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Review

Skeletal muscle fibre plasticity in response to selected environmental and physiological stimuli

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Summary. Skeletal muscle constitutes a highly adaptable and malleable tissue that responds to environmental and physiological challenges by changing its phenotype in terms of size and composition, outcomes that are brought about by changes in gene expression, biochemical and metabolic properties. Both the short- and long-term effects of nutritional alterations on skeletal muscle homeostasis have been defined as the object of intensive research over the last thirty years. This review focuses predominantly on assimilating our understanding of the changes in muscle fibre phenotype and functional properties induced by either food restriction or alternatively existing on a high fat diet. Firstly, food restriction has been shown in a number of studies to decrease the myofibre cross sectional area and consistently, it has been found that glycolytic type IIB fibres are more prone to atrophy than oxidative fibres. Secondly, in rodents, a high fat diet has been shown to induce an oxidative profile in skeletal muscle, although obese humans usually show higher numbers of glycolytic type IIB fibres. Moreover, attention is paid to the effect of prenatal maternal food restriction on muscle development of the offspring in various species. A key point related to these experiments is the timing of food restriction for the mother. Furthermore, we explore extensively the seemingly species-specific response to maternal malnutrition. Finally, key signalling molecules that play a pivotal role in energy metabolism, fibre type transitions and muscle hypertrophy are discussed in detail.

Key words: Food restriction, AMPK, PPARγ, Calcineurin, Diet, Malnutrition

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Introduction

Skeletal muscle is the most abundant human tissue comprising almost 50% of the total body mass, exhibiting major metabolic activity by contributing up to 40% of the resting metabolic rate in adults and serving as the largest body protein pool. It is a highly adaptable tissue, responding to numerous environmental and physiological challenges (e.g. mechanical loading-induced contractile activity, nutrient availability, disuse and inactivity) by changing its phenotypic profile in terms of size as well as its composition (e.g. (in)activation of the protein synthetic machinery, alteration of myofibrilar protein profiles), outcomes that are brought about by changes in gene expression (e.g. changing the transcription of structural genes), biochemical (e.g. activation of various signalling pathways that lead to enhanced activation of ribosomal machinery and changes in protein synthesis) and metabolic properties (Pette and Staron, 2001; Spangenburg et al., 2008). Skeletal muscle mass

Abbreviations. AICAR, 5-amino-4-imidazolecarboxamide riboside; a.l., ad libitum; AMPK, AMP-activated kinase; Cn, calcineurin; CPT, palmitoyl-CoA acyl transferase; CsA, cyclosporin A; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; EDL, extensor digitorum longus muscle; eEF-2, eukaryotic elongation factor 2; GLUT-4, glucose transporter-4; FOXO, forkhead box O; F.R, food restriction; GSK-3ß, glycogen synthase kinase 3ß; IGF, insulin-like growth factor; IRS-1, insulin receptor substrate 1; MEF2, myocyte enhancer factor-2; MHC, myosin heavy chain; mSin1, mitogen-activated protein kinase-associated protein 1; MCIP-2, modulatory calcineurin inhibitor protein isoform 2; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T cells; NRF-1/2, nuclear respiratory factors 1 and 2; PGC-1·, peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α; PI3K, phosphatidylinositol-3 kinase; PKB, protein kinase B also known as Akt; PPARδ, peroxisome proliferator-activated receptorδ; p70S6K1, 70-kD S6 protein kinase 1; RM, repetition maximum; TA, tibialis anterior; TAK1, TGF-ß-activated protein kinase-1; Tfam, mitochondrial transcription factor A; TSC2, tuberous sclerosis complex 2; VO2, oxygen consumption.

acquisition, maintenance or loss is essentially the outcome of a dynamic balance between the two regulatory opposing processes; skeletal muscle protein synthesis and protein degradation (Nader, 2005).

Skeletal muscle development and fibre patterning

Skeletal muscle of the body is generated in discrete phases during gestation in vertebrates starting with the formation of lineage committed muscle precursors that originate from the somites. These cells differentiate into myoblasts and can undergo proliferation and fusion to form multinucleated muscle fibres under the coordinated action of regulatory molecules (e.g. positive and negative growth factors, Stewart and Rittweger, 2006). Myofibre development occurs in two temporal phases, the first phase forming primary fibres, followed by the development of secondary fibres (Fig. 1). Primary myofibres tend to be the largest of the two types (Mallinson et al., 2007). During murine prenatal and early postnatal life the developmental myosin heavy chain (MHC) isoforms (embryonic and neonatal) account for >95% of the total MHC expression, whereas by completion of the weaning period (21 days post-partum) of the offspring, embryonic and neonatal MHC expression is markedly decreased as a percentage of the total MHC and the vast majority of hindlimb muscle fibres express adult fast isoforms (IIa, IIx and IIb) with only a minor proportion continuing to express the adult slow isoform (I, Allen and Leinwand, 2001).

Muscle fibre type plasticity

Changes in nutrition level as well as exercise affect features other than the mass of the tissue alone. A striking feature of the skeletal muscle fibre is its ability to transform and remodel in response to various environmental and physiological demands. This response takes place by means of signal transduction where an extracellular stimulus interacts with receptors at the cell surface and activates signalling pathways which ultimately remodel the fibre by eliciting changes in gene expression. To date, the most widely used scheme to delineate fibre types is based on specific myosin profiles (Pette and Staron, 2001). Classification of the muscle fibre as slow or fast has traditionally been related to the type of expressed myosin (a structural and functional protein in the muscle sarcomere) and specifically myosin heavy chain, an integral component of native myosin (Schiaffino and Reggiani, 1996; Koulmann and Bigard, 2006). Accordingly, slow muscles express mainly the slow isoform of MHC type I (e.g. postural muscles with low maximal shortening velocity and resistance to fatigue). Fast human muscles (e.g. foot extensor muscles) mainly express the fast MHC isoforms type IIa and IIx (the IIb isoform is found in rodent but not human fast muscles), exhibiting fast speed of contraction and low resistance to fatigue. Therefore before embarking on the main topic of this review, we give a brief summary of mechanisms that regulate MHC isoform transitions as these have been extensively used to explain changes in muscle phenotype following dietary manipulations.

Myosin heavy chain isoform expression

Several lines of evidence have currently established that myofibre phenotypic profiles are influenced by numerous parameters such as stretch and mechanical signals (e.g. (un)loading), altered neuromuscular activity, altered functional demands, hormonal profile (e.g. thyroid hormone, testosterone) and aging (Pette and Staron, 2001). A pre-programmed temporal sequence of myosin isoform expression in the order embryonic→neonatal→adult fast→adult slow in the rat soleus muscle was first described over twenty years ago. It appears that

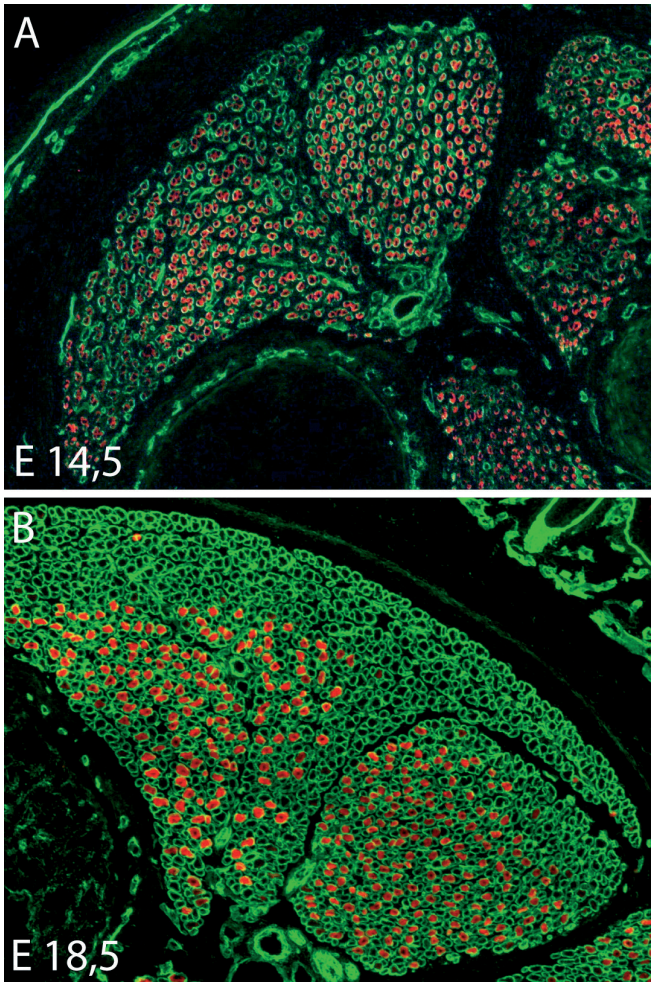

Fig. 1. Typical Immunohistochemical staining depicting the discrete phases of myogenesis: EDL and TA mouse (C57BL/6) primary myotubes (red) at E14,5 **(A)** and both primary (red) and secondary (black/negative staining) myotubes at E18,5 **(B)**. Cell membrane is shown in green.

the default pathway of muscle fibre differentiation during postnatal development and regeneration, which is controlled by thyroid hormone, is independent of neural activity and leads to the activation of a fast gene program. In contrast, the postnatal induction and maintenance of the slow gene program is dependent on slow motoneuron activity (Fig. 2; Butler-Browne and Whalen, 1984; Schiaffino et al., 2007).

