Mice without a microbiota are used to probe the role of gut bacteria in non-alcoholic fatty liver disease.

GUT-LIVER AXIS

Menace in the microbiota

Modifying the population of bacteria in the gut might help to prevent and treat non-alcoholic fatty liver disease.

BY ANDREW SCOTT

Supermarkets worldwide offer an extensive range of drinks, yoghurts and dietary supplements that are sold with the claims of being able to help populate the gut with beneficial bacteria and to displace others that may cause harm. Such probiotic bacteria could potentially be used to prevent or treat non-alcoholic fatty liver disease (NAFLD), which involves the accumulation of fat (steatosis) in liver cells, as well as a more advanced form of the condition called non-alcoholic steatohepatitis (NASH), according to Azita Hekmatdoost, a nutritionist at the University of British Columbia in Canada. She gathered evidence to support this conclusion as a co-author of a 2017 review article¹ on the role of gut bacteria in NAFLD and its progression to NASH.

“It seems that at least one of the factors involved in the pathogenesis of NAFLD is an overload of pathogenic bacteria in the gut,” Hekmatdoost says. Reducing that overload would therefore help to improve the health of the liver.

The most probable mechanism for producing this effect, says Hekmatdoost, involves lipopolysaccharides (also known as endotoxins), which are structural components of bacteria that are composed of carbohydrate and fat. These molecules, she says, enter the part of the circulatory system that takes blood from the gut to the liver. Inside the liver, they bind to receptors on the surface of cells of the immune system, inducing inflammation and resistance to insulin.

Hekmatdoost and her colleagues have conducted clinical trials that aimed to assess the potential of probiotic supplements for reversing the problematic effects of lipopolysaccharides in people with NAFLD. Probiotic bacteria, she notes, “improved the insulin resistance, hepatic steatosis and fibrosis, not only in obese patients with NAFLD, but also in lean patients”.

A HIDDEN WORLD

The human gut is home to trillions of bacteria, which collectively contain more than 100 times the number of genes that make up the human

genome. Given this enormous presence, it is unsurprising that the activities of microorganisms can exert a considerable influence on people’s health. The microbial genes often encode proteins such as enzymes that, despite not being made by the body, have an active role in its processes. These proteins, as well as the smaller molecules that they create, are crucial players in the biological drama that proceeds as food and fluid are consumed and then move through the digestive system.

Many lines of evidence suggest that gut bacteria can affect the development of NAFLD. For instance, changes in the composition of the population of bacteria — known as dysbiosis — have been associated with several damaging effects in the liver.

Comparing the combined population of bacteria found in the gut of people with NAFLD to that of healthy individuals reveals a number of differences that have the potential to influence the condition. For example, some of the types of gut bacterium that are common in people with NAFLD are more efficient at metabolic

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activities that make energy available from food. These bacteria could therefore contribute to obesity — which has been established as a main risk factor for NAFLD.

More specific and subtle problems are linked to endotoxins. Dysbiosis not only increases their release from bacteria in the gut, but also makes the wall of the intestine more permeable to these entities. As a result, a greater amount of endotoxins enter the circulatory system to reach the portal vein that leads to the liver.

Dysbiosis is also involved in creating a deficiency in a small multiprotein complex that regulates the inflammatory response to infection and damage. The disturbance in the collective microorganisms (microbiota) of the gut enhances damaging inflammation and enables more endotoxins to reach the liver.

Another consequence of dysbiosis seems to be an increase in the size of the population of bacteria that produce ethanol. So, although the name NAFLD contains the term 'non-alcoholic', the reality of the condition is more complicated — alcohol from bacteria may still play a part in its origins.

The details and relative importance of the proposed mechanisms by which dysbiosis causes liver damage are unclear. But the existence of a link between changes in the gut microbiota and NAFLD is gaining acceptance.

CAUSE AND CORRECTION

A key question concerns the direction in which the arrow of causality points. One experimental approach in animals has provided strong evidence that dysbiosis is a cause rather than a consequence of NAFLD. In 2013, researchers led by microbiologist Philippe Gérard at the Micalis Institute in Jouy-en-Josas, France, transplanted faeces containing gut bacteria from a mouse with NAFLD into mice without the condition. The recipient mice, which were of normal weight, went on to develop the symptoms of NAFLD.

