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Symposium: Signals Mediating Exercise-Induced Skeletal Muscle Remodelling

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Chair: Mark Hargreaves

Histone modifications and skeletal muscle metabolic gene expression

S.L. McGee, Department of Physiology, The University of Melbourne, VIC 3010, Australia.

Skeletal muscle oxidative capacity plays an important role in human health and performance. Impaired oxidative capacity has been implicated in contributing to the development of insulin resistance and type 2 diabetes (Mootha *et al.,* 2001). Skeletal muscle oxidative capacity is also a key determinant in endurance exercise performance, and is chiefly determined by the expression levels of a broad set of metabolic and mitochondrial enzymes, which are largely regulated at the level of transcription.

Gene transcription is highly dependent on local chromatin structure, which is related to post translational modifications of the histone proteins that form the nucleosome core. Many histone modifications have been characterised, including acetylation, methylation, ubiquitination and phosphorylation (Berger, 2007). The greatest challenge for the area of genetics in the post genome sequencing era will be to determine how these modifications interact to regulate gene expression. However, it is generally recognized that acetylation of lysine residues within histone 3 are required for transcriptional initiation (Guenther *et al.,* 2007). Histone acetylation neutralizes the positive charge carried by lysine residue side chains, thereby breaking the electrostatic interaction between histones and the negatively charged phosphate backbone of DNA. This results in unraveling of the local chromatin, allowing transcriptional regulators such as the transcription initiation complex access to DNA promoter regions. Histone acetylation is regulated by the balance in activities between histone acetyl transferase and histone deacetylase (HDAC) enzymes (McKinsey *et al.,* 2001).

Using cell systems expressing either wild type or deacetylase defective HDAC4 and 5, we have showed that the class IIa HDACs regulate the expression of metabolic and mitochondrial enzymes involved in glucose and lipid transport and substrate oxidation. As compromised oxidative capacity has been implicated in contributing to peripheral insulin resistance, we are currently testing the hypothesis that pharmacological inhibition of HDACs might protect against diet-induced insulin resistance. *In vivo*, inhibition of HDAC repressive function is regulated by either phosphorylation dependent nuclear export or ubiquitin mediated proteasomal degradation. We have recently found that the AMP activated protein kinase (AMPK) is a HDAC5 kinase. In addition, we have also found that considerable redundancy exists between AMPK and protein kinase D in signaling to HDAC5 under conditions of cellular stress. Finally, we have evidence implicating heat shock protein 70 (HSP70) in mediating proteasomal degradation of the class IIa HDACs. Importantly, HSP70 is dysregulated in insulin resistance and type 2 diabetes and through the class IIa HDACs could contribute to the oxidative dysfunction seen in these diseases.

Together, these data suggest that the class IIa HDACs are key regulators of metabolic and mitochondrial gene expression in skeletal muscle and that these enzymes could be potential therapeutic targets for the treatment of diseases such as insulin resistance and type 2 diabetes. Understanding the regulation of these enzymes will uncover mechanisms regulating oxidative capacity.

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Molecular regulation of skeletal muscle mass

A.P. Russell, The Centre for Physical Activity and Nutrition Research (C-PAN), School of Exercise and Nutrition Sciences, Deakin University, VIC 3125, Australia.

Skeletal muscle mass is increased in response to increases in functional demand as seen with resistance training (Fry, 2004). In contrast, skeletal muscle mass is dramatically reduced in diseases and conditions such as cancer, diabetes, sepsis, AIDS, denervation, ageing and muscular dystrophies (Lynch, 2001). The control of skeletal muscle mass is tightly regulated by the synergy between anabolic pathways controlling protein synthesis and catabolic pathways regulating protein degradation. These pathways are not necessarily independent of each other (Bodine *et al.*, 2001).

Advances have been made in understanding the factors controlling skeletal muscle hypertrophy and atrophy using pharmacological and genetic manipulation in cellular and rodent models. Akt (also called PKB; Protein Kinase B) has been identified as a pivotal point in the hypertrophy and atrophy signalling pathways (Bodine *et al.*, 2001). Akt phosphorylates several downstream targets including glycogen synthase kinase-3β (GSK3β) and the mammalian target of rapamycin (mTOR), promoting increases in protein synthesis and translation initiation (Rhoads, 1999) and muscle hypertrophy (Bodine *et al.*, 2001). Chronic resistance exercise results in muscle hypertrophy which is associated with increased levels of Akt and its downstream targets (Bodine *et al.*, 2001; Leger *et al.*, 2006). In contrast, acute resistance exercise is able to stimulate protein synthesis, independently of Akt phosphorylation, but in parallel with increases in mTOR and its downstream target p70s6K (Dreyer *et al.*, 2008).

