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Review

Control of muscle bioenergetic gene expression: implications for allometric scaling relationships of glycolytic and oxidative enzymes

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Summary

Muscle metabolic properties vary with body size, with larger animals relying relatively less on oxidative metabolism as a result of lower specific activities of mitochondrial enzymes and greater specific activities of glycolytic enzymes. While many have argued reasons why such relationships might be grounded in physical relationships, an explanation for the regulatory basis of the differences in enzyme levels remains unexplored. Focusing on skeletal muscle, we review potential cellular and genetic explanations for the relationship between bioenergetic enzymes and body mass. Differences in myonuclear domain (the ratio of fiber volume to nuclei number) in conjunction with constitutive expression may explain part of the variation in mitochondrial content among fiber types and species. Superimposed on such constitutive determinants are (1) extrinsic signalling pathways that control the muscle contractile and metabolic phenotype and (2) intrinsic signalling pathways that translate changes in cellular milieu (ions, metabolites, oxygen, redox) arising through the contractile phenotype into changes in enzyme synthesis. These signalling pathways work through transcriptional regulation, as well as post-transcriptional, translational and post-translational regulation, acting *via* synthesis and degradation.

Key words: allometry, scaling, metabolic rate, mitochondria, glycolysis, citrate synthase, cytochrome c oxidase, nuclear respiratory factor, peroxisome proliferator activated receptor, PGC-1.

Introduction

Allometric scaling and metabolic enzymes

For more than 100 years, physiologists have sought to explain the allometric relationship between body mass and metabolic rate. The allometric relationship is usually expressed as the equation $y=aM^{b}$, where the M is body mass and the exponent b is the scaling coefficient, estimated empirically as the slope of a log–log plot. While it is agreed that, in general, larger animals have lower mass-specific metabolic rates than small animals, the specifics of the relationship are vigorously debated. Many questions revolve around defining robust, statistically valid mathematical descriptions of data. Other questions relate to mechanistic explanations that underlie the relationships. In this paper, we discuss the relationship between the allometric patterns in the metabolic enzymes that support metabolic rate.

The aerobic metabolic rate is met by flux through the mitochondrial pathway of oxidative phosphorylation. Thus, one might predict that an allometric pattern for mitochondrial enzymes in animals would reflect the pattern of aerobic metabolism. Unlike metabolic rate, which is a whole animal phenomenon, enzyme measurements are meaningful only when assessed in individual tissues. Most studies have focused on skeletal muscle enzymes because this is the tissue that likely contributes the greatest to basal metabolic rate. While the resting metabolic rate of skeletal muscle may be lower than many other tissues, it generally contributes the greatest to whole animal mass.

Surprisingly, there have been relatively few studies that have assessed the allometric patterns in muscle enzymes. The first such study in tetrapods (Emmett and Hochachka, 1981) showed that muscle mitochondrial enzymes exhibited negative scaling; larger animals had lower mitochondrial enzyme activities (see Fig. 1A). Surprisingly, the authors found that the scaling coefficient exponent for aerobic enzymes (b=–0.11) was not as nearly as great as the exponent for aerobic metabolic rate seen in most allometric studies (b ranges from –0.33 to –0.25). These muscle enzyme analyses also revealed a second paradox. Larger animals were less oxidative, but they were also more glycolytic; glycolytic enzymes showed positive scaling, with a scaling exponent of approximately +0.2 (see Fig. 1B). In hindsight, this early study had some obvious weaknesses, such as the significance of muscle fiber types, animal activity

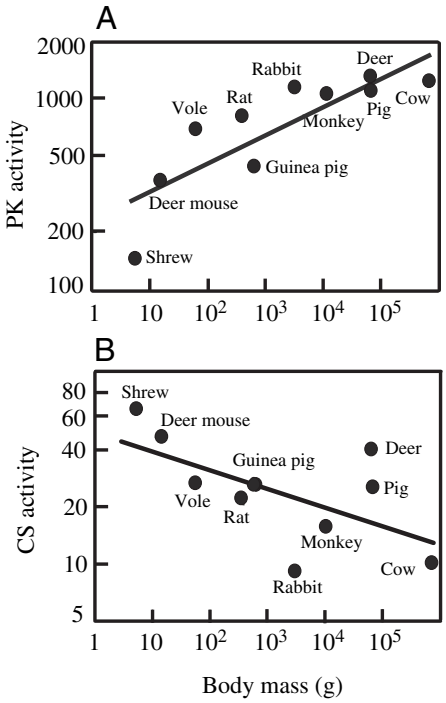

Fig. 1. Mitochondrial and glycolytic enzyme activities in muscles of mammals (adapted from Emmett and Hochachka, 1981). PK, pyruvate kinase (U g^{-1}); CS, citrate synthase (U g^{-1}); body mass (g).

patterns and phylogenetic relationships among the selected species. However, the fundamental conclusions have not been refuted; in mammals oxidative enzymes show negative scaling and glycolytic enzymes show positive scaling.

