THE FAT YOU CAN'T SEE

IN GROWING NUMBERS OF PEOPLE, THE LIVER HOLDS A HIDDEN, DANGEROUS STORE OF FAT. FINDING THE TRIGGERS IS STEP ONE.

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A SHINY, PINKISH-BROWN TRIANGLE TUCKED UNDER THE RIGHT RIB CAGE, A HEALTHY LIVER IS A MARVEL. NUTRIENT-RICH BLOOD FROM THE INTESTINES PULSES INTO ONE SIDE, AND THE LIVER GOES TO WORK REMOVING TOXINS, CONVERTING DIGESTED FOOD TO ENERGY, STORING VITAMINS AND MINERALS, AND CONTROLLING HOW MUCH FAT AND SUGAR IS SENT BACK OUT TO THE REST OF THE BODY.

Without the liver acting as a filter and energy producer, a person can't survive, and no artificial organ can perform all of its duties. But in one in three Americans—and similar numbers in other developed nations—the liver has lost its luster. It is swollen, yellowish, congested with fat, and doesn't function up to par. Over time, this condition, called fatty liver disease, can lead to inflammation, scarring, and hardening of the organ, and eventually, liver failure. In some people, it causes liver cancer.

"Fatty liver disease is the number one liver disease in this country in both adults and children," says HHMI investigator Gerald Shulman of the Yale School of Medicine. Rates of fatty liver disease have risen dramatically over the past two decades. "Furthermore, it is strongly linked to hepatic insulin resistance and type 2 diabetes," he says. Understanding these triggers and how the disease progresses may stop the uptick in its occurrence.

Scientists know that fat buildup in the liver is more common in people who are overweight, have type 2 diabetes, or drink excessive amounts of alcohol. But beyond that, not much about fatty liver disease is well understood. Does the diabetes or fatty liver come first? Do certain genes predispose people to fatty liver disease? How can the disease be detected early and treated? Do other diseases contribute to fat in the liver? These are only some of the questions that Shulman and others are pondering as they study who gets fatty liver disease and why.

"This is a slowly progressing disease," says hepatologist Rohit Loomba of the University of California, San Diego, School of Medicine. "If we want to prevent liver disease down the road, we need to act now." According to a 2008 estimate, fatty liver disease will be the leading reason for liver transplants by 2020, overtaking hepatitis C.

With a sense of urgency, scientists are pushing forward with studies on the basic biochemistry behind fatty liver. They are coming at the problem from different disciplines—genetics, endocrinology, immunology, and biochemistry—and their findings show a complex, sometimes contradictory story of what pollutes the liver with fat.

FROM FOOD AND GENES TO FAT

Fatty liver disease is rarely detected because of symptoms. Most often, a patient gets a routine blood test and the doctor notices altered levels of proteins made by the liver. Even then, there's no one test to give a definitive diagnosis of fatty liver disease. By ruling out other causes of abnormal liver proteins in the blood—such as a hepatitis infection or tumor—a doctor may conclude that the patient has fatty liver disease. If the patient is not a heavy drinker, then the diagnosis is nonalcoholic fatty liver disease (NAFLD). Today, more than three-quarters of fatty liver cases are NAFLD.

"It's an incredibly broad diagnosis," says HHMI investigator Helen Hobbs of the University of Texas Southwestern Medical Center. "It can mean anything from mild fatty liver to severe inflammation."

As recently as a decade ago, not much more was known about the prevalence of NAFLD, says Hobbs. Clinicians had a poor grasp of the overall incidence, how it progressed, or how to identify patients at risk for developing it in the first place. So when Hobbs launched the Dallas Heart Study, tracking the health of 6,000 individuals in the Dallas area, she and her colleagues included questions about all aspects of cardiovascular and metabolic health. Among topics such as cholesterol and diabetes, they also homed in on NAFLD.

