The mystery behind diabetes

Over the course of twenty years, project unravels the link between diet and insulin resistance in the body

Maria Guimarães

After twenty years of scrutinizing the biochemical mechanism of bodies that develop Type 2 diabetes, physician Mario Saad from the State University of Campinas (Unicamp) discovered that genetics is perhaps the least important part. "We are what we eat minus the amount of exercise we do plus the type of bacteria we have in our gut," he summarizes. He admits that it's a nice aphorism for a physician to use in recommending a lifestyle for patients that protects them against the disease – one that affects 350 million people all over the world and causes problems such as obesity, risk of amputation and even death – or that slows its progression.

But it is not just a contrivance for convincing patients: the conviction that the outside environment and intestinal bacteria play a more significant role than genetic protection in the emergence of obesity and diabetes is based on solid research. In an article published in the December 2011 issue of *PLoS Biology*, Saad's group revealed that the bacterial population found in any human intestine, besides aiding in digestion, may also contribute to increasing the cells' resistance to the insulin hormone, a precursor condition to diabetes. When the problem settles in, the insulin – even if present in high concentrations – loses its ability to tell the cells of muscles and other tissues that remove glucose from the blood to either

RAMESH AMRUTKAR/IMAGEBROKER/GLOW IMAGES

A villain when in excess, sugar needs to be removed from the blood

store it or convert it into energy. In experiments with mice, the Unicamp team discovered that the problem can be caused by an atypically high proportion of bacteria of the phylum Firmicutes, a group made up of dozens of species, which along with other groups of bacteria constitute the gut microbiota. These results, which were part of the doctoral research of biologist Andrea Caricilli, garnered so much attention that in the four months that followed publication of the article, the journal's site received over 13,500 hits.

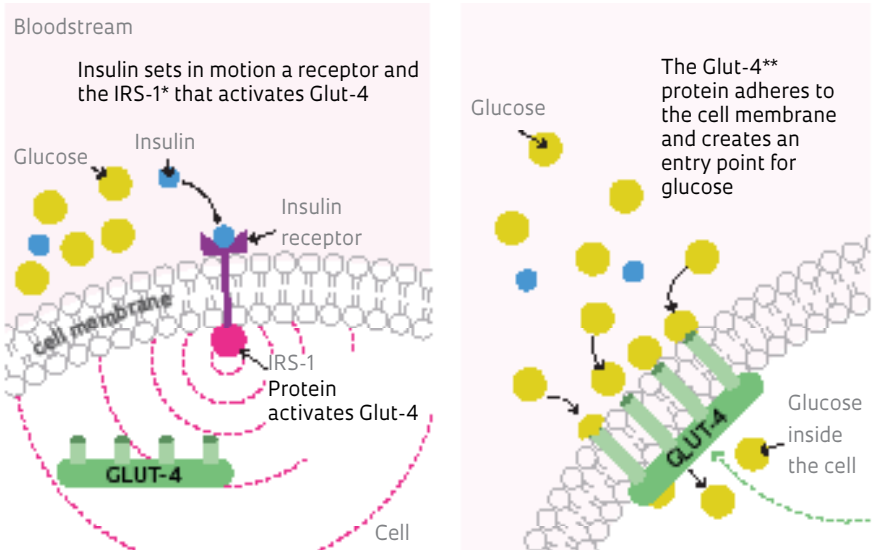
"Gut microbiota is not the only thing that causes diabetes and obesity, nor is it likely to be the most important," says Saad. "But we determined that it contributes to generating inflammation in the adipose tissue that begins a process of abnormal weight gain, which then begins to perpetuate itself." The Firmicutes seem to facilitate the passage of molecules through the intestinal walls. These molecules are released when other bacteria break open. The lipopolysaccharides (LPS), which are made up of sugars and fats, set in motion biochemical signals that activate the immune system in the rest of the body. This causes a sub-clinical (asymptomatic) inflammation that is typically seen in the obese. When obesity settles in, alterations in the intestinal wall further increase LPS permeability and reinforce the immunological reaction that in the cells of the liver, muscles and adipose tissue also trigger insulin resistance. This then adds force to the connection between diabetes and obesity.

The protagonist of the story is a protein from the family of toll-like cell receptors (TLRs) that identify molecules that are foreign to the body and stimulate the immune system reaction. In this case, the experiments were conducted on mice that did not have TLR-2. In an experiment conducted by Canadian and Swiss researchers, these mice that were raised in a sterile environment did not gain weight or develop diabetes, even when fed a calorie-laden diet. In Saad's laboratory, the opposite occurred. The mice became obese and diabetic, even though they received the same portions of food as the other rodents. The difference was in the environment. It was not sterile – a situation closer to that encountered by most bodies that live outside the laboratory, people included.

