

**AuPS Meeting - Melbourne 2008**

**Symposium: Skeletal Muscle: an Endocrine Organ**

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Chair: Mark Febbraio

## **PGC-1 $\alpha$ in muscle links metabolism to inflammation**

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Skeletal muscle has an enormous capacity to adapt to external stimuli. Most prominently, changes in protein biosynthesis and degradation rates, alterations in contractile and metabolic properties and modulation of signal transduction pathways regulate muscle fiber plasticity induced by physical activity. The transcriptional coactivator peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) is appreciated as one of the central regulators of gene expression in the exercised muscle (Lin *et al.*, 2005; Handschin & Spiegelman, 2006, 2008). In fact, ectopic expression of PGC-1 $\alpha$  is sufficient to boost mitochondrial biogenesis and function, stimulate a fiber-type switch towards oxidative muscle fibers, promote a high-endurance muscle phenotype and prevent disuse-induced muscle atrophy. Furthermore, increased PGC-1 $\alpha$  levels therapeutically ameliorate Duchenne muscular dystrophy, statin-induced fiber damage and one form of a mitochondrial myopathy in the respective rodent models. In contrast, experimental ablation of PGC-1 $\alpha$  gene expression results in lower mitochondrial gene expression, a fiber-type switch towards glycolytic muscle fibers, reduced exercise capacity, abnormal glucose and insulin homeostases and activity-dependent fiber damage.

The molecular events that mediate the protective effects of PGC-1 $\alpha$  on muscle fiber integrity remain enigmatic. Several candidate pathways have been proposed: PGC-1 $\alpha$ -mediated stabilization of the neuromuscular junction, improvement of the energy crisis, inhibition of ubiquitin ligase expression and upregulation of reactive oxygen species (ROS) detoxification potentially contribute to the therapeutic effect of PGC-1 $\alpha$  in diverse contexts of muscle wasting. We now present evidence that PGC-1 $\alpha$  in skeletal muscle also has significant anti-inflammatory properties. Pro-inflammatory gene expression in muscle is elevated and increased levels of circulating tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin 6 (IL-6) have been detected in muscle-specific PGC-1 $\alpha$  knockout mice. Importantly, these animals exhibit abnormal pancreatic islet morphology and decreased insulin secretion *in vivo* indicating an increase in detrimental circulating factors in the context of specifically ablated PGC-1 $\alpha$  gene expression in skeletal muscle. These factors subsequently lead to phenotypic alteration of the physiological functions of non-muscle tissues, including pancreatic  $\beta$ -cells. We thus propose that a pathological reduction of PGC-1 $\alpha$  levels in skeletal muscle is the molecular link between physical inactivity, persistent, low-grade inflammation and the increased risk for many chronic diseases.

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## Role of protease-activated receptors (PARs) in muscle inflammation and cytokine release

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Protease-Activated Receptors (PARs) are a newly identified family of G protein-coupled receptors that mediate a diverse range of cellular activities, particularly during inflammation. PARs are activated by serine proteases such as thrombin and trypsin. These proteases cleave the extracellular N-terminus of the receptor, resulting in a new N-terminus that acts as a tethered ligand, binding to and activating the receptor. Four PAR subtypes have been identified (PAR 1, 2, 3 & 4). PAR activation commonly activates  $Ca^{2+}$  signalling pathways within target cells, and has been shown to play a vital role in orchestrating both inflammatory and proliferative responses to tissue damage. Activation of PARs can result in production of the inflammatory cytokine interleukin 6 (IL-6) in many tissues, including airway epithelia, oral mucosa, blood vessels and connective tissue (Steinhoff *et al.*, 2005). During skeletal muscle development and regeneration, satellite cells proliferate and resulting myoblasts fuse into multinucleate myotubes, which eventually mature into adult muscle fibres. PARs are reported to play an important role in skeletal muscle development by enhancing myoblast proliferation and inhibiting myoblast apoptosis (Chinni *et al.*, 2000). However, little is known about the role of PARs in the function of myotubes and skeletal muscle. Skeletal muscle has recently been shown to be a major producer of IL-6 especially during exercise where it plays a role in glucose and lipid metabolism (Pedersen, 2007). IL-6 may also play a role in promoting the differentiation and maturation of myoblasts (Okazaki *et al.*, 1996; Baeza-Raja & Munoz-Canoves, 2004). In this study we examined the effects of PAR activation on IL-6 production in cultured  $C_2C_{12}$  myotubes.

