



SCIENCE ADVANCES
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Advances for October 2009

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Evaluating the Safety of Bariatric Surgery

The Longitudinal Assessment of Bariatric Surgery (LABS) consortium conducted a multicenter, observational study to evaluate the 30-day safety outcomes in patients who underwent an initial bariatric surgical procedure. Approximately one-third of U.S. adults are considered obese based on their body mass index or BMI (a measure of weight relative to height); these individuals have increased risk for type 2 diabetes, coronary heart disease, stroke, fatty liver disease, certain types of cancer, and other diseases. Used as a treatment for extreme obesity, bariatric surgical procedures modify the digestive tract to limit the amount of food that can enter the stomach, decrease absorption of nutrients, or both. Currently, bariatric surgery appears to be the only intervention that consistently results in substantial and sustained weight loss in people who are extremely obese, and it has been linked to remission of diabetes, decreases in cardiovascular risk factors, and a significant reduction in mortality over time. Like most surgical procedures, however, bariatric surgery presents risks of complications and death that must be considered when deciding whether to undergo the procedure.

In this study, LABS-1, the consortium followed 4,776 patients who had bariatric surgery, from before their surgery through the first 30 days following surgery, to evaluate the death and complication rates. All of the patients participating in the study were adults and were obese, and most had a BMI measurement reflecting extreme obesity. Similar to most populations undergoing bariatric surgery, the majority of the patients in the LABS study were white and female. The study took place over two years at 10 medical centers located throughout the U.S., with one center coordinating data collection and analysis. Within 30 days of surgery, 4.1 percent of patients had at least one major adverse outcome, defined as development of blood clots in the deep veins of the legs or the pulmonary artery of the lungs, repeat surgeries, not being discharged from the hospital within 30 days, or death. Mortality rates were low: less than 1 percent (0.3 percent) of patients died within 30 days. The risk of complications varied depending upon whether or not patients had certain health conditions prior to the surgery and how obese they were. Although the rate of adverse events also appeared to vary with the type of surgical procedure, differences in patient characteristics may have accounted for much of the variation in risk among the procedures. Further investigation may help clarify any such differences.

This evaluation highlights the level of short-term risks associated with bariatric surgery, an effective weight loss procedure that is increasingly popular as a treatment for extreme obesity. The safety of such surgery is an important consideration with risks examined in the context of long-term benefits. The LABS-1 study will help health care providers and patients make personalized decisions about the potential risks and benefits of bariatric surgery by taking into account a patient's characteristics. Another study being conducted by the LABS consortium, LABS-2, will follow a subset of the patients to gather longer-term data that will further inform decisions about the surgery.

The Longitudinal Assessment of Bariatric Surgery (LABS) Consortium: Perioperative Safety in the Longitudinal Assessment of Bariatric Surgery. [N Engl J Med](#). 361:445-54, 2009.

Calorie-Burning Fat Found in Lean Adults

New research has revealed that an energy-burning form of fat is active in adults—a finding which may open new avenues for efforts to combat obesity, a strong risk factor for type 2 diabetes. Unlike “white fat,” which stores energy and comprises most body fat, another type of fat, called “brown fat,” burns calories to help keep animals warm. In humans, it has been thought that brown fat is active only in babies and children. Now, using advanced imaging technology (PET-CT scans), a new study has found evidence that a significant portion of adults retain metabolically active brown fat. In this study, researchers detected substantial amounts of active brown fat in the neck region of adults. They also found some key differences among people. Older people tended to have less brown fat, but being thinner was associated more with having brown fat, especially among older people—suggesting that brown fat may help protect against age-related weight gain. Interestingly, the researchers also observed that a person’s brown fat changed with the outdoor temperature, with the most brown fat activity detectable in colder weather. This finding is consistent with two other research studies (funded in Europe) that were published at the same time, which showed that brown fat activity increased in people briefly exposed to cold. These clinical findings dovetail with recent insights in animal models into the molecular signals controlling the growth of brown fat. Together, these discoveries may help scientists develop therapeutic drug interventions to promote weight loss through increasing brown fat, or to exploit the finding that brown fat is activated by exposure to cold temperatures.

Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, and Kahn CR: Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360:1509-17, 2009.

