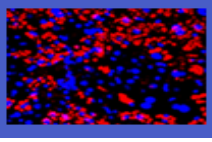

Science Advances from NIDDK

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Intensive Blood Glucose Control Reduces Kidney Disease

New results show that controlling blood glucose early in the course of type 1 diabetes yields huge dividends, preserving kidney function for decades. The landmark Diabetes Control and Complications Trial (DCCT) began in 1983, but because it can take years for early signs of diabetes complications to develop, it was not until 1993 that sufficient time had passed for the trial to prove that intensive blood glucose control reduced early signs of kidney dysfunction and other complications in people with type 1 diabetes. However, because more serious impairment of kidney function or kidney disease can take even longer to develop, researchers could not determine the effect of intensive therapy on the development of kidney disease at that time. DCCT participants were invited to join the DCCT follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, and today, nearly 3 decades after the start of the DCCT, about 95 percent of DCCT participants continue to be followed to determine the long-term effects of the therapies beyond the initial treatment period. Now, after an average 22-year follow-up, EDIC researchers reported that controlling blood glucose can prevent loss of kidney function and is likely to reduce kidney failure. Compared to conventional therapy, near-normal control of blood glucose—beginning soon after diagnosis of type 1 diabetes and continuing an average 6.5 years—reduced the long-term risk of developing kidney disease by 50 percent. This finding—along with previous DCCT/EDIC research demonstrating the benefit of intensive blood glucose control in reducing the risk of eye, nerve, and cardiovascular complications—reinforces the importance of early, intensive blood glucose control in people with type 1 diabetes. DCCT and EDIC illustrate the value of long-term studies, have revolutionized disease management, and led to greatly improved outcomes for people with type 1 diabetes.

De Boer IH, Sun W, Cleary PA, Lachin JM, Molitch ME, Steffes MW, and Zinman B. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. N Engl J Med 365: 2366-2376.

Newly Identified Muscle Hormone May Have Potential for Reducing Obesity and Type 2 Diabetes

Recent research shows that in both mice and humans, exercise induces muscle to release a newly discovered hormone, irisin, and studies in mice show that irisin promotes energy expenditure (calorie burning), and reduces obesity and type 2 diabetes. The mammalian body contains two kinds of adipose (fat) tissue: white adipose tissue (WAT), which stores fat for energy, and brown adipose tissue (BAT), which "burns" fat to help maintain body heat without shivering—thereby increasing the body's energy expenditure. Although human brown fat was initially thought to be present only newborns, recent studies have confirmed its presence and function in adults. A new study has identified a hormone, called irisin, which is produced by muscle tissue and instructs WAT to take on BAT-like characteristics. When irisin was administered to adult mice or added to mouse WAT cells, genes normally found in BAT were turned on, whereas some WAT genes were turned off. The researchers found that in mice and in human study participants, exercise led to an elevation in circulating irisin levels. When the scientists modestly increased the amounts of circulating irisin in a mouse model of type 2 diabetes, this treatment reduced obesity and improved blood glucose control without apparent side effects. These results reveal a hormone that appears to drive many of the physiological benefits of exercise. If irisin in humans works as it does in mice, administration of this hormone could be a potential new therapeutic approach for obesity and type 2 diabetes.

Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481: 463-468, 2012.

Brain Injury Associated with High-Fat Diet and Obesity

In people who are obese, and in rodents fed a high-fat diet, researchers discovered damage to an area of the brain that regulates body weight. Previously, scientists had observed inflammation in the brain of rodents with diet-induced obesity. In the current study, a team of researchers further investigated this adverse process. They began with rats and mice that were particularly genetically susceptible to obesity from a high-fat diet, and fed them this unhealthy fare. Within a day, the rodents' brains began to react as if they had suffered serious injury. Genes that promote inflammation were activated, and immune cells called microglia hastened to an area of the brain, the hypothalamus, that controls appetite and body weight. Within three days, these microglial cells had increased both in number and size. Within a week, astrocytes—another type of brain cell—had responded as well; the normally discrete projections that branch out from these cells had wrapped into a dense mass. The researchers also observed induction of a protein, Hsp72, known to help protect cells from injury. Although set in motion by a high-fat diet in the present study, these types of brain changes have also been seen in response to brain damage resulting from disruption of blood flow to the brain and even Parkinson's and Alzheimer's diseases. Some of the inflammatory and cellular changes were transient at first, as though the brain were attempting to limit adverse effects of the diet, but then reappeared as the high-fat feeding