Collectively, an increase in neuromuscular activity results in a fast-to-slow transition and conversely, a lack of activity induced through denervation, hindlimb suspension or the absence of gravity, induce a slow-to-fast transition (Pette and Staron, 2000). Cross-reinnervation by a slow nerve converts a previously fast muscle to a slow phenotype and slow muscles convert to a fast phenotype when reinnervated by a fast nerve, mainly due to specific neural impulse patterns delivered to the muscle (Pette and Staron, 2001). Moreover, accumulating data indicate that different forms of contractile activity (e.g. endurance versus resistance exercise and low frequency versus high frequency nerve stimulation respectively) induce different types of fibre phenotype adaptive responses. However, with regard to systematic endurance training, only a limited myofibre transition occurs towards slow-twitch type I fibres (in a species and dose-dependent manner) but to a less extent than the application of low frequency nerve stimulation (Pette and Staron, 2001; Koulmann and Bigard, 2006). Skeletal muscle remains undifferentiated in the early postnatal period regarding the adult MHC isoforms. For instance, fast MHC isoforms in rodents are not expressed until the age of 4-5 weeks (Butler-Browne and Whalen, 1984; Butler-Browne et al., 1984; Adams et al., 1999) and their appearance occurs through the progressive replacement of embryonic and neonatal MHC isoforms with different proportions of the adult isoforms in a muscle type dependent manner. It is believed that primary myogenesis gives rise to fibres that will go on to express slow-twitch MHC isoforms and in contrast, secondary myogenesis gives rise to fast fibres (Butler-Browne and Whalen, 1984). According to Buttler-Browne and Whalen embryonic MHC may postnatally transit to either neonatal or adult slow MHC, the neonatal MHC may further give rise to either slow or fast MHC and finally the fast isoform of MHC may transit to slow MHC. Hence, it appears that the expression of type II MHC isoforms in both slow- and fast-twitch muscles is partly related to the downregulation of the neonatal isoform (Fig. 3).

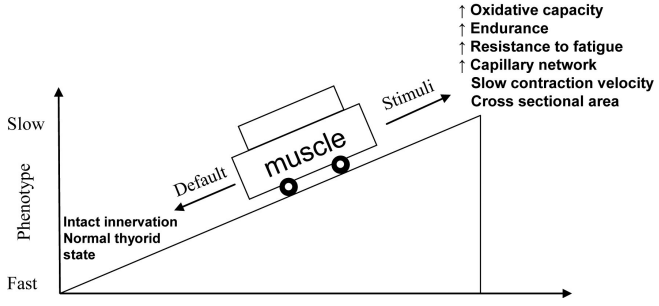

Fig. 2. Muscle fibre conversion in response to environmental stimuli. The default program of muscle fibre type development under the existence of active innervation and normal thyroid hormones status seems to be the generation of fast fibres. Contractile activity i.e. low frequency nerve stimulation favours a shift toward a slower muscle fibre phenotype.

Influence of innervation and thyroid hormone on myofibre patterning

A prerequisite for the optimal development of fast-twitch muscles is the presence of intact innervation and a normal thyroid state (Adams et al., 1999). Overall, a hypothyroid state promotes fast-to-slow fibre transitions, while a hyperthyroid state elicits slow-to-fast transitions (reviewed by Pette and Staron, 2001). Both an intact innervation and other intrinsic (e.g. hormonal: thyroid hormone) and environmental (e.g. weight-bearing activity) factors significantly contribute to the transition from neonatal to adult MHC phenotype expression postnatally, although primary myofibre development during the embryonic state and the embryonic to neonatal to adult fast transitions do not necessarily require the presence of innervation (Butler-Browne and Whalen, 1984; Esser et al., 1993; Adams et al., 1999).

However, an intact nerve is required postnatally for optimal myofibre differentiation from a developmental to adult MHC phenotype. Moreover, the type of nerve which innervates the muscle fibre determines the myosin content in adult muscles (Butler-Browne and Whalen, 1984). Furthermore, the full differentiation of slow-twitch muscles used in postural/antigravity function (e.g. soleus) precedes the differentiation of fast-twitch muscles mainly used for high power output activities (e.g. plantaris). Absence of intact innervation (e.g. studied in a denervation model) postnatally is followed by retention of neonatal isoforms, reduced expression of slow type I MHC but increased expression of fast IIx MHC in soleus muscle (Adams et al., 1999). According to these authors, dennervation of a fast muscle (plantaris) during a critical stage of development results in a reduced expression of the fast type IIb MHC isoforms and an increased expression towards both

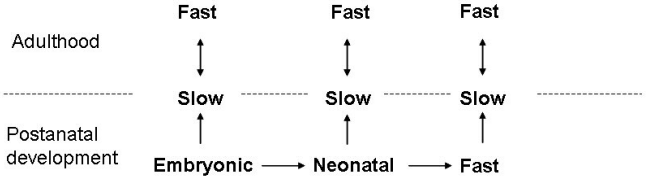

Fig. 3. Overview of the myosin isotype transitions during both postanatal develpoment and adulthood. Modified from Buttler-Browne and Whalen, (1984).

slower MHC isoforms and neonatal MHC, usually found in young neonatal muscles. Conversely, abnormal thyroid state (e.g. studied in a hypothyroid model) blunts the upregulation of the fast IIx IIb MHCs in fast muscles (e.g. plantaris) and is followed by reduced expression of IIa and enhanced type I MHC in slow muscles, leading to an even slower MHC phenotype (e.g. soleus, Adams et al., 1999). The combined effects of both denervation and hypothyroidism maintain both slow and fast muscles in a partially undeffierentiated state of MHC expression compared to the normal adult pattern. Thus, a prerequisite for the optimal expression of the fastest MHC isoform IIb as well as the transition from the neonatal condition to the adult phenotype is the presence of both intact innervation and a normal thyroid state (Adams et al., 1999).

Fibre type transformation through exercise

MHC composition of skeletal muscle is readily altered by physical exercise. Indeed possibly the most significant advances in our understanding of the mechanisms that control fibre type transformation have been made by studying the response of the tissue to differing forms of exercise. These mechanisms are now being examined in detail following changes to the diet. Therefore, we firstly summarise the influence of exercise on MHC expression. Studies in humans have shown that resistance and endurance exercise elicit an increase in MHC IIa composition and a corresponding decrease in MHC IId/x composition (Scott et al., 2001). Exercise training induces changes in skeletal muscle by increasing the myofibre oxidative metabolism through inducing a fibre-type transition from a faster to a slower phenotype according to the scheme: type IIb→IIx/b→IIx→IIa/x→IIa→I/IIa→I (Pette, 2002). Intermediate fibre types, known as hybrid fibres composed of two or more myosin heavy chain isoforms are also present in most skeletal muscles both during steady-state conditions and during fibre transformation, hence giving rise to a continuous spectrum of fibre types (e.g. Pette and Staron, 2001). Importantly, velocity of shortening is dictated by myosin heavy chain isoforms, whereas resistance to fatigue is related to oxidative enzyme content (Schiaffino and Reggiani, 1996). Hence, type I fibres show low shortening velocity and high resistance to fatigue, and type IIB fibres show high shortening velocity and low resistance to fatigue. Muscle fibre recruitment is type specific and depends on the intensity of muscle contraction. Thus, at low intensities the slow-twitch type I fibres are recruited first, while larger motor units and type II fibres respond at higher intensities, leading to increases in fibre cross sectional area and enhancements in force-generating capacity. For this reason, it has been proposed that resistance exercise should be performed at a higher loading than 70% of one repetition maximum (1RM), in order to achieve substantial increases in muscle mass (Kraemer et al., 2002). Alternatively, similar increases in strength production and muscle mass have been observed when applying a low-intensity (20% 1RM) resistance exercise load combined with a simultaneous blood flow restriction on the working musculature. The similar outcome in terms of muscle mass increase following either high resistance training or the modified low intensity regime can be reconciled since both cause an acute activation of the mammalian target of rapamycin (mTOR) signalling pathway (which promotes protein synthesis) as well as comparable hormonal production (Fujita et al., 2007).

Key signalling molecules affecting muscle phenotypic profile

A key molecule regulating skeletal muscle hypertrophy and protein synthesis is Akt1 (for review see e.g. Nader, 2005; Matsakas and Patel, 2009). It functions to prevent muscle atrophy by inhibiting the activity of forkhead box O members (FOXOs; FOXO3a plays a key role in the progression of atrophy), which in turn control the expression of members of the ubiquitin proteosome system that targets proteins for degradation. Alternatively, a decrease in the activity of signalling molecules affecting protein synthesis has been reported, based on Akt1-phosphorylation inhibition in response to a myotube atrophy model in vitro (Sandri et al., 2004; Nader, 2005). Furthermore, constitutively active Akt has been reported to be sufficient to inactivate FOXOs and reduce the expression of genes involved in the progression of muscle atrophy (e.g. atrogin-1).

Recent data have provided highly interesting novel insights relating Akt1 signalling, skeletal muscle hypertrophy and systemic metabolic regulation. Evidence has been provided that constitutive activation of Akt1 signalling in mouse muscle promotes fast type IIB muscle fibre hypertrophy in vivo, increased strength and triggered a remarkable decrease in body mass as a result of reduced visceral fat mass and white adipocyte atrophy (Izumiya et al., 2008). The reduction in adipose mass was partly attributed to an increased metabolic rate and a greater utilization of fatty acids as a fuel source. In addition, Akt1 transgene induction was shown to block diet-induced obesity and insulin-resistance. Furthermore, Akt1-mediated muscle hypertrophy compensated for the excess of adipose tissue by normalizing metabolic parameters in high fat diet-induced obese mice, suggesting that muscle growth counteracts the metabolic consequences of excess adiposity. Favourable effects of the transgene-induced glycolytic muscle growth were further manifested in the liver, as judged by the increased rates of fatty acid utilization in this tissue and a reversion of the deleterious effects of high fat diet on both liver gene expression and hepatic morphology (Izumiya et al., 2008). These results highlight a novel role for skeletal muscle as an endocrine organ, since transgene expression was confined to muscle, but the outcome resulted in modifications of the liver. Although these findings elegantly highlight the interplay between

energy supply, metabolism and muscle morphology, thus providing the molecular basis for clinical interventions, these results require further investigation. A number of questions arise from this study that need to be addressed i) it is not clear why the transgene was only expressed in some (e.g. gastrocnemius) but not other muscles (e.g. soleus and EDL), ii) despite a higher VO_2 the transgenic animals exhibited impaired endurance exercise capacity (e.g. reduced time and running distance), a decrease in expression of oxidative genes but an upregulation of glycolytic genes and iii) despite a low respiratory quotient indicating the use of fatty acids as an energy source the transgenic animals showed increased glucose uptake and lactate serum levels.

