the patients' quality of life, and patients enrolled in the study opted to continue therapy after the study's conclusion.

Also within this past year, NIDDK-supported researchers identified two genes responsible for inheritance of a second, rare inherited metabolic disease known as Niemann-Pick Type C Disease. Cells of patients suffering from this disorder have faulty cholesterol transport, resulting in accumulation of cholesterol in the brain, liver, spleen, lungs, and bone marrow. The disease is characterized by an enlarged spleen and liver, poor muscle control, impaired eye movements, slurred speech, and dementia, and is always fatal, usually by age 15. The newly-identified genes each produce proteins critical to different aspects of fat transport. In people with Niemann-Pick disease, abnormal proteins are produced. Researchers found that adding normal protein to cultured cells taken from Niemann-Pick Type C patients restores normal cholesterol transport. Scientists are now challenged with developing a therapy to use this knowledge to treat victims of the disease.

Frustratingly, even when the causative genes and faulty protein functions are known, doctors are still unable to treat some genetic metabolic disorders effectively. In FY 2002, the NIDDK will sponsor a workshop entitled "Innovative Approaches to Therapy" to attempt to identify new treatment methods for inherited disorders considered "untreatable." Participants at this workshop will also discuss so-called genetic modifiers, or genes other than the faulty gene whose presence or absence can influence the severity of an inherited metabolic disease.

Davies JP, Chen FW, and Ioannou YA. Transmembrane molecular pump activity of Niemann-Pick C1 protein. *Science* 290(5500):2295-2298, 2000.

Eng CM, Banikazemi M, Gordon RE, Goldman M, Phelps R, Kim L, Gass A, Winston J, Dikman S, Fallon JT, Brodie S, Stacy CB, Mehta D, Parsons R, Norton K, O'Callaghan M, and Desnick RJ. A phase 1/2 clinical trial of enzyme replacement in Fabry Disease: Pharmacokinetic, substrate clearance, and safety studies. *Am J Hum Genet* 68(3):711-722, 2001.

Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, Caplan L, Linthorst GE, and Desnick RJ, for the International Collaborative Fabry Disease Study Group. Safety and efficacy of recombinant human α-galactosidase A replacement therapy in Fabry's disease. *N Eng J Med* 345(1):9-16, 2001.

Ioannou YA, Zeidner KM, Gordon RE and Desnick RJ. Fabry Disease: Preclinical Studies demonstrate the effectiveness of α-galactosidase A replacement in enzyme-deficient mice. *Am J Hum Genet* 68(1):14-25, 2001.

Naureckiene S, Sleat DE, Lackland H, Fensom A, Vanier MT, Wattiaux R, Jadot M, and Lobel P. Identification of HE1 as the second gene of Niemann-Pick C disease. *Science* 290(5500):2298-2301, 2000.

Hormonal Regulation of Bone Loss

Hormones are major regulators of bone mass, and changes in hormone levels may lead to bone diseases such as osteoporosis. New developments in understanding hormonal mechanisms underlying the regulation of bone formation, on the one hand, and bone loss, on the other, make it possible to develop new therapeutic agents to properly regulate bone turnover and to potentially rebuild bone.

Bone is made up of about one third collagen and two-thirds mineral, the latter as crystals of calcium phosphate which harden the bone. About 99 percent of the body's calcium is in bone. But bone is actually a dynamic tissue, laced with the cells responsible for keeping a balance between bone formation and breakdown (resorption). When bone is formed or lost, blood calcium levels

change, signaling the parathyroid gland in the neck to restore calcium balance by secreting a hormone called parathyroid hormone, or PTH. Somewhat paradoxically, PTH can promote either the formation or loss of bone, depending upon the need; PTH also increases calcium absorption from the intestine (indirectly), and decreases the amount of calcium lost from the body in the urine. NIDDK-supported studies, highlighted in the "NIDDK Recent Advances and Emerging Opportunities (February 2001)," have elucidated how PTH is able to stimulate either bone formation or loss, suggesting the possibility for designing therapies to either suppress bone loss or increase bone formation. This was recently tested in two NIDDK-supported small-scale clinical trials, which demonstrated that using synthetic PTH for the treatment of osteoporosis can have a beneficial effect on bone mass.

