

INSIDE JEB

The biology of fat

It is hard to escape the headlines and the statistics are staggering. According to the World Health Organisation, over 1.9 million adults were classified as overweight in 2016, 650 million of whom were considered obese with a BMI of over 30. The implications for global health are sobering, with increased levels of cardiovascular disease, diabetes, musculoskeletal disorders and certain cancers. Yet, this epidemic is preventable. ‘This is one of the reasons that biologists are interested in the mechanisms involved in fat deposition and breakdown’, says Raul Suarez from the University of British Columbia, Canada. He also points out that there are various different forms of fat with diverse biological roles, not all of which are detrimental to health. ‘We used to think only in terms of white fat and brown fat: one has a storage function and the other has a thermogenic function. But now things have changed’, says Suarez. Now we are aware of other varieties of fat, the origins of these different tissues, how fat cells (adipocytes) with different functions develop and how they engage in ‘conversations’ with other cell types in the body.

‘I thought it would be really cool to put together papers by comparative physiologists and biochemists with those of biomedical researchers in a JEB issue to stimulate exchange of ideas between these communities as well as to summarise the latest developments in these fields’, says Suarez. Collaborating with the journal’s Editor-in-Chief, Hans Hoppeler, Suarez has invited 18 leaders in their fields to contribute reviews discussing topics ranging from the development of fat and the role of fat in disease to fats as heat producers, fuel, food sources and sound carriers.

Making fat in fish and humans

Understanding the mechanisms of fat development is fundamental if we are to begin tackling the obesity epidemic; however, Cristina Salmerón from the Scripps Institution of Oceanography, USA, explains that understanding the

phenomenon is also essential for food production in aquaculture (jeb161588). ‘The interest in adipose tissue came from [the need] to control excess fat accumulation experienced by some fish species in aquaculture’, she says. Focusing on the basic biology of fat in fish, Salmerón discusses the possible origins of adipose tissue, before reviewing the biochemistry of lipid metabolism. She also explains that adipose tissue grows through two mechanisms, hypertrophy – where individual cells increase in volume – and hyperplasia – where new cells are produced from stem cells – and suggests that fish and mammals probably share the same molecular pathways that regulate adipose tissue expansion. Although the mechanisms that lead to the first stage of the growth of new cells – the determination phase – are poorly understood in fish, it is clear that the differentiation phase, where preadipocytes differentiate into mature adipocytes, is essentially the same across all species. In addition, fat deposits produce hormones and other signalling molecules – adipokines, which participate in cell communication and inter-tissue communication – to regulate fat accumulation and breakdown.



Farmed Atlantic salmon. Photo credit: Institute of Marine Research.

Moving on to the impact of diet on farmed Atlantic salmon (*Salmo salar*), Nini Sissener from the Institute of Marine Research, Norway, explains that the proportion of fish oil and meal in their diet has dropped from 90% in 1990 to 30% in 2013; the deficit is made up of plant-based replacements such as rapeseed (canola) oil (jeb161521). Although

salmon are capable of synthesising some essential long-chained omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), Sissener explains that fish oil is still an essential source of these fatty acids in their diet. However, despite the recent reductions in the omega-3 content of their diet, farmed salmon are still a good source of long-chain omega-3 fatty acids for humans, as a 150 g portion of farmed salmon provides sufficient long-chain omega-3 fatty acids for 7 days.

Considering the role of fat in human disease, James Minchin, Panna Tandon and Rebecca Wafer from the University of Edinburgh, UK, discuss the development of fat. Minchin explains that the hypertrophic form of fat (where fat is stored in fewer adipocytes, which are much larger) is often associated with diseases such as type 2 diabetes and heart disease, before detailing some of the genes that are known to be involved in adipocyte enlargement (jeb164970). The team then discusses the role of the cell signalling pathway, mTORC – which regulates the size of many different cell types and coordinates nutrition with cell size – inflammation, blood stream lipids and lipid metabolism in heart disease and other obesity-related disorders.

