

Unraveling the Causes of Diabetes

As the obesity-driven epidemic of type II diabetes rages, researchers are piecing together the environmental and genetic factors behind the disease

“People cringe at the use of the word ‘epidemic’ for a chronic disease, but by all criteria, there’s [a diabetes 2] epidemic” in the United States, says Allen Spiegel, who directs the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in Bethesda, Maryland.

The number of adults with diabetes in the United States increased by 49% between 1991 and 2000, according to data from the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. Type II diabetes, formerly known as maturity-onset or non-insulin-dependent diabetes, accounts for practically all of that increase. Some 16 million to 17 million people now have the condition, and an equal number are thought to be “prediabetic,” having early symptoms but not yet the full-fledged version. Even children are no longer immune to diabetes 2, which until recently rarely affected people before middle age.

Driving this epidemic, say Spiegel and other experts, is the continuing increase in obesity that is, in turn, fueled by a relatively new development in human history: an ample food supply coupled with a sedentary lifestyle. In the past, humans who wanted food “had to grow it, harvest it, or hunt it,” says diabetes researcher Roger Unger of the University of Texas Southwestern Medical Center (UT Southwestern) in Dallas. The current overabundance of easily available food is, he adds, “a surprise to nature,” one that our bodies aren’t designed to handle.

In diabetes 2, this manifests itself primarily by the body becoming resistant to the hormone insulin, which is needed to metabolize the sugar glucose, although insulin production by the β cells of the pancreas usually becomes impaired, too. (By contrast, the much less common type I diabetes is caused by a complete inability to produce insulin due to β cell destruction.) Diabetics of both types develop serious complications, including kidney failure, blindness, damage to the feet and legs serious enough to require amputation,

and a high risk of heart attack and stroke.

Researchers are beginning to understand how obesity leads to insulin resistance and the other defects of diabetes. They have fingered several suspects, including fatty acids released by fat cells.

But there’s more to diabetes 2 than obesity. Like cancer and heart disease, it fits the profile of a complex disease: Its development is influenced both by environment—particularly by such lifestyle factors as smoking, diet, and exercise level—and by genetics—specifically the combined effects of what may be subtle alterations in several

nia School of Medicine in Philadelphia. The challenge now facing diabetes researchers, he and others note, is to sort out the contribution of each factor and then use that knowledge to design badly needed therapeutics.

Changing face of diabetes

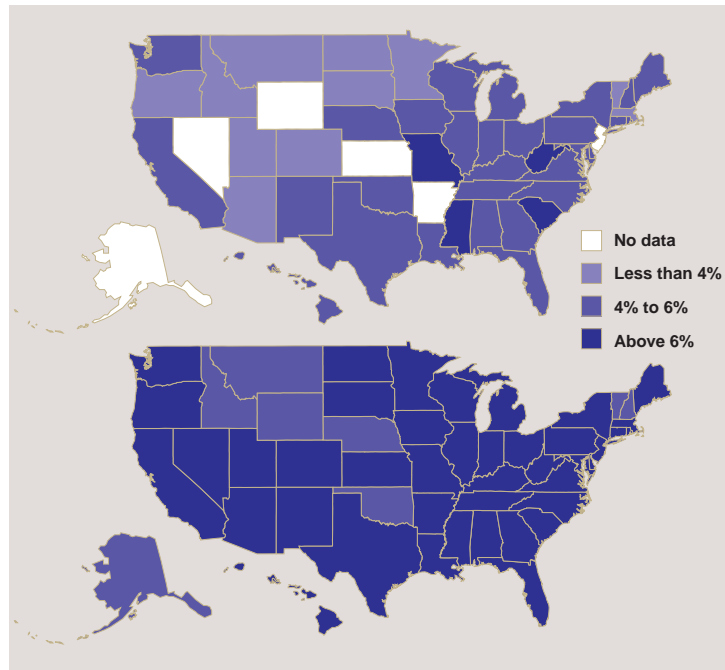
Over the years, numerous studies have pointed to obesity as a major risk factor for diabetes. “In every single racial or ethnic group, obesity raises the risk,” says David M. Nathan of Massachusetts General Hospital in Boston. Even so, the risk appears to be higher for some groups than for others. In particular, indigenous peoples tend to be hard hit. The Pima Indians of Arizona have the highest diabetes 2 incidence in the world: 50% of adults have the disease.