continued; other changes persisted unabated throughout the months of unhealthy eating. And, many of the changes began rapidly, even before the animals gained substantial body weight. The researchers then found evidence of brain cell death—specifically of brain cells called POMC neurons, which normally reduce appetite and have other functions that help prevent obesity. With fewer POMC neurons, the likelihood of obesity increases. To see whether similar brain changes occur in people, the researchers analyzed MRI images that had been taken previously, for other purposes. Close inspection of brain images from 34 people revealed differences between lean and obese individuals, with evidence of changes in the hypothalamus of the brain. Thus, this study suggests that obesity and high-fat diet consumption are associated with damage to the brain.

Thaler JP, Yi CX, Schur EA, et al. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest 122: 153-162, 2012.

Studies Shed Light on Resident Stem Cells that Repopulate Normal and Injured Intestine

Two research teams have illuminated unique roles in intestinal regeneration for two distinct stem cell populations found on the inner surface of the intestine. The inner lining of the intestine plays an essential role in absorption of nutrients and balancing absorption and secretion of water and electrolytes, as well as providing a barrier against entry of bacteria. The lining is only one cell thick and cells slough off the surface continuously, lasting only about 1 week, requiring a process of continuous regeneration. Cells are also replaced following any injury. Recently, two populations of intestinal stem cells that are necessary for regeneration were identified by their different locations in the intestinal surface, as well as the unique proteins marking their outer membranes—either Lgr5 or Bmi1. Two research teams took a closer look at these stem cells to characterize their roles in intestinal regeneration.

One of the teams conducted research as part of the NIDDK's Intestinal Stem Cell Consortium. Using a microscopic technique that highlights intestinal stem cells with fluorescent markers for Lgr5 and Bmi1 in genetically modified mice, the researchers measured proliferation of the cells under normal, healthy conditions, as well as after injury caused by radiation. They found that the stem cells with Lgr5 on their surface actively proliferated under normal conditions, but were destroyed by radiation. The stem cells marked by Bmi1 were relatively inactive under normal conditions, but resisted radiation and proliferated dramatically following the injury. The Bmi1-marked stem cells in culture could also form some Lgr5-marked stem cells, showing their capacity to repopulate the intestine with both stem cell populations following injury.

Another team examined whether the Paneth cell, another type of intestinal cell located near Lgr5 stem cells, was essential for the ability of Lgr5 stem cells to repopulate the intestinal lining as part of normal cell turnover and tissue renewal. Paneth cells are known for secreting substances, such as proteins with antimicrobial properties, enzymes, and growth factors. Considering their proximity to intestinal stem cells, Paneth cells were thought to be involved in stem cell functions. This study used genetically modified mice and fluorescent markers to identify the Lgr5-producing stem cells and Paneth cells during early intestinal development. They found that the

appearance of the intestinal stem cells preceded Paneth cells in the developmental process, and that the stem cells appeared to function normally. They then created a genetically modified mouse that lacked Paneth cells. The stem cells functioned normally in this mouse model as well, proliferating to renew the intestinal surface as usual. These experiments indicate that Paneth cells are not essential for many aspects of Lgr5-marked stem cell function, though Paneth cells may still have other beneficial effects on their neighboring stem cells.

Taken together, the work of these two research teams paints a more nuanced picture of the complementary stem cell types that renew the intestinal surface throughout life, in terms of continuing cell turnover as well as regeneration following injury. This knowledge could be applied to optimizing recovery from different forms of intestinal injury.

Kim T-H, Escudero S, and Shivdasani RA. Intact function of Lgr5 receptor-expressing intestinal stem cells in the absence of Paneth cells. Proc Natl Acad Sci USA 109: 3932-3937, 2012.

Yan KS, Chia LA, Li X, et al. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. Proc Natl Acad Sci USA 109: 466-471, 2012.