The role of systematic physical activity (i.e. exercise training) has been beautifully highlighted in the literature as an effective therapeutic intervention for enhancing insulin sensitivity and action in skeletal muscle (for review see e.g. Hawley and Lessard, 2008). Muscle contraction provides the unique natural option of stimulating skeletal muscle glucose uptake via insulin-independent mechanisms (e.g. increased GLUT-4 trafficking, activation of the AMPK pathway, Akt phosphorylation, lipid oxidation and calcium-mediated mechanisms). These would not only bypass any insulin signalling defects found in the insulin-resistant state, but also improve both the systemic and muscle insulin sensitivity (e.g. restoration of insulin receptor/PI3K signalling in the insulin-resistant muscle, Hawley and Lessard, 2008).

Nutritional aspects and skeletal muscle fibre type composition

The effect of nutritional status on muscle fibre phenotypes has been investigated for several decades but without ever being fully understood. This part of the review aims to provide an overview of studies which examined the effects of either nutritional restriction or high fat diets on skeletal muscle phenotype, bearing in mind that muscle fibres are capable of adapting their metabolism, in order to optimise protein turnover (see Table 1). The majority of the studies that address the effect of food restriction on muscle growth provide evidence that long-term malnutrition in mammals results in a decrease in both muscle mass and fibre cross sectional area (Table 1). However there is some controversy regarding its effect on muscle fibre type transitions. Conflicting reports showing either an upregulation of a slower oxidative fibre phenotype (e.g. fibre type IIA and I) as well as an increase in the numbers of fast glycolytic IIB fibres have been reported so far.

Skeletal muscle represents the major tissue of insulin-stimulated glucose uptake and storage, playing a crucial role in whole-body energy metabolism. Impaired glucose homeostasis is found in patients with non-insulin dependent diabetes mellitus, a defect that can in part be attributable to abnormalities of glucose transport in skeletal muscle. There is a clear link between diet and the development of type II diabetes and recent studies indicated that in some ethnic populations, obesity can increase the chances of developing type II diabetes by a factor of 10 (Nestel et al., 2007). Recent work has begun to establish a molecular link between diet and elevated glucose level and crucial to this is the glucose homeostatic function of skeletal muscle. For example, it has been shown that a hormone called resistin which is secreted by adipocytes and whose expression is increased by a high fat diet leads to a blunted response of skeletal muscle to take up glucose in response to insulin (Steppan et al., 2001; Pravenec et al., 2003).

High fat diet

It is known that a healthy skeletal muscle can easily switch between glucose and fat oxidation and promotes the oxidation of fat as source for energy which would favour resistance to body fat accumulation. Recent data suggest that a high fat diet changes the mouse skeletal muscle transcriptome and elicits rapid morphological alterations by increasing levels of slow MHC type I protein in the quadriceps muscle. This transformation may be brought about by peroxisome proliferator-activated receptor 1 coactivator alpha (PGC-1α), an important regulator of muscle fibre type determination, mitochondrial biogenesis, adaptive thermogenesis and fatty acid oxidation (de Lange et al., 2007; de Wilde et al., 2007). It is now well recognised that PGC-1α induces a remodelling of skeletal muscle fibre composition towards oxidative slow twitch type I fibres. On the other hand, food deprivation and exercise both exhibit a similar pattern of activation of the signalling pathways triggering metabolic adaptations toward lipid metabolism in skeletal muscle (de Lange et al., 2007). In particular, food deprivation causes a rapid transient upregulation of PGC-1α mRNA and nuclear protein as well as a sharp increase in MHCIb mRNA and protein levels, triggering a fibre shift toward the slow type I phenotype (de Lange et al., 2006) and reducing the level of oxidative stress within an aerobic organism (Sohal and Weindruch, 1996). Overall, fibre type switching involves signalling mediated by multiple factors such as the key transcription factors PCG-1α and peroxisome proliferator-activated receptor δ (PPARδ), the energy-sensing and fuel-providing role of AMPK, nitric oxide and mitochondrial fatty acid oxidation (reviewed by de Lange et al., 2007).

Several studies have established an association between skeletal muscle fibre type and the occurrence of obesity. Hence, muscle fibre type profile from both obese individuals and animals shows an increased proportion of fast twitch fibres and reduced percentage of slow type I fibres compared with lean counterparts (e.g. Krotkiewski and Bjorntorp, 1986; Abou Mrad et al., 1992; Kriketos et al., 1996; Tanner et al., 2002). On the contrary, recent findings from animal studies suggest that treatment of lean animals with high-fat diets is

Table 1. Overview of representative studies regarding the effect of dietary food restriction on skeletal muscle of various species.

Experimental intervention	Species	Muscle	Effects on muscle phenotype	Reference
Food restriction (F.R) for 3 months to achieve ~ 60% weight gain per day compared to ad libitum (a.l.)	cow	Semitendinosus (ST), biceps femoris, longissimus dorsi, triceps brachii	↑ oxidative enzyme activity, Muscle dependent effect of F.R on slow type I fibre abundance e.g. ↑ Slow fibres, ↔IIA, ↓ IIB in ST	Brandstetter et al., 1998
Food restriction (F.R) for 4 months to achieve ~ 50% weight gain per day compared to a.l.	cow	Semitendinosus	5 months post F.R: ↔Muscle mass, Body mass, ↓ Slow fibres, ↓ CSA Slow fibres, ↑ Fast IIB fibres, ↔CSA fast fibres, ↔ IIA fibres, 13 months post F.R: ↔Muscle mass or number or CSA, ↓ IIB and ↑ IIA fibres compared to a.l	Picard et al., 1995
Maternal undernutrition to 50% of a.l. before muscle differentiation	sheep	Vastus lateralis of the offspring at 2-weeks of age	↓ Fast fibres, ↑ Slow fibres, ↔Growth rate of pups	Fahey et al., 2005
Maternal undernutrition to 50% of a.l. during pregnancy	sheep	Semitendinosus muscle of the offspring at 17 or 24 weeks of age	↓ Muscle mass, ↑ Fast fibres, ↔ Slow fibres, ↔Growth rate of pups	Daniel et al., 2007
Maternal undernutrition to 50% of a.l. during pregnancy	sheep	Longisimus dorsi muscle of the offspring at 8 months of age	↓ body mass, ↔Muscle mass, ↓ Muscle fibre number, ↑ Fast IIB fibres at the expense of IIA ↑ Intramuscular triglycerides	Zhu et al., 2006
3-week food restriction to 60% of a.l.	mouse	Biceps bracchii, tibialis anterior	↓ Muscle mass, ↓ Fibre length, ↔Sarcomere length ↓ Sarcomere number per fibre, ↔Actin length ↔Myosin length	Hooper, 1984
4- to 6-week food restriction	mouse	Biceps brachii, soleus	↓ CSA with glycolytic fibres being more susceptible ↔total fibre number, ↔numbers of any fibre type	Goldspink and Ward, 1979
Maternal low-protein diet during pregnancy	rat	Soleus (S), gastrocnemius (G) of the offspring at 4-weeks of age	↓ Fast fibre number and density (S), ↓ Oxidative fibre density (G), ↔CSA	Mallinson et al., 2007
17- and 32-months of food restriction to ~ 40% of a.l.	rat	Vastus lateralis, rectus femoris, soleus	↓ muscle mass loss with age, ↓ muscle fibre number loss with age, ↓ % loss in CSA with age Retention of muscle fibres	McKiernan et al., 2004
13- to 15-month food restriction to ~ 35% and ~ 50% of a.l.	rat	Vastus lateralis	35%: ↓ fibre number, Signs of atrophy 50%: ↔ /attenuated decline of fibre number Less signs of atrophy	Lee et al., 1998
Severe maternal food restriction during gestation and lactation to ~ 70% of a.l.	rat	Soleus, lumbrical muscle	↓ body mass, ↓ Muscle fibre number	Wilson et al., 1988
Maternal undernutrition to 50% of a.l. during gestation and/or lactation (GR and GLR respectively).	rat	Psoas minor (PM), extensor digitorum longus (EDL), plantaris (P), gastrocnemius (G), biceps brachii (BB) and soleus (S)	maternal tissue : ↓ body mass, ↓ fat pads, internal organ and muscle mass, ↓ CSA, ↔fibre number (plantaris), offspring tissue : ↓ birth weight (GRL), ↓ muscle mass (PM, P, G, S), muscle mass (EDL, BB)	Glore and Layman, 1985
Long-term (varying fractions of the life span) food restriction to ~ 60% of a.l.	rat	lateral omohyoideus, soleus	Absence of striking functional changes with only minor changes in fibre composition	McCarter and McGee, 1987
6- to 36-months of food restriction to ~ 40% of a.l.	rat	lateral omohyoideus	↔Muscle mass, ↔Muscle fibre number	McCarter et al., 1982
13- and 15-months of food restriction to ~ 50% of a.l.	rat	Vastus lateralis	↓ Muscle fibre loss, ↓ Muscle fibre type changes	Aspnes et al., 1997
Food restriction for 3 to 149 weeks to ~ 50% of a.l.	rat	Soleus, extensor digitorum longus, flexor digitorum profundus	↓ Body mass, ↓ Muscle mass, ↓ Muscle fibre number, Delayed aging-induced muscle denaturing effects	Boreham et al., 1988
Severe food restriction for 4 weeks to ~ 67% of a.l.	rat	Diaphragm (D), gastrocnemius	↓ Body mass, ↔Muscle fibre type composition ↓ CSA, ↑ atrophic responses in fast vs. slow fibres ↑ Fatigue resistance (D)	Sieck et al., 1989
Food restriction for 9 to 24 months to ~ 30-35% of a.l.	rat	Soleus, extensor digitorum longus	9 mo: ↓ Body mass, ↓ Muscle mass, ↔Muscle fibre number, 24 mo: ↔Body mass, ↔Muscle mass, ↔Muscle fibre number	Daw et al., 1988
Chronic (5.5 months) food restriction to ~ 40% of a.l.	rat	Extensor digitorum longus (EDL), plantaris (P) and soleus (S)	↓ Body mass, ↓ Muscle mass, ↔CSA, ↔Muscle fibre type composition	Maxwell et al., 1992