In patients suffering from primary hyperparathyroidism, PTH levels are increased inappropriately, thus affecting bone loss and buildup. Increased PTH levels also result in excessive levels of blood calcium and can lead to kidney stones and other side effects. Primary hyperparathyroidism is diagnosed by a routine blood test that includes measurement of calcium levels, usually initiated because of the onset of symptoms ranging from weakness and fatigue, depression, or aches and pains, to loss of appetite, nausea, vomiting, constipation, confusion or impaired thinking and memory, and increased thirst and urination. When disease is confirmed by measurement of PTH levels, primary hyperparathyroidism is usually treated by surgically removing the parathyroid glands.

However, there are a large number of cases of so-called "asymptomatic primary hyperparathyroidism," in which individuals are still at risk for developing some of the negative side effects of primary hyperparathyroidism, including thinning of the bones, but often have not been diagnosed because of a lack of overt symptoms. Routine blood testing for calcium levels now identifies these patients, but what is the best treatment for mild disease? The long-term side effects of elevated PTH (and hence calcium) need to be evaluated against the long-term costs of surgery. In 1990, the NIDDK sponsored an NIH Consensus Development Conference, which deliberated how best to treat patients with asymptomatic hyperparathyroidism. The NIDDK has supported several important clinical studies addressing research questions identified at this meeting. In light of new information coming out of these studies, the NIDDK has scheduled a "Workshop on Asymptomatic Primary Hyperparathyroidism: A Perspective for the 21st Century," for April 2002, to generate an agenda for future research.

Highly Active Antiretroviral Therapy for HIV Infection—Benefits and Drawbacks

In recent years, the advent of highly active anti-retroviral therapy (HAART) has dramatically improved the survival of patients infected with the human immunodeficiency virus (HIV). HAART has been very effective in decreasing viral load, reversing the wasting syndrome, and prolonging survival in adults with HIV infection. Despite the clear benefits of the new anti-retroviral therapies, HAART has not been an unqualified success. This drug regimen, which often includes a drug known as a protease inhibitor, has been associated in many individuals with a variety of metabolic complications—including elevated levels of circulating fats and cholesterol in the blood, resistance to the actions of the hormone insulin, the development of osteoporosis and bone loss, and the abnormal distribution of body fat, or "lipodystrophy."

Lipodystrophy is a condition characterized by increased deposition of fat in the abdomen and trunk, and/or loss of fat in the face and extremities, and it appears to occur commonly in patients on HAART. These metabolic abnormalities represent major risk factors for the development of other serious diseases, such as diabetes and cardiovascular disease. Unfortunately, distress over these often disfiguring changes has caused some patients to stop taking anti-viral medications. The HAART therapy has also been increasingly associated with elevated levels of insulin in the blood and impaired glucose tolerance, ominous warning signs of possible impending diabetes.

Although HAART has allowed many HIV-positive people to prolong their lives, one of its unexpected and negative side effects is exposure to an elevated risk of diabetes and heart disease. The NIDDK is investigating the changes that HAART can cause in metabolism and is supporting studies of drug action. Current research efforts include studies designed to determine more precisely the health risks of these metabolic changes, their molecular basis, and to more fully understand the

negative role HAART can sometimes play in the treatment of HIV infection. These efforts may help to eliminate the negative side effects of HAART, which is a valuable therapy for many people with HIV infection, and, as another NIDDK study showed, can be quite beneficial to children who are HIV positive.

Increased Risk of Cardiovascular Disease in HIV-Positive Patients with Fat Redistribution Associated with Antiretroviral Therapy: In normal individuals who are not infected with HIV, elevated serum fat and cholesterol levels in the presence of insulin resistance or diabetes confer risk for the development of atherosclerotic heart disease. Because of this correlation, HAART-associated side effects are a serious potential public health concern. However, the cardiovascular risk in HIV-infected patients with this metabolic syndrome is unknown. One way to assess a person's risk of developing serious cardiovascular disease, including heart attacks and strokes, is to measure the levels of certain enzymes that circulate in the blood. Elevated levels of two particular proteins, plasminogen activator inhibitor-1 (PAI-1) and tissue-type plasminogen activator (tPA), are known to indicate elevated risk of future cardiovascular disease. NIDDK-supported scientists examined these proteins in a group of people who were HIV-positive and undergoing HAART in order to determine their relative risk of developing cardiovascular disease.