Several subsequent studies examined muscle allometric patterns in fish. The use of these animals largely avoids the confounding issue of muscle fiber type heterogeneity. Fish models also provide an opportunity to examine intraspecific variation in animals of similar geometry. In contrast to the situation in mammals, mitochondrial enzymes of most fish are independent of body mass (Somero and Childress, 1990; Burness et al., 1999; Norton et al., 2000). As in mammals, glycolytic enzymes show positive scaling, with exponents ranging from 0.15 (barred sand bass; Yang and Somero, 1996) to 0.4 (rainbow trout; Somero and Childress, 1990; Burness et al., 1999). Not all fish display the same patterns. Sea bass show complex scaling patterns over different size ranges because of the way body size influences locomotor style, hydrodynamic forces and muscle function (Norton et al., 2000). The impact of locomotor styles is also seen in interspecies comparisons. Unlike the active fish discussed previously, sedentary flatfish show negative scaling for both mitochondrial (b=–0.68) and glycolytic (b=–0.44) enzymes (Somero and Childress, 1990).

Overall, these data suggest there is no single, simple relationship between muscle enzymes and body size. Nonetheless, body size does appear to influence muscle enzyme profiles in terms of both specific activity and relative activities of glycolytic and mitochondrial enzymes. The purpose of this paper is to review what is known about the regulation of bioenergetic enzymes in muscle, focusing on explanations that may be directly relevant to allometric patterns. However, given the dearth of information on the origins of allometric patterns, we also draw on information from both genetic interventions and development models. The differences in bioenergetics among fiber types is of the same magnitude as the variation evident in allometric studies. Mitochondrial enzymes are high in slow-twitch muscles and low in fast twitch muscles; glycolytic enzymes are high in fast twitch muscles and low in slow-twitch muscles. For example, rats show a sixfold range in citrate synthase activity among skeletal fiber types, although most muscles fall within a twofold range (Delp and Duan, 1996). For an allometric relationship with a scaling exponent of –0.3, that same sixfold range would arise in animals differing 100-fold in body mass.

Why do bioenergetic enzymes vary with body size?

Mitochondrial enzyme regulation has been the subject of many recent reviews (summarized in Moyes and Hood, 2003). Based on these studies, we propose that the bioenergetic phenotype is determined directly and indirectly by the regulators that determine the contractile phenotype. We explicitly ignore the possibility that broad genomic differences are needed to account for allometric variation. Every animal has a genome that can produce tissues of differing metabolic phenotype and can modify the profile in response to regulatory changes. We argue that allometric patterns are a reflection of the plasticity inherent in all muscles. To put this assumption another way, we expect that if the nucleus of a shrew muscle were put into an elephant muscle, that shrew nucleus would express its bioenergetic genes in a manner that was appropriate to build an elephant's muscle. The environment of the elephant muscle would generate the regulatory information to instruct the nucleus on the levels of gene expression that were needed. Thus, the focus of this review is how contractile activity is linked to pathways that control bioenergetic enzymes.

Cellular roles of glycolysis and mitochondrial metabolism

If a cell controls enzyme levels in a way that matches enzyme synthesis to intracellular needs, exactly when does it sample the available information to determine if the levels of glycolytic and mitochondrial enzymes are appropriate? The most common assumption is that the levels of glycolytic and mitochondrial enzymes are maintained at levels that are needed to meet the energy demands of active muscles. Recall, however, that the pathways have roles other than energy production and also function under different activity states.

The glycolytic pathway serves purposes beyond energy production during high intensity locomotion. Glucose transport and hexokinase are needed to produce muscle glycogen. Glycolytic intermediates are needed in the pentose phosphate pathway for production of reducing energy (NADPH) and nucleotides (ribose, deoxyribose). Glycolytic pyruvate can be imported and catabolized by mitochondria (glucose oxidation).

The glycolytic pathway also produces energy under low flux conditions in the absence of oxygen. Despite these other roles, it makes sense that the levels of the muscle glycolytic enzymes are set at a level that is adequate to meet the infrequent demands of high intensity activity.

Mitochondria produce the energy for steady state muscle activity, but also play important roles in resting muscle: production of energy, metabolic intermediates, heat and reactive oxygen species. Since animals spend most of their time at rest, it is possible that mitochondria are maintained at levels primarily to meet the resting demands (see Moyes et al., 1992). For example, mitochondrial heat production is central to endotherm thermogenesis and it is intriguing that mitochondrial enzymes scale in mammals (homeotherms) but not fish (ectotherms). Since mitochondria produce more reactive oxygen species at the lowest metabolic rates (Korshunov et al., 1997), the lower mitochondrial content in muscles of large animals probably minimizes superoxide production; if an elephant's muscle had the same mitochondrial content as a mouse's muscle, its mitochondria would need to respire at rates closer to state 4, which would exacerbate reactive oxygen species production. For this reason, there may be linkage between the metabolic theory of aging and allometric patterns. Life span of animals can be increased by treatments that reduce oxidative stress, such as caloric restriction or genetic manipulations that increase antioxidant enzymes activities (see Beckman and Ames, 1998). Thus, it could be argued that the lower levels of mitochondria in muscles of large animals is part of a successful strategy for a long life span, rather than body size. With these caveats in mind, we think it is most likely that the mitochondrial content of a muscle is controlled to enable the pathways to meet the demands of steady state exercise.