↑ denotes increase, ↓ denotes decrease, ↔denotes not significant change, a.l. denotes *ad libitum* food intake.

followed by muscle fibre alterations in favour of oxidative metabolism including increased mitochondrial biogenesis, increased fatty acid oxidative capacity, increased mitochondrial respiratory chain subunit protein levels as well as increased proportions of type I fibres in skeletal muscle, which are known to be rich in mitochondria with a high oxidative capacity for carbohydrate and lipid fuel as well as greater insulin sensitivity (de Wilde et al., 2007; Turner et al., 2007). In addition, a positive correlation has been observed between the relative amount of slow type I fibres and the change of body mass index in obese subjects who undergo a weight loss intervention (Tanner et al., 2002). Taken together, these apparently opposing findings may be the sequential outcome of a complex mechanism that regulates excess of fat availability. Thus, it is possible that the initial cellular response to a high fat diet is the transcriptional activation of genes that regulate mitochondrial biogenesis (e.g. PPARs, since fatty acid are natural ligands for these receptors), subsequent increase in mitochondria and in the capacity of muscle to oxidise fat (Hancock et al., 2008). However, aberrantly high nutritional fat availability in the long-term phase is followed by increased adipocyte size concomitant with reduced muscle use of glucose and development of muscle insulin resistance, and altered adipocytokine secretion (Ronti et al., 2006). For instance, increased circulating levels of the adipocytokine resistin in obese rodents accounts for insulin resistance and contributes to impaired insulin sensitivity (Ronti et al., 2006). It has been recognised that increased fatty acid turnover interferes with intracellular metabolism of glucose in the muscle and these acids exert lipotoxic effects on pancreatic beta-cells (Keller, 2006). It appears that the functional capacity of the cell to cope with such a situation of excessive fat is limited and the occurrence of fast twitch insulin resistant muscle fibres is increased in a less clear manner so far. Since the role of adipocytokines like resistin is less clear in humans further research is needed, in order to establish novel roles of adipose tissue secreted substances in cellular metabolism.

Malnutrition

Caloric restriction influences numerous molecular, biochemical and physiological interactions that alter the aging process of skeletal muscle and is related with increased average life span in several species, probably due to impaired oxidative stress by means of altered mitochondrial metabolism (Aspnes et al., 1997; McKiernan et al., 2004). Independent of the effect of food restriction on muscle fibre type transitions, there is a general consensus that its principal effect is a reduction in fibre size i.e. cross sectional area, with fibres IIB being more vulnerable to atrophic reactions (Fig. 4, Brandstetter et al., 1998). The aging process is associated with several structural and functional alterations in skeletal muscle including decreased muscle mass, fibre number and cross sectional area. Caloric restriction attenuated but did not fully prevent the progression of sarcopenia by reducing the degree of skeletal muscle fibre loss with age in rat (McKiernan et al., 2004). Indeed, caloric restriction is known to retard age-associated fibre loss and fibre type changes, reduce the accumulation of mitochondrial DNA deletions and the incidence of mitochondrial enzyme abnormalities as well as to delay the onset of age-related fibre atrophic responses in certain rodent skeletal muscles (Aspnes et al., 1997; Lee et al., 1998). An interesting finding derived from the previously-mentioned study is the outcome of different results after the application of two nutritional restriction levels corresponding to a 35% and a 50% caloric reduction compared to the ad libitum-fed rats. Although a 35% caloric restriction resulted in a significant reduction of the muscle fibre number and a marked decrease in type I muscle fibres in vastus lateralis, a 50% caloric reduction was not accompanied by any changes in muscle fibre number, with similar values exhibited to the ad libitum fed animals and only a slight decline in type I fibres was seen when compared to the ad libitum-fed animals, that however had significantly more type I fibres than the 35% restricted group (Aspnes et al., 1997). The observation that the 50% caloric restriction preserved the fibre number and fibre type composition as opposed to the 35% reduction in calories is a paradoxical finding, which has not been initially adequately discussed. Hence, it is of interest that despite a lower caloric intake in the animals fed with 50% less calories compared to the 35% caloric restriction, their diet was in fact enriched in protein, vitamins, and minerals contents, which may serve as a probable explanation for the observed differential results in the study, although severe dietary protein restriction has been shown to reduce skeletal muscle growth rate without affecting fibre number in the weanling rat (Timson and Dudenhoeffer, 1985).

Although it has been shown that food restriction may affect the morphological characteristics of skeletal muscle of various animals, studies often provide contradictory results depending on various parameters such as species individual, muscle considered, age of the animal and whether food restriction was studied on the basis of similar age or similar body mass (see Table 1). Hence there are data supporting muscle fibre size changes in pig and cow and enhanced oxidative fibre percentages in cow, lamb and pig in response to food restriction (discussed by Gondret et al., 2000). On the other hand, Gondret et al. (2000) reported that moderate food deprivation (30% less) resulted in a significantly impaired growth rate in rabbits in the absence of changes in either muscle fibre cross sectional area or fibre type in muscles with different muscle fibre composition. Moreover, caloric restriction did not alter the change in slow-twitch type I fibre number in vastus lateralis or rectus femoris muscles, which exhibit increased type I fibre occurrence with age (Mckiernan et al., 2004).

The development of muscle in prenatal mammals is

strongly influenced by the nutrients ingested by the mother, and is a subject that has received much attention in a number of model systems (rat, mouse, lamb and pig). Primary myofibres tend to be larger than secondary fibres (Mallinson et al., 2007). While the effect of experimental manipulations on primary myotube formation is minimal, secondary myotube numbers are much more sensitive to environmental challenges and stimuli (e.g. severe maternal food restriction during gestation, Wilson et al., 1988). Hence, primary myotube numbers of the offspring lumbrical muscle were found to be unaffected in contrast however, secondary myotubes were reduced in number significantly, following a regime of severe maternal undernutrition during

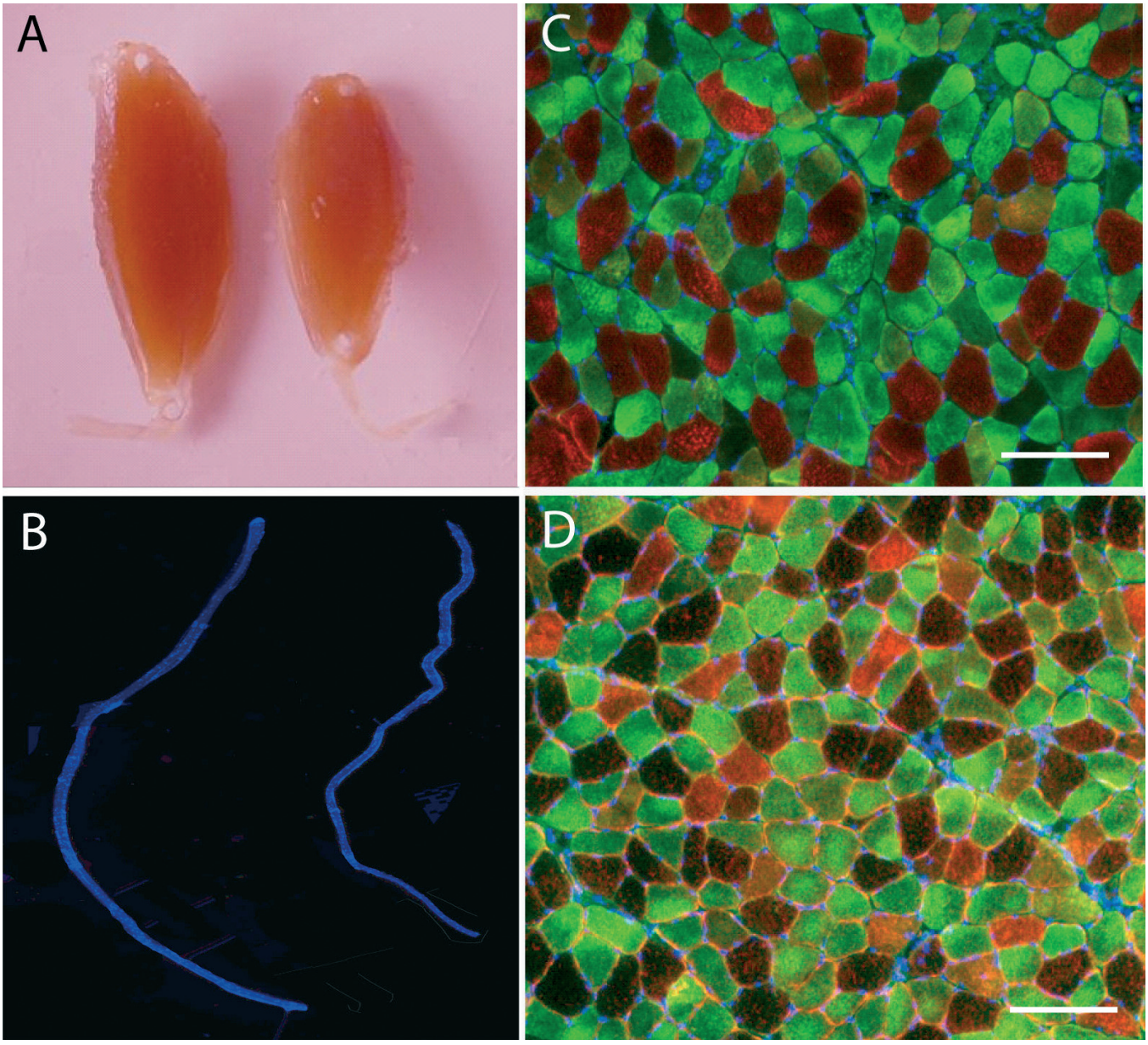

Fig. 4. Effect of dietary restriction on rodent muscle phenotype. **A.** Tibialis anterior muscle from a male 4 month old wild type C57BL/6 mouse (left) and from a another one of similar age, strain and sex subjected to a 4-week 40% food restriction of the ad libitum consumption (right). **B.** Tibialis anterior single muscle fibres from an ad libitum fed (left) and a food restricted mouse (right). Identification of different muscle fibre types by means of immunohistochemical staining for MHC type IIa (A4.74, green) and type I (A4.840, red) in the soleus muscle of a food restricted mouse **(C)** and from an ad libitum fed mouse **(D)**. Unstained fibres (black) depict MHC IIx/IIb isotypes (C, D). Scale Bars: C, D, 100 μm