"One thing we were very curious about was whether nonalcoholic fatty liver disease has a genetic underpinning," says Hobbs. As part of the study, Hobbs and her colleagues used a special type of magnetic resonance imaging (MRI) scan to quantify liver

fat in 2,349 participants. If the liver fat content was greater than 5.5 percent, the participant was classified as having NAFLD; about one-third of the total population fit the diagnosis.

"The biggest surprise for us wasn't the incidence—we had suspected it would be high," says Hobbs. "It was the differences among races."

Among Hispanics, her team found, 45 percent had NAFLD. But only 33 percent of European Americans met the diagnosis criteria, and only 24 percent of African Americans had NAFLD. Even when they factored in obesity and diabetes, there were still major differences in the rate of NAFLD among ethnic groups.

Hobbs and her colleagues delved into genetic data from the patients and tested whether any genetic mutations could explain the different frequencies of NAFLD. They discovered that one variant of a gene called *PNPLA3* seemed to predispose people to fatty liver. Moreover, the gene variant was most common in Hispanics, and least common in African Americans. It explained more than 70 percent of the differences in NAFLD incidence between races.

"Within all populations studied to date, the variation has been associated with fatty liver," Hobbs says. "If you have the risk allele of the gene, you tend to have higher triglyceride content in the liver."

In the liver, the PNPLA3 protein is responsible for breaking down triglycerides, the main building block of fats. So Hobbs suspected that the variant of *PNPLA3* stopped the protein from working, or from being expressed at all in the liver, which would lead to an accumulation of fats. In mouse studies, however, her team has shown that an excess of the variant PNPLA3 protein causes fatty liver. Hobbs' team is working to flesh out the mechanism involved. Solving the mystery will help uncover the biochemical pathways involved in fatty liver disease progression, but it's unlikely to explain the full story of fatty liver.

"This is a gene-environment interaction," says Hobbs. "If you have this gene and you're thin, you won't have fatty liver disease. But if you have this gene and you're obese, it is very likely you will."

THE DIABETES LINK

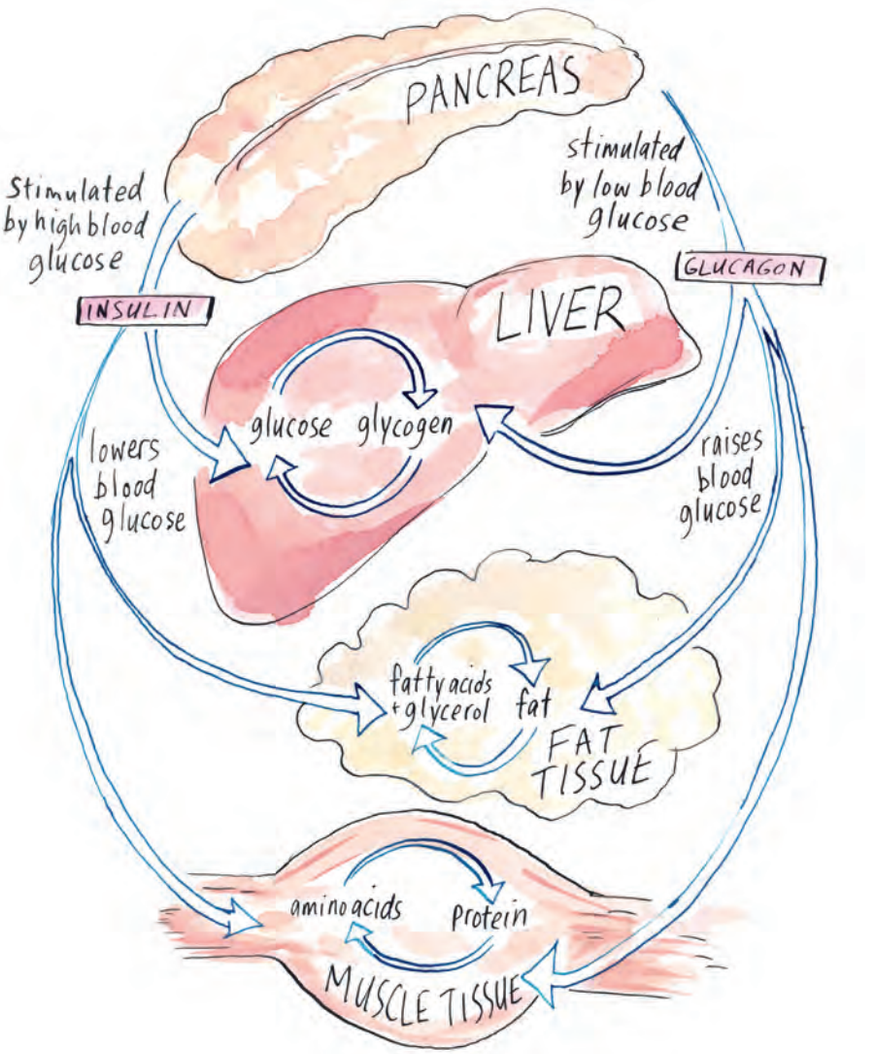
Even if there's a genetic component to NAFLD, that doesn't explain the increase in rates. The change in incidence can most likely be tracked back to changes in people's diets—particularly an increase in sugar consumption—over the past few decades, says Loomba, whose San Diego clinic sees hundreds of NAFLD patients a year.