If muscles control the bioenergetic phenotype to meet the demands of active metabolism, how does a muscle sense its own bioenergetic demand? In one scenario, which we term 'extrinsic control', the external regulatory factors (e.g. hormones, neurotransmitters) that control the fiber-type contractile phenotype also exert secondary effects on metabolic enzyme synthesis by crosstalk. Thus, if thyroid hormones or insulin-like growth factors alter contractile protein gene expression, these same extrinsic factors may trigger appropriate changes in bioenergetic gene expression. In an alternative scenario ('intrinsic control'), the contractile phenotype (e.g. activity pattern, resting Ca^{2+} levels) itself induces changes within the muscle that alter the pattern of gene expression. The contractile state may exert its effects at rest or during activity. A fiber with slow muscle excitation–contraction coupling machinery creates a resting state that induces a change in bioenergetic gene expression that is appropriate for a slow muscle. Alternately, the contractile activity of muscles at their highest intensity may create perturbations that result in changes in bioenergetic gene expression.

Consider how one potential signal – Ca^{2+} concentration – differs in slow and fast muscles. Resting Ca^{2+} concentration is higher in a slow muscle than fast muscle because of the Ca^{2+} transport machinery, yet when contraction occurs, the peak Ca^{2+} levels are greater in fast muscle. These complex patterns in the Ca^{2+} signature at rest and during activity could encode regulatory information for pathways that control bioenergetic gene expression. Thus, it could be hypothesized that the enzymatic patterns arise as a response to the contractile properties of the muscle – the way it is constructed and the way it is used.

Genetic determinants of glycolytic and mitochondrial enzyme levels

Whatever ultimately determines the enzyme requirements for a tissue, the enzyme levels are maintained by a combination of protein synthesis and degradation. Enzymes, like other proteins, are synthesized by the sequential processes of transcription, mRNA processing and export, translation (initiation, elongation, termination) and post-translational modification (folding and assembly). Many mitochondrial enzymes have the additional complication of coordinating genomic regulation in two compartments: the nucleocytoplasmic and mitochondrial. Most recent studies assessing control of metabolic gene expression focus on factors that act *via* effects on transcriptional regulation. The challenge in this field of molecular genetics is unravelling networks that involve extrinsic regulators (endocrine, paracrine, autocrine, mechanical signals), signal transduction pathways (receptors, protein kinases, protein phosphatases), transcriptional regulators (DNA-binding proteins, coactivators, histone modifying enzymes) and target genes (enhancer elements, repressor elements).

In this era of high throughput mRNA measurements, there is a temptation to focus on the role of transcriptional regulation in determining the patterns seen in enzyme levels. This predilection leads to a focus on the role of specific transcription factors. In another review on mitochondrial biogenesis (Moyes and Hood, 2003), we emphasize the potential role of other processes in determining enzyme levels: post-transcriptional, translational and post-translational events, including protein and RNA degradation. Consider the following example of the origins of the positive allometeric scaling pattern in pyruvate kinase (PK) activity of trout white muscle (Burness et al., 1999). In small fish (20–500 g), there is an excellent correlation between PK mRNA and PK activity (Fig. 2), which is what would be expected when enzyme levels are determined by transcriptional regulation. By contrast, the largest animals (800–3000 g) had much more PK than would be predicted from their PK mRNA levels. The two most obvious explanations for this observation were that (1) the half-life of the protein was greater in the larger animals, such that less mRNA was needed to maintain PK activity or (2) translational efficiency was greater in larger fish, such that more protein was produced per unit transcript. Thus, in this specific case, the muscle could achieve the expected size-dependent increase in PK with a reduction in transcription of the PK gene. The discrepancy between mRNA levels and protein levels is in fact

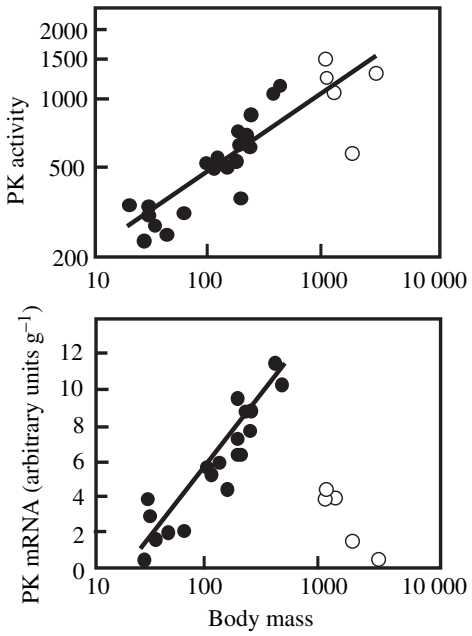

Fig. 2. Relationship between pyruvate kinase activity and mRNA. PK shows positive allometric scaling (b=0.2). Larger fish (open circles) are able to produce more protein from a lower amount of mRNA (adapted from Burness et al., 1999). PK, pyruvate kinase (U g^{-1}); body mass (g).

quite common. For example, barred sand bass display pronounced positive scaling with LDH activity yet possess LDH mRNA levels that are independent of body mass (Yang and Somero, 1996).

