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Review

Normal mammalian skeletal muscle and its phenotypic plasticity

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Summary

Since muscle mass makes up such a high proportion of total body mass, there must have been considerable selective pressure to minimize the cost of maintenance and to maximize the functionality of muscle tissue for all species. Phenotypic plasticity of muscle tissue allows the species blueprint of muscle tissue to be modified to accommodate specific demands experienced by animals over their lifetime. In this review, we report the scaling of muscle structural compartments in a set of mammals spanning five orders of magnitude (17 g woodmice to 450 kg horses and steers). Muscle mass, muscle myofibrillar volume and sarcoplasmic space were found to represent similar relative quantities in all species studies (scaling factor close to unity). Mitochondrial volumes were found to be systematically smaller in larger animals (scaling factor 0.91) and closely related to the scaling of $\dot{V}_{O_2max}$ (0.92) and were tracked by the scaling of total capillary length (0.95). In this set of species, we therefore found that maximal metabolic rate and supporting structures did not scale to the 0.75 power of body mass as generally suggested. Muscle phenotypic plasticity is reasonably well characterized on a structural and functional basis, but we still know little about the signals that cause the changes in gene expression necessary for phenotypic changes in muscle. The molecular responses of human m. vastus lateralis to endurance exercise indicate that a single bout of exercise causes specific transient transcriptional adaptations that may gradually accumulate after their translation into the (structural) modifications seen with phenotypic plasticity. Metabolic and mechanical factors are recognized candidate factors for the control of exercise-induced gene transcription in muscle. Distinct protein kinases and transcription factors emerge as possible interfaces that integrate the mechanical (MAPKs and jun/fos) and metabolic (AMPK, HIF-1α and PPARα) stimuli into enhanced gene transcription in skeletal muscle.

Key words: scaling, morphometry, mRNA, $\dot{V}_{O_2max}$, muscle, phenotype, plasticity.

Introduction

Locomotion is essential for acquiring food and to escape predation and is, thus, a key ability of most animals. Not surprisingly, we find that skeletal muscle tissue is the single most abundant tissue in an animal, representing up to 50 % of body mass in some athletic species such as the dog and the horse (Gunn, 1989). The specific condition under which skeletal muscle tissue functions in a species impacts on the structural design of muscle tissue. A dominant mechanical constraint for the function of muscle tissue is animal size. Thompson (1942) noted that, as animals increase in length, muscle force should increase with the square of the length change while animal mass should increase with the cube of the length change. Hill (1950) refined Thompson's arguments and made specific predictions as to how animal size could constrain muscle design and impact on animal performance. An important consideration in all modelling efforts is the assumption that force per unit muscle cross-sectional area is the same for animals of all sizes (Close, 1972). Hill (1950) predicted that maximum power output would increase not as body mass (M_b) but as some lower power of body mass (he proposed $M_b^{0.73}$). He further predicted that animals, irrespective of body size, should be able to move at the same maximum speeds, the advantage for larger animals being that they should be able to cover proportionately longer distances. He further noted that the rates of enzyme reactions would also have to be adjusted to body size, with smaller species requiring the metabolic machinery of their muscle cells to run faster.

As several of Hill's (1950) predictions appeared to be violated by experimental observations, McMahon (1975) refined the modelling approach and showed that animals appeared to be built such that their structures are similarly threatened by elastic failure under their own mass (elastic similarity). He found that elastic similarity predicted observed animal locomotor performance characteristics (such as stride length, stride frequency and the metabolic power required for running) better than the geometrical similarity models explored

by Thompson (1942) and Hill (1950). However, the model of elastic similarity has not remained uncontested (Heusner, 1982; Christiansen, 1999; see also Feldman and McMahon, 1983).

There has been much recent interest in deriving scaling laws from intrinsic properties of fractal networks of connectivity (blood vessels, bronchial tree; West et al., 1997). Using this approach, the 0.75 scaling power of metabolic rate observed by Kleiber (1932) is seen as a consequence of minimizing transport times and distances of internally branching hierarchical networks in systems maximized for metabolic capacity (West et al., 1999). In a more general sense, the observed scaling properties of metabolic rate in animals are seen to be general properties of efficient transportation networks relating size and flow rates in networks with local connectivity in animals and plants and even in inanimate systems such as the drainage system of river basins (Banavar et al., 1999). As steady-state locomotor performance of animals could be constrained by the availability of oxygen to skeletal muscle mitochondria (Vock et al., 1996b), these recent scaling considerations would lead us to expect that both maximal aerobic metabolic rate and muscle oxidative capacity would scale close to the 0.75 power of body mass.

