Mitochondrial metabolism: a driver of energy utilisation and product quality?

Article *in* Animal Production Science · January 2017

DOI: 10.1071/AN17322

CITATIONS
12

READS
812

6 authors, including:

Nicholas James Hudson
The University of Queensland
125 PUBLICATIONS **1,836** CITATIONS
SEE PROFILE

Walter G Bottje
University of Arkansas
203 PUBLICATIONS **5,132** CITATIONS
SEE PROFILE

Rachel Jane Hawken
Cobb Vantress
156 PUBLICATIONS **4,843** CITATIONS
SEE PROFILE

Ronald Okimoto
Cobb-vantress Inc.
25 PUBLICATIONS **525** CITATIONS
SEE PROFILE

CSIRO PUBLISHING

Animal Production Science, 2017, **57**, 2204–2215
https://doi.org/10.1071/AN17322

Review

Mitochondrial metabolism: a driver of energy utilisation and product quality?

N. J. Hudson[A,E], *W. G. Bottje*[B], *R. J. Hawken*[C], *ByungWhi Kong*[B], *R. Okimoto*[C] *and A. Reverter*[D]

[A]The University of Queensland, School of Agriculture and Food Sciences, Gatton, Qld 4343, Australia.
[B]University of Arkansas, Fayetteville, Arkansas, AR 72701, USA.
[C]Cobb Vantress Inc., Siloam Springs, Arkansas, AR 72761, USA.
[D]Commonwealth Scientific and Industrial Research Organisation, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Brisbane, Qld 4072, Australia.
[E]Corresponding author. Email: n.hudson@uq.edu.au

Abstract. High feed efficiency is a very desirable production trait as it positively influences resource utilisation, profitability and environmental considerations, albeit at the possible expense of product quality. The modern broiler is arguably the most illustrative model species as it has been transformed over the past half century into an elite feed converter. Some producers are currently reporting that 42-day-old birds gain 1 kg of wet weight for every 1.35 kg of dry weight consumed. Its large breast muscle is exclusively composed of large, low mitochondrial-content Type IIB fibres, which may contribute to low maintenance costs and high efficiency. In an effort to gain a better understanding of individual variation in chicken feed efficiency, our group has been exploring the biology of the mitochondrion at multiple levels of organisation. The mitochondrion is the organelle where much biochemical energy transformation occurs in the cell. Using Cobb-Vantress industrial birds as our primary experimental resource, we have explored the tissue content, structure and function of the mitochondrion and its relationship to growth, development, efficiency and genetic background. While much remains to be understood, recent highlights include (1) variation in muscle mitochondrial content that is associated with performance phenotypes, (2) altered muscle mitochondrial gene and protein expression in birds differing in feed efficiency, (3) variation in isolated mitochondrial function in birds differing in feed efficiency and (4) evidence for an unexpected role for the mitochondrially localised progesterone receptor in altering bird muscle metabolism. Mitochondrial function is largely conserved across the vertebrates, so the same metabolic principles appear to apply to the major production species, whether monogastric or ruminant. A speculative role for the mitochondria in aspects of meat quality and in influencing postmortem anaerobic metabolism will conclude the manuscript.

Additional keywords: feed efficiency, muscle.

Received 15 May 2017, accepted 20 July 2017, published online 23 August 2017

Introduction

Animal production can be seen as a transformation through which chemical energy stored in feed is converted into nutritious, appealing, value-added products for human consumption, such as meat and eggs. At the level of an individual production animal or bird, this transformation encompasses the biological processes of behaviour, feeding, digestive physiology (including the role of symbiotic gut microbiota) and cellular metabolism. While all are likely to play some role in the expression of animal bioenergetics including feed efficiency (FE), the last of these (cellular metabolism) is the primary focus of the present paper as it is a particular research interest of the authors. FE is calculated through relationships between dry feed intake (input) and overall wet weight gain (output). In contrast to the biomedical literature where there is a focus on obese phenotypes, efficiency in agricultural meat production specifically refers to lean-tissue deposition.

While fat accumulation can clearly be seen as the product of an 'efficient' metabolism, where the feed energy has been acquired and stored rather than liberated, it is considered wasteful in agriculture because excess fat is trimmed off animal carcasses. As is true for all biological systems, the cost of commercially valuable lean-tissue deposition in broiler chickens is ultimately paid for by the high-energy intermediate molecule, adenosine triphosphate (ATP). With this in mind, the biochemistry of ATP production and use is something we consider central to an understanding of feed efficiency, and this, in turn, has implications for the characteristics of highly feed-efficient phenotypes.

The average mammalian cell contains ~1 billion molecules of ATP, which are recycled every 2 min (Hoagland *et al*. 2001).

Humans turn over the equivalent of their entire bodyweight in ATP on a daily basis (Tornroth-Horsefield and Neutze 2008), although, at any one time, the total is ~250 g (about half a mole given a molecular mass of 507.18). Under physiological conditions, the Gibbs free energy related to ATP hydrolysis is 50–70 kJ/mol. This staggering flux of energy is in large part attributable to the 'engine-room' of the cell, the mitochondrion. In the presence of oxygen, most of ATP in most tissues most of the time is synthesised by the mitochondria, transforming multiple sources of chemical feed energy into a common, usable currency. In contrast, anaerobic metabolism tends to be a position of last resort in the live animal and even then is restricted to certain tissues and circumstances such as the skeletal musculature during vigorous exercise. Anaerobic metabolism produces a small amount of ATP very rapidly, but it comes at the expense of inhibitory end products. Anaerobic metabolism is relevant to postmortem muscle to meat conversion and will be addressed later in the paper.

To a large extent, we focus the content of the present paper on aerobic metabolism, as it is the sustainable energy-conversion system underpinning most facets of animal production. The present paper focusses on the authors' own primary research into mitochondrial structure and function and its relationship to agricultural performance phenotypes. It is not intended as a comprehensive review of feed efficiency, but rather a personal perspective on one aspect of biology that looks to play a role. A review partitioning out likely contributing physiological processes in ruminant animals can be found here (Herd and Arthur 2009). Further, the present paper is a product of an invitation to speak at the 2017 Recent Advances in Animal Nutrition in Australia (RAAN-A) where speakers were encouraged to speculate on future trends and explore new ideas, particularly across species and disciplines. In places, we have made deliberate use of analogy and non-technical language to facilitate broad readership across disciplines.

Finally, we have made an effort to introduce and explore the basic biochemistry in more detail than would be customary in a research manuscript. This is a response to an editorial request to make the manuscript accessible to non-specialists. Biochemists may wish to skip the mitochondrial overview below and move directly to the section on muscle mitochondrial content across species.

Mitochondria: an overview

Energetically depleted ADP is phosphorylated to energy-rich ATP by an enormous mitochondrial molecular motor called ATP synthase. The synthase motor is rotated by a 'downhill' flow of protons (H^+), termed the proton motive force, which has been set up across the innermost of two mitochondrial membranes. This can be compared, by way of analogy, to the energy transformation that occurs when a cyclist peddles a bicycle, with an input form of energy coupled to an output form of energy by the combination of the peddles and chain. Because protons bear a charge as well as determine pH, the flow is essentially an attempt to renormalise two imbalances (one electrical and one chemical), collectively called an electrochemical potential. It is believed that four protons are required to flow through the ATP synthase to make a single molecule of ATP.

The direct connection of this electrochemical potential to the activity of the ATP synthase complex is called 'chemiosmotic coupling' and bequeathed to its principal advocate, Peter Mitchell, a hard-won Nobel Prize (Mitchell 1961). Mitchell's ideas were originally ridiculed by the scientific community, who favoured a traditional biochemical explanation (i.e. the making and breaking of chemical bonds in a sequence of reactions exploiting high-energy intermediates). By way of contrast, Mitchell's electrochemical proposal is biophysical in nature. Unlike his contemporaries, he concentrated on thermodynamic principles and developed his theory largely in the absence of any supporting data. The psychological stress of rejection and isolation, together with Mitchell's mild-mannered personality, culminated in depression and gastric ulcers. He withdrew from the scientific community, resigned from his academic position and resorted to running experiments out of a home laboratory funded by family wealth. It would be several decades before Mitchell's view of the bioenergetic world eventually triumphed over that of his peers, but it is now widely believed that the basic tenets of his theory have proven to be fundamentally correct.

The 'uphill' supply of protons in chemiosmosis comes from reducing agents, primarily in the form of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$). The protons travel through a mitochondrial redox system set up in parallel as four enzymatic protein complexes (NADH dehydrogenase, succinate dehydrogenase, cytochrome *b* and cytochrome oxidase) termed the electron-transport chain (ETC) that are embedded in the innermost mitochondrial membrane. The combination of events involving the capacity of the ETC to drive the motor of the ATP synthase is called oxidative phosphorylation. In turn, the NADH and $FADH_2$ proton donors are a major product of the mitochondrial citric acid cycle, whose bioenergetic input is acetyl coenzyme A (CoA), a metabolite often considered to be the metabolic cross-roads.