Constitutive versus *inducible regulation*

Glycolytic and mitochondrial enzymes are frequently considered housekeeping functions, because virtually all tissues require both pathways. Yet the specific activities of both pathways differ among tissues. Muscle enzyme profile is under control of suites of transcription factors that vary among muscles to establish and modify the metabolic phenotype. Despite the tissue-to-tissue variation, cells are able to retain enzyme stoichiometries within each pathway. These factors also serve to coordinate the expression of the numerous genes that encode the enzymes and transporters needed to support glycolysis and mitochondrial metabolism.

While the maximal capacities for oxidative phosphorylation may vary among tissues, the activities of most mitochondrial enzymes co-vary within narrow ranges. Similarly, the relative activities of most glycolytic enzymes are preserved, even when the maximal capacity of the pathway differs. It is thought that this regulatory feat (maintenance of stoichiometries) is ingrained by constitutive expression. Constitutive gene expression ensures that the cell produces a predictable baseline level of vital proteins needed for cellular function. Constitutive transcriptional regulation may allow gene expression to vary somewhat but normally only within narrow ranges. Superimposed on the constitutive pathway is inducible gene expression. Complex suites of transcriptional enhancers and repressors interact to adjust gene expression across a wide range, from suppressing constitutive expression to greatly inducing expression.

Thus, the levels of glycolytic and mitochondrial enzymes are each determined by a combination of constitutive and inducible regulation. In the context of allometric scaling, or any other comparison among muscles, it is difficult to parse out which aspect of this genetic variation accounts for the phenotype. One important parameter that may alter the way constitutive regulators determine the metabolic phenotype may be ‘myonuclear domain’.

Myonuclear domain co-varies with mitochondrial content

Myonuclear domain is the ratio of cytoplasmic volume per nucleus. In comparison to slow twitch fibers, fast twitch fibers are larger, with a greater myonuclear domain (Tseng et al., 1994) and lower mitochondrial content. Thus, larger fibers have fewer mitochondria and fewer nuclei per volume of tissue. Although myonuclear domain is an ultrastructural parameter, it has ramifications for constitutive expression.

Each myonucleus of a multinucleated myofiber is thought to govern the nature of the cytoplasm within close proximity. There are no physical barriers that confine mRNA regionally and any volume of cytoplasm is under the control of both the nearest nucleus and a consortium of other nuclei in the region. There is abundant evidence for the functional relevance of myonuclear domain (see Allen et al., 1999). It stands, therefore, that in an oxidative muscle (with more nuclei per volume of cell than a glycolytic muscle) any region of cytoplasm has more nuclei within close proximity. Thus, even if their myonuclei produced mRNA at the same rate, an oxidative muscle could produce more bioenergetic enzymes per gram than would a glycolytic muscle. For example, mitochondrial enzyme activities in red *versus* white muscles of fish differ five- to tenfold when expressed per gram tissue (an index of cytoplasmic volume) but only twofold when expressed per milligram DNA (an index of nuclear content) (Dalziel et al., 2004). Differences in myonuclear domain could allow muscles to achieve differences in mitochondrial content without an alteration in constitutive gene expression.

It is not yet known how myonuclear domain varies in homologous muscles of animals that differ in body size. Since the fiber diameter, enzyme profile and contraction kinetics are intertwined, we would hypothesize that myonuclear domain might explain at least part of the allometric pattern seen with mitochondrial enzymes. The influence of myonuclear domain is intriguing because it is a way to explain some of the differences in muscle mitochondrial enzymes without invoking tissue specific gene regulators. Of course, if differences in myonuclear domain help account for mitochondrial differences, they would also increase the need for regulatory increases in glycolytic gene expression.

Coordination of glycolytic gene expression

Control of glycolytic gene expression has been studied

primarily in the context of oxygen-sensing pathways (reviewed by Semenza, 1998). When cells become hypoxic, the protein HIF-1α (hypoxia inducible factor-1α) is stabilized and rapidly accumulates. At the molecular level, hypoxia prevents the hydroxylation of a critical proline residue, which normally entices the ubiquination machinery to tag the protein for degradation. In the absence of accelerated degradation, the protein accumulates and associates with its binding partner HIF-1β. The HIF heterodimer is a DNA-binding protein that associates with regulatory hypoxia-sensitive elements to accelerate transcription. Recognition sites for HIF-1 can be found in the promoters of most glycolytic genes, as well as other genes that must respond to hypoxic conditions, such as erythropoietin. Thus, the HIF-dependent stimulation of gene expression is an elegant pathway by which a coordinated 'increase' in gene expression can be achieved. However, there is little evidence that the same pathway controls glycolytic gene expression under normoxic conditions and by extension there is no reason to believe that HIF plays a role in establishing the allometric pattern.