In addition to animal size, we expect muscle structure to reflect typical functional needs related to the 'lifestyle' of an animal. Lifestyle is variable throughout the life of an individual, so we would expect muscle to be malleable. The design of the muscle machinery should be 'optimized' such that a cheetah should be able to produce enormous power over a very short period while a gazelle should be able to cruise efficiently at high speed but with less capacity to accelerate (Hill, 1950). To characterize economic design, Taylor and Weibel (1981) coined the term 'symmorphosis'. They postulated that, by a process of regulated morphogenesis, structural elements should be designed to satisfy but not to exceed functional requirements. In this sense, the structural design of the muscular system of a species is seen as the consequence of selective pressure during evolution. Symmorphosis also encompasses the need for phenotypic plasticity of skeletal muscle tissue. The environmental conditions to which animals are exposed are not constant, so the demands that muscle tissue has to satisfy can vary over the lifetime of an animal. To respond to changing demands, skeletal muscle tissue must be able to adapt. Over the last 10 years, we have learned that, in response to environmental (or internal) cues, normal skeletal muscle tissue can alter its gene expression and thus modify its structural composition or the functional properties of its structural components. We consider this epigenetic malleability to be an important feature of the 'economic design' of skeletal muscle tissue. Under given constraints, only what is needed must be provided because structures can be modified when constraints change.

We will first review the impact of the fundamental variable body mass on the structure of skeletal muscle tissue (essentially of mammals) and then discuss the mechanisms by which an extant muscle structure is modified to accommodate differences in load characteristics experienced by an individual during its lifetime. The second part of this review reports the state of knowledge on the molecular mechanisms that are at the basis of the phenotypic malleability of muscle tissue.

Animal size and the composition of skeletal muscle tissue

For terrestrial mammals with body masses ranging from 17 g (European woodmouse *Apodemus sylvaticus*) to 450 kg (horse *Equus caballus*; steer *Bos taurus*), maximal aerobic performance capacity ($\dot{V}_{O_2max}$) and skeletal muscle morphology have been studied systematically (for reviews, see Weibel et al., 1992; Hoppeler and Weibel, 1998). In both these reviews, data relevant to oxygen transfer from the lungs to skeletal muscle mitochondria for a broad size range of mammalian species were presented. All data were obtained according to a standard experimental protocol. From all

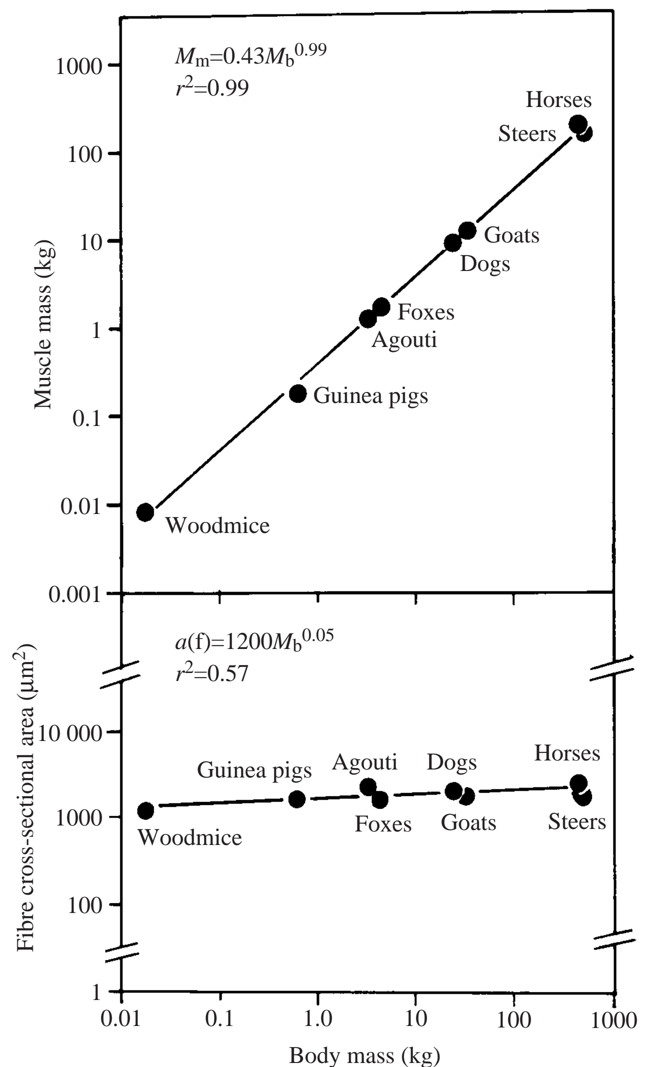

Fig. 1. Allometric plot of muscle mass (A) and fibre cross-sectional area (B) for the species reported in Table 1. a(f), mean fibre cross-sectional area; M_b, body mass; M_m, muscle mass.

species, $\dot{V}_{O_2max}$ while running was obtained together with estimates of muscle structural variables representative of the entire skeletal musculature. The technique of whole-body sampling of the musculature according to statistical principles (Hoppeler et al., 1984) was developed to estimate typical muscle 'organ' structural variables. In all studies, we sampled 15 randomly selected locations from the whole musculature of each animal. The data thus obtained can therefore be considered to be largely insensitive to species-specific or circumstantial use of individual muscles. In this review, we present and discuss the entire set of muscle morphometric data, not only those related to oxygen transfer and reported previously (Table 1). This set of data is similar, but not identical, to data published previously (Hoppeler and Weibel, 1998). For some species (goat, guinea pig and dog), we increased the number of observations by including all data for which a complete set of functional and morphometric measurements was available. This does not impact on the validity of interpretations of results published previously with the more limited data sets.

