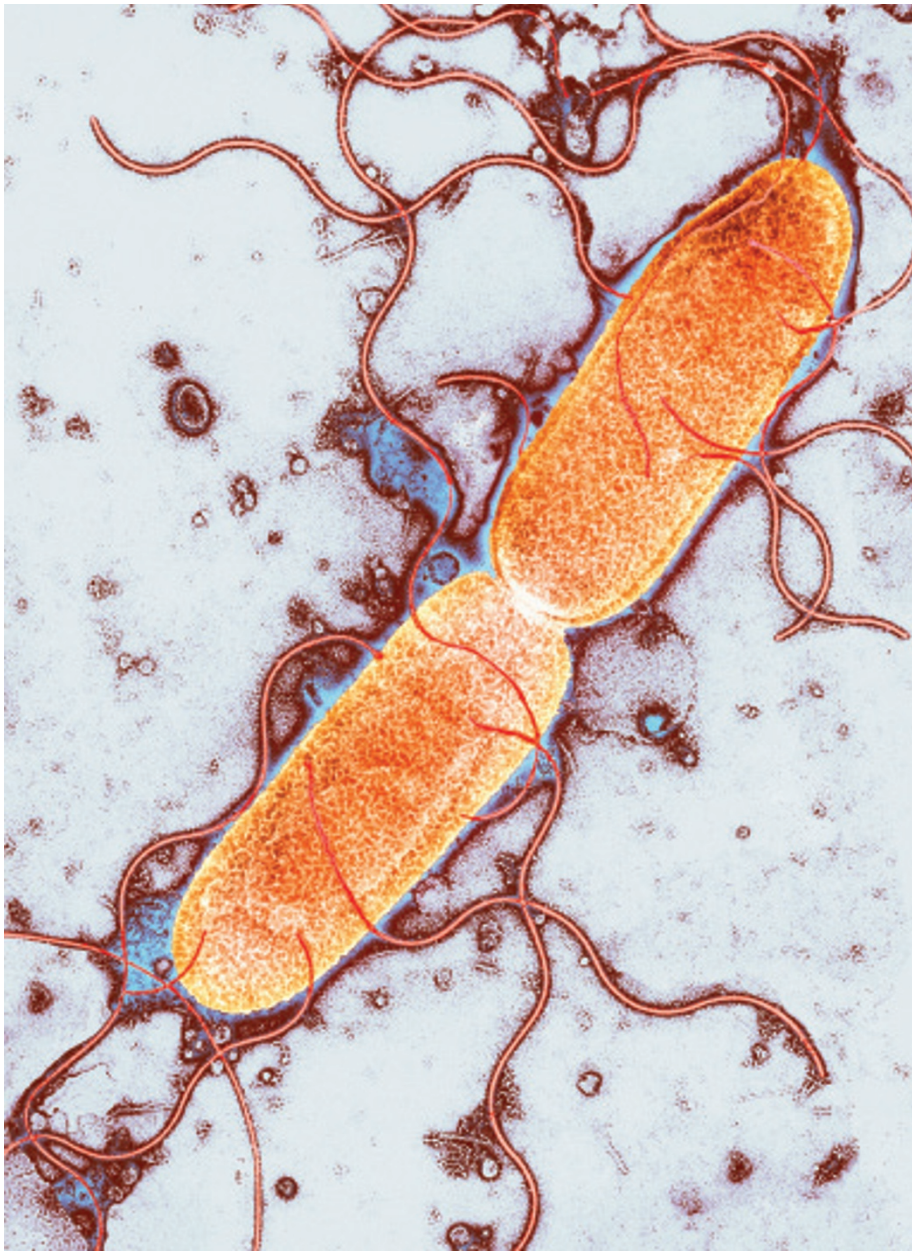

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Some of the roughly 1,000 bacterial species in the human gut help make us fat, while others keep us lean.

MICROBIOME

A complicated relationship status

Nothing is simple about the links between the bacteria living in our guts and obesity.

BY SARAH DEWEERDT

A few years ago, Liping Zhao, a microbiologist at Shanghai Jiao Tong University in China, put a man with a body mass index of 58.8 — classified as very severely obese — on a strict diet. Over the course of 6 months, the man shed more than 50 kg. In addition, a group of bacteria known as *Enterobacter* became undetectable in his stool samples, even though they had previously made up 35% of the microbes in his gut[1].

The decline and fall of a set of bacteria might seem incidental to the man's impressive weight loss, but Zhao and many other researchers say that the human gut microbiota — the assortment of 1,000 or so species of bacteria that inhabit our digestive tract — has an important role in regulating body weight.