Insulin, Metformin, and Pathways of Glucose Production in Fasting and Obesity

New research is shedding light on the ways metabolism in the liver is affected by obesity and by two of the most widely prescribed medications for people with diabetes. Insulin, produced naturally by the body in response to elevated sugar in the blood, is prescribed to all patients with type 1 diabetes because they cannot make the vital hormone themselves. It is also prescribed to many people with type 2 diabetes in cases where other medications cannot make up for lost insulin production capacity and their bodies' increased needs for the hormone. The most widely prescribed medication for type 2 diabetes, however, is metformin, which works by reducing the amount of glucose fed into the bloodstream by the liver. A hormone called glucagon triggers the liver to release glucose during periods of fasting. For reasons that have not been fully understood, liver glucose production occurs even in the absence of fasting in people with diabetes, contributing to elevated blood glucose. One of insulin's key effects is to blunt the impact of glucagon, stopping its release of liver glucose, and accounting for one of its most serious side effects. Overly high doses of insulin not only send glucose levels too low, they also limit the ability of glucagon to bring glucose back up again. The result is hypoglycemia, dangerously low blood glucose. Metformin also counteracts glucagon, but rarely causes hypoglycemia by itself, because it does not directly lower blood glucose by signaling cells to take up sugar as insulin does.

New research in obese mice fed a high fat diet clarifies the pathway that leads to excessive production of glucose from the liver in type 2 diabetes, and pinpoints the ways in which metformin and insulin interrupt the process. One group of researchers found that a complex of proteins acts to boost glucose output and is triggered both by fasting signals (glucagon) and by a cellular condition that can result from obesity, called the "ER (endoplasmic reticulum) stress response" (see feature in Cross Cutting Science chapter). Another group of researchers found that both insulin and metformin lead to a modification of one of the proteins in this complex—a protein called CBP. The modification of CBP causes the complex to fall apart so that it no longer supports glucose production. Although the impact on CBP and glucose production is the same, metformin and insulin work through different pathways to modify CBP, which helps explain why metformin is effective even in patients who are resistant to insulin's effects. Understanding the molecular pathways by which the healthy liver promotes glucose control, as well as how insulin and metformin work in disease, has the potential to help improve glucose control in diabetes patients, preventing both hypoglycemia, and the long-term complications of hyperglycemia (high blood sugar). Because metformin is currently the only approved drug in its class, this research may also help to identify new and better therapeutic strategies to help people with diabetes control their blood glucose.

Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. He L, Sabet A, Djedjos S, Miller R, Sun X, Hussain MA, Radovick S, Wondisford FE. Cell. 2009 May 15; 137(4):635-46.

The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. Wang Y, Vera L, Fischer WH, Montminy M. [Nature](#). 2009 Jul 23; 460(7254):534-7.

New Potential Therapeutic Strategy to Suppress Inflammation

Scientists have developed new technology with potential as a therapeutic strategy for inflammatory diseases. Short molecules of ribonucleic acid (RNA) can be targeted to reduce levels of specific proteins by interacting—or interfering—with the genetic material that encodes the protein, to prevent the protein from being made. This technique, known as “RNA interference,” has potentially transformative therapeutic value. The development of a safe and effective way to deliver short interfering RNA molecules (siRNA) to specific types of cells *in vivo*, however, would be required before this technique could be used therapeutically. In a recent study, scientists designed a method to orally deliver siRNA to mouse macrophages, a cell type of the immune system that is important in initiating the inflammatory response. In this novel approach, layers of RNA molecules can be encapsulated within hollow, porous, tiny (micron-sized) shells of a substance called beta1,3-D-glucan, a non-toxic material made by yeast cells. The shells are recognized by proteins found primarily on the surface of macrophages, allowing for the specific uptake of the shells by macrophages. The scientists termed these shell particles “GeRPs” or glucan-encapsulated siRNA particles.

To test this system as a potential therapeutic in animals, the investigators examined the effects of feeding mice GeRPs with siRNA to a specific inflammatory protein known as MAP4K4. They detected GeRPs inside mouse macrophages in various tissues of the mouse body, including spleen, liver, and lungs, and observed a decrease in levels of MAP4K4 in these tissues. To determine whether a reduction in MAP4K4 protein levels suppresses the inflammatory response, the mice were fed GeRPs with siRNA to MAP4K. The mice were then given a toxic chemical that mimics a bacterial infection in order to stimulate the inflammatory response. When mice without the siRNA were given the chemical, their macrophages stimulated an excessive inflammatory response which was fatal to the animals. By feeding the mice siRNA to MAP4K4, the scientists were able to halt the inflammatory response to the chemical, thus protecting the mice. This exciting result demonstrated that the orally administered siRNA was not only delivered to the correct cells—the macrophages—and carried to multiple tissues, but that the siRNA also reduced levels of MAP4K4 and thus altered the mouse inflammatory response.

