ILLUSTRATION BY JONAS BERGSTRAND

The key to endurance

The muscles of elite endurance athletes boast high numbers of extra-efficient mitochondria. Unlocking the secrets of these cellular components could yield gains for future Olympians. By Anthony King

Usain Bolt won the men's 100 metre final in the 2016 Olympic Games in 9.81 seconds and 42 strides. A few days later, Eliud Kipchoge ran 42 kilometres in 2 hours and 8 minutes to win the marathon. These extraordinary feats pose very different challenges for the human body, but the races began in much the same way.

As the starting pistol fired, Bolt and Kipchoge began to use creatine phosphate, an energy-rich molecule stored in muscle tissue, to generate the energy-carrying molecule ATP. In a few seconds, however, both athletes' stores of creatine phosphate were depleted, forcing their bodies to break down glucose to provide ATP to contracting muscle cells for a few more minutes.

For Bolt and his fellow sprinters, a few minutes seems like an age. But for marathon runners, there is much farther to go. To reach the finish line, these endurance athletes rely on a slower, but more efficient way to generate ATP that uses oxygen to burn fats and carbohydrates, in structures inside the cell called mitochondria.

Elite endurance athletes such as Kipchoge pack many more of these aerobic power plants into muscle cells than both an average person and the far-from-average Usain Bolt. "He probably doesn't need any more mitochondria than you or I," says John Hawley, an exercise physiologist at the Australian Catholic University in Melbourne. Athletes' mitochondrial engines activate quickly, within a few minutes of exercise, but even for the best, that switch doesn't flick immediately. "We can't turn on mitochondria fast enough to provide all the energy required for a sprint," says David Hood, a mitochondrial scientist at York University in Toronto, Canada.

All elite athletes push themselves to the limit in search of small advantages, and for endurance athletes the mitochondria are a target for making marginal gains. Exercise scientists are working to better understand how these structures, found inside nearly all cells, adapt to exercise, and how to improve their delivery of oxygen. Their findings are beginning

to inform training regimes, and might have therapeutic uses for non-athletes – perhaps allowing the delivery of some of the benefits of exercise through a pill.

Textbook error

The textbook picture of discrete, bean-shaped mitochondria reflects those in the liver, but in muscle cells these power factories instead form extensive tubular networks. "They are more interconnected than we had thought," says Carsten Lundby, an exercise physiologist at the Inland Norway University of Applied Sciences, who is based in Lillehammer. The structure is most akin, he says, to a mass of seaweed or spaghetti.

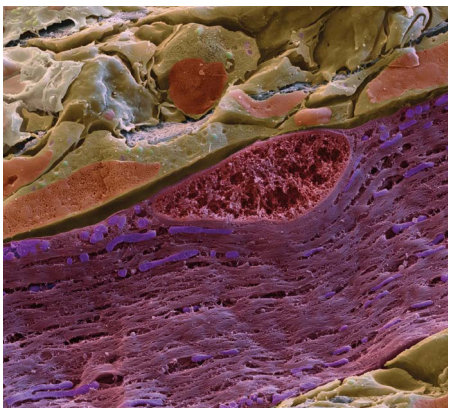
"They spread out like tendrils, efficiently dissipating energy throughout the muscle fibre," explains Christopher Perry, an exercise and health researcher at York University. The more elongated and extensively fused the mitochondria are, the better they are at generating ATP where it's needed.

Mitochondria are also highly dynamic. "In real time you would see them worming around inside a cell," says Mike Murphy, programme leader at the UK Medical Research Council's Mitochondrial Biology Unit at the University of Cambridge. "They are continually joining and separating." They are also extremely trainable. Lundby has seen the quantity of mitochondria in muscle increase after just 14 days of sustained exercise. Neither blood volume nor heart size expand in such a short time frame.

For someone who does very little exercise, going on a long run will generate physiological stress. Core temperature and heart rate increases, and the blood becomes more acidic as lactic acid builds up. Under pressure, the body responds by ramping up the expression of a large number of genes – including those for making mitochondria and mitochondrial proteins. "With the very first bout of exercise, you activate signals that promote biogenesis," says Hood. "At the same time, you accelerate the removal of old mitochondria and dysfunctional segments." This can continue for months with regular activity – great news for exercise neophytes.