Other groups in the United States also have higher than average risks. The American Diabetes Association estimates that 13% of African Americans and 10.2% of Hispanics have diabetes, compared to about 6.5% of whites. Researchers don’t yet know what accounts for these variations, but they expect that both genetic and environmental factors come into play.

Recent studies also point to some disturbing new trends. For one, diabetes is on the rise in many developing countries, as they adopt more Westernized lifestyles and diets. The World Health Organization predicts that the number of cases worldwide—now 150 million—will double by 2025. And even more alarming, obesity-driven diabetes 2 is increasingly striking younger people, including children—a

situation Spiegel describes as “potentially devastating,” because those who contract the disease early have longer to develop the sometimes deadly complications.

Some of the latest data on childhood diabetes come from Sonia Caprio of Yale University School of Medicine and her colleagues. In a study of 167 obese children, the Yale team found an early warning symptom of diabetes—known as impaired glucose tolerance—in 25% of children under age 10 and in 21% of those between the ages of 11 and 18. Four percent of the adolescents turned out to have previously undiagnosed,



A darkening scene. The percentage of adults with diabetes increased throughout the United States between 1990 (top) and 2000 (bottom).

genes. For example, not every obese person gets diabetes 2, an indication that some are more genetically susceptible than others.

Uncovering such susceptibility genes is much more difficult than identifying the single-gene defects that cause cystic fibrosis and other simple hereditary diseases. But researchers have turned up several candidates, most of which are involved in either the production of insulin or the body’s responses to it.

“Five years ago, nobody had a clue [about the causes of diabetes 2]. Now, there are almost too many ideas,” says Morris Birnbaum of the University of Pennsylva-

SOURCE: A. H. MOKDAD ET AL., DIABETES CARE 23, 1278 (2000) AND J. AM. MED. ASSOC. 286, 1195 (2001)

full-fledged diabetes 2, the team reported in the 14 March issue of *The New England Journal of Medicine (NEJM)*.

And in a vicious cycle, a long-running NIDDK study of Pima Indians has shown that diabetic parents are more likely than nondiabetics to have diabetic children. One reason, says Clifton Bogardus of NIDDK, is because diabetes susceptibility genes can be passed down from parent to child. Another is that, for unknown reasons, conditions in the wombs of diabetic mothers raise the diabetes risk of their offspring.

Some recent results have been encouraging, however. In a large, multi-center clinical trial, the Diabetes Prevention Program (DPP) Research Group found that it's possible to stave off diabetes 2 in people at high risk of getting the disease. The trial included 3234 people, who were divided into three roughly equal groups. The controls received a placebo plus standard recommendations for improving their diets and exercise regimens. A drug treatment group took an anti-diabetes 2 drug called metformin, and a second treatment group received intensive counseling about eating better and exercising regularly.

As reported in the 7 February *NEJM*, the intensive lifestyle counseling reduced the incidence of diabetes 2 by 58%, and metformin treatment produced a 31% reduction. Previous studies had shown that lifestyle changes help, but they were smaller and involved relatively homogeneous populations. In contrast, almost half the participants in the DPP trial were members of minority groups, including those at high risk such as African Americans and Hispanics. The treatments proved so effective in all groups, Nathan says, that the trial was halted a year early.

Biochemistry of obesity

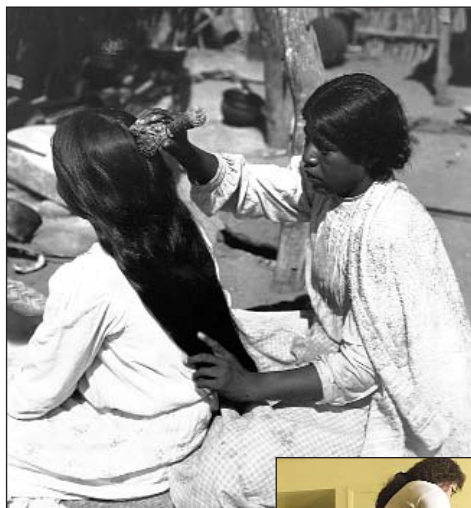
Given the firm links between obesity and diabetes 2, researchers are working hard to uncover the biochemical connections. They now have several good leads to how obesity might lead to insulin resistance and impaired glucose tolerance.