Acetyl CoA is universally produced by the digestion and catabolism of all protein, carbohydrate (by glycolysis and subsequent dehydrogenation of pyruvate) and lipid (by β-oxidation) consumed in the diet or derived from stored body reserves. In ruminants, rather than glucose, it is volatile fatty acids (particularly acetate) produced by rumen microbial fermentation that are the major source of acetyl CoA. However, whether monogastric or ruminant and irrespective of preferred substrate usage, the fundamentals of ATP synthesis (the tricarboxylic acid cycle (TCA) cycle and subsequent activity of the electron-transport chain) are highly conserved across the eukaryotes, which is well illustrated by the similarities of the mitochondria of *C. elegans* to those of mammals (Rea *et al*. 2010). With this in mind, the basic machinery of underpinning mitochondrial function can be considered largely invariant across production animals. Cellular respiration can essentially be viewed as the process by which chemical energy in food produces acetyl CoA, which produces ATP. The mitochondrion is a central player in all of the biochemical transformation after acetyl-CoA production, and in many of the events before it, such as the β-oxidation of fat (see Fig. 1 for schematic summary).

With a few rare (and functionally enlightening) exceptions, every cell contains a single, continuous branching network of mitochondria. Indeed, overall metabolic potential is largely governed by the size and structure of its mitochondria. This

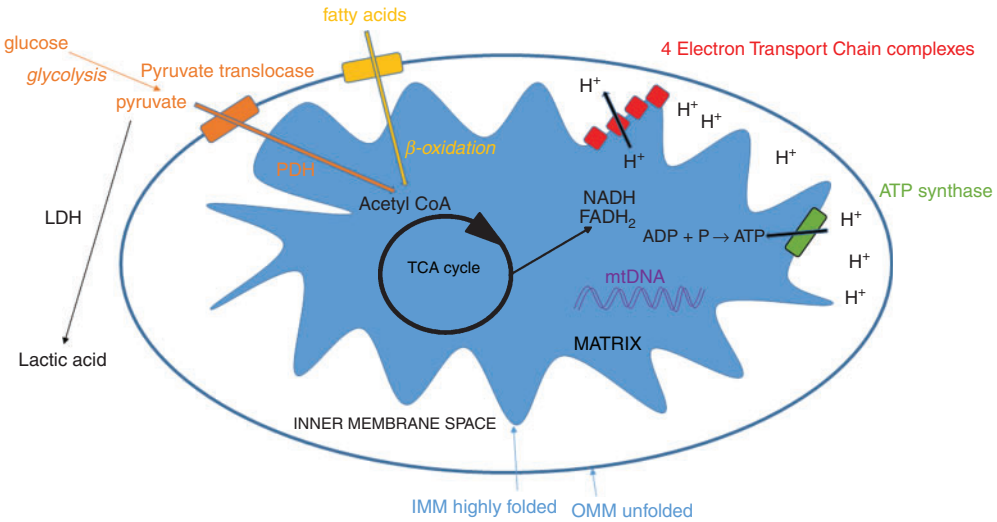

Fig. 1. A highly simplified schematic of mitochondrial function, encompassing aspects of the major pathways and processes from substrate import to adenosine triphosphate (ATP) production. The schematic has been set up to be read from left (substrate preparation and delivery to the mitochondria from the cytosol) to right (production of acetyl coenzyme A (CoA) and then ATP within the mitochondria). In general terms, the structure of a mitochondrion comprises a viscous central matrix bounded by two semi-permeable membranes. Most of the ~1000 proteins are present in the matrix. The inner membrane (IMM), in particular, is strictly regulated and contains more embedded proteins than does the outer membrane (OMM). Starting at the left-hand side, carbon skeletons from either carbohydrate (via glycolysis) or fat are imported through the membranes into the matrix. Both combustive pathways converge at acetyl CoA, which fuels the tricarboxylic acid cycle (TCA) cycle. This, in turn, yields proton donors (nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$)), which are exploited by the electron-transport chain (ETC) to collectively pump protons into the inner membrane space. This produces a proton motive force, which is used to drive the molecular motor of the ATP synthase, thereby phosphorylating ADP to make new ATP. All of the processes illustrated are under tight regulation, in part through enzyme activation and inhibition of the rate-limiting and committed steps. In brown fat, the ATP synthase is replaced by UCP1, which dissipates the proton motive force without making ATP, thereby liberating heat. Under anaerobic conditions, such as dominate in postmortem muscle-to-meat conversion, pyruvate is reduced to lactic acid in the cytosol rather than imported into the mitochondria for oxidation to acetyl CoA. In mitochondrial treason, the ETC and ATP synthase operate in reverse, consuming cytosolic ATP to maintain a trans-membrane proton gradient.

sets what is known as aerobic capacity, the upper ceiling of ATP synthesis in the presence of oxygen. Several examples will be given throughout the present paper in which there is substantial variation in the size of the mitochondrial network (aerobic capacity), which has profound functional consequences. Examples include adaptations in the muscle of human endurance athletes and hummingbirds versus human sprinters and cheetahs, metabolically profligate brown fat versus relatively inert white fat, and the near absence of mitochondria in oxygen-carrying red-blood cells.

Livestock display substantial individual variation in the aerobic capacity of muscle (Reverter *et al*. 2017) and gut (Kong *et al*. 2016*b*) tissue. In skeletal muscle, one might expect much of the individual variation to arise from variation in muscle fibre composition. However, in our work on chicken breast muscle, we observed five-fold variation in breast muscle-tissue mitochondrial content, despite an almost homogenous Type IIB glycolytic fibre composition (Reverter *et al*. 2017). In animals, such as pigs, sheep and cattle, additional variation could arise from the particular composition of mixed fibre types. In these cases, one might expect the individual variation in muscle aerobic capacity to be greater still. In non-muscle tissues, such as the cattle-gut example given above (Kong *et al*. 2016*b*), the source of the substantial individual variation in aerobic capacity they identified is not so clear. There is no compositional equivalent of mixed muscle fibre types in gut, stomach or liver tissue. Interestingly, previous authors examining liver, stomach and small intestine metabolism have suggested organ size to be a stronger driver of whole animal metabolic traits than tissue-specific metabolic activity (Burrin *et al*. 1990). This is surprising in light of the individual variation detected by (Kong *et al*. 2016*b*).

Where appropriate, comparison will be made to the wild ancestor of the domestic animal and also to less feed-efficient breeds. When analysed in standard histological cross-section, the continuous mitochondrial networks appear to be discrete oval organelles, but this is illusory. For this reason, we shall refer to the amount of mitochondria in a cell as its mitochondrial content, not number. In terms of basic structure, the mitochondrion is an organelle with a viscous centre called the matrix, bound by two semi-permeable membranes, the inner (IMM) and the outer (OMM). The matrix contains the majority of the ~1000 mitochondrial proteins, such as those engaged in the citric acid cycle and in β-oxidation. In contrast, a much smaller number of specialised transporter proteins are embedded in the two membranes, particularly the more tightly regulated IMM.

As mentioned earlier, the oxidative phosphorylation complex (four ETC complexes plus ATP synthase) and several other

protein complexes are embedded in the less permeable IMM. Various mitochondrial import (e.g. pyruvate translocase) and export (e.g. voltage-dependent anion channel) proteins are embedded in the OMM. The purpose of the OMM and associated proteins is to help provide the mitochondrion with access to fuel for combustion, but also to allow delivery of freshly minted ATP and metabolic waste to the cytoplasm for cellular consumption and disposal respectively. Furthermore, the mitochondrion maintains close physical connections with other organelles, particularly the endoplasmic reticulum and peroxisome, with which it cooperates.

Finally, the mitochondrial matrix contains its own genome. This is a vestige of its ancestry as a free-living bacterium that, in modern eukaryotes, acts symbiotically within the host cell. The presence of a mitochondrial genome means that molecular techniques (such as quantitative polymerase chain reaction (qPCR) for estimation of mtDNA copy number)) can be applied to various aspects of mitochondrial biology in an informative manner. Towards the end of this article, a fascinating example will be given in which the mitochondria's 'community service' to modern eukaryotic cells is placed in jeopardy in certain extreme metabolic circumstances. In humans, the mitochondrial genome encodes 37 genes, including two rRNAs, 22 tRNAs and 13 protein-coding genes. The regulation of mitochondrial function requires that mRNA expression from the mitochondrial genome is coordinated with that of the much larger nuclear genome.