"It's not calories alone," Zhao says, that determine whether a person is obese. To keep the weight down, "You also need to take care of the nutritional needs of beneficial bacteria in the lower gut." Similarly, some components of a healthy diet may curtail the growth of obesity-promoting bacterial strains.

Researchers are still unravelling the relationship between diet, gut microbes and body weight. "There are a lot of studies in humans, but those are only associations. There are a lot of studies of causation, but those are only in animals," says Fredrik Bäckhed, a researcher at the University of Gothenburg in Sweden who investigates the gut microbiota using mouse models.

The task now, say Bäckhed and others, is to translate results from studies of lab mice into treatments for humans in the real world.

That is far from straightforward. Last year, Zhao conducted a clinical trial of the dietary regimen that caused the dramatic weight loss in his severely obese subject, including whole grains, traditional Chinese medicinal foods and 'prebiotics' — supplements that promote the growth of beneficial gut microbes. After 9 weeks, the nearly 100 study participants had improved markers of metabolic health and lower levels of potentially harmful bacteria, including *Enterobacter*, but they only achieved a modest weight loss of about 6 kg on average[2].

But clinical trials into microbe-based interventions are just getting started, which is not surprising given the fact that serious research connecting gut microbes to obesity began scarcely a decade ago.

GUT REACTION

The first clues to this relationship came from mice that lack the gene for leptin, a hormone that regulates appetite. These mice eat insatiably, and as adults typically weigh three times as much as normal mice.

In 2005, researchers led by Jeffrey Gordon at Washington University in St Louis, Missouri, reported that the gut microbiota of leptin-deficient mice contains about 50% fewer Bacteroidetes and 50% more Firmicutes, each a major group of bacteria, compared with normal mice[3]. "This was the first direct evidence that

there were differences in the microbial communities between lean and obese mammals," says Robin Knight, a computational biologist at the University of Colorado, Boulder, who collaborated on the study.

The following year, researchers in Gordon's lab identified the same pattern in humans: obese individuals have fewer Bacteroidetes and more Firmicutes than lean people. Moreover, the proportion of Bacteroidetes increases when individuals lose weight[4]. In fact, shifts in the balance of these two types of bacteria crop up again and again in research into the gut microbiota, diet and obesity.

As DNA sequencing becomes faster and cheaper, researchers have begun to look at gut microbes in finer detail. These days, they are analysing not just the kinds of microorganisms present in the gut, but also the genes that those microorganisms carry. (Scientists generally refer to the collection of bacterial species present in the gut as the microbiota, and the collection of genes as the microbiome.)

In 2013, a group of researchers collaborated with the MetaHIT Consortium, a European effort to determine the associations between gut microbes and chronic diseases, to sequence the microbiomes of 169 obese and 123 non-obese individuals[5]. They found that people fell into two groups that differed in the diversity of the microbial genes represented in their guts. Those with fewer genes tended to have more body fat and other markers of poor metabolic health compared with people with a more diverse microbiome.

Similarly, Knight and his colleagues calculated that microbial genes sort the lean from the obese with 90% accuracy, whereas looking at human genes yields the right answer only 58% of the time. "Microbial genes are a much better readout of whether you're likely to be obese or not than human genes are," Knight says.

CAUSE OR EFFECT?

But just showing that people have different microbes is not enough. "You don't necessarily know whether the microbial changes are a cause or an effect of the obesity," Knight points out.

So another line of research is aimed at establishing causality. "The strongest pieces of evidence are the mouse studies that have been done where the microbiota of an obese mouse is transferred to a germ-free mouse," says Rosa Krajmalnik-Brown, a microbiologist at Arizona State University in Tempe.

Germ-free mice, which lack gut microbes altogether because they are delivered by Caesarean section and raised in special aseptic cages, have lower body fat than conventionally raised mice. Gordon and his colleagues have found that when a germ-free mouse is colonized with gut microbes from a normal mouse, it experiences a 60% increase in body fat over the course of 2 weeks — despite eating less food than it did before the transfer[6].

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Impact of dietary intervention on microbial genes:
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"That provided the first mechanistic evidence that something about the microbes in our gut is increasing our ability to store body fat," says Peter Turnbaugh, a systems biologist at Harvard University in Cambridge, Massachusetts, who worked as a postdoctoral researcher in the Gordon lab.

Furthermore, the microbiomes of obese individuals have a different effect than those of normal-weight mice. "They gain about twice as much body fat over the course of two weeks if you colonize them with a sample that comes from an obese donor," says Turnbaugh. "And that can be from a mouse that's obese because of a genetic mutation in leptin, or mice that are obese due to consuming a high-fat, high-sugar diet." Researchers have even shown that germ-free mice that receive gut microbes from an obese human donor gain more weight than those that receive them from a lean person[7].