However, Siegfried Ussar, Theresa Schoettl and Ingrid Fischer from the Helmholtz Zentrum München, Germany, point out that not all fat is equal. While white fat predominantly stores energy, a second form of fat, brown fat (known as brown adipose tissue) generates heat. The trio then discusses how fat deposition varies between species, pointing out that the accumulation of visceral fat, fat within muscle and pancreatic fat in humans, is associated with disease, while individuals that predominantly store subcutaneous fat are relatively healthy (jeb162958). Reviewing the factors that give rise to type 2 diabetes, the team says, ‘Local inflammation and insulin resistance in adipose tissue, together with deregulated release of free fatty acids to the liver and skeletal muscle are the events initiating obesity-associated insulin resistance and

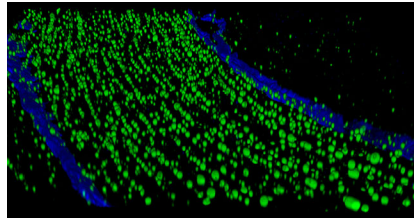
metabolic syndrome'. Ussar and his colleagues then discuss how understanding the factors regulating the development of adipose tissue and the cell types that develop into different forms of fat could help us to identify better treatments for obesity and associated diseases.

Yet, the factors that led humans to develop a tendency toward obesity remain unclear. John Speakman from the Chinese Academy of Sciences, China, and the University of Aberdeen, UK, discusses several theories, including the 'thrifty gene hypothesis', which suggests that genes which predispose individuals to obesity were selected for by famine. However, he points out that the evidence supporting this, and related theories, does not stack up. In 2008, Speakman suggested that the loss of predation pressure two million years ago may have released the selection pressure that kept the amount of fat that people accumulated in check, and named the theory the 'drifty gene hypothesis' (jeb167254). Speakman then discusses the criticism that the hypothesis has attracted before detailing the mechanisms that support it. He then goes on to suggest that instead of storing fat to protect against food shortages, animals accumulate fat to allow them to wait out periods of illness.

Fat and diet

Returning to the theme of fat stored in muscle, Matthijs Hesselink from Maastricht University Medical Centre, The Netherlands, explains that this fat is found in the form of lipid droplets. As humans become obese, the number of lipid droplets in muscle also increases and this event may indicate the early development of type 2 diabetes. However, Hesselink and colleagues point out that trained athletes also have a high lipid droplet count in their muscles, although their droplets undergo more rapid turnover and seem to be more closely associated with the energy-generating mitochondria and structures that trigger muscle contractions. The scientists then discuss the network of proteins that prepare the lipid droplet fatty acids for later use and the effects of exercise on the proteins that coat lipid droplets (jeb167015). The trio says, 'By modulating these proteins by diet, nutritional compounds or exercise, the lipid droplet phenotype can be affected such that expansion of intramyocellular

lipid can occur without negative effects on cellular function and/or insulin sensitivity', and they are optimistic that this knowledge could be applied to moderate the negative impact of lipid droplets in the muscle of obesity sufferers.



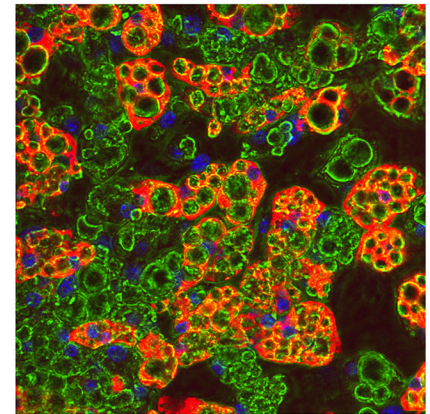
3D confocal image of BODIPY-stained lipid droplets (in green) in exercise-trained human skeletal muscle. Photo credit: Anne Gemmink.