gestation (Wilson et al., 1988). Many authors agree that there seems to be a critical period during myogenesis, coinciding with the period of secondary myotube formation, where if conformed with prolonged maternal starvation or even if maternal undernutrition precedes the muscle fibre formation period, it will then induce permanent irreversible alterations of muscle growth in the offspring (e.g. Wilson et al., 1988; Fahey et al., 2005).

Maternal nutrient restriction during pregnancy has been used as a model to study skeletal muscle formation and development in vertebrates (e.g. Daniel et al., 2007). For instance, Daniel et al. (2007) examined the effect of 50% dietary restriction during pregnancy on both skeletal muscle growth and composition of the lamb. These authors reported that although the maternal nutritional restriction did not affect lamb birth weight, there was retardation in the growing rate of the offspring lambs. Indeed, the final slaughter weight of lambs in the 24th week of age derived from restricted ewes was lighter than those from control animals, showing simultaneously lower skeletal muscle weights as well as internal organ weights such as liver, heart and lungs. Interestingly, analysis of the muscle fibre composition of semitendinosus and vastus lateralis muscles revealed that maternal dietary restriction resulted in an increased number of fast fibres (IIA and/or IIB) with no effects on slow fibre number in the offspring. On the contrary, studies in both sheep and pig having employed restricted maternal nutrition during early gestation have reported reduced fast fibre numbers in vastus lateralis muscle of the offspring and increased oxidative capacity (Bee, 2004; Fahey et al., 2005; Zhu et al., 2006; Daniel et al., 2007).

However, prenatal nutritional stress does not seem to equally affect all muscles because of their differential developmental rates during gestation (Daniel et al., 2007). These effects have been characterised as short-term effects on muscle development which may be reversed and compensated in adulthood dependent on the body mass and age of the offspring (Daniel et al., 2007). Overall, large muscles composed mainly of fast type IIA and IIB fibres are more susceptible to atrophy and fibre loss than postural muscles composed mainly of slow type I fibres, since fast glycolytic fibres have been shown to be more susceptible to undernutrition, exhibiting greater levels of atrophic responses (Fig. 5, e.g. Goldspink and Ward, 1979; McKiernan et al., 2004).

Attention has been paid recently to the molecular events that take place during early postnatal nutritional changes because of their chronic effects on glucose homeostasis in adulthood. A pivotal role has been attributed to the mTOR pathway, which has been considered as a chronic modulator of insulin-mediated glucose metabolism, serving both as a nutrient sensor and a crucial checkpoint control for integrating growth factor signalling and cellular metabolism (Khamzina et al., 2005; Tremblay et al., 2005). Recent data indicates that undernutrition during early life impairs insulin sensitivity in adult rats by affecting critical steps of insulin signalling in adipose tissue. Interestingly, maternal undernutrition was followed by activated Akt/mTOR signalling in adipocytes, implying a potential increase of glucose uptake from these cells (Garcia-Souza et al., 2008). Similarly, markedly increased activation of mTOR and its downstream target 70-kD S6 protein kinase 1 (p70S6K1) has been observed in both liver and skeletal muscle of high fat-fed obese rats, even when assessed in the fasting state. However, mTOR/p70S6K1 overactivation was found to contribute

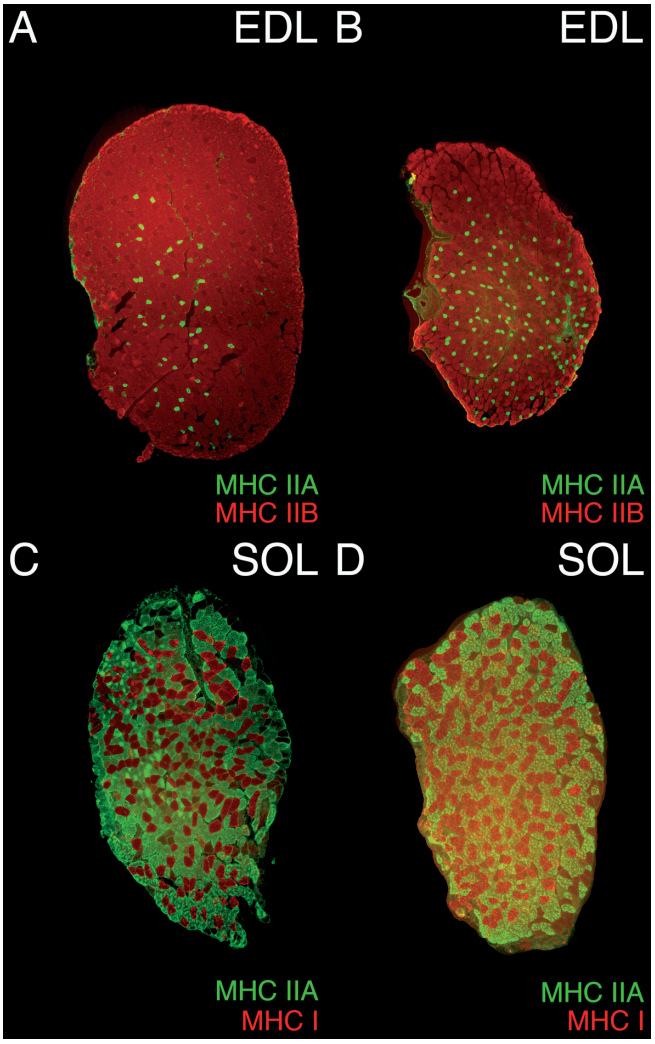

Fig. 5. Representative immunohistochemical staining of MHC isoforms from EDL and soleus of ad libitum fed (**A and C**, respectively) and food restricted (**B and D**) C57BL/6 mice. Note that the effect of food restriction on skeletal muscle mass is more prominent in the EDL (phasic muscle) rather than the soleus (tonically active muscle). The energy deprived EDL shows a higher abundance of IIA fibres than the ad libitum fed mouse in the expense of the IIX/IIB fibres, aiming to a more efficient energy utilization in a period of fuel poverty.

to elevated serine (Ser636/Ser639) phosphorylation of insulin receptor substrate 1 (IRS-1, which is considered as a molecular hallmark of insulin resistance), thus leading to impaired insulin signalling to Akt in liver and muscle (Khamzina et al., 2005). In support of this, insulin's ability to increase Akt phosphorylation was blunted in the liver of obese rats as opposed to those fed control diets.

Percent body fat has been inversely correlated to the proportion of slow type I muscle fibres in vastus lateralis from humans (Helge et al., 1999). The rationale behind this is that an augmented proportion of slow type I muscle fibres would enhance the body capacity to oxidise lipid for energy and act to reduce body fat accumulation. Hence, one may expect that upon treatment with a diet rich in fat contents, skeletal muscle fibres will adapt favouring an improvement in fat oxidation. Indeed, administration of a high-fat diet in rodents has been shown to improve both the mitochondrial enzyme activity (i.e. ß-oxidation enzymes) of the muscle cells as well as to affect the muscle fibre phenotype, by eliciting a fibre transition towards slow type I fibres (e.g. Cheng et al., 1997; de Wilde et al., 2007).

Major advances have recently been made to explain how muscle adapts to changes in diet at the molecular level. A crucial factor that plays a major role in the regulation of the mitochondrial oxidative capacity (e.g. enriched mitochondrial density), through being able to switch fuel preference from carbohydrates to lipids and reduce triacylglycerol storage is PPARδ. In general, PPARs are a group of nuclear receptors exhibiting multiple biological functions such as lipid sensors and transcription factors through binding to specific DNA elements, inducing the expression of genes important for lipid metabolism regulation and systemic fuel turnover (reviewed by Fuernsinn et al., 2007). PPARδ is preferentially expressed in skeletal muscle and is found in oxidative rather than glycolytic fibres. It plays a central role both in the control of skeletal muscle metabolism and in promoting the formation of oxidative type I slow-twitch fibres as well as the utilization of fatty acids as fuel (Schuler et al. 2006; reviewed by de Lange et al., 2007). In addition, PPARδ activation exerts numerous systemic favourable effects by increasing energy expenditure in both muscle and fat tissue, by improving insulin sensitivity and cardiovascular function and by suppressing atherogenic inflammation and mediating body mass gain, mainly due to its control of energy balance and protective role against lipotoxicity (reviewed by Reilly and Lee, 2008). GW501516-induced activation of PPARδ results in increased fatty acid oxidation and reduced glucose oxidation, glycogen synthesis, lactate release, glucose transport and triacylglycerol storage in skeletal muscle. These results suggest a fuel-switching role of PPARδ in favour of fatty acid over carbohydrate (Brunmair et al., 2006). GW501516 is the best characterized (among others e.g. GW0742and L165041) synthetic PPARδ pharmacological agonist that has been shown to induce numerous favourable lipid profile changes (e.g. decrease of circulating non-esterified fatty acids, triacylglycerols, ketones, VLDL-triacylglycerol, LDL-cholesterol, but potent raising of HDL-cholesterol) associated with a reduced risk of cardiovascular lesion (reviewed by Fuernsinn et al., 2007; Reilly and Lee, 2008). PPARδ expression is influenced by muscle activity, showing increased levels in response to an activity pattern that induces oxidative properties and decreased levels in response to an activity pattern that induces glycolytic properties. Additionally it seems to correlate with myosin heavy chain expression and high oxidative capacity in rat soleus muscle (Lunde et al., 2007). Apart from changes in PPARδ expression, the presence of natural ligands, such as fatty acids and their metabolites are essential and may tightly regulate PPARδ activity. During food deprivation or fasting, fatty acid utilization increases and is followed by a transient upregulation of skeletal muscle PPARδ levels. Hence, a low energy status elicits rapid signalling through PPARδ and a subsequent increase in oxidative capacity (de Lange et al., 2007). Furthermore, the administration of PPARδ agonists to high-fat-fed mice blunted weight gain and increased resting energy expenditure (Fuernsinn et al., 2007).