Inflammatory responses triggered by macrophages are involved in many conditions, including obesity, type 2 diabetes, inflammatory bowel disease, colitis, cardiovascular disease, atherosclerosis, and rheumatoid arthritis. The GeRP technology provides a novel oral delivery system for RNA interference to reduce levels of proteins involved in inflammation. Although further development and testing of the GeRP delivery system in animal models and in humans will be required, this study reveals the exciting potential of a new therapeutic strategy to suppress inflammation associated with numerous adverse health conditions. In addition, this technology could be used to deliver siRNA, and possibly other cargo, to other types of cells in the immune system to alter their function and therefore could be explored as a potential therapy for autoimmune diseases like type 1 diabetes.

Aouadi M, Tesz GJ, Nicoloso SM, Wang M, Chouinard M, Soto E, Ostroff GR, and Czech M: Orally delivered siRNA targeting macrophage *Map4k4* suppresses systemic inflammation. *Nature* 458: 1180-1184, 2009.

Identification of a Potential Immune System Target Antigen in Autoimmune Kidney Disease

Researchers have identified a protein that may play a key role in the development of an autoimmune form of kidney disease known as “idiopathic membranous nephropathy.” This type of kidney disease is a common cause of nephrotic syndrome in adults. It is an autoimmune disease, meaning that the body’s immune system incorrectly mounts an attack against a normally-occurring protein in the body. The disease is characterized by protein in the urine, lowered protein levels in the blood, elevated cholesterol, and swelling of the face, hands, and feet. The identification of the protein that induces this immune response (termed an “antigen”) will open new avenues of exploration in idiopathic membranous nephropathy.

To identify potential target antigens, scientists collected blood samples from patients with idiopathic membranous nephropathy, and mixed them with proteins that were obtained from kidney tissue. In 70 percent of the tested blood samples, self-reactive antibodies (autoantibodies) identified a single kidney protein that was ultimately determined to be the M-type phospholipase A₂ receptor or PLA₂R. This protein is expressed by cells in glomeruli, tiny filtering units in the kidney that are injured in this syndrome. The subtype of antibody that reacted with PLA₂R in the assay is the same kind that is found in immune deposits within the glomeruli in patients with this disease. Antibodies isolated from glomeruli of patients with idiopathic membranous nephropathy react with PLA₂R, whereas antibodies isolated from the glomeruli of patients with nephropathy arising from other causes do not. Furthermore, there is evidence to suggest that, in patients with clinically significant disease activity, autoantibodies against PLA₂R can be readily detected in the blood. In contrast, in patients in whom the disease is in remission, levels of these antibodies decline or disappear.

Fifty years ago, researchers studying a rat model of idiopathic membranous nephropathy identified a kidney protein that appeared to be an immunological target for autoantibodies; however, progress stalled when this protein was found to be absent in human kidneys. These new findings will also have important implications for patient care. For example, they may permit the noninvasive diagnosis of membranous nephropathy, as well as provide an easier way to follow the disease in response to treatment. Better understanding of the potential triggers of autoantibody production in patients with a susceptibility to idiopathic membranous nephropathy may also uncover possible new targets for preventing or treating this disease.

Beck LH, Bonegio RGB, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, and Salant DJ. M-Type Phospholipase A₂ Receptor as Target Antigen in Idiopathic Membranous Nephropathy. New Engl J Med 361: 11-21, 2009.