One major discovery involves what Mitch Lazar of the University of Pennsylvania Medical Center in Philadelphia calls "a sea change in our thinking" about fat. At one time, fatty tissue was thought to be little more than a fat storage depot. But researchers have learned that fat cells play a more dynamic role, releasing a variety of hormonelike substances that circulate in the blood and affect other tissues.

These include proteins such as leptin, which is best known for its role in suppressing appetite and obesity—effects that should inhibit diabetes development. Except in rare

cases, however, obese humans make large quantities of leptin but for unknown reasons are resistant to its antiobesity and antidiabetes effects. Researchers have recently linked other fat-produced proteins to diabetes 2.

One of these, called resistin, discovered by Lazar's group and others, apparently counteracts insulin's effects, which suggests that it contributes to resistance to the hormone. Another protein called adiponectin, identified by Philipp Scherer and his colleagues at Albert Einstein College of Medicine in New



High risk. Compared to the Pima Indians of the early 1900s (*above*), those of today (*right*) have a much more serious obesity problem—and the highest incidence of diabetes in the world.



York City, promotes insulin's effects, but its production decreases in obese persons.

Even the fatty acids released by fat cells may play a prominent role in promoting insulin resistance, as recently shown by Gerald Shulman of Yale University School of Medicine, Gunter Boden of Temple University School of Medicine in Philadelphia, and others. These researchers found, for example, that in obese people fatty acids accumulate in muscle, a prime insulin target that removes glucose from the bloodstream and stores it in the carbohydrate glycogen.

Further analysis, in which the researchers used nuclear magnetic resonance to examine the muscle tissue of living patients, showed that the fatty acids interfere with the pathway that transmits insulin signals into the muscle cell interior. As a result, glucose can no longer enter the cells and thus remains out of reach of the glycogen-synthesizing enzymes, allowing the sugar to build up in the blood—a characteristic diabetes symptom.

Recent work suggests that locally pro-

duced glucocorticoid hormones might foster diabetes development by influencing the release of fatty acids and proteins by fat cells. Last year, Eva Rask of Umeå University Hospital in Sweden and her colleagues found that the activity of a key enzyme needed to synthesize glucocorticoids is increased in the fat tissue of obese people.

To test whether local overproduction of the enzyme, known as 11 β HSD-1, might contribute to diabetes, Jeffrey Flier of Beth Israel Deaconess Medical Center in Boston and his colleagues attached the gene encoding the enzyme to a regulator sequence that allows it to be expressed only in fat. When the researchers introduced this gene into mice, glucocorticoid production went up in the animals' fat. As a result, they became obese and developed severe insulin resistance and diabetes (*Science*, 7 December 2001, p. 2166). Because the hormone remains inside the fat cells, it may have caused the diabetes indirectly by promoting the release of fatty acids and decreasing adiponectin secretion, Flier says.

Other researchers are also looking to mouse models for clues to the disease. Several teams have recently shown that they can recreate some or all of the symptoms of the disease in mice by knocking out one or another of the genes encoding proteins involved in transmitting insulin signals into the cell interior.

One such example comes from Birnbaum's team, working with Shulman's. They found that knocking out the gene for a pathway protein called Akt2 resulted in decreased glucose uptake by the animals' muscle tissue (*Science*, 1 June 2001, p. 1728). In another prime insulin target, the liver, the hormone could no longer suppress glucose synthesis as it normally would. Overall, Birnbaum says, the knockout mice have symptoms reminiscent of glucose intolerance in humans.

Focus on β cells

Although insulin resistance and the resulting impairment in glucose tolerance are early signs of diabetes, malfunction or even death of the insulin-producing β cells also contributes to the disease. Ultimately about a third of diabetes 2 patients end up having to take insulin.

