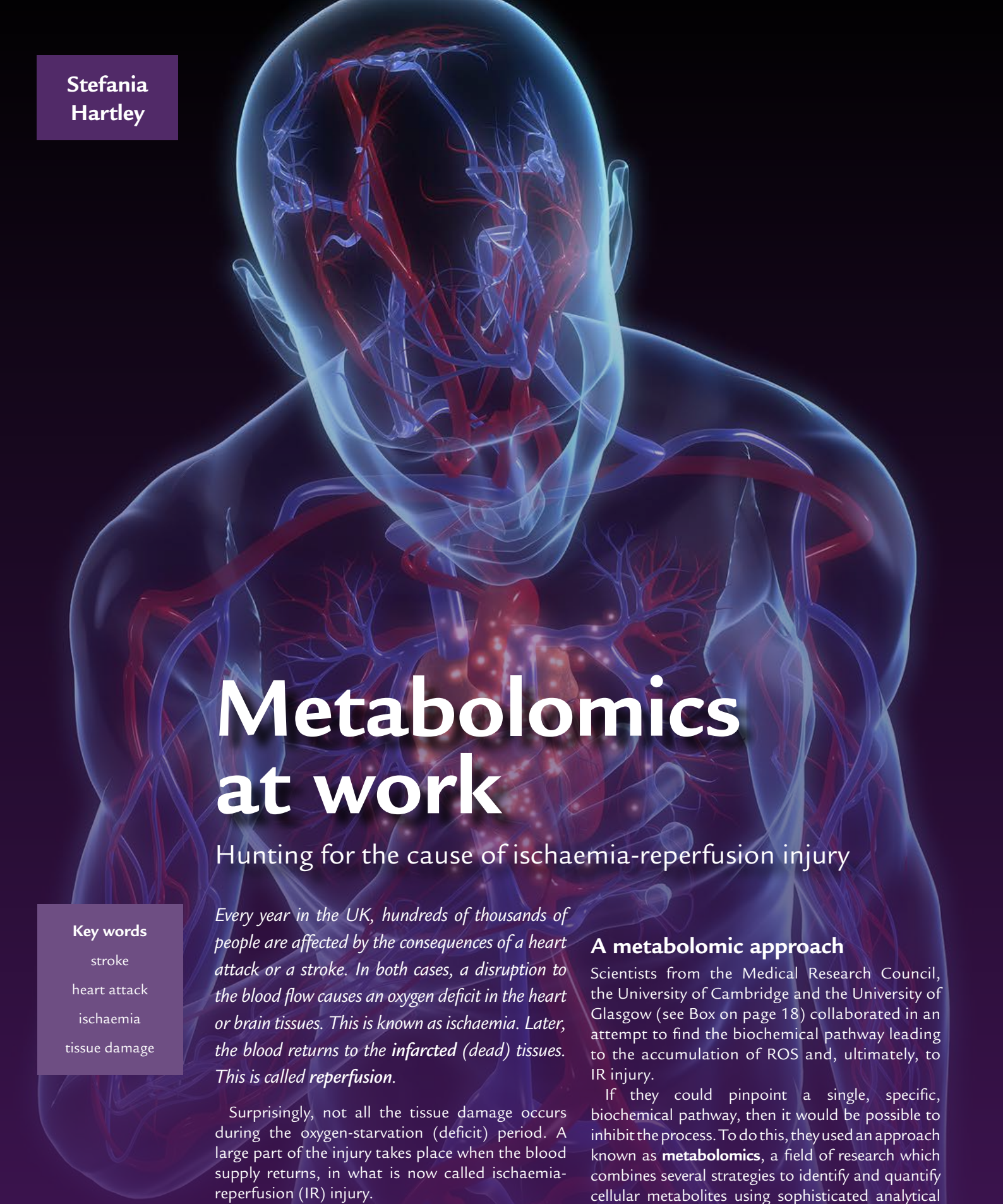
Stefania Hartley



Metabolomics at work

Hunting for the cause of ischaemia-reperfusion injury

Key words
stroke
heart attack
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tissue damage