Genetic analyses of the mice feces revealed a very different microbiota, where nearly half were Firmicutes bacteria. In the other animals, that proportion was 14%. "The toll-like

How the body uses sugars

The body uses insulin to capture glucose from the bloodstream and convert it to energy or fat



* Insulin receptor substrate-1

** Glucose-4 transporter

receptors predispose the intestinal microbiota to one or another type of bacteria," explains the physician, who had already obtained different results with TLR-4. When treated with antibiotics, the mice went back to having a normal proportion of bacterial types and recovered insulin function. "We thought we would find all the answers in the Human Genome Project, but now we need to sequence the genome of the bacteria to see how they interact with the human body."

INTERNAL REBELLIONS

Bacteria's contribution to the development of diabetes reminds us of how foreign bodies act when they invade the body. But these microorganisms are actually within a much broader context of biochemical control of sugar metabolism that can go wrong for a whole host of reasons, and this area of inquiry has been the substance of Saad's career. "What we knew was that insulin fit together with the cell receptors and that what happened had a biological effect," he jokes, summarizing the state of knowledge in the early 1990s, the time of his post-doctoral studies at the Joslin Diabetes Center, part of Harvard Medical School in the U.S. It was during his stay there that the insulin receptor substrate (IRS-1) was identified. This discovery was the first step towards understanding the molecular mechanism of hormone resistance that was responsible for balancing the level of glucose in the body.

It is partly the defects in this mechanism that cause Type 2 diabetes, where the pancreas produces sufficient amounts of insulin, but is unable to activate the cell machinery responsible for capturing glucose. Part of the problem occurs in the insulin receptors in the brain cells, more specifically, in the hypothalamus. Signaling problems in this part of the body can deregulate the appetite, for example, causing a tendency to overeat. What is now a defect in function may once have been important in human evolution, Saad explains. "Hunger and the epidemics caused by infectious diseases, which were the main causes of death among our ancestors, may have selected genes that promoted energy storage and rapid response to infections." The accumulation of fat itself can be seen as a survival mechanism in cases of food shortages, a common occurrence at the time when humans lived in caves. Today, however, the body retains the command to eat a lot, even though food is not always scarce.

More serious still is that the order to store ends up becoming chronic, partly because the mechanisms go well beyond what happens in the brain, as demonstrated over the years by studies conducted by Saad's team, which today includes groups led by researchers Lício Velloso and José Barreto Carvalheira, also from Unicamp. Just like what happens in the hypothalamus, in a period of ten days, the muscle cells in mice fed a diet rich in fats become resistant to insulin. Then the problem goes on to affect the liver and blood vessels that are also damaged by the high levels of circulating glucose in the body. But it takes more than just the extravagances of Christmas meals or some gastronomical holiday for the incapacity to respond to the hormone and capture blood glucose to spread to the adipose tissue – composed of cells that specialize in the accumulation of fat. For this, laboratory rats at Unicamp needed five months of a diet that simulated bad western eating habits with their high caloric consumption. That is what gives rise to the obesity frequently associated with diabetes.

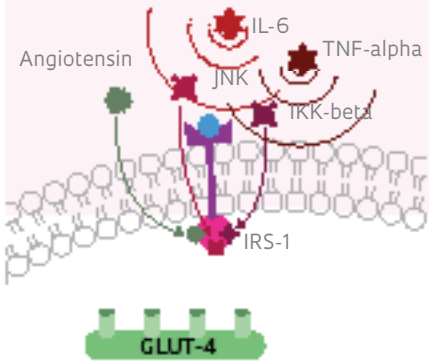
Studies increasingly find that obesity is an important risk factor that acts in the development of diabetes in several ways. One link is the angiotensinogen produced in the adipose cells. This protein is a precursor to angiotensin, the molecule that plays a central role in controlling blood pressure. In 1995, during his post-doctorate with Saad, Lício Velloso became deeply involved in studying the problem and ended up demonstrating that angiotensin also inhibits insulin action, through alterations in IRS-1. It established a clear connection between obesity, diabetes and hypertension, a trio that usually go hand in hand. In addition to this, the group also demonstrated – through the work of Carla Carvalho, another post-doctoral researcher – that anti-hypertensive drugs that reduce the proportion of angiotensin also contribute to better insulin functioning, causing an improvement in the condition of diabetes. The significance of the discovery went far from unnoticed by the scientific community. In 1996 it was published in *PNAS* and today it is the article written