Exercise not only promotes the generation of mitochondria, but also changes the structure and function of existing ones in ways that enhance physical stamina. Research shows that the mitochondria in leg muscles of endurance-trained athletes have more inner membrane folds (called cristae) than those of people who exercise recreationally, this increases the ratio of surface to mitochondrial volume[1]. These cristae are where important enzymes attach and pass on electrons during cellular respiration; more folds means more oxygen uptake in muscle. A top endurance athlete should, therefore, boast more-efficient mitochondria than everyone else.

The enzymes themselves can adapt to regular exercise too, becoming better organized. Francesca Amati, an exercise physiologist at the University of Lausanne in Switzerland, and her colleagues, studied the effect of exercise training on 26 sedentary older adults[2]. After four months of three workout sessions per week, proteins in the mitochondria clustered together to form complexes that allowed them to pass electrons more efficiently. The bodies of highly trained athletes seem to adapt even further, she adds, by storing energy in the form of fat globules closer to their mitochondria. She compares this to having a fridge in the kitchen rather than in the living room – the fuel is nearer to where it is needed.



Mitochondria (purple) inside a muscle cell.

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Adaptations such as these take time to develop – the amount and intensity of training is crucial to the quantity and quality of mitochondria, Hood explains. They are also not permanent: muscle mitochondria have a half-life of only one or two weeks, so in the absence of constant activity, the number present in the muscle can quickly fall from Olympian to unimpressive levels. "It is really a matter of use it or lose it," says Hood.

Even if athletes keep up the training, however, it is not easy to keep making gains. As their bodies adapt, the work required for further advances increases. "It is very difficult for elite athletes to continue to get improvements," says Perry. They must find ways to push their bodies to ever greater extremes.

A stressful road

Conventionally, coaches and athletes have developed training techniques through trial and error, and scientists have jogged along behind to investigate how and why they work. "The athletes have taught the scientists more than scientists have taught athletes," says Hawley. But now he and others think that the tables could turn, with basic research into mitochondria providing a scientific foundation for improving elite conditioning regimes.

"The quality of training and the type of training does impact mitochondria," confirms Hood. One approach is to deplete the muscle of glycogen, the body's glucose store, through high-intensity exercise, and to continue training with the glycogen fuel tank empty. This stress has been shown to stimulate mitochondrial breakdown and regeneration. "The point of training really is to disturb homeostasis in muscles," says Hawley.

Another popular way to push athletes' bodies to adapt is altitude training. The practice largely arose after the 1968 Olympics, which was held in Mexico City at an altitude of around 2,300 metres. Even champion endurance runners struggled with the lower levels of oxygen in their blood as a result of the thin air. Athletes began training at altitude, and interest in using it to stress the bodies of elite competitors continues today. "One of the latest ways to do training is to sleep at high altitude, but then to train at normal tempo down at sea level," says Hawley. Some coaches and athletes, however, are taking the opposite approach: training at high altitude and living nearer sea level. Lundby, meanwhile, plans to try doing everything at altitude by bringing a group of Norwegian cyclists to the Swiss Alps for one month to live and train at 3,500 metres. Most coaches would consider this altitude "bonkers", he admits. But he thinks there will be benefits.

An athlete's body acclimatizes to high-altitude training by increasing blood volumes, red blood cell count and heart output, all of which allow muscles to get the oxygen they need and boost mitochondria. "We previously showed that altitude exposure, for one month, increases mitochondrial volume density by 6–8%," Lundby says[3]. However, he thinks that another form of physical stress might be even more effective. "It seems that heat training could have greater effects than altitude training, but it is still early days." Heat is a key stress during endurance events, with higher temperatures impairing performance. Running speed in elite marathons declines as ambient air temperature rises above about 12 °C.

In November 2020, Lundby began an experiment with 50 high-level amateur cyclists to test the effect of heat training. One group cycled in a chamber at 38 °C, another cycled at ambient temperatures and another wore suits designed to retain body heat. The training was split into one 50-minute session per day, 5 days a week for 5 weeks, and was in addition to their normal training. Body suits to retain heat have been shown to boost athletic performance, says

Lundby. He now aims to determine whether some of these gains can be pinned on changes to mitochondria.