Scientists studied 86 HAART-treated HIV-positive patients who had experienced recent changes in body fat distribution. Thirty-three of these patients were found to have impaired glucose tolerance or elevated insulin levels in their blood, two risk factors for the development of type 2 diabetes. These patients also had elevated levels of PAI-1 and tPA, suggesting that they were at elevated risk for cardiovascular disease. A subset of these individuals was then enrolled in a study designed to determine whether the drug metformin, which is currently used in the treatment of type 2 diabetes, could be beneficial to HIV-infected patients with metabolic complications of HAART. After three months, patients who received metformin demonstrated significant reductions in the levels of PAI-1 and tPA as well as a return of their circulating insulin to a lower level. This result suggests that metformin may lower the cardiovascular disease risk in HIV-infected patients who develop metabolic complications of HAART.

Effect of the Protease Inhibitor Indinavir on Import of Glucose into Muscle: How might the metabolic effects of HAART be mediated? Scientists are studying this question in two ways. The body largely regulates circulating levels of glucose through the controlled uptake and release of the sugar in skeletal muscle and liver tissue. As described in preceding sections, the hormone insulin signals tissues to take up glucose from the blood after a meal to either use it or store it; later, when blood glucose levels have fallen, other hormones signal these tissues to release glucose into the blood. If HAART were somehow upsetting this balance, it would be a clue as to why some HIV-positive individuals on HAART develop metabolic complications, resistance to insulin, and, in some cases, type 2 diabetes.

In order to determine the impact, if any, of HAART on the import of glucose into skeletal muscle, NIDDK-supported scientists studied whole muscles isolated from rats. By incubating the muscles in a solution containing radioactive glucose, the researchers were able to monitor the rate at which the sugar was imported into the muscle by measuring the radioactivity incorporated into the tissues. The addition of insulin dramatically increased the amount of glucose imported into the muscle, a result in agreement with what happens in the body. When the muscles were incubated in a solution containing the protease inhibitor indinavir, a common component of many HAART regimens, the import of glucose following the addition of insulin decreased by 40 to 58 percent. Did the drug somehow interfere with the ability of insulin to signal the cell? When the scientists examined, at a molecular level, how the protease inhibitor diminished glucose uptake by the muscle, they found that insulin signaling was normal. However, when the cell attempts to import the sugar, it cannot; therefore, indinavir inhibits the transport of the glucose molecules across the outer cellular membranes but does not interfere with the ability of insulin to signal the cell.

Further evidence for a role of the protease inhibitor indinavir in glucose metabolism comes from a study of ten HIV-negative men who were given the drug for four weeks to determine its effect on glucose metabolism in healthy individuals. Indinavir therapy resulted in significant increases in fasting blood glucose levels, higher insulin levels, higher insulin-to-glucose ratios, and increased insulin resistance.

The cause of the HAART-associated metabolic syndrome is unknown. These two studies indicate that,

even in healthy individuals, the protease inhibitor indinavir can significantly impair glucose metabolism, suggesting that protease inhibitors may contribute to the metabolic changes seen in patients receiving HAART. This seems to be a result—at least in part—of impaired import of glucose in skeletal muscle. These studies offer important insights into the understanding of the physiological causes of HAART-associated metabolic complications and may lead to the refinement of HIV therapeutic approaches.

Protease Inhibitors Impair Fat Cell Development: Fat cells develop from an immature precursor cell that, under certain conditions, differentiates into a mature fat cell known as an "adipocyte." Using the appropriate signals, it is possible to coax pre-adipocytes to mature into adipocytes in culture, and such controlled differentiation is a powerful technique for studying the steps in the cellular differentiation process. Scientists have used this approach to study how protease inhibitors might influence the development of fat cells. Researchers found that, in the presence of the protease inhibitor nelfinavir, immature pre-adipocytes failed to differentiate. Moreover, when the drug was added to a culture of already-mature fat cells, they died. The results clearly demonstrate that, while nelfinavir is not toxic to pre-fat cells, it seems to diminish the number of mature fat cells through two mechanisms: by inhibiting the differentiation of pre-adipotyces and by promoting the death of mature adipocytes. Understanding the molecular basis of this and other metabolic changes associated with HAART may lead to the development of safer, more effective anti-HIV therapies that do not have the unwanted side effects on adipocytes that may play a role in the development of lipodystrophy.