Various experimental settings have shown that PPARδ activation is followed by multiple molecular (e.g. repression of lipid absorption genes, induction of lipid oxidation and cholesterol efflux genes and repression of inflammatory genes), cellular (e.g. reduced intestinal cholesterol uptake, improved insulin sensitivity, reduced cellular lipid accumulation in muscle, liver and fat tissue, increased cholesterol transfer to HDL and amelioration of the inflammatory state) and metabolic (e.g. improved glucose homeostasis, increased mitochondrial capacity, increased systemic fat burning both in muscle and adipose tissue and energy dissipation and improved plasma lipid profile) responses, hinting the functional adaptations of regular physical activity (Fuernsinn et al., 2007). Skeletal muscle overexpression of PPARδ or administration of PPARδ agonists in mouse resulted in improved running performance (time and distance), increased number of type I muscle fibres and a higher resistance to obesity and improved metabolic profiles, even in the absence of exercise (Wang et al., 2004). On the contrary, selective ablation of PPARδ in mouse skeletal muscle is accompanied by impaired muscle oxidative capacity and phenotype as well as subsequent development of obesity and diabetes (Schuler et al., 2006). Interestingly, supportive evidence has recently accumulated showing that PGC-1α is both directly controlled by PPARδ and may act as a co-activator of PPARδ to control mitochondrial biogenesis and muscle fibre type plasticity (Wang et al., 2004; Schuler et al., 2006). However, the majority of the above-mentioned findings remain to be established in humans given that PPARδ has been proposed as potential therapeutic target for treating the

whole spectrum of metabolic diseases.

Malnutrition and exercise

It has long been shown that food restriction prevents rat hindlimb hypertrophic adaptations in response to exercise (Maxwell et al., 1992). However, energy intake (e.g. the amount of food consumed the day after exercise) appears to greatly influence the rate of protein degradation during the recovery period after a bout of exercise. In particular, exercise has been reported to exhibit a muscle protective role by preventing increased rate of total protein degradation caused by food restriction in the rat (Kasperek et al., 1992). In addition, this study noted that electrically stimulated muscle contractions elicited a decrease in the rate of both myofibrilar and total protein degradation. It seems that a not fully understood mechanism works to conserve muscle protein when energy reserves run low and probably neural activity may play a role. Furthermore the protective influence of muscle activity under food restrictive conditions is conserved during evolution to include invertebrate species. In support of this, in vivo data shows that neural input control muscle proteolysis, a process that is mediated by acetylcholine signalling and stimulation of muscle nicotinic acetylcholine receptors even in *Caenorhabditis elegans*, a free-living nematode (roundworm, Szewczyk et al., 2000).

However, the protective effect of exercise is only apparent under certain dietary conditions. Ballor et al. (1990) examined the effects of exercise training on lean mass conservation during moderate and severe dietary restriction in rats. These authors showed that exercise training resulted in the conservation of lean mass at the moderate but not severe levels of dietary restriction. For instance, gastrocnemius muscle mass was conserved only at the moderate dietary restriction level, implying that the level of dietary restriction appears to affect the ability of exercise training to elicit conservation of both total lean mass and muscle mass during diet-induced body mass reduction. Similarly, it has been shown that acute exercise during pregnancy can have detrimental effects on fetal development only if dietary glucose is severely restricted (Cobrin and Koski, 1995). In addition, hindlimb glucose uptake rate in sedentary food restricted rats has been found to be similar to that of swim trained rats and greater than that of the sedentary ad libitum fed rats, while food restriction itself does not inhibit glycogen deposition in the liver or skeletal muscle in rats (Taylor et al., 1974; Ivy et al., 1991). Accordingly, despite the energy depletion during exercise in the starved state, it seems that skeletal muscle contraction has a favourable effect on the tissue attenuating the undesirable effects of reduced energy levels and warrants further research.

Celullar energy metabolism

Molecules that play an important role in the control of mitochondrial biogenesis include AMPK, PGC-1α, nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2), mitochondrial transcription factor A (Tfam), PPARα and PPARγ. Muscle mechanical loading induced by either endurance exercise or electrical stimulation elevates these transcriptional regulators in muscle (Winder et al., 2006).

AMPK signalling and mitochondrial biogenesis

AMPK is a highly conserved heterotrimeric protein kinase containing a catalytic α-subunit and regulatory ß- and γ-subunits, exhibiting distinct regulatory properties and functions. Skeletal muscle appears to be the only tissue able to express all subunit isoforms (Hardie and Sakamoto, 2006; McGee et al., 2008). AMPK is considered as a key factor in the maintenance of energy homeostasis and a major molecular sensor for cellular AMP levels by regulating fuel and energy balance at not only the cellular level but also systemically (Hardie and Sakamoto, 2006). Upon activation during energetic stress such as hypoxia, glucose starvation or physical exercise, AMPK stimulates catabolic pathways and suppresses anabolic pathways in an effort to supply ATP for cell survival. AMPK can inhibit protein translation by at least two mechanisms: i) by phosphorylation of the eukaryotic elongation factor 2 (eEF2) and ii) by phosphorylation of tuberous sclerosis complex 2 (TSC2, also known as tuberin) on Thr1227 and Ser1345 (Inoki et al., 2003), two components of the insulin-like growth factor (IGF) signalling pathway. Moreover, AMPK activation has been suggested to play an important role in the inhibition of protein synthesis by suppressing the function of multiple translational regulators including p70S6K and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) in response to energy starvation and low metabolic conditions (Horman et al., 2002; Inoki et al., 2003).

Recent studies indicate that the activation of AMPK may be important in regulating the specificity of adaptation to different types of exercise training (e.g. endurance vs. resistance; Winder et al., 2006). One should bear in mind that the decrease in muscle protein synthesis during resistance exercise is associated with an increase in AMPK activity and a reduction in the phosphorylation of 4E-BP1 at Thr36/47, a downstream effector of the mTOR pathway and a key event in controlling translation initiation, although the precise mechanisms by which AMPK may attenuate 4E-BP1 phosphorylation at Thr36/47 remain to be fully elucidated (Dreyer et al., 2006). Hence, activation of AMPK, which occurs primarily as a response to endurance exercise, elicits both acute metabolic changes in muscle including increased glucose transport and activation of fatty acid oxidation due to direct phosphorylation of metabolic enzymes as well as chronic effects through altering gene expression of multiple downstream targets (e.g. upregulation of PGC-1α a key regulator of mitochondrial biogenesis,

glycogen synthase phosphorylation on Ser9 and hence inactivation of the enzyme and mTOR inhibition via TSC2 phosphorylation (Hardie and Sakamoto, 2006). AMPK seems not to be required for anaerobic metabolism of endogenous glycogen but is required instead for the switch to aerobic oxidation of blood-borne fuels (Hardie and Sakamoto, 2006).

Pharmacological intervention and exercise both activate AMPK which phosphorylates and inactivates the mitochondrial membrane associated acetyl-CoA carboxylase 2, thus lowering malonyl-CoA. This relieves the inhibition of palmitoyl-CoA acyl transferase (CPT) isoform 1, allowing fatty acids to enter mitochondria as carnitine esters. They are converted by CPT2 back to fatty acyl-CoA esters in the mitochondrial matrix, where they are oxidized to generate ATP and serve as an energy source (Hardie and Sakamoto, 2006). In addition, once phosphorylated and activated, AMPK stimulates transcription of PGC-1α, which in turn induces transcription of various factors, including PPARγ and genes involved in lipid metabolism (de Lange et al., 2007). An exercise training-induced upregulation of AMPK activity may potentially improve insulin sensitivity favouring muscle glucose uptake and metabolism (Hawley and Lessard, 2008).

Glycogen reduction and cellular stresses that result in a drop of the ATP:ADP ratio and ATP depletion followed by an increase in the AMP:ATP ratio are indicative of a compromised energy status and are considered as main AMPK activators during exercise or food deprivation. However, additional factors have been proposed to activate AMPK in skeletal muscle such as the upstream kinases LKB1 tumour suppressor protein kinase, the calmodulin-dependent kinase kinases CAMKKα and ß, the TGF-ß-activated protein kinase-1 (TAK1), the cytokine interleukin-6 and the adipokines leptin and adiponectin (reviewed by Hardie and Sakamoto, 2006; de Lange et al., 2007; McGee et al., 2008). For instance, LKB1 is considered as a primary upstream kinase, given that neither muscle contraction nor treatment with the chemical compound 5-aminoimidazole-4-carboxamide 1-ß-D-ribofuranoside 5-aminoimidazole-4-carboxamide riboside (AICAR), an AMP analogue that activates AMPK, activate AMPK in extensor digitorum longus and tibialis anterior muscles from muscle-specific LKB1 knockout mice (Sakamoto et al., 2005). Upon energy deprivation the tumour suppressor LKB1 in conjunction with AMP have been reported to activate AMPK, which in turn phosphorylates and activates TSC2, leading to the inhibition of mTORC1 signalling, in order to downregulate energetically demanding processes like protein synthesis and stimulate ATP-generating processes such as fatty acid oxidation (Wullschleger et al., 2006; Matsakas and Patel, 2009).