A major contributor to the obesity epidemic in Europe and North America has been the dramatic increase in sugar consumption over the last century. However, it is the increase of fructose, which occurs naturally in sucrose and in high-fructose corn syrup – which is used extensively as a sweetener – that concerns Luc Tappy from the University of Lausanne, Switzerland. Explaining how fructose must be converted into glucose, lactate or fatty acids before it can be used or stored by the body, Tappy warns that the sugar increases blood lipid levels, in addition to raising the amount of fat in the liver and around the visceral organs (jeb164202). However, he explains that fructose consumed in combination with glucose during exercise seems to be beneficial, as it is converted into lactate in the liver before it is transported to the muscle, where it is oxidised for energy production. Tappy suggests that the ability to convert fructose into other energy sources, and the distinct mechanisms that transport fructose-based metabolites, may have been advantageous for early humans, allowing them to store energy when available. However, he warns that these benefits could now prove disastrous in an environment where fructose pervades the modern diet.

Fat as fuel and other uses

The release of energy from fat stores is tightly choreographed in tune with the body's ever-fluctuating energy demands. Martin Klingenspor from the Technical University of Munich, Germany, explains that the process of fat breakdown is governed by the autonomic nervous system – which innervates adipose tissues. Release of the neurotransmitter noradrenaline by these nerves triggers a

chain of events in the fat cell that results in the release of lipids from fat stores into the blood stream. Outlining our current understanding of the role of neurotransmission, Klingenspor and colleagues point out that many other non-neuronal control systems have been found, including hormones produced by the fat itself and other organs (jeb165381). The team also discuss the role of these regulatory molecules in heat production in brown fat cells and the 'browning' of white fat – when white fat accumulates heat-producing mitochondria – which may have clinical benefits.



Cultured fat cells (green) stained for UCP1 (red) and cell nuclei (blue). Photo credit: Yongguo Li.

While the obesity epidemic is a recent human scourge, Christopher Guglielmo from Western University, Canada, explains that the accumulation of fat is a vital component of the natural history of many migratory birds. Subcutaneous fat stores of some migratory species prior to departure can comprise over 50% of the body mass with 75–90% of the lipid stores composed of medium-length fatty acids. Explaining that migratory birds gorge during their preparations and can gain weight at a rate of 15% per day, Guglielmo also points out that migrants prefer diets with unsaturated fatty acids (which contain one or more double bonds in the fatty acid chain, in contrast to saturated fatty acids, which have no double bonds), which suggests that increased fatty acid unsaturation may be beneficial for migration (jeb165753). Referring to migratory birds as 'fat-burning machines', Guglielmo explains that in addition to raising the activity of the enzymes that oxidize fat to release energy in mitochondria, the animals also increase fatty acid transport mechanisms into the muscle cells and the mitochondria. Comparing the physiology

of migratory birds with that of bats, which evolved flight independently, Guglielmo explains that migrating bats also appear to use fat, in contrast with other mammals, which depend on glycogen for intense exercise. Guglielmo says, ‘These animals show us that fatty acids are indeed fantastic high-energy fuels that can be used to power the highest aerobic metabolic rates, given the right adaptations to the energy supply system’.



A fully-fuelled sanderling (*Calidris alba*) weighing 99 g. The usual lean body mass is about 50–55 g. Photo credit: Chris Guglielmo and Brock Fenton.

Fat is also key to the survival of many overwintering insects, which do not feed while enduring sub-zero temperatures. However, Brent Sinclair, also from Western University, Canada, and Katie Marshall from the University of Oklahoma, USA, explain that low temperatures pose a specific set of challenges for animals that depend on fat for fuel. They discuss how these insects accumulate lipids from their diet, while producing others internally, in addition to reducing their metabolic rate to conserve reserves while food is unavailable. However, many fats solidify at low temperatures, so the insects convert solid saturated fatty acids into unsaturated fatty acids, which remain fluid at low temperatures. In addition, overwintering insects accumulate lipid-derived antifreeze compounds, including glycolipids, to ensure that they do not freeze and the enzymes that break down fats continue to function at low temperatures (jeb161836). Yet, Sinclair and Marshall point out that our understanding of lipid metabolism, based on this small group of insects, remains incomplete and the duo is keen to learn more about the insect’s regulation of lipid use and how they use them in sub-zero temperatures.