Every year in the UK, hundreds of thousands of people are affected by the consequences of a heart attack or a stroke. In both cases, a disruption to the blood flow causes an oxygen deficit in the heart or brain tissues. This is known as ischaemia. Later, the blood returns to the ***infarcted*** *(dead) tissues. This is called* ***reperfusion****.*

Surprisingly, not all the tissue damage occurs during the oxygen-starvation (deficit) period. A large part of the injury takes place when the blood supply returns, in what is now called ischaemia-reperfusion (IR) injury.

Reactive oxygen species (ROS) have long been recognised as the culprits for IR injury. These chemicals contain positively-charged oxygen ions which can cause damage in several different ways, such as by damaging DNA and by deactivating specific enzymes. The question, though, is why are ROS so abundant in reperfused tissues?

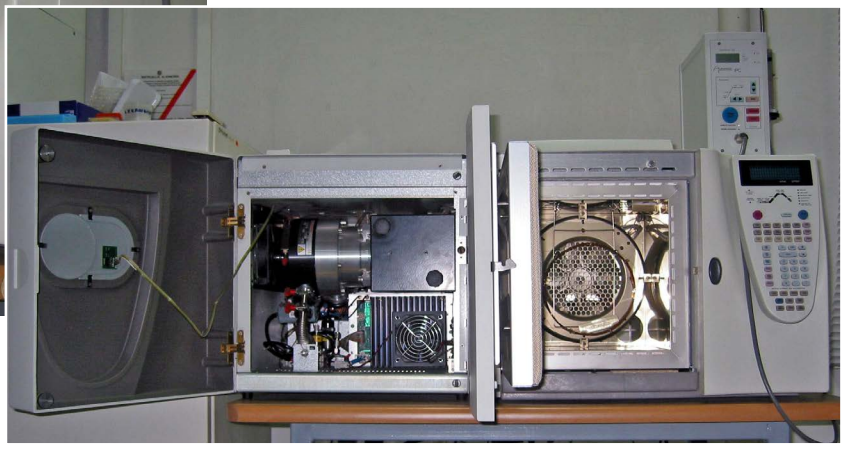
metabolite an intermediate in a sequence of chemical reactions in living cells

A metabolomic approach

Scientists from the Medical Research Council, the University of Cambridge and the University of Glasgow (see Box on page 18) collaborated in an attempt to find the biochemical pathway leading to the accumulation of ROS and, ultimately, to IR injury.

If they could pinpoint a single, specific, biochemical pathway, then it would be possible to inhibit the process. To do this, they used an approach known as **metabolomics**, a field of research which combines several strategies to identify and quantify cellular metabolites using sophisticated analytical technologies.

The teams used Liquid Chromatography and Mass Spectrometry to measure and compare levels of different metabolites in ischaemic and reperfused mouse kidneys, livers, hearts and brains, together with ones in which the concentration of oxygen was normal.

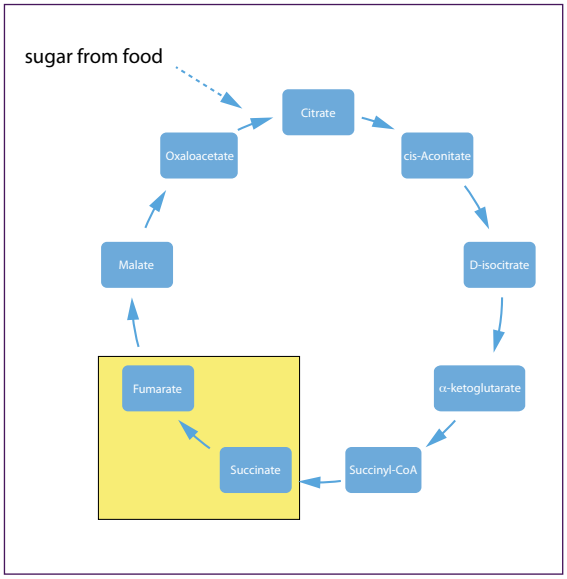


A liquid chromatography mass spectrometer (LCMS). The sample to be analysed is inserted at top right. The different substances in it are separated in the chromatography column in the oven on the right. They then pass into the mass spectrometer on the left which identifies each compound by determining its molecular mass.

