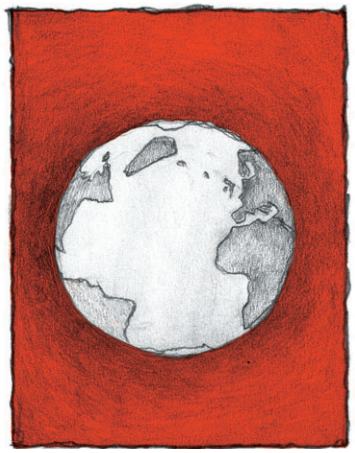
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

HYPOXIA TOLERANCE



LYMPHATICS ASSIST CIRCULATION DURING HYPOXIA

The vertebrate lymphatic system is a network of vessels that collects excess fluid from tissues, delivers the fluid to lymph nodes and then ultimately returns the fluid back to the circulatory system for redistribution. The system plays a critical role in the immune response, fluid balance and the transport of fatty acids. Moreover, the lymphatic system is guilty of transporting cancerous cells throughout the body, spreading cancer to new tissues. As such, many people are interested in studying the lymphatic system to better understand the development and treatment of cancer.

Recently, scientists have shown that lymphatic and circulatory vessels are connected in adults. However, the structure and function of these connections, called arterial-lymphatic conduits (ALCs), are unknown. Lasse Dahl Ejby Jensen from the Karolinska Institute in Stockholm, Sweden, together with colleagues from Sweden and Denmark examined ALCs in zebrafish and glass catfish and studied how hypoxia (low levels of oxygen) affects these connections since cancerous tissue is often hypoxic.

The research team used various microscopy techniques to examine the lymphatic system and the structure of the ALCs. Fortunately, zebrafish and glass catfish are completely transparent, so the authors also used video and photography to non-invasively observe the flow of blood and lymphatic fluid. They found that zebrafish and glass catfish have similar lymphatic systems and that ALCs often look like tangled corkscrew-shaped vessels, directly connecting lymphatic to arterial vessels.

Next, Jensen and colleagues compared fish exposed to hypoxia with those held in normal oxygen levels in order to better understand the functional relationship between the circulatory and lymphatic systems. Remarkably, the authors found that blood, containing red blood cells, flowed into the lymphatic vessels during hypoxia. This incredible finding suggests that the lymphatic system can act as a back-up for the circulatory system. This is a quick and easy way to ameliorate a hypoxic situation, with the lymphatic system stepping in to expand the circulatory system so that it does not have to grow new blood vessels and make new red blood cells.

The authors then wanted to figure out how blood enters the lymphatic system. Again using microscopy and video technology, they observed that during hypoxia, ALCs dilate and straighten, indicating that blood is entering the lymphatic system *via* the ALCs. Finally, Jensen and colleagues sought to determine which molecular players control this phenomenon. Nitric oxide (NO) is known to act on vascular smooth muscle cells to relax and dilate them. The authors used various chemicals to test whether NO induces the ALCs to relax during hypoxia and found that NO is indeed behind the hypoxia-induced flow of blood into the lymphatic system.

Jensen and colleagues have uncovered a new function for the lymphatic system: a back-up circulatory system in times of hypoxia. They demonstrated that ALCs act as gatekeepers for blood flow to the lymphatic system during hypoxia. These gates are controlled by NO, which causes ALCs to relax and straighten, allowing blood to enter. Findings from this study could be used to better understand how cancerous tumours, which can be hypoxic, could enter the lymphatic system from the circulation.

10.1242/jeb.036434

Jensen, L. D. E., Cao, R., Hedlund, E.-M., Söll, I., Lundberg, J. O., Hauptmann, G., Steffensen, J. F. and Cao, Y. (2009). Nitric oxide permits hypoxia-induced lymphatic perfusion by controlling arterial-lymphatic conduits in zebrafish and glass catfish. *Proc. Natl. Acad. Sci. USA* **106,** 18408-18413.