"If you take any normal, healthy person and do an MRI of their liver, and then start giving them three cans of soda a day, you can scan their liver again two weeks later and see liver fat already going up."

Soda doesn't have fat in it, but a diet high in sugar changes the way the body deals with nutrients, including fat. Normally, the hormone insulin produced by the pancreas after eating a meal causes the liver to store sugars—taken up from the blood

Shulman: Brian Park Hobbs: David Cresham / UT Southwestern Medical Center Flavell: Yale University

HHMI investigators Gerald Shulman, Helen Hobbs, and Richard Flavell are each studying fatty liver disease from different perspectives and finding unique roads into the complex disease and its causes.



Normal Energy Storage & Use

WHEN YOU EAT, INSULIN PRODUCED BY THE PANCREAS PUTS YOUR BODY INTO STORAGE MODE TO SAVE ALL THE ENERGY YOU'VE INGESTED (LEFT SIDE OF DIAGRAM). IN THE LIVER, GLUCOSE IS CONVERTED TO GLYCOGEN FOR LONG-TERM STORAGE. IN FAT DEPOSITS AROUND THE BODY, MOLECULES COME TOGETHER TO FORM FATS. AND IN MUSCLES, THE BUILDING BLOCKS OF PROTEINS ASSEMBLE. ONCE YOU'VE DIGESTED THE FOOD, GLUCOSE AND INSULIN LEVELS DROP AND THE MOLECULES THAT HAVE BEEN ASSEMBLED FOR ENERGY STORAGE START BEING BROKEN DOWN AS YOUR BODY NEEDS THEM.

in the form of glucose and fructose—for later (see diagram). And some of the glucose is repackaged into fat molecules. But a diet high in sugar can lead to insulin insensitivity, or insulin resistance. The body, including the liver, becomes less efficient at responding to insulin's signals. Eventually, it stops responding at all. And rather than send the fat molecules into the blood, the liver retains the fat it produces.

"The liver is clearly central to normal glucose homeostasis," says Shulman, who is determined to sort out the intricate interplay between sugar and fat metabolism in the liver. The critical questions, he says, are what factors lead to the development of NAFLD and how do alterations in the liver's metabolism of fat contribute to insulin resistance in the liver.