The likely culprit

Only three substances were found to increase in abundance in tissues after ischaemia. One of these was succinate, which was the most interesting, because it is a mitochondrial metabolite. Succinate levels increased three- to nineteen-fold across all tissues tested, and so could be used as a chemical signature of ischaemia.

Further metabolomics tests showed that succinate accumulated in areas that were subsequently affected by IR injury and that concentrations were proportional to the duration of ischaemia. This provides confirmation of the involvement of succinate in IR injury. But where did the succinate come from?



The Citric Acid Cycle (CAC) showing the placing of succinate and fumarate

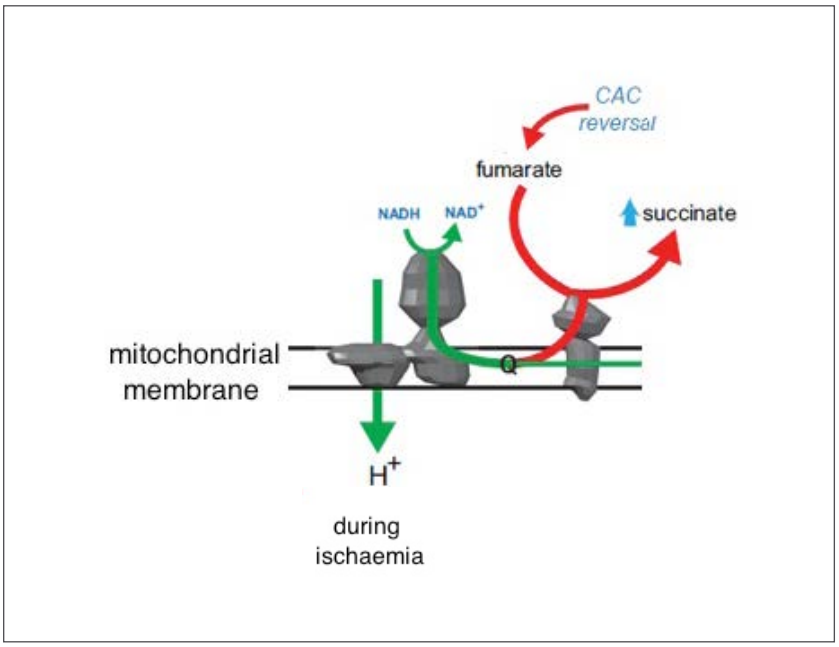
If succinate were part of the mechanism that led to IR injury, finding succinate's origin would be a step towards preventing IR injury. Succinate is an intermediary in the Citric Acid Cycle (CAC) which takes place inside mitochondria and is essential for the release of energy from food. The possibility was that, in anaerobic conditions, succinate dehydrogenase, which during the Citric Acid Cycle turns succinate into fumarate, might work in reverse. The team tested the hypothesis by inhibiting succinate dehydrogenase in mice. As expected, they found a decrease in the accumulation of succinate, as well as an improvement in the mice's scores in post-stroke neurological tests.