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EAT LESS TO BURN MORE FAT

Over 70 years ago, a team of scientists discovered that a reduction of daily caloric intake can prolong lifespan in rats. Since then, a moderate caloric restriction has been associated with increased longevity in a variety of organisms ranging from yeast to mice. Animals subjected to caloric restriction typically exhibit reduced occurrence of age-related disorders such as cardiovascular diseases, diabetes and oxidative damage, resulting in an overall longer life expectancy. Although the exact mechanisms promoting these health benefits remain elusive, it has been argued that caloric restriction could reduce oxidative stress in cells, therefore preventing excessive oxidative damage. Furthermore, when carbohydrates are oxidized in the mitochondria they tend to produce more oxidative stress than lipids, so it has been further hypothesized that an increased reliance on fatty acids *vs* carbohydrates could mediate the health benefits of caloric restriction. However, the nutrient composition of the caloric restriction diet is similar to that of a regular diet; that is, the two diets have the same relative lipid content. Therefore, this hypothesis cannot hold unless calorie-restricted animals are synthesizing more lipids than their satiated counterparts. In this study, Matthew Bruss and his colleagues from the University of California at Berkeley and the Children's Hospital in Oakland (USA) sought to test this hypothesis by examining fuel selection patterns in calorie-restricted mice.

The team reduced the caloric intake of mice by 30% and measured their oxygen consumption and CO_2 production to examine their fuel preferences over several weeks. The authors initially discovered that the calorie-restricted mice wolfed down their entire daily food ration within an hour of the food arriving and essentially fasted for the next 23 h. Monitoring the fuel sources that the animals used while fasting, the team found that during the first 6 h after being fed, the mice used carbohydrates for their energetic needs and synthesized their own fatty acids. During the rest of the day, the rodents relied almost exclusively on lipids as metabolic fuels, burning four times more lipids than if they were on a normal diet, and three times more than their actual daily fat intake.

As these results strongly suggested that calorie-restricted mice synthesized more lipids, Bruss and his colleagues further examined changes in the animals' lipid production. The team confirmed that calorie-restricted mice exhibited elevated rates of lipid synthesis and retention. Furthermore, they found that shortly after their meal the mice started producing and storing the majority of these lipids in their adipose tissues.

Using classical physiological techniques, Bruss and colleagues elegantly demonstrated unique metabolic adjustments in calorie-restricted mice. The team clearly showed that the calorie-restricted mice switched to a predominant reliance on their own newly synthesized fatty acids as oxidative fuels and there was a pattern to the timing of this shift, which should most definitely be kept in mind for future experimental designs. The next logical step is probably to ask the question: is burning more fat the key to living a long, healthy, calorie-restricted life?

10.1242/jeb.036418

Bruss, M. D., Khambatta, C. F., Ruby, M. A., Aggarwal, I. and Hellerstein M. K. (2009). Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. *Am. J. Physiol. Endocrinol. Metab.* **298**, E108-E116.

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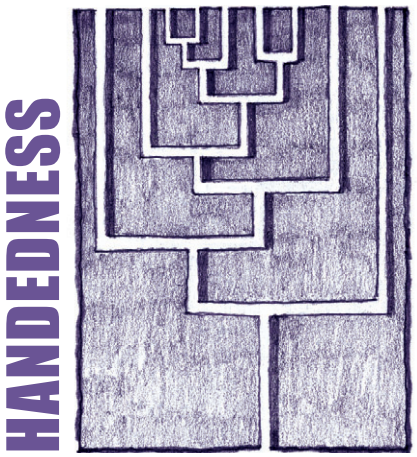

HOW THE SNAIL SHELL GOT ITS TWIST

Handedness – asymmetrical anatomy or behaviour – is a fundamental feature of many animals, but how it occurs remains a mystery. Snails can also show 'handedness' – some individuals have shells that spiral in a right-handed direction, others have left-handed shells. Scientists call this twisted form of handedness 'chirality'. Differences in shell twist can help produce new species, as individuals of opposite chirality cannot mate.

The genetic basis of this variability can be remarkably simple – in *Lymnaea stagnalis* chirality is determined by a single gene or a small set of genes that is inherited from the mother, with the right-hand twist form of the gene being dominant. However, neither the gene involved nor its developmental pathways are known.

In an astonishing piece of experimental dexterity, a group of Japanese scientists, led by chirality chemist Reiko Kuroda, have used a combination of genetics and physical manipulation to reveal exactly how the snail shell gets its twist.

In its first stages, the snail embryo shows no sign of chirality; however, after the embryo's third cleavage, when it grows from four to eight cells, information about the future shell twist is present. Kuroda and her colleagues demonstrated this by literally poking about in the embryo.

They used minute glass rods to push around cells in the eight-cell embryo, making the cells establish new connections typical of embryos with the opposite twist to that expected from the embryo's genes. In over 75% of cases, they were able to successfully 're-programme' snail embryos, from right-handed to left-handed twist, and *vice versa*. If they carried out the same experiment at an earlier stage, when there were only two cells, it had no effect.

To see whether successfully manipulated embryos would produce re-twisted snails, they reared the embryos to adulthood and studied their external and internal anatomy. In every case, the snails were fully reversed in every respect compared with the handedness expected from their genes.