These results led the researchers to reach two main conclusions²: first, gut bacteria can indeed promote NAFLD; and second, this transmission can occur even in mice that were not obese. Gérard has also participated in a study that focused on alcohol-related liver disease, in which researchers transplanted gut bacteria from affected people into healthy mice. The team "reproduced the disease", suggesting that there is a more general link between the gut microbiota and liver disease.

To assist the development of potential treatments in people, Gérard is trying to learn more about the bacteria in the body — "identifying the good ones and bad ones", as he puts it.

Questions remain about the finer details of the microbial changes in the gut that are associated with NAFLD. Although many studies have recorded differences in the gut microbiota of people with NAFLD in comparison to healthy people, the changes are inconsistent between studies. Yet some patterns can

Bacteria (green, yellow, red) in the mouse gut (blue).

be found. Obese people with NAFLD have increased numbers of particular bacteria from the phylum Firmicutes, for example. People in which NAFLD has progressed to NASH, however, have a lower abundance of certain Firmicutes but a higher abundance of bacteria from the genus *Parabacteroides* and the Firmicutes *Allisonella*. The importance of these associations is still being explored. Another interesting piece of the puzzle is that some children and adolescents with NAFLD or NASH show patterns of microbial changes that differ to those found in adults.

Further studies are needed to pin down the connection between the gut microbiota and NAFLD, and Hekmatdoost and her colleagues

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plan to contribute to that effort. "We are running a project to investigate the microflora in obese patients with NAFLD, lean patients with NAFLD, obese healthy people and lean healthy people to compare them," she says.

Given the evidence for a causative link between changes in the gut microbiota and NAFLD, many researchers are asking why these changes happen, and whether anything can be done about them.

"It is more complicated than just explaining it by poor diet, but diet is the most important modifiable factor" says Hekmatdoost.

It can be difficult to get people to change their dietary habits, even when they understand the changes that are needed and the reasons behind them. However, Hekmatdoost thinks that there are more direct options for intervention. "We can recommend probiotic supplements," she says, and she also points to the role of prebiotic supplements — components of the diet that encourage the growth of beneficial bacteria

such as those found in probiotic supplements. Hekmatdoost emphasizes that soluble dietary fibre is an effective prebiotic substance, and suggests that the consumption of foods rich in such fibre, including legumes, fruit and whole grains, is increased gradually.

But evidence to support the benefits of these interventions for NAFLD and NASH in people is patchy. Some studies, including Hekmatdoost's work, have been completed and more are under way or being planned. But such studies are scarce, as Hekmatdoost and her co-authors acknowledged in their 2017 review. They emphasized the need for a more thorough investigation of what is still a slow-moving area of research, despite its clear potential.

In 2013, Vincent Wong and colleagues at the Chinese University of Hong Kong completed a proof-of-concept study that used probiotic bacteria to treat people with NASH³. They found that there was sufficient improvement to recommend further and larger studies, and are continuing to investigate the findings. The team has already shown that the level of endotoxins that enter the liver from the gut is associated with the severity of NAFLD. Wong says he is now conducting a randomized controlled trial to test the effectiveness of faecal transplants in obese people — research that he hopes will clarify whether directly manipulating the composition of the gut microbiota could be a useful treatment for both obesity and NAFLD.

In work reported in January 2017, Kamal Adel Amin and Hessah Mohammed Almuzafer at Imam Abdulrahman Bin Faisal University in Saudi Arabia examined the effect of adding probiotic bacteria to the diet of rats⁴. The supplement prevented rats on a high-fat, high-sucrose diet from developing NAFLD, which would have occurred on the same diet without the bacteria. This finding suggests that probiotic supplements may offer hope to people who find it difficult to modify their diet.

Several major initiatives to study the human microbiome (the collective genomes of bacteria in the body) may help to speed research. In April 2017, research organization Verily (formerly Google Life Sciences) of Mountain View, California, announced a study of 10,000 people in the United States that will include analysis of the participants' microbiomes. And in August, IBM launched the Microbiome Immunity Project, describing it as "the largest study to date of the bacteria in the human microbiome, starting with the gut." Interest in the bacteria that live in the gut is growing, and NAFLD may eventually become less of a problem as a result. ■

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