Shulman's lab group has been studying the development of insulin resistance in muscle and the liver for nearly three decades. They want to know the precise sequence of events that occur when patients develop muscle insulin resistance, liver insulin resistance, NAFLD, and type 2 diabetes. So they have developed

novel magnetic-resonance scanning techniques to noninvasively measure the concentrations of metabolites within liver and skeletal muscle in patients who are prone to develop type 2 diabetes and in those with well-established type 2 diabetes. Using this approach, they have found that healthy, young, lean, insulin-resistant offspring of parents with type 2 diabetes, who have a high likelihood of developing type 2 diabetes, have insulin resistance only in skeletal muscle and not in the liver.

"We have shown that selective insulin resistance in skeletal muscle—the earliest defect we can observe in these otherwise young, healthy, lean individuals—can predispose them to hyperlipidemia, NAFLD, and liver insulin resistance by diverting ingested carbohydrate away from muscle, where it is normally stored as glycogen, to the liver, where it is converted to fat," says Shulman. "By screening patients for muscle insulin resistance, and understanding the progression to fatty liver and diabetes, researchers may be able to uncover new ways to stop these diseases in their early stages," he says.

In a separate study, Shulman and his colleagues studied healthy, young, lean individuals in the New Haven, Connecticut, community from five different ethnic groups and found a striking increase in the prevalence of NAFLD associated with insulin resistance in the lean Asian-Indian male volunteers. "Just about everyone will develop NAFLD if they become obese, independent of ethnicity," says Shulman. "But there appears to be something going on in the Asian-Indian men that predisposes them to develop NAFLD and hepatic insulin resistance at a much-lower body mass index."

To further investigate this question, Shulman's group teamed up with HHMI investigator Richard Lifton at Yale and found a different mutation than Hobbs' team had uncovered. They pinpointed mutations in a gene called *APOC3*. While Hobbs' PNPLA3 is involved in triglyceride breakdown within the liver, the APOC3 protein regulates the breakdown of triglycerides in the blood for storage in fat cells. Increased plasma concentrations of APOC3, which these gene variants have been shown to cause, will predispose these individuals to both NAFLD and increased triglyceride concentrations in the blood.

In transgenic mouse studies, Shulman's team found that mice overexpressing human *APOC3* developed both fatty liver and hepatic insulin resistance when fed a high-fat diet, supporting his theory that increased plasma concentrations of APOC3 predisposes an individual to the development of NAFLD, and that fatty liver can cause hepatic insulin resistance and contribute to the development of type 2 diabetes.

"I think the *APOC3* variants that we describe predispose lean individuals to NAFLD and hepatic insulin resistance reflecting a gene-environment interaction. Just having high plasma concentrations of APOC3 will not do anything in itself, as reflected by the lack of fatty liver and hepatic insulin resistance in the *APOC3* transgenic mice fed a regular chow diet," says Shulman. "But if you add a little bit of fat to their diet, they get fatty liver and hepatic insulin resistance." When the young, lean, Asian-Indian men with NAFLD lose a relatively small amount of weight, Shulman's team has found, their fatty liver and hepatic insulin resistance can be reversed.

Hobbs' and Shulman's evidence of two different genes with profound effects on NAFLD hints at the complexity of this metabolic disease. More research will be needed to sort out how these genes, and likely others, affect the development of this devastating syndrome.

BACTERIA'S ROLE

Doctors in the clinic need a way to identify patients who are at risk of developing worse forms of the disease—inflammation and scarring of the liver. After all, some people with fatty liver do just fine without treatment (although if Shulman is right, their fatty liver could be contributing to diabetes). In other patients, however, the liver becomes inflamed, clogged with immune molecules, and eventually begins to harden and stop working.

"We need to find out who is at risk for developing full-on liver disease and who isn't," says Loomba. "And then we need to focus our energy on stopping liver disease in those at risk."

Patients with more risk factors—diabetes, smoking, poor diet, and alcohol consumption—are more likely to develop worse stages of liver disease. But even without those risk factors, some patients end up worse off than others.

Now, a theory is emerging out of left field on why some patients may develop more severe inflammation in the liver. HHMI investigator Richard Flavell at Yale studies bacteria that inhabit people's guts. He recently discovered problems in the lining of the gut that lead to the body's inability to control these types of bacteria in mice with susceptibility to inflammatory bowel disease (IBD).