PGC-1α controls the expression of genes involved in adaptive thermogenesis, mitochondrial biogenesis, insulin secretion and fuel metabolism and has been characterised as a crucial factor in regulating muscle fibre type determination (Lin et al., 2002; Puigserver, 2005). PGC-1α is preferentially expressed in slow-twitch type I fibres which contain a high concentration of mitochondria and use oxidative metabolism as energy source (de Wilde et al., 2007). New lines of evidence suggest that AMPK may directly phosphorylate PGC-1α on Thr177 and Ser538, eliciting an increase in PGC-1α-dependent activation of its own promoter and further initiating many of the important gene regulatory functions of AMPK in skeletal muscle (Jäger et al., 2007). This is in line with previous reports showing that PGC-1α may regulate its own gene expression in muscle, in a positive feedback loop (Handschin et al., 2003). These AMPK-mediated phosphorylations provide an option to modulate the ability of PGC-1α to bind transcription factors or affect the function of other cofactors in the PGC-1α coactivator complex (Jäger et al., 2007).

In vitro and in vivo studies have shown the necessity of PGC-1α as a mediator of AMPK activity. Treatment of primary muscle cells from wild type and PGC-1α deficient mice with AICAR (a pharmacological activator of AMPK) resulted in increased AMPK activation in both genotypes but increased PGC-1α mRNA expression only in the cells from wild type mice. Similar results were obtained in vivo (Jäger et al., 2007). Taken together these data indicate that AMPK requires PGC-1α protein for many of its most important effects on mitochondrial gene expression in skeletal muscle (Jäger et al., 2007). Accumulating evidence shows that the gene program for muscle turnover in response to acute endurance exercise is followed by a higher induction of genes associated with muscle protein breakdown, as opposed to resistance exercise (Louis et al., 2007).

Calcineurin signalling

Depolarization of the sarcolemma in response to motor neuron firing triggers a release of Ca^{2+} from the sarcoplasmic reticulum and its binding to troponin C, initiating a cascade of protein interactions that allow actomyosin binding, cross-bridge cycling, sarcomeric shortening and subsequent contraction of the fibre (Michel et al., 2004). Calcium is considered as a putative primary messenger that transduces the mechanical signal of contraction upon neural activation (Fig. 6, Coffey and Hawley, 2007). In addition, there are three calcium-triggered regulatory pathways that transduce cytosolic calcium concentration changes to target genes acting through calcineurin, calcium-calmodulin-dependent protein kinases and calcium-dependent protein kinase C (Koulmann and Bigard, 2006). Elevation of intracellular calcium increases the activity of a heterodimeric, ubiquitous serine/threonine phosphatase (which removes phosphate groups from targets) called calcineurin or protein phosphatase 2B, comprised of a calmodulin-binding catalytic A subunit and calcium-binding regulatory B subunit. Signalling is initiated upon binding of released calcium to calmodulin and subsequent

activation of calcineurin via the regulatory subunit (Bassel-Duby and Olson, 2006). Activated calcineurin dephosphorylates and thereby activates the transcriptional promoter nuclear factor of activated T cells (NFAT), resulting in translocation of NFAT to the nucleus, where in association with other transcription factors it induces the expression of calcium-dependent target genes. In addition, activated calcineurin dephosphorylates and activates the transcription factor myocyte enhancer factor-2 (MEF2), which plays a major role both in muscle cell formation and myofibre transformation.

While overexpression of calcineurin has been found to upregulate endogenous oxidative proteins and drive fast to slow muscle fibre transitions in the direction IIB→IIX→IIA→I (or alternatively attenuate postnatal disappearance of slow fibres). Furthermore various strategies aiming to abolish calcineurin activity (for review see Bassel-Duby and Olson, 2006; Schiaffino et al., 2007) resulted in the induction of glycolytic enzymes, a decrease in myosin heavy chain type I and IIa and an upregulation of type IIx with a transformation towards a fast phenotype as well as a decrease in the number or even total lack of slow type I muscle fibres. Hence, the vast majority of the studies concluded that activated calcineurin is associated with increased abundance of oxidative slow twitch type I fibres and suggest that calcineurin is involved in fibre-type plasticity and is essential for the induction and nerve activity-dependent maintenance of the slow muscle gene program during postnatal muscle growth (Stewart and Rittweger, 2006).

Although the role of calcineurin signalling in maintenance of slow fibre type I gene program is well established, there does not seem to be consensus regarding its role in skeletal muscle fibre growth. The role of calcineurin in regulating skeletal muscle hypertrophy appears to be controversial. The majority of studies employed administration of the immunosuppressive drug cyclosporin A (CsA) and FK506 to inhibit calcineurin-dependent signalling pathways, in order to examine skeletal muscle hypertrophic responses. Dunn and colleagues (1999) described a calcineurin-dependent muscle hypertrophy effect in mice in vivo by demonstrating that a 2-4 week administration of CsA completely prevented plantaris hypertrophy in response to overload. Conversely, multiple studies failed to confirm that skeletal muscle hypertrophy is dependent on calcineurin activity (reviewed by Bassel-Duby and Olson, 2006). Even analysis of the inhibition of calcineurin signalling for 2 weeks in phenotypically distinct skeletal muscles (soleus and plantaris) of the same animals resulted in differential effects; the fast-twitch plantaris was more severely affected by CsA than the slow-twitch soleus, with regard to maintenance of normal muscle size as well as growth after atrophy (Mitchell et al., 2002). Furthermore, overexpression of calcineurin in transgenic mice was not followed by any evidence of muscle hypertrophy (Naya et al., 2000). Table 2 summarizes the effects of calcineurin signalling on the skeletal muscle growth. The vast majority of the studies regarding the role of calcineurin on skeletal muscle hypertrophy have focused on the administration of calcineurin inhibitors in various animal models in order to observe whether muscle hypertrophy is blunted or not. Given any intra-species differences and inter-study variability (e.g. experimental design, dosage of a drug etc.) there does not seem to be a consensus regarding the role of calcineurin on skeletal muscle hypertrophy regulation with the report of controversial results (see Table 2). In addition, genetic manipulations aiming at a loss of function or gain of function of calcineurin failed to establish a causal-relationship between calcineurin and skeletal muscle hypertrophy, implying that other signalling molecules and pathways interfere or play a more crucial role (e.g. Naya et al., 2000; Parsons et al., 2004). For instance, pharmacological inhibition of calcineurin is accompanied by reduced myostatin mRNA levels (Michel et al., 2007).

A possible reason for the observed discrepancies of the studies aimed to elucidate the role of calcineurin in muscle mass, which make the interpretation of the data more difficult might be the dose of the agent administered, the length of the treatment, intraspecies differences, mice strains and gender differences, the appropriate time-points chosen for analysis, different muscle types, the different developmental stages of the animals as well as the different experimental approaches (Mitchell et al., 2002; Schiaffino and Serrano, 2002). An interesting suggestion has been proposed by Mitchell et al (2002), who investigated the molecular components involved in the calcineurin signalling pathway, such as

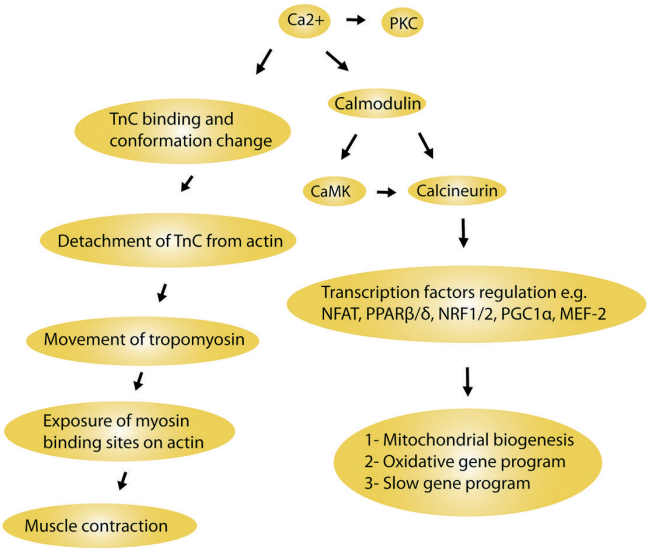

Fig. 6. Overview of the role of Ca^{2+} in muscle contraction control and gene expression.

the regulatory subunit (Calcineurin B), the catalytic subunit (Calcineurin A) as well as the expression of cyclophilin isoforms A, B and C, which mediate CsA effects. Interestingly, these authors reported that although calcineurin A is expressed at higher levels in the plantaris, calcineurin B which is required for activity of calcineurin A is expressed at lower levels in the same muscle, meaning that the proportion of calcineurin capable of being enzymatically active in plantaris is lower compared to the soleus. Moreover cyclophilins, a group of proteins that can bind to the immunosuppressant CsA, exhibit a differential efficiency at which they inhibit calcineurin upon binding to CsA. Given that cyclophilin B (cyclophilins constitute natural cell ligands for CsA) levels are higher in plantaris, one may speculate that certain muscles may be protected from the effects of CsA by low levels of cyclophilin B, whereas other muscles may be more susceptible due to higher levels of cyclophilin B, demonstrating a complexity in mammalian skeletal muscle growth (Mitchell et al., 2002). Another speculation regarding the CsA-induced changes in both body and muscle mass is based on preliminary observations which relate a depressed growth rate and muscle mass with impaired food intake (Koulmann et al., 2008).