The relationship between HIF activities, HIF-1α levels and body size remains to be addressed experimentally. But if HIF does not determine glycolytic enzyme levels under normal conditions, then what does? Table 1 summarizes the transcription factors that are thought to regulate each of the glycolytic genes. As is the case with many genes, glycolytic genes share sensitivity to the transcription factors of the Sp1 (specificity protein 1) family. The most important members – Sp1 and Sp3 – bind to the same GC-rich element in the proximal promoter. When Sp1 or Sp3 attach to DNA, they also bind proteins of the general transcriptional machinery, which must assemble to initiate transcription. Sp1 binding usually stimulates transcription whereas Sp3 binding can stimulate or inhibit transcription. Regulation by Sp1 and Sp3 is complex because of their interactive effects and multiple modes of post-translation modification (reviewed by Li et al., 2004). For example, Sp3 binds the promoter as a monomer, whereas Sp1 can form multimers that allow Sp1 sites to act synergistically. If Sp3 binds to the promoter, it prevents Sp1 multimers from binding multiple sites, disrupting their synergy. Truncated forms of Sp3 can also bind the same element; they do not enhance transcription, thereby acting as repressors. Members of the Sp1 family can be modified by phosphorylation, glycosylation and acetylation – modifications that may promote or impair their transcriptional effects.

The complexity of Sp1 signalling makes it difficult to assess if it is responsible for the patterns of glycolytic enzymes among tissues and species, including allometric variation. Within a given muscle nucleus, a particular Sp1 family profile may have different effects on each glycolytic gene. First, the affinity of Sp1 and Sp3 for their element depends on the context (the sequence of DNA that flanks the element). Thus, a particular nuclear ratio of Sp1/Sp3 could enhance a gene with a single Sp1 site, where both Sp1 and Sp3 act in a similar fashion, but inhibit the expression of a gene with multiple tandem Sp1 sites, where Sp3 disrupts the synergistic action of Sp1 multimers. As a given Sp1 profile can have different effects on genes within the same nucleus of a muscle, it is difficult to predict the consequences when the Sp1 profile changes in phenotypic adaptation or differs among fiber types. When genomic/interspecies differences are included in the mix, a formidable task approaches the impossible. Two species could, in principle, differ in the promoter context of each glycolytic gene, as well as the transactivational properties of the different

Table 1. *Glycolytic gene regulator in muscle*

Protein (EC number)	*Gene* (quaternary structure)	Transcriptional regulators
Glucose transporter 1	*SLC2A1*	CRE, TRE[1], Sp1/Sp3[2], p53[3], HIFα[4]
Glucose transporter 4	*SLC2A4*	p53[3], MEF2/GEF[5], KLF15/MEF2A[6], MEF2, TRα1, MyoD family[7]
Hexokinase 2* (EC 2.7.1.1)	*HKII*	CRE-binding protein/ATF-1[8], SREBP-1c[9]
Glucose-6-phosphate isomerase (EC 5.3.1.9)	*GPI* (homodimers)	Sp1[10]
Phosphofructokinase* (EC 2.7.1.11)	*PFKM* (homotetramer)	Sp1[11]
Aldolase A* (EC 4.1.2.13)	*ALDOA* (homotetramer)	HIF1α[12,13], Sp1/Sp3[14], TR2[15]
Glyceraldehyde 3-phosphate dehydrogenase* (EC 1.2.1.12)	*GAPD* (homotetramer)	HIF1α[16]
Phosphoglycerate kinase* (EC 2.7.2.3)	*PGK1*	HIFα[12,13,17]
Phosphoglycerate mutase 2* (EC 5.4.2.1)	*PGAM2* (homodimer)	p53[18], Mef2[19]
β-Enolase* (EC 4.2.1.11)	*ENO3* (homodimer)	Sp1/Sp3[20]
Pyruvate kinase* (EC 2.7.1.40)	*PKM2* (homotetramer)	Sp1/Sp3[10], HIF1α[12]
Lactate dehydrogenase* (EC 1.1.1.27)	*LDHA* (homotetramer)	HIF1α[12,13]

*For those proteins that possess multiple isoforms, we only focused on the most prevalent isoform in skeletal muscle.

GEF, glucose transporter 4 enhancer factor; KLF15, Krüppel-like factor 15; TRα1, thyroid hormone receptor; SREBP-1c, sterol regulatory element binding protein-1c; TR2, orphan nuclear receptor TR2.

[1]Murakami et al., 1992; [2]Fandos et al., 1999; [3]Schwaerzenberg-Bar-Yoseph et al., 2004; [4]Ebert et al., 1995; [5]Knight et al., 2003; [6]Gray et al., 2002; [7]Moreno et al., 2003; [8]Osawa et al., 1996; [9]Gosmain et al., 2004; [10]Claes et al., 1994; [11]Johnson and McLachlan, 1994; [12]Semenza et al., 1994; [13]Semenza et al., 1996; [14]Netzker et al., 1999; [15]Chawnshang et al., 1997; [16]Graven et al., 1999; [17]Okino et al., 1998; [18]Ruiz-Lozano et al., 1999; [19]Nakatsuji et al., 1992; [20]Disher et al., 1998.

Sp1 family members. While the structure of transcription factors tends to be conserved across broad taxonomic comparisons (Hsia and McGinnis, 2003), there are important examples of evolutionary variation in the Sp1 gene family (Kolell and Crawford, 2002). Thus, there are many experimental obstacles to assessing genetic explanations for the allometric variation in glycolytic genes across phylogenetically diverse taxa. Furthermore, it remains unknown how a muscle might transduce a metabolic signal into a change in Sp1 transactivational activity.