Several factors seem to be involved in β -cell dysfunction, including some of the same culprits implicated in insulin resistance. For example, in experiments per-

formed on the Zucker rat, a rodent model of obesity and diabetes, Unger's group at UT Southwestern has found that fatty acids can trigger a form of cell death called apoptosis in β cells.

The fatty acids work indirectly, the UT Southwestern team found: They are first converted in β cells to toxic compounds known as ceramides. That suggests to Unger that the β -cell loss can be prevented. "If we block that [ceramide-producing] pathway, we can block apoptosis," he says.

Unger also suggests that this fatty acid toxicity may result from the body's insensitivity to leptin. In his view, that hormone's job is to keep fatty acids from accumulating in cells that aren't designed to handle them, such as β cells and muscle.

But β cells don't have to die to contribute to diabetes 2 pathology: They can simply fail to secrete the insulin needed to handle all the glucose the body takes in. At least in mouse models, researchers can duplicate that type of malfunction.

For example, a team led by Ronald Kahn of the Joslin Diabetes Center in Boston and Mark Magnuson of Vanderbilt University School of Medicine in Nashville, Tennessee, found that they could prevent the increase in insulin secretion that normally occurs in response to glucose ingestion by specifically inactivating the insulin receptor in the β cells of mice. As a result of the consequent block in insulin activity, glucose can't get inside the cells to trigger release of the hormone.

Work by Bradford Lowell's team at Beth Israel Deaconess Medical Center points to another possible way of interfering with glucose sensing by the β cell and thus disrupting insulin secretion. Working with mice, they found that uncoupling protein 2 is a negative regulator of insulin secretion, presumably because it decreases production by the mitochondria of adenosine triphosphate, the ultimate signal for the hormone's release. Conversely, the researchers found that production of the protein is elevated in another rodent model of obesity and diabetes, the ob/ob mouse, indicating that it might contribute to development of diabetes.

Susceptibility genes

Although this and other animal work has uncovered many potential candidates for diabetes susceptibility genes, researchers still need to show that they contribute to the

problem in humans. At the same time, other researchers are searching for human genes that confer susceptibility to diabetes 2—a search that illustrates the problems posed by diseases involving multiple genes. What's "most worrisome," Birnbaum says, "is that the disease is caused by a series of insults individually so small that they will escape detection."

Indeed, so far researchers have had the greatest success with a rare, single-gene

Research Institute in London, Ontario, reports that a mutation in one MODY gene, which encodes a transcription factor called HNF-1- α , contributes to the high incidence of diabetes 2 in the Oji-Crees, an indigenous population of roughly 30,000 people in northwestern Ontario. About 40% of adult Oji-Cree have diabetes 2, and Hegele and his colleagues have been searching for the culprit genes for several years.

The HNF-1- α gene turned up unexpectedly when the Ontario team analyzed a series of candidate genes, looking to see whether people with the disease carry mutations in them. In a different type of analysis, the researchers had found several "hot spots" in the genome that seem to be linked to diabetes 2 in the Oji-Crees. But Hegele says that the HNF-1- α gene isn't located in any of those sites. The researchers examined the gene in addition to other candidates, he adds, "just so we could say we looked at all the usual suspects."

The discovery illustrates another problem in pinning down the causes of complex diseases. The Hegele team found the HNF-1- α mutation only in the Oji-Crees. Something similar has been seen with a gene that Graeme Bell of the University of Chicago, Polonsky, and their colleagues linked to diabetes 2 in a different high-diabetes population: the Mexican-Americans of Starr County, Texas. Genetic linkage studies by this team fingered a gene encoding a protein-splitting enzyme

called calpain-10. But the finding has been controversial, partly because the researchers as yet have no idea how a calpain-10 mutation might lead to diabetes 2, and partly because the linkage doesn't show up in all study populations. For example, it's been found in some French populations but not others.

However, a team led by Michael Garant and Alan Shuldiner of the University of Maryland School of Medicine reported in the January issue of *Diabetes* that mutations in the gene could account for 25% of the diabetes 2 susceptibility of African Americans. Bell doesn't find this variability in gene impact in different populations at all disconcerting. It is, he says, "what you expect in these [susceptibility] genes for complex diseases."