Studies in muscle cell culture systems provided additional mechanistic information by showing that IGF-I may induce calcineurin-mediated activation of the transcription factor GATA-2, which serves as a marker of skeletal muscle hypertrophy (Musaro et al., 1999). These authors have elegantly shown that hypertrophic myocytes overexpressing IGF-I induce calcineurin-mediated dephosporylation of NFATc1 and the subsequent induction of GATA-2. Calcineurin is considered by various researchers to act as a co-regulator of muscle hypertrophy with IGF-I and may also contribute to myogenic proliferation and differentiation of satellite cells during skeletal muscle regeneration (reviewed by Coffey and Hawley, 2007). Supporting data for this hypothesis have recently shown that a NFAT-rich regulatory element in the IGF-I exon 1 promoter flanking region is responsive to calcineurin signalling and NFAT activation in skeletal myocytes. Both IGF-I and calcineurin/NFAT signalling are implicated in skeletal muscle development and mutagenesis of either pathway in mice results in compromised skeletal myogenesis. The identification of a calcineurin/NFAT-responsive element in the IGF-I gene may represent a potential mechanism of intersection of these signalling pathways in the control of muscle development and homeostasis (Alfieri et al., 2007). Although the regulatory relationship between

Table 2. Effect of calcineurin signalling on skeletal muscle mass.

Experimental design	Hypertrophic Response	Reference
Expression of a dominant-negative calcineurin (Cn) mutant or treatment with inhibitors (CsA: 3-5 μM) in a mouse cell culture	Both interventions repressed hypertrophy	Musaro et al., 1999
Treatment with Cn inhibitors (CsA: 1 μM, FK506: 100 ng mL^{-1}) in a mouse cell culture system	Suppression of hypertrophy	Semsarian et al., 1999
Administration of Cn inhibitors (CsA: 25mg k^{-1}, FK506: 3mg kg^{-1}) in mice for 1-4 weeks	Inhibition of Cn signalling prevents overload-induced hypertrophy in plantaris	Dunn et al., 1999
Administration of Cn inhibitors (FK506: 3-5mg kg^{-1}) in rats for 4 weeks	Inhibition of Cn signalling attenuated muscle maintenance and inhibited regrowth during reloading in soleus	Miyazaki et al., 2006
Treatment with Cn inhibitor (CsA: 5 μM) in a rat cell culture system with overexpression of the IGF-IR	Inhibition of Cn did not inhibit myotube hypertrophy	Quinn et al., 2007
Administration of Cn inhibitors (CsA: 15mg k^{-1}, FK506: 3-5mg kg^{-1}) in rats for 2-4 weeks	Inhibition of Cn signalling did not blunt overload-induced hypertrophy in plantaris	Bodine et al., 2001
Genetic manipulations leading to loss of Cn in mice	Loss of Cn does not block muscle hypertrophy in plantaris	Parsons et al., 2004
Administration of Cn inhibitors (CsA: 25mg kg^{-1}) in rats for 2 weeks	Inhibition of Cn signalling did not inhibit the beneficial effects of muscle-maintaining interventions, nor did it change muscle mass in control or atrophied muscles	Dupont-Versteegden et al., 2002
Administration of Cn inhibitors (CsA: 12, 25 or 50mg kg^{-1}) in mice for 1-2 weeks	Inhibition of Cn signalling provided differential results on skeletal muscle growth depending on muscle type (soleus vs. plantaris)	Mitchell et al., 2002
Administration of Cn inhibitors (Cs: 7mg kg^{-1}) in rats for 4 weeks	Unaffected cross sectional areas in the treated muscles (diaphragm, EDL, soleus)	Biring et al., 1998
Administration of Cn inhibitors (CsA: 5mg kg^{-1}, FK506: 1mg kg^{-1}, transfection with a Cn peptide inhibitor) in rats for 1 week	Cn inhibitors do not block the increase in fibre size induced by nerve activity in regenerating soleus	Serrano et al., 2001
Overexpression of Cn in the muscle of transgenic mice	Absence of hypertrophic responses	Naya et al., 2000

IGF-I and NFAT appears to be complicated, it is clear that the embryonic expression of IGF-I is responsive to NFATc3 and that the IGF-I exon 1 promoter region contains a conserved NFAT-responsive regulatory region. Besides, it appears that muscle gene expression during myogenic differentiation is promoted by NFATc3, which in turn is negatively regulated by glycogen synthase kinase 3ß (GSK-3ß, van der Velden et al., 2008). However, the regulatory relationships between calcineurin and IGF-I signalling mechanisms in skeletal muscle differentiation and hypertrophy have not been completely established.

Utrophin A, a cytoskeletal protein that accumulates at the neuromuscular junction and plays a role in the maturation of the postsynaptic apparatus and myostatin, a potent negative regulator of muscle development have both recently been postulated to function as probable downstream effectors of the calcineurin signalling, providing novel avenues for experimentation (reviewed by Michel et al., 2007). For instance, utrophin A expression has been shown to be both suppressed in animals treated with calcineurin inhibitors and upregulated in transgenic animals constitutively expressing active calcineurin, indicating that utrophin A expression is calcineurin -dependent. Activated calcineurin in cell culture studies followed by nuclear translocation and interaction with other transcription factors, coactivators and the utrophin A promoter causes synergistic activation of utrophin A expression in myocytes, showing that regulation of utrophin A in vivo is controlled by multiple signalling pathways, of which calcineurin/NFAT is an important component (Michel et al., 2007). Alternatively, myostatin mRNA levels have been reported to be lower in muscles from transgenic animals with compromised calcineurin activity and higher in transgenic animals with enhanced calcineurin signalling. On the other hand, pharmacological inhibition of calcineurin is followed by reduced myostatin mRNA levels and myostatin expression downregulates calcineurin signalling via NFATc1, implying thus that myostatin acts as a downstream target gene of the calcineurin pathway affecting muscle growth remodelling and may itself regulate calcineurin expression and activity (reviewed by Michel et al., 2007). However, further research is required in order to clearly establish the interplay between calcineurin and other signalling pathways (i.e. IGF-I) and to identify important effectors and downstream targets.

The effect of muscle mechanical loading by using different exercise modalities has recently been addressed. Very recent data provide evidence for a causal link between the extent of myofibre transitions and the modulation of calcineurin activity in mouse skeletal muscles in response to different exercise paradigms, namely a high frequency and amplitude exercise (swimming) and a low frequency and amplitude exercise (running) for a 6- and 12-week period (Grondard et al., 2008). These authors found an exercise- and muscle-specific effect of training on the myofibre phenotype transitions followed by exercise-induced changes in calcineurin phosphatase activity in a biphasic manner, originating in an initial increase in activity and a subsequent inhibition phase which was due to the inhibition of the catalytic subunit gene expression on one hand and to the enhancement of the modulatory calcineurin interacting protein I gene transcription, on the other.

A recent study examined both the respective and combined effects of calcineurin inhibition by CsA and hypothyroidism on myosin heavy chain isoform transition in the rat soleus muscle, two interventions with known opposite effects on muscle phenotype (Koulmann et al., 2008). CsA administration induced a slow to fast MHC transition which was blocked in the hypothyroid state, whereas hypothyroidism markedly reduced both the expression of fast MHC isoform and oxidative capacity independent of calcineurin inhibition. In addition, CsA and/or thyroid deficiency decreased the expression of modulatory calcineurin inhibitor protein isoform 2 (MCIP-2), a read-out of calcineurin transcriptional activity, implying that thyroid hormone is a prerequisite for CsA effects on soleus muscle phenotype and regulates calcineurin activity by controling levels of MCIPs mRNA. These data provide supportive evidence that thyroid hormone is necessary for the expression of fast MHC isoforms acting upstream of calcineurin activity (Koulmann et al., 2008).

A future avenue of research could investigate the link between the adaptation of the muscle following a high fat regime, which results in an increased oxidative capacity and whether this response is anyway mediated by the calcineurin signalling pathway.

Conclusions and perspectives

Advances in molecular biology and physiological experimentation methodologies have led to a plethora of recent studies into the phenotypical adaptation of skeletal muscle to various stimuli, providing novel findings about the molecular mechanisms involved in the multicomplexed cellular processes. Work from animal models allowing the application of genetical engineering techniques enables us to study the systemic function of key regulatory molecules that affect the skeletal muscle phenotype and homeostasis.

The timing of food restriction whether implemented before or after weaning seems to elicit differential changes in skeletal muscle in a species and muscle specific manner, with tonically active muscles (e.g. soleus) showing less atrophic susceptibility than phasic muscles (e.g. EDL). Hence, pre- or post-natal energy restriction before weaning seems to elicit long-term effects on muscle phenotype and is followed by a decreased proportion of slow type I fibres and increased fast IIB fibres in the rat but not in cattle, while postnatal undernutrition after the weaning period is accompanied by a decreased proportion of fast IIB fibres and an increased number of IIA fibres in various species, thus

providing supportive evidence for a positive relation between dietary energy status and the proportion of fast glycolytic fibres (Goldspink and Ward, 1979; Bedi et al. 1982; Brozanski et al., 1991; Picard et al., 1995). Apparently, favouring oxidative metabolism seems to be the cells adaptive response for efficient ATP generation and energy supply in a period of energy starvation. However, impaired endocrine action of the thyroid gland during a period of energy restriction provides a plausible explanation for the decreased occurrence of fast type IIB fibres and offers an attractive starting point for further research (Brozanski et al., 1991). To this end, recent data on thyroid hormone signalling cascades indicate that thyroid hormone rapidly activates p38 and AMPK and increases PGC-1α mRNA contents in skeletal muscle in vivo (Irrcher et al., 2008).

Finally, although recent considerable progress has been made regarding the role of calcineurin in skeletal muscle signalling, controversial findings still cloud its precise role in the regulation of muscle hypertrophy and further investigation is required.

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