To remain consistent with previous data sets (see Hoppeler and Weibel, 1998), we have opted to disregard the phylogenetic relationship of the species evaluated. As the species analysed share a variable part of their evolutionary history, they cannot be considered independent *sensu stricto* from a statistical point of view. The possibility that taking phylogeny into consideration might have had some impact on the scaling of the functional and structural variables under scrutiny cannot therefore be excluded (Garland, 1999; Garland and Ives, 2000).

Muscle mass and fibre size

As noted previously (e.g. Hoppeler, 1990), relative muscle mass measured by complete dissection of the carcass of an animal is size-invariant (muscle mass scales to $M_b^{0.99}$; Fig. 1A). A similar scaling exponent of close to unity (1.05) was found to relate white muscle mass to body mass in an intraspecific comparison in rainbow trout *Oncorhynchus mykiss* (Goolish, 1989). In general, we find that active species in any size class tend to have a larger relative muscle mass than sedentary species (Kayar et al., 1989; Weibel et al., 1992). More surprisingly, we find that fibre cross-sectional area (measured as mean fibre cross-sectional area, obtained from low-power electron micrographs) is found to be body-mass-independent at least for animals down to the size of a 16 g woodmouse (Fig. 1B). There is no

Table 1. *Body-mass-specific maximal oxygen uptake rate and structural variables from eight mammalian species obtained by statistically sampling the entire skeletal musculature*

Species	*n*	M_b (kg)	$\dot{V}_{O_2max}$ (ml kg^{-1} min^{-1})	M_m (kg)	V_V(mc,f) (%)	V_V(ms,f) (%)	V_V(mt,f) (%)	V_V(li,f) (%)	V_V(fi,f) (%)	V_V(re,f) (%)	N_A(c,f) (mm^{-2})	$\bar{a}$(f) (μm^2)
Woodmouse *Apodemus sylvaticus*	4	0.017±0.002	264±9.6	0.00836±0.0077	9.99±1.2	1.98±1.3	11.97±2.2	0.279±0.32	79.1±4.0	8.93±3.1	1270±277	1256±201
Guinea pig *Cavia porcellus*	21	0.607±0.12	55.8±6	0.178±0.025	3.97±1.1	2.12±0.9	6.09±1.4	0.105±0.098	79.6±2.7	14.3±1.7	821±157	1693±334
Agouti *Agouti paca*	1	3.22	102	1.31	4.36	1.26	5.62	0.030	81.0	13.3	581	2367
Fox *Alopex lagopus*	3	4.27±0.35	217±29	1.67±0.2	9.13±0.2	3.59±0.25	12.72±0.81	NA	80.9±0.049	6.30±0.31	827±88	1726±136
Goat *Capra hircus*	12	32.4±8.3	59.8±9.2	12.0±7.6	3.29±0.48	0.72±0.31	4.01±0.77	0.0655±0.078	83.3±2.3	13.1±2.6	625±253	1809±243
Dog *Canis familiaris*	6	24.3±1.8	127±37	9.04±2.3	6.08±1.4	1.88±0.89	7.96±2.1	0.305±0.2	76.5±4.9	15.3±3.7	776±491	2070±467
Steer *Bos taurus*	3	474±21	51.2±2.1	165±5.1	2.96±0.41	0.597±0.12	3.56±0.53	0.045±0.007	79±0.47	18.1±5.7	727±9.6	1933±194
Horse *Equus caballus*	3	447±61	136±6.7	191±28	4.85±0.36	2.62±0.62	7.47±0.94	0.243±0.051	73.2±2.2	19.1±1.5	928±62	2498±241

Values are means ± S.D.

NA, not available.

M_b, body mass; $\dot{V}_{O_2max}$, maximal oxygen consumption; M_m, muscle mass; V_V(mc,f), volume density of central mitochondria; V_V(ms,f), volume density of subsarcolemmal mitochondria; V_V(mt,f), V_V(mc,f)+V_V(ms,f); V_V(li,f), volume density of intracellular lipid droplets; V_V(re,f), volume density of remaining sarcoplasmic components; N_A(c,f), capillary density; $\bar{a}$(f), mean fibre cross-sectional area.

indication that active animals have larger fibre sizes than inactive ones.

Myofibrils constitute the major compartment in muscle cells, comprising 73.2 % of muscle fibre volume in horses to 83.3 % of muscle fibre volume in goats. Total myofibrillar volume is directly proportional to muscle mass (scaling factor 0.98). Skeletal muscle sarcomeres are built very similarly in all mammalian species, so the total number of cross-bridges that the myosin heads can form with actin is directly proportional to muscle mass. Deviations of the ATP demand for muscle contraction from direct proportionality to body mass must therefore depend mainly on size-dependent differences in cross-bridge cycling rates.

Structural and functional variables related to oxidative metabolism

We have reported the scaling of structures determining muscle oxygen demand (mitochondria) and muscle oxygen supply (capillaries) with body mass and on the relationship between these structures and $\dot{V}_{O_2max}$ (Hoppeler et al., 1981). With the current set of data and considering all species, we observe that $\dot{V}_{O_2max}$ scales to $M_b^{0.92}$, as previously reported for a similar set of animals (Fig. 2; Hoppeler and Weibel, 1998). This is greater than the scaling exponent of 0.809 reported for 22 wild and domestic mammalian species reported by Taylor and Weibel (1981). This difference in scaling is probably due to the inclusion of 'athletic' species (foxes, dogs and horses) in the larger size classes. If the regression for the current data set is calculated without these species, $\dot{V}_{O_2max}$ scales to $M_b^{0.86}$.