"Once we had found this problem in the gut, it occurred to us that it was unlikely to be limited to just the intestines," says Flavell. Researchers knew that the blood vessels between the intestines and liver of people with IBD often had leaks, letting unwanted material through.

"The function of these blood vessels is to carry food to the liver," says Flavell. "But we hypothesized that there could be a way in which the bad components of bacteria—or even whole bacteria—were traveling to the liver."

So he took mice with mutations that predispose them to IBD and put them on diets that usually lead to NAFLD. "We immediately saw that if the mice don't have this pathway in the intestines working, they get much worse fatty liver," he says.

The experiment, which included help from Shulman, was the first to suggest that NAFLD isn't just a metabolic disease, but could have a link to bacteria. The gut bacteria, while likely not causing fatty liver in the first place, could explain the inflammation that can lead—in a fraction of NAFLD patients—to more severe liver disease. Flavell plans to study how the bacterial populations in the intestines of people with NAFLD vary from those in healthy people. It's an angle of the disease that may someday lead

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to ways to identify patients at risk for severe liver disease, or to distinct treatment methods from those targeting obesity and blood sugar.

For now, though, scientists agree on the best way to treat—and reverse—NAFLD: weight loss, exercise, and a balanced diet.

In the same way three cans of soda a day can quickly lead to fatty liver, Loomba says, weight loss and exercise can rapidly reverse it. He's seen patients, within weeks of beginning an exercise regimen, show improvement in both liver fat levels and insulin resistance in liver cells.

"If we get patients with type 2 diabetes to lose relatively small amounts of weight by diet alone, we can cure most cases of fatty liver, hepatic insulin resistance, and type 2 diabetes," says Shulman. "But unfortunately getting patients to lose weight and keep it off is one of the most challenging clinical endeavors."

There are multiple drugs in clinical trials for treating later stages of liver disease—the inflammation and scarring—but no pill to cure early NAFLD. And because of the complex interconnections between diabetes, weight, and liver fat, a single cure-all drug targeting only the liver is unlikely to be discovered. But with each discovery of genes, molecular pathways, and bacteria that contribute to the disease, scientists feel they are getting closer to being able to identify patients most at risk and help lessen the severity of their disease.

"I think we have probably just moved from the infancy to the adolescence of understanding this disease," says Loomba. "The hope is that our understanding of fatty liver is really going to explode in the next five years. I think we are on the cusp of something big." ■

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(THE VIEW FROM HERE)

genetically induced changes. He aims to learn how neural circuits associated with those genetic variations produce and control specific animal behaviors.

Jensen is pushing computational reconstruction of reality in pursuit of scientific understanding in yet another direction. He and visualization experts in his laboratory have been transforming their electron cryotomography data into mechanism-revealing animations. Says Jensen, "If a picture is worth a thousand words, then an animation is worth a million."

"We have done movies where we fly into cells and look at things from different points of view," says Jensen. "We have tried to illustrate mechanisms of how things work inside cells." He believes animations can be powerful educational tools. "Some people still don't believe that HIV is the causative agent of AIDS, for instance, so they decline treatments that could help them," says Jensen. "Our animations of HIV's molecular transformations will help them understand that we are not making this up."

As researchers move through the early part of Schnitzer's third phase of biological imaging, Jensen is already imagining what might be a fourth phase. "The ultimate would be a microscope where you could see down to individual atoms while they were being arranged and rearranged inside a cell in its living state," Jensen muses. "If you could develop an imaging tool like that, the study of cell biology would be all but over," Jensen says, noting, however, that it would take a century or more to digest such a comprehensive portrait of life. An outlandish vision for revealing biology's deepest structures and mechanisms, to be sure, but that is just the sort of thinking it takes to unveil molecular biology in all of its elaborate minutiae. ■