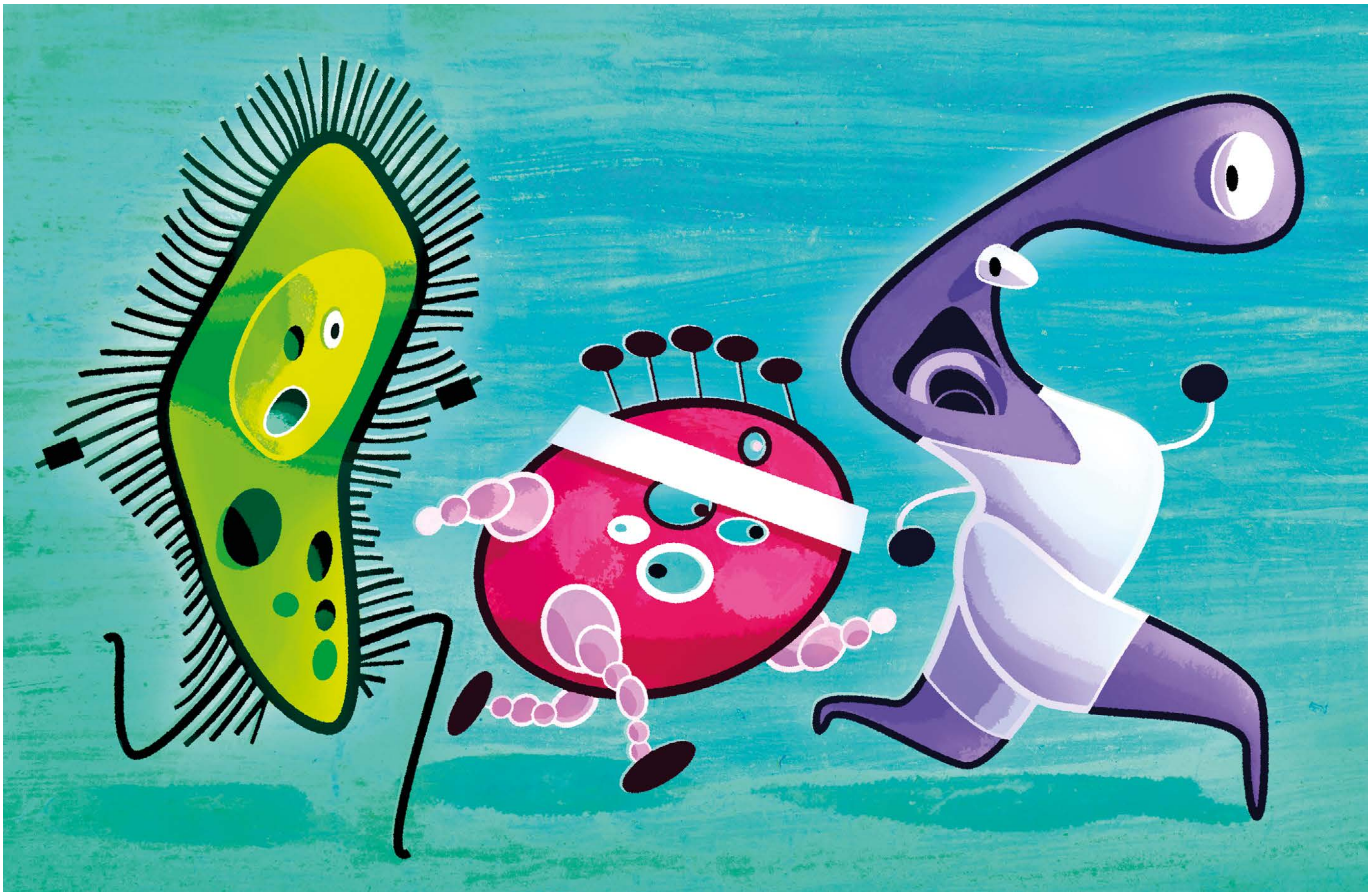


ILLUSTRATION BY JONAS BERGSTRAND

A winning gut

Reports about the microbiome's influence on athleticism are building. **By Simon Makin**

A week before the 2015 Boston Marathon, Jonathan Scheiman, then a postdoctoral researcher in the lab of geneticist George Church at Harvard Medical School in Boston, Massachusetts, was racing around the city in a hire car collecting faeces. He gathered stool samples from runners planning to participate, as well as those from non-runners – mostly fellow postdocs. After the race, he revisited his donors to collect more samples. Four years later, his efforts were rewarded with a paper describing one of the first attempts to establish a causal link between the symbiotic community of microorganisms living in our guts and athletic performance[1].