Coordination of mitochondrial gene expression

Mitochondrial biogenesis requires coordinated transcription of nuclear and mitochondrial genes (Garesse and Vallejo, 2001; Kelly and Scarpulla, 2004; Scarpulla, 2002). Coordination of gene expression is achieved through several transcriptional regulators that regulate suites of genes: nuclear respiratory factor 1 (NRF1), NRF2, the peroxisome proliferator activated-receptors (PPARs) and the PPARγ coactivator-1 α (PGC-1α). The reader is directed to the many excellent reviews that discuss these transcriptional regulators in more detail.

NRF1 is a homodimeric DNA-binding protein that was first identified as an activator of the rat somatic cytochrome *c* gene and has since been shown to regulate multiple genes involved in mitochondrial oxidative phosphorylation and proliferation (see Kelly and Scarpulla, 2004).

NRF2, a member of the ETS family of transcription factors, is a heterotetramer composed of two DNA-binding subunits (α) and either two β subunits or two γ subunits (see Kelly and Scarplla, 2004). NRF2 was identified as an activator of rat COXIV but has since been shown to regulate multiple genes associated with mitochondrial biogenesis and oxidative metabolism.

The PPARs are a subfamily of the nuclear hormone receptor superfamily composed of three members: α, β/δ and γ (for reviews, see Berger and Moller, 2002; Gilde and Van Bilsen, 2003). The PPARs heterodimerize with a retinoid acid receptor. In the presence of agonists, the heterodimer binds PPAR response element in the promoter sequence of target genes to activate transcription. PPARα and β/δ are the most prevalent isoforms in striated muscle, where they regulate genes related to lipid metabolism.

The PGC-1α family of transcriptional coactivators includes PGC-1α, PGC-1β and PGC-1-related coactivator (PRC) (for reviews, see Knutti and Kralli, 2001; Puigserver and Spiegelman, 2003). PGC-1α is recruited by transcription factors that are bound to the promoter of target genes. The complex then recruits other effectors, increasing the transcriptional activity. PGC-1α was first identified as a cold-inducible coactivator of PPARγ in adipose tissue (Puigserver et al., 1998). It has since been shown to interact with several of the transcriptional regulators that control mitochondrial gene expression and is now considered to be a master coordinator of mitochondrial biogenesis. PGC1α has also been shown to stimulate the expression of genes encoding slow muscle contractile proteins, providing a potential link between the contractile and bioenergetic phenotypes (Lin et al., 2002). Recently, a PGC1α mouse was created and displayed 30–60% shortfalls in muscle mitochondrial gene expression, energetic defects and a deficiency in shivering thermogenesis (Lin et al., 2004). While this attests to the importance of PGC1α, even the null mutants were viable and possessed mitochondria, perhaps reflecting the role of other PGC1 family members.

These transcriptional regulators serve to coordinate the many hundreds of genes required for mitochondrial biogenesis. There is natural variation in the levels of these transcriptional regulators among muscle fiber types that correlates with mitochondrial content (see Moyes and Hood, 2003). In most cases, transgenic studies that over-expressed one of these transcriptional regulators led to an increase in the levels of selected mitochondrial precursors (see Table 2). However, the importance of these transcriptional regulators in determining allometric relationships has not been studied. Given the complexity of the mechanisms (multiple members of gene families, complex heterodimerization patterns, ligand-dependent interactions), it would be challenging to use a comprehensive transcription factor profile to predict a resulting bioenergetic profile.

Linking bioenergetic enzyme gene expression with the contractile phenotype

Earlier in this review we distinguished between intrinsic and extrinsic determinants of bioenergetic gene expression. Intrinsic pathways begin when a muscle senses an intracellular condition that triggers a change in the synthesis or degradation of enzymes. Extrinsic regulators exert effects on muscle development to establish an appropriate metabolic phenotype to support the contractile phenotype. Most studies that investigate the roles of these potential regulators focus on a single pathway. In the context of allometry, we are on the lookout for a factor that could trigger reciprocal changes in glycolytic and mitochondrial gene expression.

Intrinsic control of bioenergetic gene expression

How does a muscle cell sense a need to alter gene expression? Most recent research has focused on the roles of four surrogates of muscle activity: redox status, hypoxia, Ca^{2+} and AMP. While there is some merit in hypotheses that implicate these regulators in determining metabolic gene expression, there is no evidence that a single regulator causes reciprocal changes in glycolytic *versus* oxidative enzymes, as seen in allometric relationships.