Skeletal muscle atrophy is characterized by an up-regulation of the muscle specific E3-ligases, atrogin-1 and MuRF1, in numerous models of muscle wasting (Glass, 2005). Atrogin-1 and MuRF1 are often increased by Forkhead (FoXO) transcription factors (Sandri *et al.*, 2004) with a concomitant increase in proteasomal and lysosomal protein degradation (Zhao *et al.*, 2007). Over-expressing Akt (Sandri *et al.*, 2004) or peroxisome proliferator-activator receptor co-activator gamma-1α (PGC-1α) (Sandri *et al.*, 2006) can inhibit the FoXO dependent increase in atrogin-1 and MuRF1 and attenuate muscle wasting.

Akt and PGC-1α signalling appear to be key elements regulating skeletal muscle mass. Determining how these proteins can be therapeutically manipulated in skeletal muscle *in vivo* may help with attenuating skeletal muscle wasting.

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Calpains and skeletal muscle function

R.M. Murphy and G.D. Lamb, Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia.

Skeletal muscle fibres contain ubiquitous (μ-calpain and m-calpain) and muscle-specific (calpain-3), Ca^{2+}-dependent proteases. Their physiological roles are not well understood, although ubiquitous calpains have been associated with apoptosis and myogenesis and calpain-3 has been suggested to be involved in sarcomeric remodeling. A defect in the expression of calpain-3 results in limb-girdle muscular dystrophy type 2A. Contrary to the dogma published from biochemical experiments that described calpain-3 as undergoing spontaneous autolysis (and hence activation) (Sorimachi *et al.*, 2006), we have shown that this protease is stable unless exposed to [Ca^{2+}] above resting physiological levels (> 50 nM). Our work has characterized the Ca^{2+}- and time-dependencies of μ-calpain and calpain-3 in muscle homogenates, importantly with physiological ionic conditions preserved. During normal activity, skeletal muscle undergoes frequent episodes of high intracellular [Ca^{2+}] and to understand how calpains are regulated during such periods, we have investigated various properties (such as diffusibility, binding and autolysis) of μ-calpain and calpain-3 using mechanically-skinned single fibres (Murphy, Venburg & Lamb, 2006). In addition, we have seen that overall the calpains were found not to be activated immediately following sprint, endurance or eccentric exercise in healthy human subjects (Murphy, Snow & lamb, 2006; Murphy *et al.*, 2007). Notably, we found that a substantial proportion of calpain-3, but not μ-calpain, was activated 24 h after the eccentric exercise bout, which could possibly be explained by the small but sustained increase in intracellular [Ca^{2+}] that occurs following eccentric contractions (Lynch, Fary & Williams, 1997) being both high and long enough to result in calpain-3 activation.

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A novel role for β-adrenoceptor signalling in the regulation of skeletal muscle mass

J.G. Ryall and G.S. Lynch, Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia.

Skeletal muscle mass is maintained via a careful balance between protein synthesis and degradation, processes which until recently were though to be regulated in an independent manner (Sandri, 2008). The insulin-like growth factor-I (IGF-I) is perhaps the best documented of the growth factors implicated in the regulation of skeletal muscle mass, which it does predominantly through Akt-mediated activation of mammalian target of rapamycin (mTOR), resulting in an increase in protein translation. In addition to its role in increasing protein synthesis, the IGF-I/Akt signalling pathway has been implicated in the regulation of protein degradation, presumably through Akt-mediated phosphorylation (and subsequent nuclear exclusion) of the forkhead box O transcription factors FoxO1 and FoxO3 (Sandri, 2008). While IGF-I has long been considered the master regulator of Akt activation, a study by Kline and colleagues (2007) implicated a second IGF-I-independent growth pathway involving the activation of β-adrenoceptors and subsequent hypertrophic signalling via mTOR.

While the importance of β-adrenergic signalling in the heart has been well documented and continues to receive significant attention, it is only more recently that we have begun to appreciate the importance of this signalling pathway in regulating skeletal muscle growth and development (Lynch & Ryall, 2008). In addition to the findings of Kline *et al.* (2007), work from our laboratory has identified a novel role for β-adrenoceptors in regulating skeletal muscle regeneration (Beitzel *et al.* 2004; 2007). Further confirmation of the important role these receptors play in regulating skeletal muscle mass has come from our recent investigations utilising transgenic mice that lack β-adrenoceptors ($Adrb1^{-/-}$/$Adrb2^{-/-}$).

These studies have provided important insight into the role of β-adrenoceptor signalling in regulating skeletal muscle size. Importantly, a clearer understanding of the pathways that regulate skeletal muscle mass may lead to the identification of novel therapeutic targets for the treatment of muscle wasting conditions.

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