In our collective research, we have mainly focussed on mitochondria located in the skeletal musculature. This is on the grounds that, in addition to its commercial value, muscle (1) contributes a substantial amount (~50%) to animal mass, (2) accounts for ~25% of resting metabolism (Henriksson 1990), and a much greater proportion of energy expenditure in an active animal, (3) usually exhibits substantial diversity in fibre composition, which sets up the clear possibility of substantial between-individual variation even after corrections for tissue mass, (4) is the primary tissue we consume and (5) is highly plastic and responsive to environmental cues. This combination of high mass, high responsiveness and high potential for individual variability is a unique feature of the skeletal musculature of vertebrates. It also opens up the possibility for manipulation by environmental stimuli (namely activity, ambient temperature and diet), which are known to influence mitochondrial biology and could potentially be managed in commercial production settings.

With regard to the chicken experiments discussed here, except for the experimental data section on broiler mitochondrial content and broiler genetics, we made repeated use of an unusual feed-efficiency resource, namely, two groups of birds ($n = 8$ in each group) from within a single genetic line and from the same generation, with one group expressing a ~1.5-fold higher feed efficiency (HFE) than the other group (LFE) from 42 to 49 days of age. These birds were phenotyped on the basis of the industrially accepted measure of FE. In our case, the HFE birds exhibited 0.65 ± 0.01 g wet weight gain per gram of dry feed consumed, whereas the LFE birds exhibited 0.46 ± 0.01 g wet weight gain per gram of dry feed consumed. This animal resource was not the product of divergent selection, meaning that we can rule out the role played by functionally irrelevant genetic founder effects. However, before discussing production-animal mitochondria in particular, it is important to consider muscle mitochondria and animal function in general terms.

Muscle mitochondrial content in different species

Contrasting the mitochondrial phenotypes of species that are extreme metabolic performers is one useful approach for understanding what is achievable with mitochondrial adaptation. This may not be a common analytical strategy in traditional animal science. Nevertheless, comparative biology is a useful approach here as it provides a broad perspective in which to embed discrete individual, breed and species data. Comparative physiology has a particularly rich history in metabolic research because it is the basis for the allometric relationships that describe basal (White and Seymour 2003) and field metabolic rates (Nagy 2005) and how they scale with animal mass. Similarly, comparative genomics can be used to identify gene expansions and pseudogenic degeneration that are species specific. In both cases, whether comparative physiology or comparative genomics, the extra layer of data can provide fresh biological insight.

For example, to take the big-picture viewpoint on muscle mitochondria, the highly athletic hummingbird has a pectoralis muscle mitochondrial content of ~35% (Mathieu-Costello *et al*. 1992). In the late 1970s, the much more sedentary broilers were found to contain a pectoralis mitochondrial content nearly 1/10 of that, i.e. 4% (Kiessling 1977). The current mitochondrial content of broiler breast muscle is unknown, but one of us (NJH) predicts that it is now likely to be even lower than 4% for reasons that will be made apparent. To the best of our knowledge, sprint-adapted cheetahs possess the lowest measured value for muscle mitochondrial content, ~2% (Williams *et al*. 1997). In the case of the cheetah, the very low muscle mitochondrial content is a sprint adaptation. In muscle geared to high-power output supported by anaerobically produced ATP (sprinting), the space that would be taken up by mitochondria is more effectively consumed by the contractile filaments themselves. This is because the cross-sectional area of the available contractile apparatus relates to the power output of the whole muscle. This argument follows the principle of allocation as applied to individual cells (or muscle fibres). More broadly, it is an example of an economic-design principle being applied to biological systems, an idea from comparative physiology called 'symmorphosis' (Weibel *et al*. 1998).

Muscle mitochondrial content is plastic and responsive to environmental as well as genetic drivers. It can be increased by endurance training, cold ambient temperature (Bruton *et al*. 2010; Shabalina *et al*. 2010) and various diets (van den Broek *et al*. 2010) and tends to decline during ageing. For example, in the elderly even simple tasks may require the bioenergetic system to resort to physically uncomfortable bouts of anaerobic metabolism to meet ATP demand (Young 1997). Measurements of mitochondrial content across the skeletal musculature of numerous animals tend to show two patterns. First, there is a well established general decrease in content with an increasing animal mass (Mathieu *et al*. 1981). Second, superimposed on this trend is the tendency for production of animal muscle mitochondrial content to be particularly low even

after mass has been accounted for (Hudson 2009). Further to this, a deeper exploration of particularly efficient production breeds has shown a consistent tendency towards higher proportions of low mitochondrial content fibres, such as the white, Sprint type IIB and IIX glycolytic fibres. In these instances, mitochondrial content has not normally been measured directly, but has been assumed from knowledge of the biochemistry of the different fibre types.

Examples of breeds with a muscle fibre composition transition consistent with a reduction in mitochondrial content include numerous feed-efficient myostatin mutant cattle, callipyge sheep and Large White pigs among others (Jackson *et al*. 1997; Deveaux *et al*. 2001; Lefaucheur *et al*. 2004; Bouley *et al*. 2005; Lehnert *et al*. 2007; Lefaucheur *et al*. 2011). In these cases, a combination of antibodies selectively staining the fast versus slow myosin heavy-chain isoforms in addition to oxidative (citrate synthase and isocitrate dehydrogenase), glycolytic and anaerobic (lactate dehydrogenase) enzyme activities are typically used to characterise the muscle fibre transition. The pig example is particularly noteworthy. The entire structure of the skeletal musculature of modern pig breeds has changed so dramatically compared with the wild boar (Essén-Gustavsson and Lindholm 1984; Rehfeldt *et al*. 2008) that they are sometimes now characterised as being the second white meat after chicken rather than the third red meat after beef and lamb.

If one accepts that the mitochondrion is the engine-room of the cell, then mitochondrial content determines engine size. By this reasoning, the modern broiler, in particular, and feed-efficient production breeds, in general, possess a small engine. This observation is additional to the simple reductions in the mass of vital organs previously observed in myostatin-mutant cattle (Fiems 2012). These tissue-level changes, which have also been argued to drive enhanced FE, can also be characterised as examples of a small engine and are analogous to the organelle-level arguments that form the focus of this manuscript.

In the case of the broiler, the small-engine phenotype is partly a consequence of the ancestral species, the red jungle fowl, bearing a very unusual metabolism already dominated by low mitochondrial content Sprint type IIB muscle fibres, even in the wild, free-ranging state. In fact, a low muscle mitochondrial content appears common to all ground-dwelling bird species in the Phasianidae taxonomic group, such as turkeys, pheasants, grouse and quail. This reflects their wild physiological ecology as they use short-burst flights reliant on anaerobic ATP production to escape predators, as reviewed by (Askew and Marsh 2002). In the case of mammalian production species, the small engine has been inferred from changes in muscle fibre composition towards the low mitochondrial-content white, glycolytic fibres.

Why should modern production animals possess a low muscle mitochondrial content in general, and why should this be present in feed-efficient breeds in particular? Mitochondrial content has implications for bioenergetics at rest as well as during activity because the proton motive force cannot be allowed to dissipate. This creates substantial maintenance costs for 'idling' mitochondria that have to be ultimately paid for by feed energy. Along these lines, physiological spare-capacity arguments (Diamond and Hammond 1992) suggest that building in unnecessary spare capacity is inefficient. Capacity comes at a cost.

This is another example of the symmorphosis economic-design principle borrowed from comparative physiology (Weibel *et al*. 1991). One can apply this thinking to mitochondrial content; an efficient feed converter has enough, but not too much, fuel-burning capacity (Hudson *et al*. 2008; Hudson 2009). To take a vehicular analogy, fuel-efficient, small-engine Toyota Corollas consume less fuel than do high-performance drag racers, even at common speeds. The common speed part of the argument is important here because production animals need to satisfy only the bioenergetic requirement of production. From the perspective of the skeletal musculature and cardiovascular system, the energy demand for production is far less than that required during elite athletic performance.

A more formal explanation of symmorphosis and its application to low mitochondrial content relates to the bioengineering costs of construction, maintenance and load. In the context of mitochondrial function, these would be reflected in the reduced cellular costs of (1) mitochondrial biogenesis, (2) creating and maintaining the proton motive force and (3) space occupied by the mitochondria that could be used for other cellular apparatus. This economic-design argument explains in part why 'drag-racing' hummingbirds must feed almost constantly while on the wing, and enter a specialised metabolic hibernation to fend off the threat of starvation while inactive every night. A starker contrast to the sedentary, highly feed-efficient broiler is hard to imagine.