Where diabetes comes from

Insulin resistance has many causes

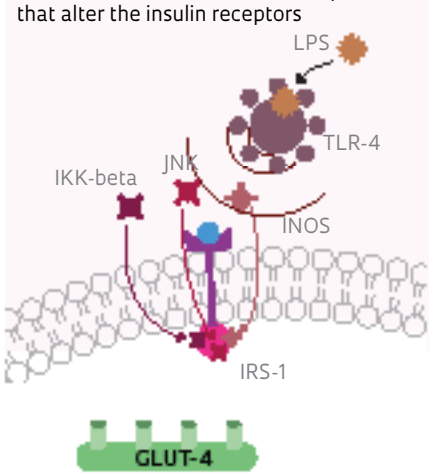
ADIPOSE TISSUE

Inflammatory factors (IL-6 and TNF-alpha) activate proteins that adversely affect the functioning of the IRS-1. Angiotensin contributes to inactivation of the receptors



INTESTINE

The LPS* released by bacteria set in motion the TLR-4** that activate proteins that alter the insulin receptors



* Lipopolyssacharides ** Toll-like receptor

INFOGRÁFICO TIAGO CIRILLO, FONTE: MARIO SAAD/UNICAMP

by Saad's group most often cited in academic publications.

Fat cells are also known to be the stage for assaults on the immune system that generate the characteristic inflammation seen in the obese. "'You have a slight inflammatory condition' may be the politically correct way of saying that a patient is obese," jokes Saad. He adds that the adipose tissue produces cytokines – inflammatory factors such as interleukin-6 and TNF-alpha – that attract macrophages, which are the immune system cells responsible for destroying invader particulates through phagocytosis (in generic terms, they swallow and digest these unwanted visitors). Although they do not cause pain or detectable changes in temperature – both common symptoms of inflammatory reactions – these substances activate the JNK and IKK-beta enzymes, which in turn alter the configuration of IRS1, which is another factor that causes insulin resistance.

Showing that these relationships are never linear and that the body operates as a highly complex network, these enzymes – as well as inducible nitric oxide synthase (iNOS) – are also activated by the cell membrane proteins TLR-4. They in turn respond to the lipopolysaccharides released by the gut microbiota dominated by Firmicutes, the same bacteria that contribute to obesity and diabetes. The Unicamp group demonstrated the TLR-4 protagonism with experiments using mutant mice whose receptor for this protein is not functional: these animals were able to eat their fill of a fatty diet and still not gain weight. Just the opposite occurred in the same laboratory, years later, with mutant animals and TLR-2. Even when produced in large quantities, the LPS did not cause problems. The significance of TLR-4 in the link between a diet full of fried foods and the development of diabetes was part of the doctoral study of Daniela Tsukumo. Its findings, published in the journal *Diabetes* in 2007, were those by Saad's group most cited in academic articles.

This discovery of the toll-like receptor family role is important for understanding how the disease works, but still falls short of developing therapies to control diabetes. "A TLR-4 blocker may help, but it plays an important role in controlling infections so we can't block the molecule entirely," Saad explains. For now, the physician celebrates an achievement that although simpler, brings real relief to his patients: an insulin-based cream that speeds wound healing, reducing the risks of common amputations in advanced cases of diabetes. The description is about to be published in *PLoS One*.

Saad compares this intricate mystery to the poem entitled "Truth," by Carlos Drummond de Andrade. The truth is always divided into two halves that do not fit perfectly together. Those who seek it will have access to only one of the halves, and if they try to select the 'most attractive' one, they will choose wrong. The determined physician (from Minas Gerais State like Drummond) continues to fit together the pieces of truth that constitute the metabolism of diabetes, to perhaps one day find more solutions. ■

PROJECTS

1. *Insulin resistant molecular mechanisms in the hypothalamus and peripheral tissues* – No. 2001/03176-5 (2002-2006)
2. *National Institute of Obesity and Diabetes* – No. 2008/57952-5 (2009-2014)

GRANT MECHANISM
1. and 2. Thematic Project

COORDINATOR
1. and 2. Mario José Abdalla Saad – FCM/Unicamp

INVESTMENT
1. R$1,116,696.66
2. R$3,241,614.71

SCIENTIFIC ARTICLES

1. VELLOSO, L. A. *et al.* Cross-talk between the insulin and angiotensin signaling systems. PNAS. v. 93, n. 22, p. 12490, 1996.

2. TSUKUMO, D. M. L. *et al.* Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes. v. 56, n. 8. p. 1986-98, 2007.

3. CARICILLI, A.M. *et al.* Gut microbiota is a key modulator of insulin resistance in TLR2 knockout mice. PLoS Biology. v. 9, n. 12, e1001212, 2011.

FROM OUR ARCHIVES

From obesity to diabetes
Issue no. 82 – December 2002

High-risk diet
Issue no. 140 – October 2007

Visceral connections
Issue no. 193 – March 2012