Redox regulation is a term used in multiple contexts (reviewed by Leary and Moyes, 2000). In some cases, oxidative stress can trigger changes in gene expression through affects on the transcription factor NF-E2-related factor-2. The acronym for NF-E2-related factor-2 is Nrf2, which is unfortunately quite similar to that of an unrelated transcription factor, nuclear respiratory factor 2, or NRF 2. It binds to

Table 2. *Interventions that modify striated muscle bioenergetics*

Intervention	Aerobic metabolism	Glycolytic metabolism
Regulatory enzymes		
CaN: Activated form of CaN driven by muscle-specific promoter[1]	In plantaris muscle, an increase in SDH activity in type II but no change in type I	Type II fibers of plantaris show reduced alpha glycerophosphate dehydrogenase activity (no change in type I)
CaMK IV: Constitutively active in type II fibers[2]	Increased expression of PGC-1α; threefold increase in mRNA levels of a broad range of mitochondrial proteins	Not measured
Transcriptional regulators		
PPARδ: Targeted overexpression of an constitutively active form in mouse skeletal muscle[3]	Twofold increase in gastrocnemius type I fibers; increase in myoglobin, mtDNA, mRNA for nuclear and mtDNA genes	Not measured
PPARδ: Targeted overexpression (sixfold) of a native PPAR delta in mouse skeletal muscle[4]	50% increase in citrate synthase and HOAD mRNA in tibialis anterior; twofold increase in fatty acid binding protein	No change in mRNA for LDH and alpha glycerophosphate dehydrogenase
PGC-1α: Muscle specific transgene[5]	Increases in mRNA for many mitochondrial genes	Not measured
Mef2a: Heart of knockout mouse[6]	Reduction in mtDNA, mt mRNA and cytochrome oxidase activity; no change in mRNA of nuclear encoded mitochondrial genes	Not measured
Myogenin: Transgene with fast muscle-specific promoter[7]	2–3-fold increase in mitochondrial enzymes	40–70% reduction in glycolytic enzymes
TFAM: Mitochondrial transcription factor A; skeletal muscle knockout mouse[8]	Increase in electron transport enzymes	Not measured
TFAM: Cardiac knockout mouse[9]	Decrease in expression of TCA enzymes and fatty acid enzymes	Expression of some glycolytic enzymes increased, others decreased, most did not change
Myopathies		
ADNT: Adenine nucleotide translocase; knockout mouse[10]	Proliferation of defective mitochondria	Not measured
MnSOD: Heart and skeletal muscle of knockout mouse[11]	Reduction in mitochondrial enzymes, likely as a result of oxidative damage	Not measured
Lipoprotein lipase: Muscle specific[12]	Increased SDH and peroxisomal enzymes	Not measured
Type II diabetes[13]	Decrease in oxidative enzymes	An increase in glycolytic enzymes
Obesity[13,14]	Decrease in oxidative enzymes	An increase in glycolytic enzymes

[1]Talmadge et al., 2004; [2]Wu et al., 2002; [3]Wang et al., 2004; [4]Luquet et al., 2003; [5]Lin et al., 2002; [6]Naya et al., 2002; [7]Hughes et al., 1999; [8]Wrendenberg et al., 2002; [9]Hansson et al., 2004; [10]Graham et al., 1997; [11]Li et al., 1995; [12]Hoefler et al., 1997; [13]Hickey et al., 1995; [14]Tanner et al., 2002.

antioxidant response elements in the promoters of many genes related to antioxidant protection. This type of redox regulation is intended to compensate for cytotoxic effects of xenobiotics, but another type of oxidative stress is thought to be important in the regulation of bioenergetic genes. When mitochondria are defective or inhibited, there can be an increase in the production of superoxide at complex I and III. This type of oxidative stress may contribute to neurodegenerative diseases, but it may also signal the nucleus that there are mitochondrial shortfalls (see Leary and Moyes, 2000). A third connotation of redox regulation refers to the metabolic redox state, whereby changes in NADH/NAD^+ may be communicated to regulatory enzymes. Recently, an NAD^+-dependent histone modifying enzyme, Sir2, was shown to be sensitive to NADH/NAD^+ ratios, which may contribute to control of gene expression in response to metabolic conditions (Fulco et al., 2003).

Hypoxia stimulates glycolytic gene expression through increases in HIF-1, as discussed earlier in this review. HIF-dependent gene regulation probably plays a role in stimulating muscle remodelling in intense exercise, more so at high altitude (Vogt et al., 2001). While HIF may induce adaptive remodelling, it is unlikely to be responsible for establishing basal levels of glycolytic enzymes under normoxic conditions. There is no evidence that mitochondrial genes are responsive to HIF, although it alters the expression of proteins that facilitate oxygen delivery and could affect mitochondrial metabolism indirectly.

Ca^{2+} plays an important role in control of contraction, but it may also help tailor muscle bioenergetic gene expression. The nature of the Ca^{2+} signal is complex because of the temporal, spatial and quantitative variation in Ca^{2+}, known as the Ca^{2+} signature. Changes in the Ca^{2+} signature influence a multitude