Weight Loss and Increased Fitness Slow the Decline of Mobility in Adults

New research has shown that weight loss and increased physical fitness reduce the risk of losing mobility in overweight or obese adults with type 2 diabetes. Older adults with type 2 diabetes are more likely to have reduced mobility than those without this disease, and obesity increases the risk for mobility-related health problems. As part of the Look AHEAD (Action for Health in Diabetes) clinical trial, researchers investigated whether a lifestyle intervention program could slow the reduction of mobility. Look AHEAD is determining whether a lifestyle intervention designed to promote weight loss can improve health outcomes in overweight or obese people with type 2 diabetes. Participants were randomly assigned to either an intensive lifestyle intervention group (ILI) or a diabetes support and education group (DSE). Among the tests done in the trial, the researchers measured participants' weight, and they assessed the participants' fitness with a treadmill test. When the Look AHEAD trial began, nearly two-thirds of participants reported mild, moderate, or severe restrictions in mobility. After 4 years of the study, participants in the ILI group did not lose as much mobility as those in the DSE group. The ILI intervention slowed decline in mobility by 48 percent compared to DSE. Moreover, 20.6 percent of ILI participants reported severe disability compared to 26.2 percent of participants in the DSE group. Similarly, 38.5 percent of those in the ILI group reported good mobility, whereas the rate was 31.9 percent in the DSE group. Weight loss was a slightly stronger predictor of better mobility than was improved fitness, but both contributed significantly to the observed reduction in risk. These results are consistent with previous analyses of four-year outcomes, which showed that participants in the ILI group lost significantly more weight than those in the DSE group, and also had improved fitness, glucose control, blood pressure, and HDL cholesterol with less use of medication. These findings show that intensive lifestyle intervention programs can slow the decline of mobility in overweight or obese people with type 2 diabetes, which may have significant implications for their functional ability and for reducing health care costs as people age.

Rejeski WJ, Ip EH, Bertoni AG, et al. Lifestyle change and mobility in obese adults with type 2 diabetes. N Engl J Med 366(13): 1209-17, 2012.

Kidney Damage: New Insights into Initiation, New Targets for Therapy

Multiple recent studies have provided important insights into the origin of scar tissue that is seen in some forms of kidney disease. "Fibrosis"—the term that describes the deposition of large amounts of collagen-rich connective tissue that can lead to organ damage—is seen in many conditions related to inflammation and, unchecked, can diminish the ability of an organ to perform its normal functions. In the kidney, fibrosis is a common final pathway for many diseases. It may arise as the result of a brief, severe injury to the kidney—causing acute kidney failure—or from a slowly-progressing, chronic condition. Extensive kidney fibrosis, and the scar tissue that can sometimes arise, can impair the removal of toxins and excess fluid from the blood, cause irreversible organ damage and, in severe cases, lead to kidney failure. These new reports shed more light on the origins of kidney fibrosis and identify multiple potential new targets for therapy.

One study focused on molecular regulators of gene expression (or the extent to which gene functioning is on or off), and how these regulatory factors might influence the deposition of fibrous tissue following kidney injury. In this study, researchers examined two different mouse models of kidney fibrosis, and sought to identify regulators of gene expression that were elevated in the presence of scarring. The researchers focused on one molecule, microRNA 21 (miR-21) that was found to be highly elevated in two mouse models of kidney disease soon after injury but before fibrosis appeared. This molecule is also found in humans with kidney injury. Mice engineered to lack the miR-21 gene showed diminished fibrosis in response to kidney injury; similar results were observed in normal mice that had been treated with an inhibitor of miR-21. This molecule represents a potential target for antifibrotic therapies in kidney disease.

Another research group identified a cell surface protein, activin-like kinase 3 (Alk3), that is present at elevated levels following kidney injury. Deletion of this protein in certain areas of the kidney leads to increased fibrosis, suggesting that it plays a protective role in the organ. The scientists developed a small, synthetic protein that bound to and activated Alk3. This agent suppressed inflammation and reversed established fibrosis in five different mouse models of kidney disease. Molecules such as this synthetic protein may be able to treat, and possibly reverse, kidney fibrosis.