Another species that endures a lengthy fast is the northern elephant seal. However, in addition to fuelling their own metabolism, many females also sustain milk production while raising their pups. As lactation is extraordinarily costly for mothers, female elephant seals ration their fat reserves, which comprise 36% of their body fat when they deliver their young, to fuel their own metabolism while directing lipids to produce energy-rich milk for their young. Melinda Fowler from Springfield College, USA, Cory Champagne from Old Dominion University, USA, and Daniel Crocker from Sonoma State University, USA, discuss how the females mobilise medium-length mono-unsaturated fatty acids and saturated fatty acids to fuel their own metabolism while directing long-chain mono-unsaturated fatty acids into milk production. They also explain that a reduction in the amount of insulin in the blood stimulates the release of fat into the blood (jeb161554).



Female elephant seal (right) with her pup [National Marine Fisheries Service Permit (NMFS) #786-1463]. Photo credit: Sarah Ann Thompson.

Although fat is widely used as an energy repository, Heather Koopman from the University of North Carolina Wilmington, USA, explains that a small number of aquatic mammals exploit the ability of fat to carry sound (jeb161471). Toothed whales produce a specialised class of waxy fats, which are thought to contribute to the buoyancy, energy storage and insulation properties of blubber beneath the skin, and can comprise as much as 60–99% of the blubber in beaked and sperm whales. Koopman explains that, in contrast, the waxes that occur in the whale’s head are involved in sound transmission. According to Koopman, the low-density waxy fats, which transmit sound at low speeds, are located in the centre of the melon structure, while higher density triacylglycerol lipids that transmit sound faster are distributed around the waxy core to focus sound for

echolocation. Suggesting that these waxes evolved first in the head for their acoustic properties, Koopman is keen to learn why belugas and narwhals have virtually eliminated them from their acoustic system and how wax deposition in the blubber is regulated as the animals grow and develop.

Fat as furnace

Shifting focus to the mitochondria-packed form of fat known as brown fat or brown adipose tissue, which generates heat, Matthew Andrews from Oregon State University, USA, and Mallory Ballinger from the University of California, Berkeley, USA, explain how hibernating mammals have tailored this tissue to generate warmth rapidly when they periodically emerge from torpor during hibernation. Building up their reserves of brown fat in the months leading up to winter, hibernating animals depend on a unique protein, uncoupling protein 1 (UCP1), found in the mitochondria of brown fat, which diverts energy from ATP production into heat (jeb162586). Andrews and Ballinger explain that, during hibernation, brown adipose tissue is maintained in a state where heat production can be triggered rapidly by the sympathetic nervous system when animals emerge from hibernation. However, instead of maintaining RNA production in readiness for the expression of proteins that are essential for heat production, mitochondria in brown adipose tissue maintain the proteins involved in membrane transport, oxidation and components of the electron transport chain, ready to generate heat rapidly.



A hibernating thirteen-lined ground squirrel. Photo credit: Matthew Andrews.