Overall, the present data confirm the observation that $\dot{V}_{O_2max}$ closely tracks total mitochondrial volume, V(mt). For V(mt), we find a scaling factor of 0.91 for all species (0.86 for the 'sedentary' subset). If we subdivide the mitochondrial population into central and subsarcolemmal mitochondria, we find that subsarcolemmal mitochondria represent between 16 and 35 % of the total mitochondrial population, with no apparent systematic relationship to the activity level of the species. The volumes of both subsarcolemmal and central mitochondria and total mitochondrial volume scale similarly to body mass. Capillary length J(c) can be calculated from capillary density [(Na(c,f); Table 1)] using the formula published previously with a tortuosity factor of 1.24 (Conley et al., 1987). J(c) scales as $M_b^{0.95}$ for all species and as $M_b^{0.92}$ for the sedentary subset. These scaling factors are very close to values reported previously for individual muscles (diaphragm, semitendinosus, longissimus dorsi and vastus medialis) in 21 wild and domestic species (Hoppeler et al., 1981). The difference in capillary length between athletic and sedentary species is smaller than that observed for the volume of mitochondria because oxygen supply is helped in athletic species by the greater haemoglobin concentration in their circulation (Conley et al., 1987). The current data set supports the hypothesis of an extremely close match between oxidative capacity and capillary supply from a scaling perspective (Hoppeler and Kayar, 1988).

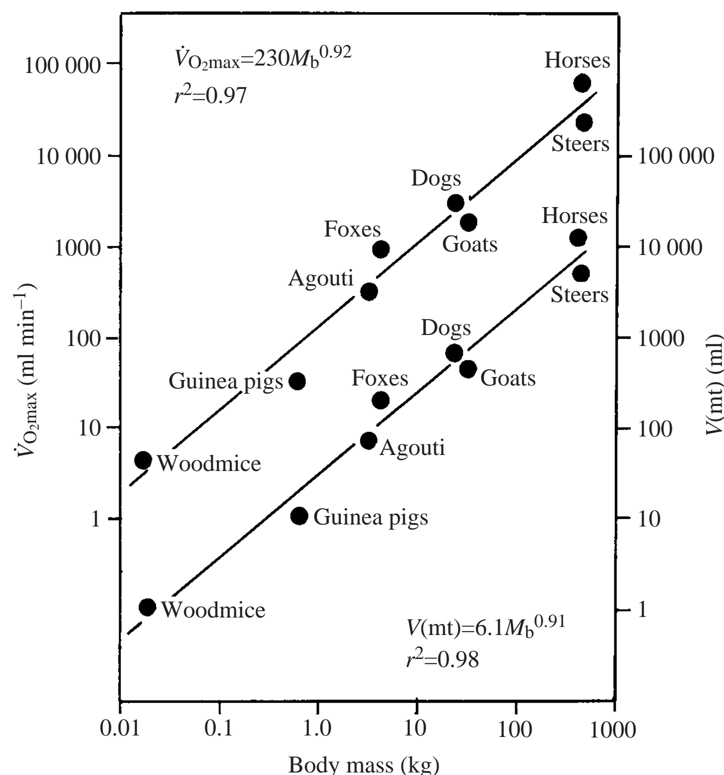

Fig. 2. Allometric plots of $\dot{V}_{O_2max}$ and total volume of mitochondria, V(mt), for the species reported in Table 1. V(mt) was calculated by multiplying the muscle mass by the volume density of mitochondria and the density of muscle tissue (1.06 g ml^{-1}). M_b, body mass.

We observe that maximal aerobic capacity and the quantities of muscle structural elements determining this function (mitochondria and capillaries) scale with scaling factors larger than 0.75, as predicted by recent theoretical considerations (West et al., 1997). In view of the proposed generality of scaling laws, this seems surprising. The number and selection of species considered in this review and the size range may be too limited and might skew the relationship between body mass and the investigated variables. However, alternative explanations for scaling factors differing from 0.75 have been proposed and may have to be considered in this context. On the basis of metabolic control theory, one would expect scaling of metabolic rate to depend on multiple-site control and to show different exponents depending on whether basal or maximal metabolic rate is considered (Darveau et al., 2002). Bejan (2000) showed that a scaling factor of close to 0.875 would be expected for a bioengineering approach to scaling of metabolic rate encompassing both the fractal transportation network concept (West et al., 1997) and heat transfer considerations.

Intramyocellular lipid stores

Morphometry lends itself well to determining intramyocellular lipid concentrations in the form of lipid droplets (IMCLs) found in contact with mitochondria (Vock et al., 1996a; Howald et al., 2002). We find the

scaling of IMCLs (Fig. 3) to be very similar to the scaling of $\dot{V}_{O_2max}$ and of mitochondrial volume. There seems to be a strong tendency for athletic species to have larger intramyocellular (lipid) substrate reserves, as was previously noted by Vock et al. (1996a).