Two other studies investigated the role of various cell types within the kidney, and tried to identify the source of the collagen-producing cells that can lead to fibrosis. One focused on pericytes, a type of stem cell that is usually associated with blood vessels, in kidney injury and fibrosis. Previous research indicated that kidney fibrosis appears to arise through a pathway involving cells derived from pericytes. It found that kidney pericytes increased their levels of the enzyme ADAMTS1, which plays a role in remodeling the tissue surrounding kidney cells, and downregulated an inhibitor of this enzyme, TIMP3, following kidney injury. Mice engineered to lack TIMP3 were more susceptible to kidney injury-induced fibrosis. Together, these results

suggest central roles for regulators of enzymes that can modify networks of blood vessels in the kidney following injury.

In a second project focused on the role of a particular type of kidney cell, scientists used a new mouse model to study acute kidney injury and fibrosis. This study involved selective injury to the proximal convoluted tubules, part of the nephron, the basic structural and functional unit of the kidney. These tubules resorb about two-thirds of the fluid generated by the glomeruli, the filtering units within the kidney's nephrons. After inducing a one-time injury in a specific region of these tubules, the scientists observed the proliferation of tubular cells and the appearance of inflammatory cells. Following this single injury, the kidney recovered completely. However, when the researchers induced three injuries at one-week intervals, they observed diminished cellular repair, with resultant blood vessel damage and fibrotic damage to both the kidney tubules and the glomeruli. This study shows that repeated injuries, even to a portion of the nephron, can lead to more widespread kidney damage, similar to that associated with chronic kidney disease.

Researching another form of kidney disease, a team of scientists used computational and systems biology approaches to examine signaling molecules that regulate gene expression in a mouse model of HIV-associated kidney disease. They identified the protein HIPK2 as a key regulator of kidney fibrosis. Levels of this protein were found to be elevated in both the mouse model and in patients with various forms of kidney disease. Deletion of the gene encoding HIPK2 in the mouse model improved kidney function and reduced the severity of fibrosis. HIPK2 may be a potential target for novel therapies to address kidney fibrosis.

These five studies illuminate the complex system of regulation surrounding kidney fibrosis following injury, and identify multiple potential targets for further strategies aimed at preventing and possibly reversing kidney fibrosis, thereby preserving kidney function. Understanding the cellular and molecular mediators of kidney fibrosis is a high priority for scientists studying kidney disease. The identification of the factors that play a key role in this process might identify new targets for treatment aimed at preventing or reversing fibrosis. Furthermore, a better understanding of fibrosis in general could yield insights into how this process unfolds in other tissues and organs, potentially opening up new avenues to therapy for a range of diseases.

Chau BN, Xin C, Hartner J, et al. MicroRNA-21 Promotes Fibrosis of the Kidney by Silencing Metabolic Pathways. Sci Transl Med 4: 121ra18, 2012.

Grgic I, Campanholle G, Bijol V, et al. Targeted proximal tubule injury triggers interstitial fibrosis and glomerulosclerosis. Kidney Int 82: 172-183, 2012.

Jin Y, Ratnam K, Chuang PY, et al. A systems approach identifies HIPK2 as a key regulator of kidney fibrosis. Nat Med 18: 580-588, 2012.

Schrimpf C, Xin C, Campanholle G, et al. Pericyte TIMP3 and ADAMTS1 Modulate Vascular Stability after Kidney Injury. J Am Soc Nephrol 23: 868-883, 2012.

Sugimoto H, LeBleu VS, Bosukonda D, et al. Activin-like kinase 3 is important for kidney regeneration and reversal of fibrosis. Nat Med 18: 396-404, 2012.

Searching for a Way to Prevent HIV Transmission

Researchers recently investigated whether two drugs that have been used to treat infection with HIV-1, the virus that causes AIDS, might also be used to prevent transmission of the virus. For years, a combination of drugs termed "highly active antiretroviral drug therapy" (HAART) has been used to treat people infected with the human immunodeficiency virus, HIV-1. The use of drugs that are part of HAART in individuals before they are exposed to HIV-1 has been considered as a possible strategy to prevent the transmission of this virus. Scientists have now examined the metabolism of two drugs used in HAART and have described their distribution in different tissues.