Lundby thinks that training in the heat or wearing the suit causes the athletes to produce heat-shock proteins (HSP). Several HSPs are known to be involved in adaptations to endurance and exercise performance, and some, such as HSP72, encourage the synthesis of new mitochondria. In a mouse study[4], for example, 15 sessions of heat stress over 3 weeks had more than twice the impact on HSP72 content in muscle as did endurance training. Lundby plans to take some of the cyclists who underwent heat training to the Swiss Alps for altitude training, and will then compare the results.

To better understand the effect that training has on athletes' mitochondria, sports scientists need to take a look inside the competitor's muscles. That is no simple task. "A key barrier to mitochondria research in elite athletes", says Perry, "is getting access to their mitochondria."

The best way to study a person's mitochondria is to take a biopsy of their muscle tissue. The procedure involves using a pencil-sized probe to extract 50–100 milligrams of tissue. Researchers can then measure not only the tissue's oxygen consumption, but also the activity of enzymes, such as citrate synthase, that are proxies for mitochondrial biogenesis.

The problem for sports scientists is that elite sportspeople shy away from anything that they think might impair their performance, and this includes safe procedures such as muscle biopsies. For athletes at the very top of their game, "you are not going to get a biopsy", Hawley concludes. Breaking this impasse requires new techniques. Sports scientists, says Perry, "need breakthroughs in *in vivo* assessments of mitochondrial metabolism that do not require a biopsy".

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Near-infrared spectroscopy is one promising approach for measuring muscle oxygen consumption without needles. In 2020, researchers in the Netherlands demonstrated this method's effectiveness for comparing muscle recovery after exercise in older and younger adults[5]. And in 2019, Brian Glancy, a muscle-energetics researcher at the National Institutes of Health in Bethesda, Maryland, and his colleagues took advantage of the natural fluorescence of the energy molecule NADH – which is mostly found in mitochondria – to determine oxygen use[6]. The rate of production and use of NADH in muscle is almost directly proportional to the oxygen being consumed. As a result, the imaging technique allowed the researchers to observe mitochondrial function in the muscles of living mice, and it could lead to a better understanding of disease states. But the technology is not ready for *in vivo* human studies. "We cannot image the muscle cells without first removing the surrounding skin and connective tissue," Glancy explains.

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Cyclists take part in an experiment to test the effects of training in the heat.

The right stuff

An appreciation of the extreme physiologies of elite athletes might also provide information that is useful for understanding illness. "Studying the edges of biology can show us what is possible, but also maybe understand how they get there," says Glancy.

Perry thinks that the mitochondria of elite athletes could suggest new therapeutic approaches in muscle disease – his current focus – and other disorders. "It is amazing how many diseases have a mitochondrial dysfunction," he says. Researchers are increasingly recognizing the part played by faulty mitochondria in diabetes, obesity and neurodegenerative diseases such as Alzheimer's. And there are signs that mitochondria fail in the course of many other diseases, including cancer, cardiopulmonary disease and heart disease. "Just like mitochondria respond to the stress of exercise and improve, they respond to the stress of disease and fail," Perry explains.

Understanding the pathways through which exercise stimulates higher mitochondrial volume in athletes could, for example, lead to therapeutics that target those pathways in non-elite individuals, especially older people or those who are unwell. Indeed, the dietary metabolite urolithin A – reported[7] to increase muscle function and endurance in aged mice and running capacity in young rats – has also shown signs in clinical trials of improving the mitochondrial health of older people with sedentary lifestyles[8]. But, so far, these studies have been small; there is much still to uncover.

For endurance athletes at the top of their field, researchers are sceptical that taking a biopsy to determine an athlete's mitochondria status is of any value to an individual – for now. The muscles of all these athletes are already jammed with efficient mitochondria; any insights are unlikely to influence medal prospects at the next Olympics.

The value of mitochondria in sport might lie mainly in evaluating the efficiency of different training and conditioning regimens. "There is still a tonne to learn in how mitochondria function and respond to training," says Glancy. But where there is the potential to eke out a marginal gain from insights into optimizing mitochondria, athletes and coaches are likely to be receptive to new ideas. And in the long race to perfecting athletic achievement, researchers are ready to accept the baton.

Anthony King is a science writer in Dublin.

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