The key developmental change, which takes place as the embryo grows from four to eight cells, involves the twist-determining gene, which affects the way the protein Nodal is expressed, leading to handedness in the way the snail is organized.

Exactly how this happens and above all how cells 'remember' exactly which way they are supposed to be twisting remain unknown. The delicate micromanipulation at the heart of this elegant study provides a vital tool for investigating the origins and nature of handedness, not only in spiral organisms like snails but also in other animals.

10.1242/jeb.036426

Kuroda, R., Endo, B., Abe, M. and Shimizu, M. (2009). Chiral blastomere arrangement dictates zygotic left-right asymmetry pathway in snails. *Nature* **462**, 790-794.

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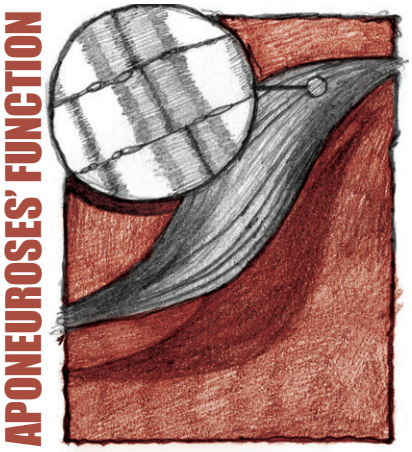

CONTRACTING MUSCLES STIFFEN THEIR APONEUROSES

We're all taught that tendons connect muscles to bones, but like most generalities this statement, while true, doesn't quite capture the complexity involved in the musculoskeletal system. For example, many limb muscles have broad collagenous sheets, or aponeuroses, that lie in between their fibers and the more rope-like tendons that transmit forces to the skeleton. These aponeuroses can be quite large, enveloping much of the distal muscle belly, and yet we have little sense of their purpose. Manny Azizi and Tom Roberts of Brown University wondered whether muscle shape changes during contraction might deform aponeuroses, altering their stiffness and adding some functional versatility to the system. The key to their idea is that unlike tendons, which are almost exclusively stretched along their long axis, aponeuroses likely deform in a more complicated fashion and in so doing might perform a previously unappreciated role when muscles are active.

To test their idea, Azizi and Roberts used an *in situ* preparation of the turkey lateral gastrocnemius (a major ankle extensor), which possesses a large superficial aponeurosis. Using a variety of techniques, they were able to stimulate the muscle to contract, as well as measure and control its force production and length changes. They attached 15–20 small steel markers in an array throughout the aponeurosis so that its deformations could be tracked in the longitudinal and transverse dimensions during stimulated contractions. An experiment consisted of subjecting the muscle to 10 contractions over a broad range of forces, all the while using 3D video fluoroscopy to measure marker movements in the aponeurosis. Following these active contractions, the muscle was driven through a series of passive sinusoidal length changes while the aponeurosis deformations were again tracked.

During active contractions, as muscles generated force and began to shorten, the aponeuroses stretched longitudinally. The longitudinal strains were relatively small and linearly related to the muscle's force production, as one might expect of tissue connected directly to a contracting element. Perhaps more surprising was the fact that the aponeuroses were also stretched in the transverse direction, and these strains were always quite high. Moreover, this transverse stretching altered the mechanical properties of the aponeurosis: the greater the transverse strain, the higher its longitudinal stiffness. The simultaneous stretching of the aponeurosis in orthogonal directions was clearly a function of the muscle actively contracting because during passive length changes in unstimulated muscles, longitudinal aponeurotic strains were out of phase with transverse strains (i.e. stretching in one direction led to contraction in the other).

So why does an actively contracting muscle stretch its aponeurosis transversely (i.e. orthogonal to the muscle force's line of action)? As the muscle's fibers shorten longitudinally, it must expand transversely to maintain a constant volume, and because the aponeurosis is so intimately connected to the muscle belly, it too expands and thus stretches transversely.

Why should I care? Well, as aponeurotic tissue is stretched transversely, its longitudinal stiffness is increased. Recall that aponeuroses act as liaisons between muscles and their tendons, and if their stiffness can be modulated, so too can their effectiveness at transmitting forces from muscles to bones and storing elastic energy. Effective (and variable) force transmission and elastic energy storage potential seem important considerations when studying or modeling the musculoskeletal system in the context of locomotion. Given their size and abundance in limb musculature, it isn't necessarily surprising that aponeuroses have functional relevance, but it is very nice to begin to understand what this relevance might be.

10.1242/jeb.036442

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