Allometric scaling of RNA and DNA concentrations

We have so far discussed the size-dependent structural design of mammalian skeletal muscle tissue and have expanded this discussion to the compartments that can efficiently be quantified by electron microscopic morphometry. For oxidative enzymes such as citrate synthase, it has been shown that activity decreases per gram muscle tissue in accordance with the morphometric data on mitochondria (Emmett and Hochachka, 1981; Hochachka et al., 1988). In contrast, the activities of enzymes associated with anaerobic metabolism, such as lactate dehydrogenase and pyruvate kinase, increase with increasing body mass (Somero and Childress, 1980; Emmett and Hochachka, 1981). For these enzymes, there is no established structural correlate. The opposite scaling of aerobic and anaerobic enzyme concentrations cannot be explained by overall regulation of protein synthesis or degradation (Yang and Somero, 1996). Explanations must therefore be sought at the transcriptional or translational level.

Little is known as to the allometric scaling of RNA and DNA concentrations and, hence, whether and how the observed size-dependencies of protein concentrations (ultimately estimated morphometrically as structural quantities) are achieved at the molecular level. This problem has been addressed by using fish as a model organism (Yang and Somero, 1996; Burness et al., 1999), an approach that allows large intraspecific size differences to be included. The scarce data on fish show a complex pattern in which not only size but also age and growth rate seem to influence DNA and mRNA concentrations (Burness et al., 1999), with translational regulation being implicated in the regulation of the concentration of glycolytic enzymes (Yang and Somero, 1996). The molecular mechanisms by which basic levels of protein concentrations are established and controlled in muscle thus remain largely unexplained.

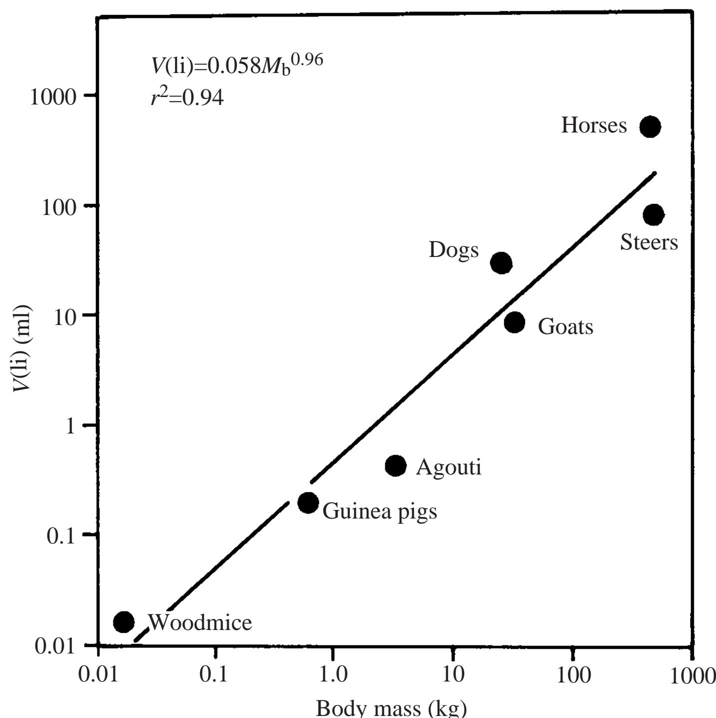

Fig. 3. Allometric plot of the total volume of lipid V(li) in skeletal muscle for the species reported in Table 1. M_b, body mass.

Basis of molecular plasticity of skeletal muscle tissue

As indicated in the Introduction, we perceive malleability to be a fundamental design feature of skeletal muscle tissue. The capacity to adapt muscle tissue phenotypically to specific stresses is superimposed on a standard building plan of muscle tissue for a species, this building plan being determined mainly by body size and athletic prowess (related to the lifestyle of a species). To respond to specific challenges in the environment, muscle tissue rapidly and specifically adapts to variables such as exercise, temperature and nutritional conditions. As a consequence of muscle malleability, muscle structural and functional modifications are reversible: the modifications are maintained as long as the stimulus persists. The most studied muscle modifications are probably those related to exercise training.

Different forms of exercise such as ‘high repetitive, low load’ (endurance) exercise and ‘low repetitive, high load’ (strength or resistance) exercise induce specific and distinct structural and functional modifications in muscle fibres. Classical endurance training interventions of 6–8 weeks duration in previously untrained human subjects can lead to an elevation of mitochondrial volume density of up to 40 %, while capillary density may be increased by close to 30 % (Hoppeler et al., 1985). In response to strength training of similar duration, myofibrillar volume selectively increases by as much as 20 % (Luethi et al., 1986). These training responses involve changes in gene expression. The set of mRNAs expressed provides a basic instruction for the palette of translated proteins that is finally manifest as a characteristic structural adaptation to exercise training. Endurance training influences gene expression in skeletal muscle within a matter of 30 min to some hours (regulatory genes) or as a consequence of weeks of systematic training (structural genes). In contrast, little is known about transcriptional adaptations in response to resistance exercise (Kadi and Thornell, 2000; Booth et al., 1998).