Because HIV-1 is often transmitted through sexual contact, it was important that the researchers accurately measure levels of the active forms of the drugs in genital and colorectal mucosal tissue. The investigators gave 15 healthy men and women a single oral dose of a combination of two antiretroviral drugs, tenofovir (TFV) disoproxil fumarate and emtricitabine (FTC), and subsequently measured the concentration of these drugs over the next 14 days in the volunteers' blood and genital secretions, as well as in their vaginal, cervical, and rectal tissues. The drugs were detected in the blood and genital secretions for the full 14-day duration of the study and were present at higher concentration in the genital secretions, with a particularly high concentration of FTC in these samples. The biologically active metabolites of the drugs were detected in the vaginal, cervical, and rectal tissues for varying durations and at different levels. The active form of TFV was found at high levels for all 14 days of the study in rectal tissue, but was present at much lower levels in vaginal and cervical tissue. The active form of FTC was present at higher levels in vaginal and cervical tissues than in colorectal tissues, but could be detected for less than 2 days.

The wide range of tissue exposure to an orally-administered drug reported in this study illustrates the need for more detailed studies of the pharmacology of drugs currently used to treat HIV infection as possible agents to prevent transmission of the virus. Ultimately, the success of drug therapy to prevent the spread of HIV-1 will depend on selecting the proper combination of drugs and their doses.

Patterson KB, Prince HA, Kraft E, et al. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. Sci Transl Med: 3: 112re4, 2011.

Induced Pluripotent Stem Cells Hold on to Past Identity

Researchers found genomic marks in human induced pluripotent stem (iPS) cells that currently limit their scientific and therapeutic potential, but also suggest opportunities to improve the development of these cells. Researchers initially developed iPS cells with the hope of overcoming challenges posed by other types of stem cells. Human embryonic stem (ES) cells, for example, hold promise in the treatment of disease because they are "pluripotent," meaning that unlike most other cells, they have the ability to form virtually any cell type and thus could generate cells for repair of human tissues and organs. The use of human ES cells, however, is controversial because their isolation entails the destruction of early-stage human embryos, and

ES cells have other limitations as well. In recent years, scientists developed strategies to reprogram cells, such as blood or skin cells, to revert from their specific cell types back to an ES cell-like state, with the potential to form not only new cells of their original type, but also stem cells and a multitude of different cell types. These pluripotent, reprogrammed cells, called iPS cells, could potentially be used to study diseases and to generate cells to treat specific diseases, potentially with a tissue match for the recipient (avoiding transplant rejection).

For reasons that have been poorly understood, iPS cells generated to-date are significantly less pluripotent than ES cells; they are more easily able to form the cell type from which they were originally derived than to form cells of other types. To understand why this might be the case in human iPS cells, researchers analyzed the cells' DNA, based on previous findings in mouse cells. Mouse iPS cells retain a pattern of chemical modifications on their DNA characteristic of their past cell type, rather than a pattern characteristic of ES cells. Although this modification does not alter the sequence of the genetic code, it can affect the cell's ability to turn genes on or off. The combination of genes that are active and inactive characterizes a cell type, therefore this important modification has the effect of helping a cell to "remember" its identity. In this new research, scientists sought to determine whether human iPS cells retained the chemical modifications of their past cell type, like the mouse iPS cells did. They produced iPS cells from both blood and skin cells. The researchers found, as expected, that blood-derived iPS cells were more likely to form blood cells, and skin-derived iPS cells were more likely to become skin cells. By comparing the patterns of chemical modifications of iPS cells to ES cells, the researchers determined that the iPS cells retained patterns characteristic of their original cell types. Current techniques to generate iPS cells, therefore, do not fully erase the cell's memory, limiting its potential to become another cell type. Scientists will continue research to develop new techniques that may be able to erase the residual patterns more fully. In the meantime, scientists may be able to take advantage of the bias of iPS cells toward their original cell type in the study of and development of therapies for diseases associated with those cell types. Cautious optimism continues for the eventual, wider use of iPS cells.

Kim K, Zhao T, Doi A, et al. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nat Biotech 29: 1117-1119, 2011.

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