As mentioned above, ATP foots the bioenergetic bill in all living systems. Notably expensive (ATP demanding) cellular processes include the maintenance of trans-membrane ion gradients, particularly the Na^+K^+ plasma membrane transporter, which, on its own, typically consumes 20–30 of every 100 ATP molecules produced by any given cell (Jorgensen and Pedersen 2001). This explains why organisms considered to be energetically frugal (equivalent to a feed-efficient phenotype), such as tailed amphibians and lungfish, both of which persist in oxygen-poor environments, are built of individually very large cells (Szarski 1983). For surface area-to-volume reasons, they possess less total plasma membrane across which the Na^+ and K^+ gradients must be maintained. This would be expected to lessen their ATP demand on a per-unit-tissue basis. Along these lines, the Type IIB fibres that exclusively constitute the breast muscle of broilers have the largest cross-section of all the possible sprint and endurance muscle fibre types, thereby presumably achieving the same energy-saving goal. A similar energy-saving argument has been made in the context of Antarctic fish, which also possess individually large muscle fibres (Johnston *et al*. 2003). Putting all this to one side, whether energetically profligate like a hummingbird or frugal like a broiler, all living systems must ensure that ATP demand is met by adequate supply. Failure to do so quickly leads to either pathology (in the short-term) or death if left uncorrected. This failure is discussed in later sections dealing with white striping and wooden breast in live birds and aspects of postmortem muscle-to-meat conversion.

Taken together, these data clearly point to mitochondrial content as a fundamental point of overall bioenergetic regulation. Endurance-training effects take place over weeks

and months rather than days or hours. For example, Farrar *et al*. (1981) found that in rats endurance trained for 16 weeks, what they described as 'large' muscle mitochondria showed evidence of biogenesis only in the second 8 weeks of the regimen. This implies that mitochondrial content is a fairly stable phenotype, at least on a day-to-day basis. In practice, genetics and environmental factors combine together to generate the outcome.

The molecular mechanism of action is not always clear, but, in mammalian muscle, the transcriptional co-activator peroxisome proliferator-activated receptor gamma coactivator 1 α (*PPARGC1A*) is considered a major player in integrating and transducing the various signals. Several dietary compounds have been identified that can upregulate mitochondrial content in mammalian tissues, including high-fat diets in general (Jain *et al*. 2014), ketone esters (Srivastava *et al*. 2012), curcumin (Wang *et al*. 2015) and pyrroloquinoline quinone (PQQ; Rucker *et al*. 2009), to name a few. These dietary influences contribute to the plasticity of muscle mitochondrial content, along with other environmental effectors, such as endurance training and cold stress.

Mitochondrial content and function in metabolically adapted non-muscle tissues

It is also valuable to examine mitochondrial performance in tissues (within a single organism) with highly adapted forms of metabolism. A fine example comes from comparing brown versus white fat, being two tissues composed of adipocytes. The former is adapted for profligate heat production and is energetically wasteful and inefficient. The latter is adapted to store energy and, from a strict bioenergetic perspective, is relatively inert and efficient. Thus, while some of the bioactive molecules produced by white fat (such as the adipokines adiponectin and leptin) are biologically influential, the oxygen consumption of the tissue itself is very low. The two opposite functions of white versus brown fat are largely mitochondrial in origin. First, unlike white fat, brown fat has a very high mitochondrial content, giving it its brown colour. Second, in brown fat, the ATP synthase complex is replaced by a specialised membrane pore protein called uncoupling protein 1 (UCP1; Nicholls *et al*. 1978). This serves to wastefully dissipate the proton motive force without producing ATP, a process called the 'futile cycle' (Brand *et al*. 1994, 1999).

This mitochondrial adaptation is analogous to taking the chain off a bicycle so that the kinetic energy in the cyclist's muscles is no longer coupled to the wheels turning. In both the cyclist and the brown-fat cell, the original source of energy is ultimately liberated (lost) as heat. Mitochondrial uncoupling is also a consequence of other factors, sometimes collectively called proton leak, which can apply in the mitochondrion across a multitude of tissues including skeletal muscle (Brand *et al*. 1994). These factors include mitochondrial membrane lipid composition, and possibly the impact of gene orthologs of UCP1, but the evidence for the latter is debatable (Brand *et al*. 1999). These homologues include UCP2, which has a broad expression pattern across many tissues, and UCP3, which is restricted to the skeletal musculature. Extent of uncoupling (whether driven by UCP proteins, membrane lipid composition or something else) has potential implications for feed-conversion efficiency. It provides a mechanism for determining the proportion of feed energy (in the form of metabolised acetyl CoA) that is made available for lean growth (in the form of ATP to support muscle protein synthesis and related processes) versus that wasted as heat.

Put another way, increasing uncoupling wastes more feed energy on heat production, leaving a smaller proportion of feed energy available for ATP production that could service the cellular needs of productive tissues. This would be expected to reduce feed-conversion efficiency at the level of the whole animal. For this reason, DNA polymorphisms in genes encoding UCPs have been of interest to both the biomedical (Krauss *et al*. 2005) and agricultural communities (Liu *et al*. 2007) because of associations to metabolic traits. There are not many clear examples of DNA variation in uncoupling proteins being associated to variation in feed efficiency, although a relationship ($P = 0.03$) has been identified between the avian uncoupling protein allele Ala118Val and high feed efficiency in broilers (Sharma *et al*. 2008). We shall explore physiological measurements of mitochondrial uncoupling efficiency and its possible relationship to feed efficiency later on in the paper.

Finally, red blood cells, particularly in mammals, and to a lesser extent in birds, are devoid of mitochondria. This reflects their specialised role as carriers of oxygen rather than consumers of it. As an illuminating aside, between 1933 and 1938, there was short-term interest in consumption of 2,4,dinitrophenol (DNP) as a potent weight-loss agent in humans (i.e. promoting an inefficient metabolism). DNP is a proton ionophore that can shuttle protons across the IMM, thereby dissipating the proton motive force without ATP production. Taking DNP does promote weight loss, but is dangerous and its appeal was short-lived. Death by hyperthermia (body temperatures rising to 44°C) was a common endpoint following overdose.

Broiler muscle mitochondrial content

Despite the fact that muscle mitochondrial content looks to be a promising source of individual variation in a population of production animals, no production species had been screened until our recent work in Cobb-Vantress broilers (Reverter *et al*. 2017). There are several ways of quantifying mitochondrial content, with electron microscopy based morphometric analysis on serial sections being the most direct. Another approach is to count the number of copies of mitochondrial DNA (mtDNA) in a given tissue sample. This method is convenient as it means that an unbiased DNA extraction (which is simple and cheap) contains the compositional information. We have designed a duplex high-throughput qPCR assay to quantify the number of mtDNA copies per nucleus in broilers (Reverter *et al*. 2017). The correction for nDNA yields a 'per cell' (or in the specific case of multinucleate muscle fibres, a 'per unit tissue mass') expression.

Using this method, we detected substantial variation in the order of five-fold across both breast and thigh muscle in 80 birds, despite the animals being members of a single genetic line and generation. Furthermore, we found that any bird with a particularly low breast mitochondrial content also tended to possess a low thigh content (correlation coefficient 0.61; $P < 0.0001$), being consistent with systemic regulation of

mitochondrial content across the bird musculature. To the best of our knowledge, this cross-tissue relationship has never been observed in any other species.

In terms of commercial phenotypes, birds with particularly low breast mitochondrial content were more muscular, possessing a higher breast muscle yield (–0.27; $P = 0.037$) and higher carcass yield (–0.26; $P = 0.045$). These low mitochondrial-content birds also possessed higher abdominal fat content (–0.31; $P = 0.017$). Unfortunately, we did not have FE phenotypes for this particular resource, so the relationship of bird FE to muscle mitochondrial content remains a focus of future work. However, the genetic-evaluation study of (Rekaya *et al*. 2013), using commercial Cobb-Vantress Inc. broilers, reported than improvements in FE could also improve growth, carcass yield and composition. Therefore, in light of the growth, relationships reported above selection for decreased breast mitochondrial content might be expected to increase FE.

While composing this paper, another research group detected a clear negative relationship between *longissimus* muscle mitochondrial activity and FE in Yorkshire boars (Fu *et al*. 2017), supporting the basic tenet articulated here. In these pigs, the more efficient pigs had both a higher average daily gain (0.99 ± 0.03 kg versus 0.87 ± 0.03 kg; $P = 0.013$) and a lower daily feed intake (2.18 ± 0.08 kg versus 2.64 ± 0.11 kg; $P = 0.004$) than did the less efficient pigs. It is also worth mentioning in passing that, in cattle, low mitochondrial content in rumen epithelium has recently been shown to be modestly associated with more feed-efficient animals ($r = 0.21$; $P = 0.03$; Kong *et al*. 2016*b*).