These difficulties haven't stopped researchers from looking for the genes. Bogardus and his colleagues, for instance,

SOME CANDIDATE DIABETES 2 GENES

Mutated Gene	Function	Effect	Linked to
HNF-4- α , HNF-1- β IPF-1, NeuroD1	Transcription factors	↓ Insulin secretion	MODY (human)
HNF-1- α	Transcription factor	↓ Insulin secretion	MODY Oji-Cree diabetes
Glucokinase	Glucose metabolism	↓ Insulin secretion	MODY
Calpain-10	Protease	Unknown	Diabetes 2 in Mexican and African Americans
PPAR- γ	Transcription factor	↓ Insulin sensitivity	Diabetes 2
Insulin receptor	Transmits insulin signals into cell	↓ Insulin sensitivity and secretion	Human diabetes (rare); mouse models
IRS1 and -2	Insulin signaling	↓ Insulin sensitivity	Mouse models
Akt2	Insulin signaling	↓ Insulin sensitivity	Mouse models
11- β -HSD	Glucocorticoid synthesis	↑ Blood lipids, ↓ insulin sensitivity	Mouse models
UCP2	↓ ATP synthesis	↓ Insulin secretion	Mouse models
Resistin	Fat cell "hormone"	↓ Insulin sensitivity	Mouse studies
Adiponectin	Fat cell "hormone"	↑ Insulin sensitivity	Mouse, human studies

form of the disease called MODY (for maturity-onset diabetes of the young), although this has led them to a susceptibility gene in a larger population. Studies of MODY patients have uncovered some half-dozen genes, each of which can, when mutated, cause MODY. "The genes involved in this syndrome all cause abnormalities of β cell function," says Kenneth Polonsky of Washington University School of Medicine in St. Louis, one of the researchers studying the genes. Five of them encode transcription factors that regulate genes involved in insulin production, and the mutations turn down secretion of the hormone.

Only 2% or 3% of diabetes 2 patients have MODY. But in a paper published online on 19 March by the *Proceedings of the National Academy of Sciences*, a team led by Robert Hegele of the John P. Robarts

have found several hot spots in a genomewide scan for susceptibility genes in the Pimas and are now trying to pin down the genes involved.

There are encouraging signs that susceptibility genes picked up by these scans could provide good targets for antidiabetes

drugs. Certain variations in the gene for a transcription factor called PPAR- γ have been linked to a modest increase in diabetes risk, and researchers now know that members of a relatively new class of drugs, known as the thiazolidinediones, work at least partly by stimulating PPAR- γ activity.

Yet other drugs are urgently needed to treat the diabetes epidemic, because people are unlikely to cut back on food intake and start exercising anytime soon. Indeed, CDC has just found that more than half of the U.S. population exercises little or not at all.

—JEAN MARX

NEWS

Lupus: Mysterious Disease Holds Its Secrets Tight

Caused by an unruly immune system, lupus manifests itself in a variety of symptoms; researchers are beginning to learn what the triggers are

Lupus. Even the origin of the name is uncertain. According to one tradition, the disease was named lupus—wolf in Latin—because people afflicted with it had lesions that resembled wolf bites. According to another, a classic rash on the face created a wolfish appearance. It was not until 1851 that a physician gave it a medical appellation: systemic lupus erythematosus. Today, this complex disease remains a mystery in more than name.

The deepest puzzle lies at its core: Something in the lupus patient causes the immune system to go awry and turn its armamentarium of cell-killing forces against the host. For the more than 1 million people in the United States with lupus, symptoms can appear in a bewildering variety of forms, ranging from mild to lethal. The damage can affect almost any organ in the body, causing arthritis, fatigue, blood clots, heart disease, osteoporosis, kidney failure, and other life-threatening illnesses. Symptoms flare and recede over time, and more often than not, the disease produces a slow decline, including cognitive loss. Even professionals have trouble diagnosing it, and by the time a diagnosis is confirmed, the patient may have developed irreversible kidney damage.