Although the gut microbiome has been implicated in numerous aspects of health and disease, links with athleticism are much less studied. Interest is growing, however, helped by advances over the past decade that enable researchers to reveal not just which microbes the gut harbours but also what they do. Such work suggests that the enormous diversity of organisms that make up a person's gut microbiome – each as unique as a fingerprint – might converge on a smaller number of functions, which, in turn, could suggest candidate mechanisms. And although elite athletes and their coaches might hope to be the first to benefit, a deeper understanding of the link between the gut microbiome and physical fitness might instead benefit the health of the wider population.

A box of puzzles

Of the trillions of microorganisms that live on and inside us, most are found in the gastrointestinal tract. Some are pathogens, but many are beneficial, and the overall community is essential to human health. Disruption of the microbiome is directly linked to gastrointestinal conditions, such as inflammatory bowel disease, and has been implicated in diabetes, cancer, heart disease, obesity and even mental health disorders. The clearest indication of the microbes' importance comes from laboratory mice conditioned to carry none at all. "They've got metabolic issues, immune issues, neurological issues – you name it," says Aleksandar Kostic, a microbiologist at Harvard and a senior author on Scheiman's study. "Introduce a normal mouse microbiome to them, and many of these phenotypes are rescued," he adds.

Work to unpick the mind-boggling complexity of the gut microbiome has been greatly aided by technical advances over the past two decades, and more affordable genetic sequencing. To investigate a community, researchers must sequence the genetic code of hundreds of organisms simultaneously, drawn from a cast of millions. To identify the microbes present in a sample – usually faecal matter in the case of the gut microbiome – researchers could simply grind it up and sequence everything. Historically, this had two big drawbacks: it was very expensive, and it was difficult for researchers to work out which genes belonged to which microbes. It has been likened to emptying many jigsaws into a box, throwing away the lids, and then trying to solve the puzzles.

To avoid this problem, researchers use a technique known as marker-gene sequencing. This involves sequencing a single gene common to every microbe – specifically, the gene the codes for the 16S rRNA of the ribosome, part of the cellular machinery that translates RNA into proteins. Some parts of the gene are identical no matter which microbe it is from, allowing researchers to easily locate it in a sample. But other regions vary from one microbe to another – differences that make it possible to distinguish which organisms are present. The technique enables researchers to rapidly characterize microbiome composition at low cost. However, because it only uses one piece of each organism, the information it provides is limited. The variations in the 16S gene typically only permit classification of the microbes to the level of genus, not species.

Now, however, many researchers are fronting up to the challenge of sequencing everything. The plummeting cost of sequencing over the past decade or so, as well as the development of databases of microbial genomes, which serve as the puzzle-box lids that enable researchers to match genes to microbes, have made this approach much more tractable. Known as shotgun sequencing, this technique provides more detailed insights than 16S rRNA gene sequencing – microbes can be distinguished beyond even their species, right down to the strain, and researchers can more easily characterize their potential functions on the basis of known genes. As sequencing costs continue to fall, the technique is coming within reach of more researchers. "Within the next few years the price is going to be more competitive with 16S," says nutrition scientist Alex Mohr at Arizona State University in Tempe.

A relationship blossoms

The make-up of the gut microbiome is influenced by many factors, including how the person was born (vaginal or caesarean delivery), the use of drugs (especially antibiotics), smoking habits, alcohol consumption, stress levels, age and – most obviously – diet. Remarkably little, however, is known about how exercise affects the microbiome, or vice versa. Although the field is in its infancy, researchers are beginning to glimpse a relationship, along with potential mechanisms.

A frequent finding is that fitness is associated with greater microbial diversity. One 2016 study[2] that used 16S rRNA gene sequencing found that, in 39 healthy adults, cardiorespiratory fitness correlated with microbial diversity. This was the case even after factors such as diet were accounted for. Variation in cardiorespiratory fitness was better than sex, age, body mass index (BMI) and diet at predicting the degree of species diversity in participants' guts.

The team did not find specific genera or families of microbes that were significantly associated with fitness. But they were able to use a microbial database to infer the likely functions of the microbes associated with cardiorespiratory fitness, these included microbial mobility and fatty-acid synthesis. One role of gut bacteria is to help break down complex carbohydrates by fermentation, producing short-chain fatty acids (SCFAs) such as butyrate, acetate and propionate as by-products. SCFAs are emerging as a potentially important component of the link between the microbiome and fitness. Of the most common SCFAs, "propionate and butyrate can only be produced by gut bacteria," says Kostic. These are thought to act as energy sources in muscle, he adds. The team found that fitter participants had higher levels of butyrate in their faeces. "Butyrate is interesting because it's one of the principal fuel components for the cells that line your gut," Mohr says. "Increasing their health is obviously important for gastrointestinal health overall."