But not everyone finds these data convincing. Germ-free mice given obesity-associated microbiota gain weight, but they do not actually become obese themselves, points out Eric Martens, a microbiologist at the University of Michigan Medical School in Ann Arbor. "The magnitude of the change never really comes back to anything above what a normal mouse would have," Martens says. "You're not looking at transplantable obesity."

Moreover, diet is a major factor in obesity, and diet also shapes the microbiota. Often, changes in the levels of gut microbes produced by healthy or unhealthy diets are broadly similar to the differences seen in lean versus obese individuals. "So you already have this diet-to-microbiota relationship that's difficult to disentangle from the microbiota-to-obesity relationship," Martens says.

Others say the tight coupling of diet and the microbiota is the point. Zhao has shown that mice colonized with *Enterobacter cloacae* B29, a bacterial strain isolated from his obese patient, become obese themselves if they are fed a high-fat diet, but not if they are fed a normal diet. "As microbiologists we know for a pathogen to cause a disease you need many things," Zhao says. "First you need the pathogen, but then you also need the right environmental condition to trigger the problem."

Demonstrating similar causality in humans will require additional work, however. "What we really need is prospective studies where we see an altered microbiome before the disease onset," says Bäckhed. Several groups are now beginning these investigations.

A BUG'S LIFE

Meanwhile, these questions have not stopped researchers from starting to look at microbe-based approaches to treating obesity. But for this to be more than educated guesswork, scientists will have to figure out the precise molecular and biochemical mechanisms that link diet, gut microbes and body weight. They will also need to identify the particular bacteria, at species level, that may be involved.

Early work in this area suggested that the genes and biochemical pathways characteristic of obesity-associated microbiomes are more efficient at extracting energy from food than are those of normal-weight individuals. Essentially, an obese mouse gets more calories out of a cup of mouse chow than a lean mouse does. "Our hypothesis at that time was basically about energy harvest, that the microbiome helps to digest carbohydrates that would otherwise be indigestible to the host," recalls Bäckhed, who worked on these questions as a postdoc in the Gordon lab.

But the picture soon got more complicated, as Bäckhed and others found that germ-free mice do not become obese when fed a Western-style diet high in fat and simple sugars — components of food that gut microbes have only a minor role in digesting. "The germ-free gut was still protecting these mice against obesity, suggesting that there are other mechanisms at play as well," says Bäckhed.

"Microbial genes are a much better readout of whether you're likely to be obese or not than human genes are."

Researchers have since shown that the gut microbiota can affect the body's signalling systems related to hunger and feeling full[8], and even how quickly food passes through the gut[9]. Other studies have traced how diet and microbes can interact to produce inflammation and an impaired gut barrier, or 'leaky gut', which may contribute to obesity[10].

In mice, it is possible to reverse many of these effects with prebiotics[11], the most widely used of which is oligofructose, a type of indigestible carbohydrate found in foods such as bananas, garlic and Jerusalem artichokes. "We found that mice fed with oligofructose had an improved gut barrier function," says Patrice Cani, a researcher into metabolism and nutrition at the Catholic University of Louvain in Belgium. The mice that were given prebiotics also had improved metabolic markers, reduced fat mass and reduced inflammation, Cani adds.

OF MICROBES AND HUMANS

It is not clear, however, how well these outcomes translate to humans. Last year, Cani and his colleagues reported that obese women who took a supplement of oligofructose and a similar substance called inulin every day for three months showed a slight decrease in fat mass and a reduction in blood levels of an inflammation-promoting molecule[12]. But the results "were not really equivalent to the ones we observed in mice", Cani says.

Prebiotics are only one of several strategies to manipulate the gut microbiota. Other

LEAN OPERATION

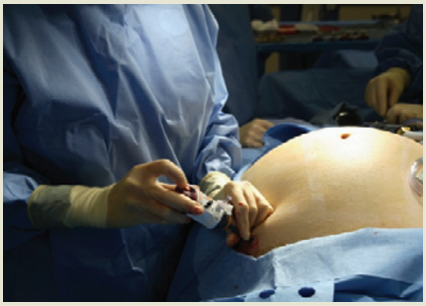
Does the microbiota determine the success of gastric surgery?

One of the most drastic approaches to weight loss in obese individuals is gastric bypass surgery. Surgeons make the stomach smaller and reroute the small intestine, reducing the body's ability to absorb nutrients from food.