The complexity of the disease also impedes clinical research. One symptom may be “cured,” only to be replaced by another that may be worse. Clinical trials are tough because it is hard to accumulate significant data if each patient seems unique, and clinicians grumble that drug developers are leery of lupus trials because the patients may have unrelated medical problems that look like side effects. Doctors

have been able to offer relatively few therapies, and those that are available, including corticosteroids and cytotoxic compounds, are also very risky.

But an explosion of new data promises to bring lupus research out of the doldrums. Molecular biology has unlocked a trove of information about factors that regulate the immune system. Using new mouse models of the disease, researchers have begun to identify the biochemical mechanisms by which lupus causes tissue damage, and they have identified a series of candidate genes that appear to be involved in lupus. Desperately needed money for clinical trials may also be on the way. In the past 2 years, new lupus organizations have opened shop, vowing to put all their money into peer-reviewed science (see sidebar on p. 690).

“We’re going to see a lot of activity” in lupus research, predicts Peter Lipsky, scientific director at the National Institute for Arthritis and Musculoskeletal and Skin Dis-

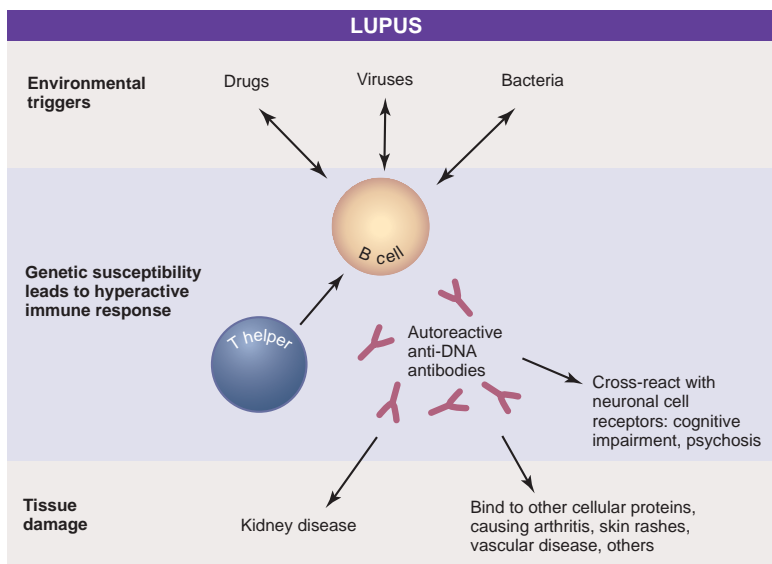
eases (NIAMS). He also predicts an intensified focus on emerging drug targets. Equally encouraging, Lipsky notes, is that scientists from disciplines other than immunology are entering the field, drawn by genetic and physiological discoveries. “You have a lot of new people in the mix: nephrologists, cardiologists, neurologists, hematologists.” A few biotech companies are also testing the waters, raising hope that less toxic drugs may soon be available.

War within

Anyone who investigates lupus encounters a striking fact, says Michael Lockshin, an immunologist who directs the Barbara Volcker Center for Women and Rheumatic Disease at the Hospital for Special Surgery in New York City: 90% of the patients are women. Black women are three times as likely to get lupus as white. And lupus strikes primarily between the ages of 15 and 40, during peak fertility. Because of this pattern, estrogen, the female sex hormone, has long been considered a key risk factor. But Lockshin says he has heard too many simple arguments blaming estrogen. Men get autoimmune diseases, too, Lockshin points out—including lupus. In some autoimmune diseases, males and females are equally affected. In others, males predominate. “There are so many anomalies” in the patterns of autoimmune disease, says Lockshin, that researchers should look beyond sex hormones.

Although scientists have proposed a smorgasbord of causes—and debate them endlessly—they agree on some fundamentals. Environmental factors such as estrogen and viruses are important, but just as critical are inherited genetic traits that make an individual’s immune system susceptible to dysregulation. Among twins of lupus patients, for example, monozygotic twins are about 10 times more likely to get the disease than dizygotic twins.

Animal studies suggest several ways this complex interaction between environment and genetics might lead to chronic disease. The dominant view



Self-destruction. Environmental factors such as viruses interact with inherited risks to create a flood of “self” antibodies that harm tissues.