These experimental findings collectively support the theoretical physiological spare-capacity arguments previously applied to metabolic efficiency in humans and production animals (Hudson *et al*. 2008; Hudson 2009), not only in muscle but also in the context of gut tissue where one might not expect to observe much individual variation in terms of cellular composition and structure. This is not to say that gut physiology is unimportant in the phenotypic expression of FE, but that it is not as clear where any observed sources of individual variation may come from in the animal tissue structure (other than in absolute changes in organ size) as there is no visceral equivalent to muscle fibre types.

Muscle mitochondrial structure

In comparing species with very different aerobic capacities (i.e. mitochondrial contents), it has been found that the shape and structure of the organelle membranes are largely invariant, i.e. the OMM is not folded, and the extent of IMM folding is largely constant, except in small, highly oxidative endotherms such as hummingbirds. The increased folding in organisms such as hummingbirds provides a simple mechanism for connecting more of the central matrix to the IMM (shortening diffusion distance) and also for embedding more IMM proteins per volume of matrix. In principal, this could allow higher rates of ATP production, without having to pay the cost of maintaining more matrix (although the maintenance costs of the additional IMM would still need to be met). IMM folding can be visualised using transmission electron microscopy (TEM). We have not analysed broiler mitochondria by using TEM, so we do not know for sure whether IMM (or even OMM) architecture has been modified by domestication or subsequent genetic selection. There is scope for more basic research in this area.

Using the independent FE resource described above (HFE and LFE), Iqbal *et al*. (2004) used a western blotting antibody-based strategy to probe for a set of IMM proteins in breast muscle. They discovered that ADP/ATP translocase 1, cytochrome *b*, cytochrome c, Core II, Core I and COXII were all lower ($P < 0.05$) in the HFE birds. No differences were detected for IMM proteins NAD3, NAD4, NAD5, NAD6, NAD7, 70S and α-ATPase. The protein abundances were expressed per unit muscle tissue, not per unit mitochondria. Given that we might expect increased IMM folding to house more IMM proteins *across the board*, this combination of data tends to indicate that the HFE birds have a subtle change in IMM composition, favouring a reduction in certain IMM proteins. To complicate matters, a recent mass spectrometry-based proteomic study on the same tissue samples (Kong *et al*. 2016*a*) suggested a trend towards increases, not decreases, in several ETC proteins. The apparent conflict remains unresolved at this time.

Muscle mitochondrial function and activity

The definitive way to measure mitochondrial function is to measure respiratory performance as unintrusively as possible. Traditionally, cells or tissues are homogenised and then isolated mitochondrial preparations are assayed spectrophotometrically under various conditions. Using this traditional approach with the HFE and LFE birds, Iqbal *et al*. (2004) found that HFE birds had significantly higher activity in all four ETC complexes, with a particularly large increase in complex IV activity. These data were expressed per unit mitochondrial protein, implying a change in mitochondrial function independent of any change in content. Furthermore, the HFE birds showed greater coupling efficiency between IMM membrane potential and ATP synthesis.

Collectively, these data imply that proton pumping across the IMM through the ETC is higher in the HFE birds and that the electrochemical gradient produced is more efficiently coupled to ATP synthesis. This suggests an increased potential for ATP production on a per unit mitochondrial basis. Recently, a new high-throughput technology called the Seahorse flux analyser has become available that enables mitochondrial and non-mitochondrial sources of ATP production to be assayed using intact cells with minimal disruption, but this approach has not been applied to primary cells of broilers, or any other production animal, divergent in FE.

To gain more molecular detail into how mitochondrial metabolism and other aspects of muscle structure may differ with FE, we have used genome-wide transcriptome (mRNA) screening and mass spectrometry-based proteomics on total breast-muscle homogenates. We were encouraged to find that both technologies identified a tendency for the HFE birds to express less slow-muscle contractile isoforms and associated machinery (Kong *et al*. 2016*a*; Bottje *et al*. 2017). This suggests a muscle fibre composition shift towards the whiter, Type IIB phenotype, in line with prior expectation. However, surprisingly, both approaches (mRNA and protein) indicated a subtle, but coordinate increase in the mitoproteome (including

but not limited to ETC proteins) in HFE birds. This suggests increased mitochondrial content and/or activity in the HFE birds.

This conclusion appears somewhat contrary to the lower IMM proteins in HFE birds, on the basis of antibody detection (Iqbal *et al*. 2004), being at odds with the muscle fibre composition changes observed in numerous highly efficient production species, and entirely contrary to the physiological spare-capacity argument, i.e. small engine animals are more feed efficient. We are currently working on reconciling these issues. Interestingly, the rumen mitochondrial-content work of (Kong *et al*. 2016*b*) has shown that the more efficient animals possess a lower mitochondrial content but a higher mitochondrial mRNA expression level, indicating that the relationship between the two parameters may not be simple and one cannot be assumed from the other.

At this point in time, we should emphasise that voltage-dependent anion channel (VDAC), resident in the OMM, was among the most upregulated mitochondrial proteins in HFE birds, on the basis of proteomic data. VDAC helps shuttle ADP in and ATP out of the mitochondria, a clear driver of overall energetic flux and homeostasis. One promising analytical approach for the future is to try to better disentangle mitochondrial content versus structure at a molecular level by treating matrix, IMM and OMM mitochondrial proteins independently when dealing with ranked output from the genome-wide screening analyses. Concurrent assessments using electron microscopy also offer promise in this context.

Furthermore, we used two different approaches to predict upstream causal regulation from downstream patterns of differential expression, whether mRNA or protein. Gratifyingly, both identified perturbations in progesterone signalling as a likely driver in the muscle of HFE birds (Kong *et al*. 2016*a*; Bottje *et al*. 2017). We also showed in a quail-muscle cell line that the progesterone receptor localises to the mitochondrion. This functional link between progesterone signalling and avian mitochondria further implicates the mitochondrion as a player in broiler FE. The progesterone signalling prediction is interesting, given that progesterone is added to combination-mix hormone growth promotants (testosterone and oestrogen) that collectively increase FE by 20% in cattle, but very little is known about the exact role that progesterone plays in the hormone mix, nor its particular role in bird-muscle biology.

Genetic selection for genes encoding mitochondrial proteins

Drawing on a genetic resource of ~200 birds in each of four Cobb lines, we have used an in-house -etection algorithm to find segments of DNA apparently under selection in one or more of the lines (Hudson *et al*. 2017). This identified aspects of the IGF-1 signalling pathway in growth-selected lines, and the gonadotropin-releasing hormone pathway in fecundity-selected lines. We specifically explored the 507 mitochondrial proteins (of a total 1045) for which we could find gene matches in the broiler 50K single nucleotide polymorphism (SNP) data. Among other findings, we detected dramatically different allele frequencies for two SNPs in the gene region encoding *AGK*, which encodes mitochondrial acylglycerol kinase (Cobb Vantress Inc., unpubl. data). For one of these the effect was particularly striking. The two lines possessing an elevated FE had the AA allele in the highest frequency, whereas the two lines possessing the lower FE had BB as the highest-frequency allele. *AGK* has recently been identified as a possible functional candidate within FE-quantitative trait loci in Cobb broilers (Reyer *et al*. 2015), using a bird population independent from those we analysed.

Metabolic flux control

It will become apparent in this section that there is considerable scope to 'activate' the physiology of mitochondria to meet pressing energetic needs such as occurs during aerobic exercise, but it is important to note that the upper ceiling on this activation is limited by overall mitochondrial content. According to (Conley 2016), there are three control points in muscle ATP flux in regard to meeting the enhanced demand during intense exercise *for a given mitochondrial content*, including electron transport-chain activity, coupling efficiency and ATP synthesis. These can be exploited in the immediate short term to increase muscle ATP production 50-fold compared with the same tissue at rest. Some of these control points can apparently be modified by diet, at least in mammals. For example, dietary nitrate, through beetroot-juice consumption, apparently elevates mitochondrial ATP synthesis per oxygen uptake by increasing coupling efficiency (Jones 2014). Second, the mitochondrially targeted antioxidant SS-31 was able to not only improve coupling efficiency, but also increase capacity for ETC flux (Siegel *et al*. 2013). Both dietary treatments were rapid, apparently working within 1 h of administration.

At the molecular level, there are several known endogenous flux control points. These include isocitrate dehydrogenase and citrate synthase, the rate-limiting enzymes in the citric acid cycle, and regulation of the pyruvate dehydrogenase complex to control the flux of acetyl CoA derived from carbohydrate. Outside of the mitochondrion, in the cytosol, resides AMP kinase whose job is to monitor overall ATP supply versus demand. If mitochondrial ATP supply is failing to meet cellular demand, AMP kinase sets in motion a chain of events to accelerate mitochondrial or cytosolic ATP production, depending on oxygen availability.