It is a profound reorganization of the digestive system. Yet recent studies suggest that a major portion of the weight loss triggered by bypasses — commonly 4.5–9.0 kg per month for the first year — may be attributable to changes in the gut microbiota that occur after surgery.

A study of three normal-weight individuals, three obese individuals and three people who had undergone gastric bypasses provided the first evidence for this idea. "We analysed the bacterial communities in the three groups, and they were really different," says study co-leader Bruce Rittmann, a professor at the Biodesign Institute at Arizona State University in Tempe. "Each community was relatively similar within the group, but the groups were very different from each other."

Despite the study's small size, the differences were statistically significant. "Patients with a gastric bypass had a higher population of Gammaproteobacteria," says Rosa Krajmalnik-Brown, a microbiologist at Arizona State University and the other co-leader. This large group of microbes includes, for example, the familiar gut bacterium *Escherichia coli*. "That was one of the most striking differences."



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It is not yet clear why these shifts occur, but researchers hypothesize that the changes in gut anatomy, together with different dietary habits post-surgery, may favour the growth of different bacteria in the gut. Bolstering the observations in humans, similar changes have been found in the microbiota of rats and mice that undergo gastric bypass operations. In fact, if gut microbes from a post-surgery mouse are transferred to a mouse lacking gut microbes, the recipient mouse loses weight[13].

That is particularly striking because such mice, without any gut microbes to help them extract energy from food, start off with a lower than normal percentage of body fat. "It's surprising that given the right configuration of microbiota you can actually drive their weight even lower," says study team member Peter Turnbaugh, a systems biologist at Harvard University who works on gut microbes.

But some individuals who undergo gastric bypass surgery regain much of their weight after a few years. In an ongoing study, Rittmann and Krajmalnik-Brown are analysing the gut microbiota of up to 40 individuals several years after gastric bypass surgery to try to identify differences in the microbiota of successful and unsuccessful patients.

The team also plans to follow about ten new gastric bypass patients to track the changes in their gut microbiota after the surgery. "We're trying to find a biomarker," Krajmalnik-Brown says. "That would be really great if we could increase the success rate of surgery just by knowing the characteristics of the gut microbiota that are linked to success." Perhaps one day these patterns will yield insights into how to manipulate the microbiota to produce weight loss even without surgery. ***S.D.W.***

possibilities include faecal transplantation — in which communities of bacteria from one individual are given to another — or consuming beneficial bacteria as probiotics.

But so far, these other approaches have also worked better in mice than in humans. For example, a small faecal transplantation study found that people who received microbiota from a lean donor experienced improvements in insulin sensitivity, but no change in body mass index.

The top candidate for a bacterial species that could be given as a prebiotic to decrease body weight is *Akkermansia muciniphila*. This bacterium is found in most people's guts, but at lower numbers in obese individuals. When people lose weight after gastric bypass surgery (see 'Lean operation'), *A. muciniphila* flourish.

Mouse studies suggest that *A. muciniphila* strongly affects body weight. "If you take a normal mouse and give it a high-fat diet, it becomes obese. That's not rocket science," says Willem de Vos, a microbiologist at Wageningen University in the Netherlands, who was part of the team that discovered the species in 2004. "But if you give a high-fat diet and you give *Akkermansia* at the same time, there's no obesity."

Now, de Vos and his collaborators are beginning a clinical trial in which obese patients will be given *A. muciniphila* to see if the bacterium can help them to lose weight.

It is probably too soon to get excited. Even if the science is straightened out, it will be hard to develop an effective microbiota-based weight intervention for humans. Lab mice, after all, live tightly controlled lives, whereas we are constantly surrounded by temptation. "It's so easy to be a nibbler," as Bäckhed puts it.

People are also a lot more genetically diverse than lab mice. As a result, the effectiveness of diet and exercise interventions for obesity varies greatly between individuals, and the same is likely to be true for microbe-based treatments. "If you put two people on the same diet they're not going to come out with the same intestinal microbiota," says Krajmalnik-Brown. A person's initial microbiota, metabolism and even their gut anatomy may influence the results of a prebiotic or probiotic regimen.

> ***"Lab mice live tightly controlled lives, whereas we are constantly surrounded by temptation."***

It may eventually be possible to analyse a person's existing microbiota to predict the effectiveness of various treatments. Already scientists have shown that individuals with a gene-rich microbiome respond differently to diets than those with fewer genes in their gut.

But that is still far away. "What we really don't know in humans is the question that is most relevant for a person who is obese, which is how much of your body fat is really caused by gut microbes," says Turnbaugh. "And that might vary a lot from person to person." So microbes may punch above their size, but the relationship between them and us is what finally matters, and that remains, well, complicated. ■

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