of signalling pathways in cardiac muscle (see Bers, 2002) and skeletal muscle (see Michel et al., 2004). Increases in Ca^{2+} in L6 cultured myoblasts triggers an increase in expression of several transcription factors implicated in mitochondrial gene expression, including NRF-1, NRF-2 and PGC-1α (Ojuka et al., 2003). When at rest, oxidative muscles tend to have higher levels of Ca^{2+} (100–300 nmol l^{-1}) than do glycolytic muscles (50 nmol l^{-1}) (Chin and Allen, 1996). This difference in resting Ca^{2+} levels may induce the changes in expression of bioenergetic genes, acting through CaN (Michel et al., 2004) and CaMK (Wu et al., 2002). While both CaN and CaMK can each trigger a transformation of fast skeletal muscle fibers to slow skeletal muscle fibers (e.g. Talmadge et al., 2004; Wu et al., 2002), they can exert subtly different effects on bioenergetic enzymes, at least in heart (Schaeffer et al., 2004). CaN activates expression of fatty acid oxidizing genes and selected mitochondrial proteins, without affecting glycolytic genes. by contrast, CaMK transfection has little effect on fatty acid oxidizing genes but increases the expression of mitochondrial genes as well as the glucose transporter 4 and phosphofructokinase. While both CaN and CaMK increase PGC-1α expression, only CaN increases PPARα expression (Schaeffer et al., 2004).

The final metabolic regulator we consider is the adenylate pool. Under conditions of high ATP turnover, AMP levels increase due to the activity of myokinase (2ADP ↔ ATP + AMP). When AMP:ATP ratios increase, AMP-activated protein kinase (AMPK) is activated, phosphorylating many proteins that are involved in intermediary metabolism (see Hardie et al., 2003), including skeletal muscle mitochondrial enzymes (Winder et al., 2000). There is also evidence that AMPK activity can be modulated by phosphorylation, independent of AMP:ATP ratios (see Carling, 2004). Several studies have shown that AMPK can affect gene expression (see Hardie et al., 2003), but the exact signalling pathways have not yet been identified.

Extrinsic factors determine bioenergetic profile in muscle differentiation and development

In contrast to adaptive remodelling, the metabolic profile that arises during muscle differentiation and development is likely under the control of extrinsic factors. Undifferentiated muscle cells (myoblasts) derive approximately 80% of their energy from glycolysis. Serum starvation triggers the myogenic program, initiated by autocrine production of insulin-like growth factor II. Within the first couple of days of differentiation, the myocytes shift their metabolic poise and derive almost 80% of their energy from mitochondrial metabolism, however the metabolic rate is not altered. Two weeks post-differentiation, myotubes have increased mitochondrial content about fivefold, but glycolytic enzymes do not change (Moyes et al., 1997). The reason for this metabolic shift in the first few days of differentiation remains enigmatic because it occurs well before major changes in mitochondrial enzymes (Lyons et al., 2004). In any case, the changes in mitochondrial enzymes during myogenesis appear to arise independent of metabolic changes, arguing against a role for the intrinsic regulators in myogenic mitochondrial proliferation.

Once the muscle has matured, many hormonal regulators can trigger muscle remodelling. Transgenic studies that employ active forms of CaN and CaMK implicate these enzymes in muscle remodelling, but activation in normal muscle would require a favourable change in Ca^{2+}. As mentioned, fast and slow muscles have different Ca^{2+} levels (Chin and Allen, 1996) as a result of the contractile phenotype (Ca^{2+} transporters, Ca^{2+} sequestration, parvalbumin). Thus, the question ultimately is what causes the change in the contractile phenotype. Muscle fiber-type transformation can arise in response to innervation patterns, mechanosignalling pathways, autocrine IGF-II production and thyroid hormone state (see Baldwin and Haddad, 2002). Understanding the patterns of allometric scaling of enzymes then would need to focus on the hormones and other signals that determine the contractile phenotype, which then affects the metabolic phenotype.

Perhaps more provocative are the interventions that lead to reciprocal changes in mitochondrial and glycolytic enzymes, analogous to the differences seen with body size. Hughes et al. (1999) showed that overexpression of the myogenic factor myogenin induced reciprocal changes in mitochondrial and glycolytic enzymes of fast muscle, but without a change in the contractile phenotype, as indicated by myosin heavy chain profile. Unlike the other studies summarized in Table 2, myogenin is one of the few transgenes that can cause reciprocal changes in the two pathways. Similarly, metabolic disorders including obesity and type II diabetes trigger reciprocal changes in glycolytic enzymes (Hickey et al., 1995; Tanner et al., 2002).

Future directions

Many recent studies have identified signalling enzymes and transcriptional regulators that control mitochondrial gene expression. Regrettably, few of these studies measure the effects of the transgene on glycolytic enzymes. At the same time, enough is known about these regulators to perform comparative analyses in the context of allometric variation. Unfortunately, those species that differ most in body size are only distantly related, complicating analyses that may be sensitive to genomic variation. We see progress in this area dependent upon several related approaches. (1) More studies of muscle biochemistry in specific fiber types of animals of different sizes. There are many studies that have assessed allometric patterns in metabolic rate of tetrapods, very few studies have explored muscle biochemistry in this context. (2) More fish studies linking metabolic rate and muscle biochemistry. Muscle biochemistry in relation to body size is better studied (and perhaps best studied) in fish, but in these animals metabolic rate measurements are more problematic. (3) Molecular genetics of bioenergetic gene expression. As we learn more about the control of both mitochondrial and glycolytic genes, it will become possible to explore the

regulatory basis of comparative patterns of muscle gene expression. While studies will likely focus on the role of transcriptional regulation, other factors such as myonuclear domain, translational and post-translational regulators must also be considered.

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