A study published the following year[3] looked at differences between the microbiomes of amateur and professional cyclists, using the more sensitive shotgun sequencing technique. The researchers didn't find systematic differences between amateurs and professionals, but they did find that self-reported exercise duration was associated with levels of the bacterial genus *Prevotella*. Levels of *Prevotella* also correlated with certain processes in the gut, including the metabolism of carbohydrates and branched-chain amino acids.

"This was one of those results where a light bulb goes off, and it just makes sense."

Some press coverage of the study, however, was problematic. The possibility that boosting the level of a specific microbe in a cyclist's gut could increase performance led to stories trumpeting the imminent arrival of 'poop doping'. But not only was the sample used in the study too small to draw such strong conclusions, it also wasn't random. The authors recruited friends, who probably shared many other things in common, such as geographic location, social interactions and diet, which could result in similarities in their microbiomes. "That's a huge confounding factor," says Jonathan Eisen, director of the Microbiome Special Research Program at the University of California, Davis, who was one of several researchers who pushed back against the media coverage.

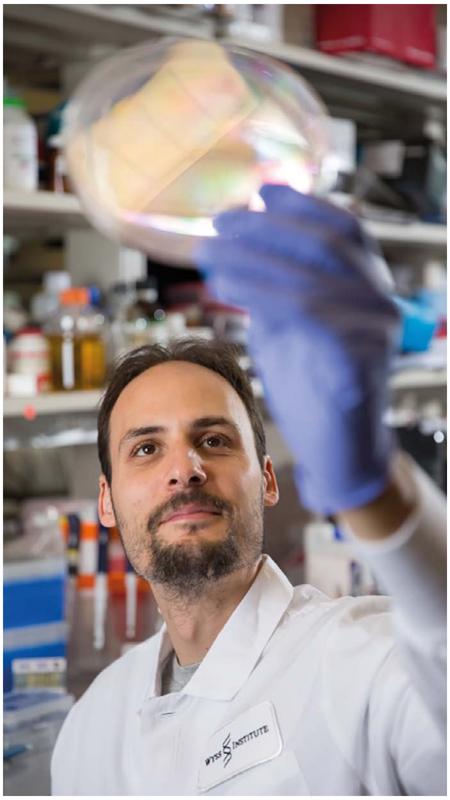
The difficulty of untangling the contribution of diet was highlighted in a 2014 study[4] of professional rugby players. Using 16S rRNA gene sequencing, the authors found greater diversity in gut microbiota in the players than in non-athletes matched for age, gender and BMI. However, the team also noted that there were extreme dietary differences between the two groups. This was especially true of protein intake, which also correlated with microbial diversity. "We couldn't quite separate exercise from the accompanying dietary changes, to see which was having the greater effect," says one of the authors, gastroenterologist Fergus Shanahan at University College Cork, Ireland.

In 2018, Shanahan and his colleagues analysed the same samples again, but this time they used shotgun sequencing[5]. The researchers found a clearer separation between athletes and non-athletes in terms of gene function, including differences in carbohydrate metabolism, amino-acid synthesis and the production of SCFAs. The study illustrates the power of shotgun sequencing for assembling the puzzle of microbiome function. "It's very much a product of technology development," says Kostic. "Even though bacteria might be taxonomically very diverse, oftentimes their basic functions are quite similar."

A marathon effort

Although studies such as these show a link between fitness and the gut microbiota, they can only demonstrate associations. "A big issue is drawing causation from observational, cross-sectional studies," says Mohr. "We really need some longitudinal and, importantly, experimental work."

Scheiman's Boston marathon study was longitudinal from the outset. Samples were collected daily, before and after the marathon, in an effort to study the acute effects of exercise on the microbiome. The researchers used 16S rRNA sequencing, and one genus in particular caught their attention. "The thing that really jumped out was a spike in *Veillonella* abundance immediately after the marathon," says Kostic. "We also noticed that *Veillonella* was generally higher amongst the runners, relative to sedentary controls." With just 15 runners and 10 control participants, the sample size was limited, but the team also replicated the finding in an independent cohort of elite rowers and runners using shotgun sequencing. The task then was to work out why.



WYSS INST. AT HARVARD UNIV.

Jonathan Scheiman co-founded Fitbiomics.