Unraveling the Genetic Causes of Type 1 Diabetes

Several recent studies are contributing to understanding the genetic underpinnings of type 1 diabetes. Scientists in the NIDDK-supported Type 1 Diabetes Genetics Consortium (T1DCG) studied over 2,400 families and discovered that variants in the *UBASH3A* genetic region were associated with the disease. They also confirmed previously reported associations with three other genetic regions (*INS*, *IFIH1*, and *KIAA0305*). A study by a different group of scientists analyzed several patient populations to follow-up on results of recent genome-wide association studies (GWAS). These populations included people enrolled in the T1DGC, as well as participants from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study, which is a long-term NIDDK-supported study of people with type 1 diabetes. These researchers also identified *UBASH3A* as being associated with type 1 diabetes, and in addition discovered an association in the *BACH2* genetic region. *UBASH3A* is predominantly found in immune system cells called T cells, and *BACH2* is thought to be a regulator of the immune system's antibody response. Because type 1 diabetes is an autoimmune disease, it is plausible that defects in these genes could contribute to type 1 diabetes, although more research will help determine how these genes may play a role. In another study, scientists examined an independent patient population to confirm the association between variants in the *IFIH1* genetic region and type 1 diabetes. The *IFIH1* gene was found to be more strongly expressed (turned on) in some immune system cells from people carrying variants of the gene associated with type 1 diabetes. This observation suggests that higher amounts of the protein encoded by the *IFIH1* gene may be linked to increased risk for type 1 diabetes, but additional studies in more people are necessary to confirm the finding. *IFIH1* is thought to play a role in the immune system and has also been linked to two other autoimmune diseases. In another study, T1DGC scientists combined data from a new GWAS with data from previous studies to discover that over 40 different genetic regions influence a person's risk of developing type 1 diabetes. That number includes the genetic regions described above, as well as several novel regions.

Scientists are also building on recent genetics findings to understand how other genetic factors contribute to risk for type 1 diabetes. Researchers looked at previously-identified type 1 diabetes susceptibility genes to determine their impact on an early stage in type 1 diabetes onset—the development of autoimmunity—in children participating in the NIDDK-supported Diabetes Autoimmunity Study in the Young (DAISY). These children were originally enrolled in DAISY because they carry variants for a different diabetes susceptibility gene (*HLA*) that put them at high genetic risk for developing type 1 diabetes. The scientists discovered that a variant in the *PTPN22* gene region increased the risk of developing autoimmunity in children with a family history of the disease. In contrast, a variant of the *CTLA-4* gene region increased the risk of autoimmunity in children without such a family history.

These studies are shedding new light on genetic factors that underlie type 1 diabetes, and may lead to enhanced ways to predict who is at high risk for the disease, and potentially inform new intervention approaches. They also demonstrate how new knowledge is stemming from long-term, NIDDK-supported research studies based on new and emerging genetics technologies.

Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, and Rich SS; The Type 1 Diabetes Genetics Consortium: Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet* 41: 703-707, 2009.

Concannon P, Onengut-Gumuscu S, Todd JA, Smyth DJ, Pociot F, Bergholdt R, Akolkar B, Erlich HA, Hilner JE, Julier C, Morahan G, Nerup J, Nierras CR, Chen WM, and Rich SS; Type 1 Diabetes Genetics Consortium: A human type 1 diabetes susceptibility locus maps to chromosome 21q22.3. *Diabetes* 57: 2858-2861, 2008.

Grant SF, Qu HQ, Bradfield JP, Marchand L, Kim CE, Glessner JT, Grabs R, Taback SP, Frackelton EC, Eckert AW, Annaiah K, Lawson ML, Otiemo FG, Santa E, Shaner JL, Smith RM, Skraban R, Imielinski M, Chiavacci RM, Grundmeier RW, Stanley CA, Kirsch SE, Waggott D, Paterson AD, Monos DS; DCCT/EDIC Research Group, Polychronakos C, and Hakonarson H: Follow-up analysis of genome-wide association data identifies novel loci for type 1 diabetes. *Diabetes* 58:290-295, 2009.

Liu S, Wang H, Jin Y, Podolsky R, Reddy MV, Pedersen J, Bode B, Reed J, Steed D, Anderson S, Yang P, Muir A, Steed L, Hopkins D, Huang Y, Purohit S, Wang CY, Steck AK, Montemari A, Eisenbarth G, Rewers M, and She JX: IFIH1 polymorphisms are significantly associated with type 1 diabetes and IFIH1 gene expression in peripheral blood mononuclear cells. *Hum Mol Genet* 18: 358-365, 2009.

Steck AK, Zhang W, Bugawan TL, Barriga KJ, Blair A, Erlich HA, Eisenbarth GS, Norris JM, and Rewers MJ: Do non-HLA genes influence development of persistent islet autoimmunity and type 1 diabetes in children with high-risk HLA-DR,DQ genotypes? *Diabetes* 58: 1028-1033, 2009.