Benefits of Antiretroviral Therapy in HIV-positive Children: Although HAART is not without negative side effects, this therapy has been responsible for some remarkable successes. Both survival time and quality of life have improved dramatically in HIV-positive children with the introduction of HAART. The growth patterns in HIV-positive children prior to the introduction of HAART indicate that they have similar birth weights compared with non-infected children but that they fall behind in both weight and height within the first months of life. Scientists have therefore been interested in determining whether HAART, and particularly the protease inhibitor component of HAART, could have a positive impact on these and other growth parameters in HIV-infected children.

Researchers monitored the growth of a group of HIV-positive children who were treated with drug therapy that included at least one HIV-1 protease inhibitor. After two and one-half years, treatment with protease inhibitors resulted in significant increases in weight, weight-for-height, and arm muscle circumference compared to status prior to therapy. A smaller effect was seen on height alone. Protease inhibitor therapy also reduced levels of HIV in the children's blood by nearly 80 percent. The use of protease inhibitors in the treatment of children with HIV infection therefore has a beneficial effect, resulting in improvement of several growth parameters. Whether the metabolic complications sometimes seen in adult HIV-positive patients receiving HAART with protease inhibitors might also appear in children will be monitored closely and will also be a topic of future studies.

NIDDK and NIH Research Efforts: Despite the clear benefits of the new anti-retroviral therapies, the metabolic abnormalities induced by the HAART regimen represent major risk factors for the development of other serious diseases, such as diabetes and cardiovascular disease, as well as bone fractures. Initial attention was focused on the protease inhibitors as the possible cause of these metabolic complications; however, the protease inhibitors are frequently used in combination with several other medications, making it difficult to pinpoint the "offending" agent. In addition, metabolic complications have emerged in patients who are not being treated with protease inhibitors.

Several large epidemiologic studies are currently ongoing with an eye towards producing a better description of the metabolic changes associated with HAART and understanding whether particular drugs, or classes of drugs, are the causative agents of these changes. In addition, a large research effort is aimed at understanding the molecular mechanisms by which anti-retroviral drugs might lead to these metabolic abnormalities. A long-term goal of this research is the development of new, highly active anti-HIV drugs that lack these adverse metabolic consequences.

In the meantime, it is essential to develop strategies to improve lipid levels and insulin sensitivity, to restore normal body fat distribution, and to minimize bone loss in patients treated with HAART therapy, in order to enhance patient compliance and to decrease the risk for

future disease. To foster research in this area, the NIDDK and the National Heart, Lung and Blood Institute (NHLBI) have announced a research program that seeks to develop and test strategies for treating the metabolic complications associated with anti-retroviral drug therapy in patients with HIV infection. The expectation is that clinical studies initiated under this program will both test the effectiveness of agents currently approved for the treatment of dyslipidemia, insulin resistance or diabetes, and osteoporosis, and also develop and test novel treatment approaches to the HAART-associated metabolic changes.

Dowell P, Flexner C, Kwiterovich PO, and Lane MD. Suppression of preadipocyte differentiation and promotion of adipocyte death by HIV protease inhibitors. *J Biol Chem* 275(52):41325-41332, 2000.

Hadigan C, Meigs JB, Rabe J, D'Agostino RB, Wilson PWF, Lipinska I, Tofler GH, Grinspoon S. Increased PAI-1 and tPA antigen levels are reduced with metformin therapy in HIV-infected patients with fat redistribution and insulin resistance. *J Clin Endocrinol Metab* 86(2):939-943, 2001.

Miller TL, Mawn BE, Orav EJ, Wilk D, Weinberg GA, Nicchitta J, Furuta L, Cutroni R, McIntosh K, Burchett SK, and Gorbach SL. The effect of protease inhibitor therapy on growth and body composition in human immunodeficiency virus type 1-infected children. *Pediatrics* 107(5):E77, 2001.

Nolte LA, Yarasheski KE, Kawanaka K, Fisher J, Le N, and Holloszy JO. The HIV protease inhibitor indinavir decreases insulin- and contraction-stimulated glucose transport in skeletal muscle. *Diabetes* 50(6):1397-1401, 2001.

Noor MA, Lo JC, Mulligan K, Schwarz J-M, Halvorsen RA, Schambelan M, and Grunfeld C. Metabolic effects of indinavir in healthy HIV-seronegative men. *AIDS* 15(7):F11-18, 2001.