This second part of this article will briefly review the most striking transcriptional adaptations seen in exercised skeletal muscle and provide a model aimed at linking these events to physiological signals induced by exercise. A particular focus will be on transcriptional adaptations to endurance-type exercise in human skeletal muscle since these have been best characterized.

Table 2. *Transcriptional changes in human m. vastus lateralis in response to exercise*

Response	Change	Protocol	Time of (maximum) response	Stimulus		Reference
				Mechanical	Metabolic	
Second messengers						
JNK phosphorylation	Eightfold	30 min treadmill, 5 mmol l^{-1} lactate	4–30 min post exercise	con/ecc	Lactate	5
JNK kinase activity	5.9-fold	60 min ergometer, 70 % $\dot{V}_{O_2max}$	<10 min post-exercise	con		2
JNK kinase activity	3.5- to 15.4-fold	Maximal knee extension, 10 sets, 10 repetitions	<10 min post-exercise	ecc>con		3
ERK phosphorylation	Eightfold	60 min ergometer, marathon	<10 min post-exercise	con		1, 14
p38 phosphorylation	4.4-fold	Marathon 3–4.5 h		con/ecc		14
α2-AMPK kinase activity	3–4-fold	60 min ergometer, 70 % $\dot{V}_{O_2max}$	<10 min post-exercise		Lactate	13
Immediate early genes						
c-fos, fosB, fra-1, c-jun, junB, junD mRNA	3- to 20-fold	30 min treadmill, at aerobic threshold, 5 mmol l^{-1} lactate	4–30 min post-exercise	con/ecc	Lactate	9
c-jun mRNA	1.8-fold	60 min ergometer, 70 % W_{max}	<10 min post-exercise	con	Lactate	2
HSP-70 mRNA	Fourfold	30 min treadmill, aerobic threshold	4 min to 3 h post-exercise	con/ecc	Lactate	8
VEGF mRNA	2.1-fold	45 min knee extensor	30 min post-exercise	con	Lactate	4
VEGF mRNA	7- to 17-fold	5 min warm-up, max. test (50 % max. work rate)	1 h post-exercise	con	Normoxia/P_{CO_2}	10
Late genes						
LPL, PDK4, HKII, UCP-3, HO-1 transcription	2- to 4-fold	4 h ergometer, 50–60 % $\dot{V}_{O_2max}$	1–2 h post-exercise	con		6
LPL, CPTI, HKII, GYS, PDK4, UCP-3, HO-1mRNA	2- to 5-fold	60–90 min ergometer, 70% 2 min maxr, fifth day of training	>1 h mRNA post-exercise	con		6
LPL mRNA	1.3-fold	60–90 min ergometer, 55–70 % peak oxygen, fifth day of training	4 h post-exercise	con		11
Chronic events						
CPT I mRNA	Twofold	60–90 min ergometer per day for 4 days, 70 % 2 min maxr		con		6
MCAD mRNA	1.5-fold	5× per week 30 min ergometer at 67 % relative W_{max}		con		12
PFK, HSP-70, COX4 mRNA	1.5- to 2.5-fold	5× per week 30 min ergometer at 67 % relative W_{max}		con	Lactate	12
VEGF mRNA	1.5-fold	5× per week 30 min ergometer at 58 % relative W_{max}		con	Lactate+P_{CO_2}	12
HIF-1α mRNA	1.8-fold	5× per week 30 min ergometer at 52 % relative W_{max}		con	P_{CO_2}	12
COX1, COX4, NADH6, 16S, SDH, fumarase mRNA	1.5- to 1.9-fold	Years of systematic endurance training				7

1, Aronson et al. (1997); 2, Aronson et al. (1998); 3, Boppart et al. (1999); 4, Gustafsson et al. (1999); 5, Heider et al. (1995); 6, Pilegaard et al. (2000); 7, Puntschart et al. (1995); 8, Puntschart et al. (1996); 9, Puntschart et al. (1998); 10, Richardson et al. (1999); 11, Seip et al. (1997); 12, Vogt et al. (2001); 13, Wojtaszewski et al. (2000); 14, Yu et al. (2001);

con, concentric exercise; ecc, eccentric exercise; W_{max}, maximal power; $\dot{V}_{O_2max}$, maximal rate of oxygen uptake; maxr, maximal resistance; P_{CO_2}, cellular oxygen partial pressure.

The transcriptional response of a variety of genes to a single bout of exercise is transient (Seip et al., 1997; Pilegaard et al., 2000). Depending on the time course of appearance of the maximal response to a single bout of exercise, these transcriptional events can be grouped into early and late responses, i.e. occurring within 0.1–1 h and within 1–4 h, respectively (see Table 2). Many of the genes induced early correspond to the classically defined immediate early genes involved in transcriptional and hormonal cell regulation. The later-responding genes mostly have particular functions in cellular metabolism. For example, 30 min of treadmill running and ergometer training above the aerobic threshold induces expression of the jun/fos family of transcription factors in human m. vastus lateralis (Puntschart et al., 1998; Aronson et al., 1998). Furthermore, 45 min of knee-extensor ergometer exercise increases the mRNA level of the angiogenic factor VEGF (Richardson et al., 1999; Gustafsson et al., 1999). Ergometer training of more than 60 min near the aerobic threshold induces expression of the metabolic genes LPL, CPTI, PDK4, GYS and UCP-3 in human m. vastus lateralis (Pilegaard et al., 2000; see Table 2).