Further to this, our gene-expression data in multiple species and circumstances has pointed to the mRNA encoding the mitochondrial enzymes pyruvate dehydrogenase kinase 4 (*PDK4*), mitochondrial creatine kinase (*CKMT1A*) and uncoupling protein 3 (*UCP3*) as particularly responsive to, or responsible for, metabolic challenges (Hudson *et al*. 2013; Bottje *et al*. 2017). These genes encode mitochondrial proteins with the following functions. First, PDK4 is a fuel decision-making enzyme that inhibits the pyruvate dehydrogenase complex and, therefore, production of acetyl CoA from carbohydrate. This inhibition forces the acetyl CoA supply to come from β-oxidation of fat. Second, CKMT1A transfers high-energy phosphate from ATP to creatine, so as to replenish cytosolic phosphocreatine. Third, UCP3 is thought to export excess fatty acids out of the mitochondria and into the cytosol during times of dietary fuel excess. Further to this, coenzymes (such as the vitamin-like coenzyme Q10) work as activating co-factors for various enzymatic components of the ETC. Coenzyme Q10 is

endogenously synthesised, as well as being partially derived from the diet.

Mitochondria and muscle-to-meat conversion

When an animal is slaughtered, the muscle system is quickly divorced from the rest of the carcass, including the lungs and cardiovascular system. However, the muscle tissue is still 'alive' in the sense that the biochemical pathways and enzymes aimed at sensing and maintaining ATP homeostasis (such as AMP kinase and the rate limiting enzymes of glycolysis) are operational for some time. This means that the muscle tissue will continue to sense its ATP status (via AMP kinase) and then drive substrate catabolism to try to meet that ATP demand. The same cellular processes that demand ATP in the muscle of a live animal (namely, trans-membrane ion gradients, gene and protein expression and so on) also demand ATP in a freshly euthanased carcass, with the exception of cycles of gross muscle contraction and relaxation, which will obviously cease.

The lack of perfusion by oxygenated blood very quickly forces the muscle to become more reliant on anaerobic methods of ATP generation. This form of metabolism is unsustainable and accumulates inhibitory end products. This means that the attempt of the muscle to maintain bioenergetic homeostasis is ultimately doomed to failure. In biochemical terms, the pyruvate produced by glycolysis will be anaerobically reduced to lactate by cytosolic lactate dehydrogenase, rather than being transported into the mitochondria for conversion into acetyl CoA and aerobic combustion (refer to Fig. 1). In a live animal, any muscle lactate produced during short-term intense exercise (such as a 400-m race) is released into the blood stream, dealt with by the liver and sent back to the muscle as glucose in an inter-organ process called the Cori Cycle; this is clearly no longer operational post-slaughter.

The production of lactic acid from pyruvate yields (indirectly) the protons that cause lactic acidosis. The associated pH decline, both its rate and ultimate value, is important in determining meat product quality in the 24 h or so postmortem. Because fat exists in a highly reduced state and its chemical energy can be released only by aerobic combustion, it presumably plays little role in the postmortem situation. Rather, the dependence of postmortem pH decline on the amount of stored carbohydrate that can be turned into lactate (via both glycolysis and subsequent reduction of pyruvate) has led to the concept of 'glycolytic potential' (Monin *et al*. 1987). Generally speaking, the greater the glycolytic potential, the greater the pH decline, although some authors have found contrary evidence (England *et al*. 2016). Too much pH decline produces pale, soft, exudative meat, whereas too little produces dry, firm, dark meat. Both are rejected by consumers, with the middle ground producing the products with the most desirable organoleptic properties.

Given these anaerobic circumstances, what possible role could there be for the mitochondria, the seat of aerobic, not anaerobic, metabolism? The answer lies in the unusual behaviour of mitochondria under anoxic conditions. These mitochondrial behaviours have been observed in certain specialised metabolic situations, such as the metabolic depression exhibited by painted turtles hibernating under ice-covered ponds. In an effort to maintain the IMM proton gradient, whose collapse spells the demise of the organelle, the mitochondria become net consumers of ATP rather than producers (St-Pierre *et al*. 2000). They achieve this mercenary goal by taking in cytosolic ATP and using it to run the mitochondrial ATP synthase in reverse. This reversal in flux has been called 'cellular treason.' When metabolic circumstances get very challenging, mitochondria take a position that appears to benefit their own function over the fate of their symbiotic host cell. Because this behaviour accelerates ATP depletion, it presumably leads to anaerobic cytosolic ATP production pathways (glycolysis followed by lactate production) being pushed even harder to meet the demand. If we return to the bicycle analogy one final time, treason is like peddling backwards with the chain on; the ETC system is still coupled to ATP handling, but it is operating in reverse to a destination potentially at odds with the metabolic goals of the host cell.

As was previously pointed out (Hudson 2012), cellular treason has possible implications for meat science, as it has the potential to accelerate postmortem pH decline curves in a manner commensurate with muscle mitochondrial content. This provides a possible explanation for the fast rates of pH decline sometimes observed in red, mitochondrial-rich muscle types, which do not fit the prevailing glycolytic potential model very well (Devine *et al*. 1984). It may also provide some insight into the Hampshire pig breed, which is particularly vulnerable to producing acid pork. This animal has a mutation in one of the subunits of AMP kinase and its musculature possesses a higher mitochondrial content than that of other modern pig breeds (Scheffler *et al*. 2011). Moreover, a fascinating *in vitro* model of meat pH-decline dynamics has empirically detected a role for the mitochondria (Scheffler *et al*. 2015), but the limitations of this model in explaining the metabolic behaviour of an intact piece of meat remain unclear.

A final point of interest relating mitochondrial function and meat quality is that, in broilers, several muscle pathologies are now appearing in the live animal. 'White striping' is diagnosed by adipocyte and connective tissue infiltration and necrotic lesions in the muscle, and 'wooden breast' is identified by a superficial hard feeling to the muscle. The two syndromes often co-exist. The cause is unknown, but given that lactic acidosis has been noted in the tissue of afflicted birds, and that those afflicted birds are usually large, muscular birds grown rapidly (Kuttappan *et al*. 2012), one possibility is that a combination of very low capillarity and mitochondrial content results in inadequate aerobic ATP production. In our mitochondrial content estimates, we found that low mitochondrial content was negatively correlated with breast muscle yield (Reverter *et al*. 2017). The particularly low mitochondrial content in the muscle system of large rapidly grown birds could leave them vulnerable to prolonged anaerobic metabolism.

We know that muscle anaerobic metabolism cannot be sustained. Low mitochondrial content, thus, presents a hypothesis for the tissue damage (necrosis and infiltration of non-muscle tissues) observed in white striping birds. The prevalence of white striping in broilers, but not in other production animals, may relate to broilers having the most extreme skeletal musculature, with the breast muscle being almost entirely composed of very low mitochondrial-content Type IIB fibres.

The road ahead

Revisiting this body of work reinforces a need to better structurally characterise production animal mitochondria in terms of both content and morphology. A basic TEM analysis quantifying the folding of the IMM and OMM with regard to the mitochondrial matrix (mitochondrial structure) would help interpretation of the mountain of molecular data we are currently analysing. It would also help check the status of muscle aerobic capacity (mitochondrial content) in modern versus previous generations of animals, such as the broilers analysed by Kiessling back in the late 1970s. Inclusion of wild red jungle fowl in this particular context would clarify several questions relating to the consequences of domestication and subsequent selection on growth and efficiency.

The relationship between variation in mitochondrial content and production-animal feed efficiency still needs further empirical justification, with both more species and more tissues likely to be informative. Finally, mitochondrial content and function is highly responsive to exercise, temperature and diet as well as genetics. In mammals, *PPARGC1A* is the master regulator coordinating the cellular response to these influences, but the gene and protein are not so well characterised in birds. There appears to be some room to explore the basic biology of the mitochondrion as well as an opportunity to inform current industry practices. Possible paths to industry impact include informing existing genomic predictions for animal breeding purposes or, alternately, developing novel feedstuffs containing bioactives relevant to mitochondrial function.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank the RAAN-A committee for the invitation to speak and prepare this paper. This paper is largely based on a previous conference paper written for the Australian Poultry Science Symposium held in Sydney, February 2017. Additional material includes the generalisations across production species and the new section on muscle to meat conversion. Three reviewers made comments that have been used to improve the manuscript. We thank Cobb Vantress, the US Department of Agriculture (USDA-NIFA #2013-01953) and CSIRO for supporting various aspects of this work. In 2012, Walter Bottje visited Nick Hudson on a CSIRO McMaster Fellowship, which led to the development of some of the functional genomic research explored here.

References

Askew GN, Marsh RL (2002) Muscle designed for maximum short-term power output: quail flight muscle. *The Journal of Experimental Biology* **205**, 2153–2160.