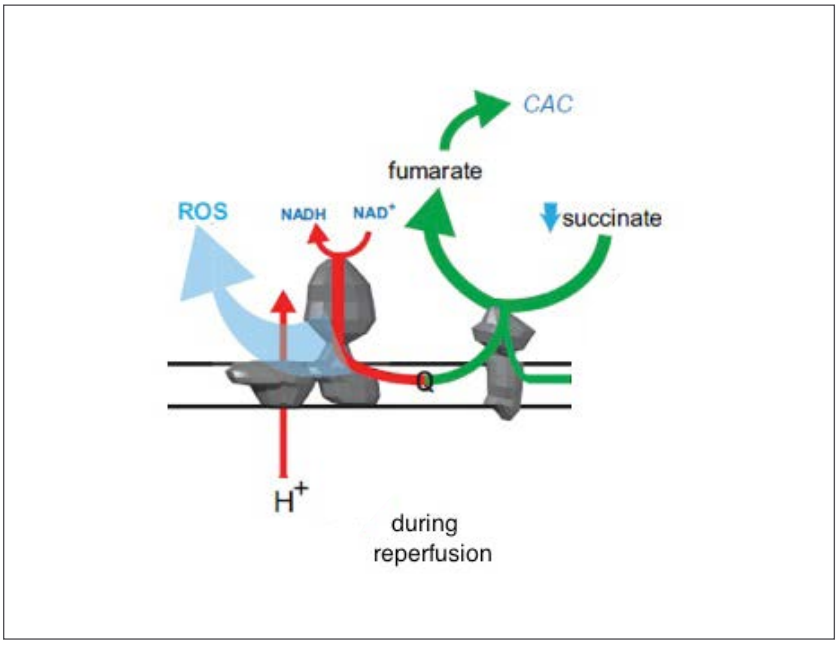
From succinate to ROS

Once it was established that the extra succinate – produced by the enzyme succinate dehydrogenase working in reverse – was somehow responsible for IR injury, the team needed to make the final connection between succinate and ROS.

The answer was to be found in the last stages of mitochondrial respiration, where a series of electron transporters bring about the production of ATP by the ATP synthase.

Computer simulations showed that, straight after reperfusion, succinate dehydrogenase turned succinate into fumarate at a speed such that the electron transporters couldn't keep up, generating an excess of protons inside the mitochondria. These attach themselves to oxygen atoms, resulting in the oxygen ions which are what make ROS so reactive.





During ischaemia the CAC (citric acid cycle) goes into reverse, generating a build up of succinate. During reperfusion the build up of succinate is removed but this leads to a build up of protons (H^+) on the wrong side of the mitochondrial membrane, thus causing the production of excess ROS and leading to all the IR damage.

Further experiments involved increasing the levels of succinate and testing for ROS with fluorescent probes. The results further confirmed the relationship between succinate levels and ROS production at reperfusion. The pieces of the puzzle had finally come together.

The good news is that inhibiting succinate production by succinate dehydrogenase is quite straightforward and cheap. The identification of a single pathway leading to IR injury has opened the way for a new therapeutic approach to dealing with IR injury.

Stefania Hartley is a science teacher living in Singapore.

Interview with Dr Richard Hartley

Dr Richard Hartley, Professor of Chemical Biology and Organic Synthesis at the University of Glasgow, is one of the co-authors of the research paper.

Which aspect of the research was contributed by your lab?

A molecular probe from my lab, MitoB, helped answer one big question in this research: dimethyl malonate prevents IR injury, but does it really do this by reducing mitochondrial ROS? To answer this, the teams needed a way of measuring mitochondrial ROS produced in vivo, and only MitoB can do this.

What are the consequences of the discovery of succinate as a key player in the onset of IR injury?

Before this work, it was believed that IR injury was a general consequence of oxygen returning to the heart or brain when the blood flow was restored. This would make it very difficult to prevent. Now we know that there is a single biochemical pathway causing the damage and that it can be stopped! At last we can see how to give people the best chance of good health after a stroke or heart attack. The race is now on to find compounds that are even better than dimethyl malonate.

Is the image of the lone scientist making new discoveries a thing of the past?

There is a place for research in a single field, but this work on IR injury needed experts in everything from chemistry to medicine. It could not have been achieved in any other way and it's a testament to Dr Murphy and Prof. Krieg that they gathered and co-ordinated such a team. I think this is a pattern for future success: big discoveries will come from scientists working together.