An increase in muscle activity causes acute perturbations of the physical environment and the chemical constitution of skeletal muscle fibres, suggesting that metabolic (lactate, oxygen, disturbances in ATP turnover) and mechanical factors are the signals sensed and integrated into the transcriptional adaptations seen in exercised skeletal muscle. This concept is supported by the observation that increasing the contribution of metabolic and of mechanical factors to the physiological stimuli modulates the transcriptional response. For example, VEGF mRNA is induced concomitant with (but is not correlated to) reduced oxygen tension (Richardson et al., 1999). In addition, the VEGF response is reported to be proportional to the increase in plasma lactate level incurred during exercise (Gustafsson et al., 1999). The role of mechanical factors is best supported by animal model studies demonstrating that mechanical factors (stretch) modulate transcriptional induction of the c-jun and c-fos genes within 1 h (Dawes et al., 1996). *In situ* hybridization experiments with human m. vastus lateralis show an increase in levels of c-fos and c-jun mRNA after running exercise in a patchy expression pattern, indicating that factors other than metabolic products related to fibre recruitment contribute to increased c-fos gene transcription (Puntschart et al., 1998). Overall, these observations emphasize that metabolic and mechanical factors together influence the steady-state level of mRNA, possibly by modulating the rate of gene transcription as well as through pathways of RNA degradation.

The proposal that mechanical factors are involved in the regulation of the mRNA concentration in response to exercise training is supported by observations in mechanically stressed skeletal muscle. These observations indicate that mechanisms known to contribute to pre-transcriptional control are affected concomitantly with transcription of downstream target genes. In particular, exercise can induce all three limbs of the MAP-kinase pathways (ERK1/2, JNK and p38) in human m. vastus lateralis (Aronson et al., 1997, 1998; Yu et al., 2001; Boppart et al., 1999; Widegren et al., 2001). Activation of these kinases is known to affect transcription factors of the jun/fos and ATF/CREB family (Hunter and Karin, 1992), which bind to the promoter of many genes, e.g. c-jun, c-fos and the gene for cytochrome *c*, that are affected synchronously by exercise (Hood, 2001). *In situ* studies have demonstrated that mechanical factors, i.e. the degree of tension in rat muscle fibres, control JNK and ERK1/2 differently and directly (Martineau and Gardiner, 2001). In contrast, the p38 pathway in skeletal muscle is responsive only when mechanical stress (stretch, exercise) is applied *in vivo* (Boppart et al., 2001), indicating that p38 is presumably indirectly (possibly endocrinally) controlled by mechanical factors. Furthermore, activation of p70S6 kinase may relate to the indirect effects of mechanical or metabolic stimuli that are responsible for muscle hypertrophy by influencing translation factors (Nader and Esser, 2001). Mechanically induced release of growth factors (MGF, IL-6) has been recognized as another signalling route in exercised skeletal muscle and may represent an important link between contracting skeletal muscles and exercise-related metabolic changes (Goldspink, 1999; Pedersen et al., 2001). Activation of all these mechano-transduction events is potentially linked to mechano-sensation *via* integrins and associated kinases (Gordon et al., 2001; Carson and Wei, 2000; Chiquet and Flück, 2001).

It is not clear which factors sense and integrate the metabolic signals to skeletal muscle. Candidate factors that are potentially activated by metabolic factors such as reduced AMP, oxygen or fatty acids include 5′-AMP-activated protein kinase (AMPK), transcription factors such as hypoxia-inducible factor 1 alpha (HIF-1α) and peroxisome-proliferator-activated receptor-alpha (PPARα). Ergometer exercise has been demonstrated to activate α2-AMPK, but not the α1 form, in human m. vastus lateralis in an intensity-dependent manner (Wojtaszewski et al., 2000). The AMPK complex is involved in the regulation of skeletal muscle metabolism during exercise and was recently implicated in the control of transcription of the glucose transporter glut-4 in mouse skeletal muscle (Zheng et al., 2001). HIF-1α and PPARα are known to promote transcription of genes involved in carbohydrate metabolism, oxygen delivery and fatty acid oxidation through binding to specific promoter regions (Escher and Wahli, 2000; Semenza, 2001). Both these transcription factors are largely controlled by post-translational events.

In many tissues, reduced oxygen tension (tissue hypoxia) instantaneously stabilizes the normally degraded HIF-1α (Jewell et al., 2001) by a process involving reduced hydroxylation of particular residues (Wenger and Bauer, 2001). The resulting increase in HIF-1α levels promotes the transcription of VEGF and glycolytic genes (Semenza, 2001). The reduced oxygen tension in response to a single bout of exercise (Richardson et al., 1999) suggests that the concomitant increase in levels of mRNA for HIF-1α-dependent VEGF and glycolytic genes in exercised m. vastus lateralis (Pilegaard et al., 2000; Richardson et al., 1999) may

be brought about by a hypoxic stabilization of HIF-1α. Support for a sensitivity to exercise of HIF-1α in skeletal muscle is provided by the increase in HIF-1α mRNA levels in m. vastus lateralis in response to repeated bouts of exercise in hypoxia (Vogt et al., 2001). Similarly, the abundance of PPARα and the transcript levels of genes whose transcription is known to be regulated by PPARα were concomitantly increased in trained human m. vastus lateralis (Horowitz et al., 2000).