Bottje W, Kong BW, Reverter A, Waardenberg AJ, Lassiter K, Hudson NJ (2017) Progesterone signalling in broiler skeletal muscle is associated with divergent feed efficiency. *BMC Systems Biology* **11**, 29. doi:10.1186/s12918-017-0396-2

Bouley J, Meunier B, Chambon C, De Smet S, Hocquette JF, Picard B (2005) Proteomic analysis of bovine skeletal muscle hypertrophy. *Proteomics* **5**, 490–500. doi:10.1002/pmic.200400925

Brand MD, Chien LF, Ainscow EK, Rolfe DF, Porter RK (1994) The causes and functions of mitochondrial proton leak. *Biochimica et Biophysica Acta* **1187**, 132–139. doi:10.1016/0005-2728(94)90099-X

Brand MD, Brindle KM, Buckingham JA, Harper JA, Rolfe DF, Stuart JA (1999) The significance and mechanism of mitochondrial proton conductance. *International Journal of Obesity and Related Metabolic Disorders* **23**(Suppl. 6), S4–S11. doi:10.1038/sj.ijo.0800936

Bruton JD, Aydin J, Yamada T, Shabalina IG, Ivarsson N, Zhang SJ, Wada M, Tavi P, Nedergaard J, Katz A, Westerblad H (2010) Increased fatigue resistance linked to Ca2+-stimulated mitochondrial biogenesis in muscle fibres of cold-acclimated mice. *The Journal of Physiology* **588**, 4275–4288. doi:10.1113/jphysiol.2010.198598

Burrin DG, Ferrell CL, Britton RA, Bauer M (1990) Level of nutrition and visceral organ size and metabolic activity in sheep. *British Journal of Nutrition* **64**, 439–448. doi:10.1079/BJN19900044

Conley KE (2016) Mitochondria to motion: optimizing oxidative phosphorylation to improve exercise performance. *The Journal of Experimental Biology* **219**, 243–249. doi:10.1242/jeb.126623

Deveaux V, Cassar-Malek I, Picard B (2001) Comparison of contractile characteristics of muscle from Holstein and double-muscled Belgian Blue foetuses. *Comparative Biochemistry and Physiology. A. Comparative Physiology* **131**, 21–29. doi:10.1016/S1095-6433(01)00459-7

Devine CE, Ellery S, Averill S (1984) Responses of different types of ox muscle to electrical stimulation. *Meat Science* **10**, 35–51. doi:10.1016/0309-1740(84)90030-5

Diamond J, Hammond K (1992) The matches, achieved by natural selection, between biological capacities and their natural loads. *Experientia* **48**, 551–557. doi:10.1007/BF01920238

England EM, Matarneh SK, Oliver EM, Apaoblaza A, Scheffler TL, Shi H, Gerrard DE (2016) Excess glycogen does not resolve high ultimate pH of oxidative muscle. *Meat Science* **114**, 95–102. doi:10.1016/j.meatsci.2015.10.010

Essén-Gustavsson B, Lindholm A (1984) Fiber types and metabolic characteristics in muscles of wild boars, normal and halothane sensitive Swedish landrace pigs. *Comparative Biochemistry and Physiology. A. Comparative Physiology* **78**, 67–71. doi:10.1016/0300-9629(84)90094-X

Farrar RP, Mayer LR, Starnes JW, Edington DW (1981) Selected biochemical parameters of two sizes of rat skeletal and heart muscle mitochondria at selected intervals of a 16-week endurance training program. *European Journal of Applied Physiology and Occupational Physiology* **46**, 91–102. doi:10.1007/BF00422181

Fiems LO (2012) Double muscling in cattle: genes, husbandry, carcasses and meat. *Animals* **2**, 472–506. doi:10.3390/ani2030472

Fu L, Xu Y, Hou Y, Qi X, Zhou L, Liu H, Luan Y, Jing L, Miao Y, Zhao S, Liu H, Li X (2017) Proteomic analysis indicates that mitochondrial energy metabolism in skeletal muscle tissue is negatively correlated with feed efficiency in pigs. *Scientific Reports* **7**, 45291. doi:10.1038/srep45291

Henriksson J (1990) The possible role of skeletal muscle in the adaptation to periods of energy deficiency. *European Journal of Clinical Nutrition* **44**(Suppl. 1), 55–64.

Herd RM, Arthur PF (2009) Physiological basis for residual feed intake. *Journal of Animal Science* **87**, E64–E71. doi:10.2527/jas.2008-1345

Hoagland M, Dodson B, Hauck J (2001) 'Exploring the way life works: the science of biology.' (Jones and Bartlett Publishers: Canada)

Holloszy JO (1982) Muscle metabolism during exercise. *Archives of Physical Medicine and Rehabilitation* **63**, 231–234.

Hudson NJ (2009) Symmorphosis and livestock bioenergetics: production animal muscle has low mitochondrial volume fractions. *Journal of Animal Physiology and Animal Nutrition* **93**, 1–6. doi:10.1111/j.1439-0396.2007.00791.x

Hudson NJ (2012) Mitochondrial treason: a novel driver of pH decline in postmortem muscle? *Animal Production Science* **52**, 1107–1110. doi:10.1071/AN12171

Hudson NJ, Lehnert SA, Harper GS (2008) Obese humans as economically designed feed converters: symmorphosis and low oxidative capacity

skeletal muscle. *Medical Hypotheses* **70**, 693–697. doi:10.1016/j.mehy.2007.05.042

Hudson NJ, Lyons RE, Reverter A, Greenwood PL, Dalrymple BP (2013) Inferring the in vivo cellular program of developing bovine skeletal muscle from expression data. *Gene Expression Patterns* **13**, 109–125. doi:10.1016/j.gep.2013.02.001

Hudson NJ, Hawken RJ, Okimoto R, Sapp RL, Reverter A (2017) Data compression can discriminate broilers by selection line, detect haplotypes, and estimate genetic potential for complex phenotypes. *Poultry Science.* doi:10.3382/ps/pex151

Iqbal M, Pumford NR, Tang ZX, Lassiter K, Wing T, Cooper M, Bottje W (2004) Low feed efficient broilers within a single genetic line exhibit higher oxidative stress and protein expression in breast muscle with lower mitochondrial complex activity. *Poultry Science* **83**, 474–484. doi:10.1093/ps/83.3.474

Jackson SP, Green RD, Miller MF (1997) Phenotypic characterization of rambouillet sheep expressing the callipyge gene: I. Inheritance of the condition and production characteristics. *Journal of Animal Science* **75**, 14–18. doi:10.2527/1997.75114x

Jain SS, Paglialunga S, Vigna C, Ludzki A, Herbst EA, Lally JS, Schrauwen P, Hoeks J, Tupling AR, Bonen A, Holloway GP (2014) High-fat diet-induced mitochondrial biogenesis is regulated by mitochondrial-derived reactive oxygen species activation of CaMKII. *Diabetes* **63**, 1907–1913. doi:10.2337/db13-0816

Johnston IA, Fernandez DA, Calvo J, Vieira VL, North AW, Abercromby M, Garland T Jr (2003) Reduction in muscle fibre number during the adaptive radiation of notothenioid fishes: a phylogenetic perspective. *The Journal of Experimental Biology* **206**, 2595–2609. doi:10.1242/jeb.00474

Jones AM (2014) Influence of dietary nitrate on the physiological determinants of exercise performance: a critical review. *Applied Physiology, Nutrition, and Metabolism* **39**, 1019–1028. doi:10.1139/apnm-2014-0036

Jorgensen PL, Pedersen PA (2001) Structure-function relationships of Na(+), K(+), ATP, or Mg(2+) binding and energy transduction in Na,K-ATPase. *Biochimica et Biophysica Acta* **1505**, 57–74. doi:10.1016/S0005-2728(00)00277-2

Kiessling KH (1977) Muscle structure and function in the goose, quail, pheasant, guinea hen, and chicken. *Comparative Biochemistry and Physiology* **57**, 287–292.