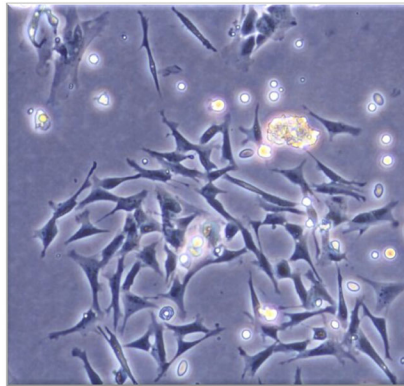
Intrigued by the evolution of this unconventional tissue, Martin Jastroch, also from the Helmholtz Zentrum München, Germany, and colleagues outline the lessons that have been learned

from a variety of non-model organisms (jeb169425). It has been essential to explore the tissue in a wide range of animals from fish to monotremes and marsupials to learn about the evolution of brown fat. Jastroch explains that the UCP1 protein evolved in fish before animals emerged onto land and remains in modern mammals and amphibians, despite its loss in birds and reptiles. Focusing on brown fat in afrotherians (which include tenrecs and aardvarks), Jastroch and his colleagues suggest that instead of evolving in mammals to generate heat during the move to colder regions, brown fat initially evolved in placental animals for the purpose of offspring incubation. They suggest that it may have allowed the early ancestors of modern placental animals to survive the short-term global cooling caused by dust from the meteorite impact that led to the extinction of the dinosaurs.

Good and bad fat: regulation and dysregulation

How animals manage their metabolism throughout life can have profound implications for health. Féaron Cassidy and Marika Charalambous from Queen Mary University of London, UK, discuss the role of a selection of genes, known as imprinted genes – where only one inherited parental copy of the gene is functional – on the metabolism of animals from conception through to adulthood and motherhood. Explaining that the products of imprinted genes act in specific tissues, Charalambous and Cassidy list the imprinted genes that are involved in energy regulation through the nervous and hormonal systems during pregnancy and their role in white and brown fat development (jeb164517). They say, ‘As abnormal birthweight correlates with adverse adult metabolic health, including obesity and cardiovascular disease, it is crucial to understand how the modulation

of this dosage-sensitive, epigenetically regulated class of genes can contribute to fetal and postnatal growth, with implications for life-long health and disease’.



Cultured preadipocytes of gilthead sea bream (*Sparus aurata*). Photo credit: Cristina Salmerón.

While the genetic makeup of an animal has major implications for the amount and distribution of different forms of fat around the body, Kristin Stanford and Adam Lehnig from The Ohio State University Wexner Medical Center, USA, discuss how exercise also has an important effect, altering white and brown fat in rodents and humans (jeb161570). They say, ‘Studies consistently demonstrate that exercise training has marked effects on mitochondrial gene expression and activity in human white adipose tissue’, adding that training has a similar effect in rodents. And when researchers from Stanford’s team tested the effects of transplanting white fat from fit mice into couch-potato mice, they were impressed to see that glucose metabolism, which is symptomatic of type 2 diabetes, in the unfit mice improved, despite their inactive lifestyle. In addition, exercise affects the hormones and signalling factors that are released by white fat,

which regulate appetite and metabolism, as well as altering the lipid content of subcutaneous fat. However, they explain that the impact of exercise on brown adipose tissue is less certain. As exercise clearly has beneficial effects on white fat, they suggest, ‘Elucidation of the adaptations to adipose tissue with exercise may ultimately lead to more effective exercise intervention programmes and therapeutic remedies aimed at combating the growing epidemic of metabolic diseases’.

Concluding the collection, Laura Musselman from the State University of New York, Binghamton, USA, and Ronald Kuhnlein from the University of Graz, Austria, review what we have learned about obesity and metabolic disease from the humble fruit fly, *Drosophila melanogaster* (jeb163881), thanks to the extensive assortment of molecular tools that allow scientists to investigate fundamental mechanisms in these insects. Explaining that a mutation in a single gene, which also occurs in humans, can cause the insect to become obese, the duo reviews other parallels between human and fruit fly obesity. Listing hormones that regulate the accumulation of fat and the mechanisms that regulate the accumulation and breakdown of fats, Musselman and Kuhnlein also discuss how obese fruit flies suffer many of the same complications experienced by overweight humans, including heart disease and dangerously high blood sugar levels, but there are still many lessons that this tiny, but powerful, insect can teach about the biology of fat.

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