Unsaturated long-chain fatty acids which are released from adipose tissue during exercise serve as ligands for PPARα and stimulate PPARα-activated gene transcription (Kliewer et al., 1997). Recently, we observed an increase in PPARα mRNA levels in response to training in human m. tibialis anterior, indicating that this factor is also controlled by transcriptional events (B. Schmitt, J. Décombaz, M. Flück and H. Hoppeler, unpublished observations). There is evidence that contractile-exercise-induced transcription of mitochondrial transcription factor A (Tfam) and nuclear respiratory factor-1 (NRF-1) is involved in the coordinated expression of the nuclear and mitochondrial genomes and may be a link to mitochondrial biogenesis as a result of enhanced metabolic flux (Hood, 2001; Bengtsson et al., 2001). Thus, distinct protein kinases and transcription factors appear to be the interface that integrates mechanical (MAPKs and jun/fos) and metabolic (AMPK, HIF-1α and PPARα) stimuli into enhanced gene transcription.

The steady-state level of RNA is determined by the balance of gene transcription and RNA degradation (Booth and Thomason, 1991). Contractile activity modulates the level of factors interacting with the cytochrome *c* mRNA (Yan et al., 1996) in a region of the 3′ untranslated region (3′UTR) known to determine the degradation of mRNA (Sachs, 1993). These observations indicate that mRNA degradation is a mechanism effective in the control of transcript level in (human) skeletal muscle. The involvement of RNA degradation and chromatin structure (Felsenfeld et al., 1996), controlling the recruitment and assembly of transcription factors and polymerase complexes for individual gene promoter regions, in determining exercise-modulated mRNA levels in human skeletal muscle is not understood.

Changes in gene expression result in an incremental adaptation in protein level and activity determined by the spatial organization of the corresponding protein and its biological half-life. The transient nature of transcriptional adaptation following a single bout of exercise indicates that detectable distinct structural (and functional) adaptations may reflect the gradual accumulation of discrete post-transcriptional micro-adaptations of the corresponding protein (Vogt et al., 2001). Evidence for such a scenario comes from the increase in angiogenic VEGF mRNA level concomitant with an increased capillary-to-fibre ratio and the concomitant modulation of RNAs coding for proteins involved in oxidative phosphorylation (Puntschart et al., 1995). The increased steady-state levels of VEGF, CPTI and mitochondrially encoded RNAs (COX 1, NADH reductase subunit 6, 16S rRNA) and in nuclear-encoded RNAs (COX 4, SDH, fumarase) in trained human m. vastus lateralis (Vogt et al., 2001; Pilegaard et al., 2000; Puntschart et al., 1995) indicate that transcriptional changes are responsible for the typical structural changes observed in response to endurance-type exercise.

The distinct pattern of changes in the steady-state mRNA level of genes involved in carbohydrate and fatty acid metabolism in response to exercise training demonstrates that expressional changes are specific for the pattern of physiological stimuli applied. For example, increases in mRNA levels for medium-chain acyl-CoA dehydrogenase, which is involved in oxidative metabolism, in m. vastus lateralis are seen only in response to exercise at low intensity (Vogt et al., 2001), when oxidation of fatty acids predominates over that of carbohydrates (Brooks and Mercier, 1994). In contrast, phosphofructokinase mRNA levels were found to increase only in response to high-intensity training, while HIF-1α mRNA levels were found to increase under conditions of hypoxia (Vogt et al., 2001). Furthermore, the differences in the response of VEGF and CPTI mRNA levels in m. vastus lateralis to a single bout of training indicate that endurance training modulates the sensitivity of expressional responses to exercise stimuli (Richardson et al., 1999; Pilegaard et al., 2000). A main issue for future research is to elucidate the extent to which the specific structural and functional adaptations of skeletal muscle in response to defined external stimuli, i.e. the combination of mechanical and metabolic stimuli, is due to specific modifications in the gene profile.

In conclusion, the present data support and complement previous reports on the scaling of the structural variables of skeletal muscle tissue with body mass. Muscle mass and myofibrillar volume are found to represent constant fractions of body mass, while structures related to the oxidative metabolic capacity of muscle tissue have scaling factors smaller than unity (ranging from 0.86 to 0.95). These values, however, seem to be consistently larger than the scaling factor of 0.75 proposed from general considerations related to the design of fractal networks of connectivity such as circulatory systems. In the context of the basic design properties of skeletal muscle, malleability is considered to be an important feature of the 'economic design' of muscle tissue. Future research will therefore concentrate on the molecular basis of the phenotypic plasticity of skeletal muscle tissue. There is evidence that transient transcriptional regulations after exercise perturbations occur in response to repetitive stimuli and eventually lead to specific modifications of the transcriptome and eventually of the proteome. We propose that muscle tissue can sense and respond to both mechanical and metabolic disturbances, with the two stimuli acting through distinct signalling pathways.

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