Kong BW, Lassiter K, Piekarski-Welsher A, Dridi S, Reverter-Gomez A, Hudson NJ, Bottje WG (2016*a*) Proteomics of breast muscle tissue associated with the phenotypic expression of feed efficiency within a pedigree male broiler line: I. Highlight on mitochondria. *PLoS One* **11**, e0155679. doi:10.1371/journal.pone.0155679

Kong RS, Liang G, Chen Y, Stothard P, Guan LL (2016*b*) Transcriptome profiling of the rumen epithelium of beef cattle differing in residual feed intake. *BMC Genomics* **17**, 592. doi:10.1186/s12864-016-2935-4

Krauss S, Zhang CY, Lowell BB (2005) The mitochondrial uncoupling-protein homologues. *Nature Reviews. Molecular Cell Biology* **6**, 248–261. doi:10.1038/nrm1592

Kuttappan VA, Brewer VB, Apple JK, Waldroup PW, Owens CM (2012) Influence of growth rate on the occurrence of white striping in broiler breast fillets. *Poultry Science* **91**, 2677–2685. doi:10.3382/ps.2012-02259

Lefaucheur L, Milan D, Ecolan P, Le Callennec C (2004) Myosin heavy chain composition of different skeletal muscles in Large White and Meishan pigs. *Journal of Animal Science* **82**, 1931–1941. doi:10.2527/2004.8271931x

Lefaucheur L, Lebret B, Ecolan P, Louveau I, Damon M, Prunier A, Billon Y, Sellier P, Gilbert H (2011) Muscle characteristics and meat quality traits are affected by divergent selection on residual feed intake in pigs. *Journal of Animal Science* **89**, 996–1010. doi:10.2527/jas.2010-3493

Lehnert SA, Reverter A, Byrne KA, Wang Y, Nattrass GS, Hudson NJ, Greenwood PL (2007) Gene expression studies of developing bovine *longissimus* muscle from two different beef cattle breeds. *BMC Developmental Biology* **7**, 95. doi:10.1186/1471-213X-7-95

Liu S, Wang SZ, Li ZH, Li H (2007) Association of single nucleotide polymorphism of chicken uncoupling protein gene with muscle and fatness traits. *Journal of Animal Breeding and Genetics* **124**, 230–235. doi:10.1111/j.1439-0388.2007.00654.x

Mathieu O, Krauer R, Hoppeler H, Gehr P, Lindstedt SL, Alexander RM, Taylor CR, Weibel ER (1981) Design of the mammalian respiratory system. VII. Scaling mitochondrial volume in skeletal muscle to body mass. *Respiration Physiology* **44**, 113–128. doi:10.1016/0034-5687(81)90079-7

Mathieu-Costello O, Suarez RK, Hochachka PW (1992) Capillary-to-fiber geometry and mitochondrial density in hummingbird flight muscle. *Respiration Physiology* **89**, 113–132. doi:10.1016/0034-5687(92)90075-8

Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* **191**, 144–148. doi:10.1038/191144a0

Monin G, Mejenes-Quijano A, Talmant A, Sellier P (1987) Influence of breed and muscle metabolic type on muscle glycolytic potential and meat pH in pigs. *Meat Science* **20**, 149–158. doi:10.1016/0309-1740(87)90034-9

Nagy KA (2005) Field metabolic rate and body size. *The Journal of Experimental Biology* **208**, 1621–1625. doi:10.1242/jeb.01553

Nicholls DG, Bernson VS, Heaton GM (1978) The identification of the component in the inner membrane of brown adipose tissue mitochondria responsible for regulating energy dissipation. *Experientia. Supplementum* **32**, 89–93. doi:10.1007/978-3-0348-5559-4_9

Rea SL, Graham BH, Nakamaru-Ogiso E, Kar A, Falk MJ (2010) Bacteria, yeast, worms, and flies: exploiting simple model organisms to investigate human mitochondrial diseases. *Developmental Disabilities Research Reviews* **16**, 200–218. doi:10.1002/ddrr.114

Rehfeldt C, Henning M, Fiedler I (2008) Consequences of pig domestication for skeletal muscle growth and cellularity. *Livestock Science* **116**, 30–41. doi:10.1016/j.livsci.2007.08.017

Rekaya R, Sapp RL, Wing T, Aggrey SE (2013) Genetic evaluation for growth, body composition, feed efficiency, and leg soundness. *Poultry Science* **92**, 923–929. doi:10.3382/ps.2012-02649

Reverter A, Okimoto R, Sapp R, Bottje WG, Hawken R, Hudson NJ (2017) Chicken muscle mitochondrial content appears co-ordinately regulated and is associated with performance phenotypes. *Biology Open* **6**, 50–58. doi:10.1242/bio.022772

Reyer H, Hawken R, Murani E, Ponsuksili S, Wimmers K (2015) The genetics of feed conversion efficiency traits in a commercial broiler line. *Scientific Reports* **5**, 16387. doi:10.1038/srep16387

Rucker R, Chowanadisai W, Nakano M (2009) Potential physiological importance of pyrroloquinoline quinone. *Alternative Medicine Review* **14**, 268–277.

Scheffler TL, Park S, Gerrard DE (2011) Lessons to learn about postmortem metabolism using the AMPKgamma3(R200Q) mutation in the pig. *Meat Science* **89**, 244–250. doi:10.1016/j.meatsci.2011.04.030

Scheffler TL, Matarneh SK, England EM, Gerrard DE (2015) Mitochondria influence postmortem metabolism and pH in an *in vitro* model. *Meat Science* **110**, 118–125. doi:10.1016/j.meatsci.2015.07.007

Shabalina IG, Hoeks J, Kramarova TV, Schrauwen P, Cannon B, Nedergaard J (2010) Cold tolerance of UCP1-ablated mice: a skeletal muscle mitochondria switch toward lipid oxidation with marked UCP3 up-regulation not associated with increased basal, fatty acid- or ROS-induced uncoupling or enhanced GDP effects. *Biochimica et Biophysica Acta* **1797**, 968–980. doi:10.1016/j.bbabio.2010.02.033

Sharma P, Bottje W, Okimoto R (2008) Polymorphisms in uncoupling protein, melanocortin 3 receptor, melanocortin 4 receptor, and pro-opiomelanocortin genes and association with production traits in a commercial broiler line. *Poultry Science* **87**, 2073–2086. doi:10.3382/ps.2008-00060

Siegel MP, Kruse SE, Percival JM, Goh J, White CC, Hopkins HC, Kavanagh TJ, Szeto HH, Rabinovitch PS, Marcinek DJ (2013) Mitochondrial-targeted peptide rapidly improves mitochondrial energetics and skeletal muscle performance in aged mice. *Aging Cell* **12**, 763–771. doi:10.1111/acel.12102

Srivastava S, Kashiwaya Y, King MT, Baxa U, Tam J, Niu G, Chen X, Clarke K, Veech RL (2012) Mitochondrial biogenesis and increased uncoupling protein 1 in brown adipose tissue of mice fed a ketone ester diet. *The FASEB Journal* **26**, 2351–2362. doi:10.1096/fj.11-200410

St-Pierre J, Brand MD, Boutilier RG (2000) Mitochondria as ATP consumers: cellular treason in anoxia. *Proceedings of the National Academy of Sciences, USA* **97**, 8670–8674. doi:10.1073/pnas.140093597

Szarski H (1983) Cell size and the concept of wasteful and frugal evolutionary strategies. *Journal of Theoretical Biology* **105**, 201–209. doi:10.1016/S0022-5193(83)80002-2

Tornroth-Horsefield S, Neutze R (2008) Opening and closing the metabolite gate. *Proceedings of the National Academy of Sciences, USA* **105**, 19565–19566. doi:10.1073/pnas.0810654106

van den Broek NM, Ciapaite J, De Feyter HM, Houten SM, Wanders RJ, Jeneson JA, Nicolay K, Prompers JJ (2010) Increased mitochondrial content rescues *in vivo* muscle oxidative capacity in long-term high-fat-diet-fed rats. *The FASEB Journal* **24**, 1354–1364. doi:10.1096/fj.09-143842

Wang S, Wang X, Ye Z, Xu C, Zhang M, Ruan B, Wei M, Jiang Y, Zhang Y, Wang L, Lei X, Lu Z (2015) Curcumin promotes browning of white adipose tissue in a norepinephrine-dependent way. *Biochemical and Biophysical Research Communications* **466**, 247–253. doi:10.1016/j.bbrc.2015.09.018

Weibel ER, Taylor CR, Bolis L (1998) 'Muscle energy balance in sound production and flight.' (Cambridge University Press)

Weibel ER, Taylor CR, Hoppeler H (1991) The concept of symmorphosis: a testable hypothesis of structure-function relationship. *Proceedings of the National Academy of Sciences, USA* **88**, 10357–10361. doi:10.1073/pnas.88.22.10357

White CR, Seymour RS (2003) Mammalian basal metabolic rate is proportional to body mass2/3. *Proceedings of the National Academy of Sciences, USA* **100**, 4046–4049. doi:10.1073/pnas.0436428100

Williams TM, Dobson GP, Mathieu-Costello O, Morsbach D, Worley MB, Phillips JA (1997) Skeletal muscle histology and biochemistry of an elite sprinter, the African cheetah. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology* **167**, 527–535. doi:10.1007/s003600050105

Young A (1997) Ageing and physiological functions. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **352**, 1837–1843. doi:10.1098/rstb.1997.0169