Veillonella is known to metabolize lactate, a by-product of anaerobic respiration that is responsible for the aching sensation in the muscles that can follow intense exercise. When the team analysed samples from the second cohort, it found post-exercise spikes in the expression of genes that encode proteins involved in the metabolic pathway that *Veillonella* uses to convert lactate into propionate. "This was one of those results where a light bulb goes off, and it just makes sense," says Kostic. Lactate is a metabolite that would be produced in abundance during a marathon, and that *Veillonella* can use for energy. And the propionate produced by *Veillonella* promotes muscle function. The presence of these bacteria might, therefore, create a feedback loop that benefits athlete and bacteria alike. "Someone exercising regularly is creating this metabolic niche for lactate-utilizing bacteria like *Veillonella*," says Kostic.

The importance of microbes that produce SCFAs has been hinted at by several studies, but only ever as an association. So, with a strong theory to test, the team conducted some experiments. First, it isolated a *Veillonella* strain from a runner, inoculated mice with it and then measured how long the rodents could run on a treadmill. Compared with mice inoculated with a bacterium that doesn't metabolise lactate (*Lactobacillus bulgaricus*), the *Veillonella*-supplemented mice ran for 13% longer. It is possible that the control bacterium was having a detrimental effect, rather than *Veillonella* having a positive one, but a final experiment showed that administering propionate directly to mice had a similar effect on performance, compared with a saline control. "This study stands out" in taking the crucial step from correlation to causation, says Mohr.

It doesn't, however, stand as definitive proof. The experiments cannot rule out the possibility that the effects of propionate were due to providing energy to mice that were fasted. That aside, work is also still needed to see if the effect translates to people. "The human gut and the mouse gut are really different, and the laboratory mouse gut even more so," says Eisen. "It's great they're doing experiments to test this, but their result isn't convincing even that this is broadly applicable to mice, let alone translatable to humans." Eisen thinks that the hypothesis that gut microbes affect athletic performance "seems totally plausible", but he is wary of premature or overly simplistic claims. "More studies in a wider diversity of animals, with more natural, complicated microbiomes, would be helpful," he says, adding that "at some point you have to do human studies."

Marketing microbes

Encouraged by their work, Scheiman and his colleagues founded a start-up called Fitbiomics in New York City nearly three years ago. Their aim is to benefit human health, rather than the performance of athletes. Scheiman, who is chief executive of the firm, says that the company's goals are to "decode the biology of the fittest people in the world, understand what makes them unique, then translate that information into nutritional modalities that could benefit the masses". Eisen supports their efforts, but cautions against thinking there will be a silver bullet. "I'll bet it's going to take a complicated manipulation of the microbiome," he says.

Scheiman expects that the mechanism proposed in their study will be one of many linking the microbiome to athleticism. "We hope to identify not just one or two, but dozens to hundreds of these, for different functional applications," he says. "*Veillonella* is just one example; we're now looking to [conduct preliminary tests] in humans, then move towards clinical trials."

One potential application of the *Veillonella* mechanism is for diabetes. Exercise is encouraged as a treatment for people with the disease and those at high risk of developing it, but a 2020 study[6] found that around one-third of people at risk fail to derive any metabolic benefit from exercise – a phenomenon called exercise resistance. The researchers found that in those who do benefit, levels of butyrate and propionate rose sharply after exercise, suggesting that the microbiome might be involved. "We reanalysed this data and found that *Veillonella* was significantly reduced in the people that exhibited exercise resistance," Kostic says, referring to unpublished work. "If we can identify people with exercise resistance we can potentially restore microbes that might help."

Because probiotics are treated as supplements, regulation is light. "Unless they make specific health claims, the probiotic field is not regulated like drugs, so while there are dozens that are real there are hundreds that are bogus," Eisen says. Biochemist Ralf Jaeger, a consultant based in Milwaukee, Wisconsin, who is advising Fitbiomics, is keenly aware of the problem. "You're always going to see products containing strains that are not scientifically-backed," he says. Microbes with as little as 70% DNA in common can belong to the same species, so Jaeger stresses the importance of specifying strains. "We have probiotic strains that belong to the same genus and species, but have totally different effects," Jaeger says. "Benefits are strain-specific."

To provide guidance to industry and consumers, Jaeger and colleagues authored a paper outlining the position of the International Society of Sports Nutrition on probiotics and the evidence related to their use in the health and performance of athletes. The review[7], of which Mohr and Scheiman were also co-authors, concluded that specific strains have been shown to improve the integrity of the intestinal barrier, which can be adversely affected by intense, prolonged exercise; and that anti-inflammatory strains might improve recovery of damaged muscle. It also mentions preliminary evidence of other potential benefits, including normalizing hormone levels, reducing lactate and upregulating neurotransmitters, but states these require more vigorous validation. This tallies with Eisen's position on the *Veillonella* findings. "It's nice that they have an experimental result, but if I was going to train for the Olympics, I wouldn't buy this stuff," he says. "It's not at that point yet."

Simon Makin is a freelance writer in London.

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