Experiments were performed on cultured  $C_2C_{12}$  myoblasts and myotubes grown in Dulbecco's modified Eagles medium with 20% and 2% foetal calf serum, respectively. PARs were activated with thrombin (activates PAR-1, 3 & 4 isoforms) trypsin (activates PAR-2) or specific PAR activating peptides mimicking the tethered activating ligand of the cleaved PAR receptor.  $Ca^{2+}$  was measured using the fluorescent  $Ca^{2+}$  indicator fura-2. Cytokine levels were detected by ELISA. IL-6 levels were determined by ELISA.

In untreated myotubes, exposure to thrombin resulted in a rise in intracellular  $Ca^{2+}$  (mean amplitude;  $0.38 \pm 0.03 \mu M$ ) in only 14% of cells tested ( $n=14$ ). This success rate was increased by pre-exposure of myotubes to inflammatory mediators such as  $TNF\alpha$  (10 nM) (success rate 57%,  $n=12$ ) and thrombin (5 U/ml) (success rate 92%,  $n=12$ ). An investigation into the effect of serine protease exposure on myokine production showed that exposure to thrombin for 24 hours increased the production of IL-6 by 40% in untreated myotubes compared to controls ( $n=8$ ,  $p<0.01$ ). Thrombin increased IL-6 production by 80% compared to controls after thrombin pre-exposure (1 hr exposure to 10U/ml thrombin 12 hours before experimentation) ( $n=8$ ,  $p<0.001$ ). Exposure to trypsin also increased IL-6 secretion by 20% compared to controls in untreated myotubes ( $n=8$ ,  $p<0.001$ ). This secretion was not enhanced by pre-exposure to trypsin.

These results suggest that inflammatory mediators may up-regulate PAR receptor expression in skeletal muscle. Increased IL-6 production induced by PAR activation could play a role in skeletal muscle development and regeneration by increasing myoblast differentiation into myotubes during skeletal muscle development and/or regeneration.

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## Myokines and metabolic regulation

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Regular physical activity is known to have multiple health benefits. Of note, exercise is associated with increased insulin stimulated glucose uptake in the immediate post exercise period, while chronic physical activity enhances insulin sensitivity. However, the precise mechanisms by which physical activity confers protection against metabolic disease are not fully understood. Approximately five years ago, we identified skeletal muscle as a cytokine-producing organ demonstrating that the metabolic and physiologic effects of exercise also may be mediated by muscle derived humoral factors (Pedersen & Febbraio, 2008). We have identified that both interleukin-6 and brain derived neurotrophic factor are "myokines" that are up-regulated by muscle contraction and released from contracting skeletal muscle where they play important roles in lipid and glucose metabolism in other metabolically active tissues such as liver and adipose tissue. These discoveries were made serendipitously, but it is likely that contracting skeletal muscle produces many myokines that positively act on the metabolism of other organs, presenting novel targeted therapeutics for the treatment of obesity related type 2 diabetes. We are currently using the well established quantitative method, namely Stable Isotope Labelling by Amino Acids in Cell Culture (SILAC) which allows the direct and unbiased quantification of protein expression/secretion to identify novel myokines. SILAC utilizes the capability of the cells to be grown in defined media, containing "normal" amino acid or a stable isotope-labelled "heavy" versions of the same amino acid thereby encoding the entire proteome of a given cellular population. Using triple encoding SILAC in combination with highly advanced mass spectrometry we are currently identifying the proteins secreted by the myoblast C2C12 cells during contraction. The identification of novel myokines that play a biological role in energy metabolism may aid in the development of identifying new drug targets to treat obesity related disorders.

Pedersen BK & Febbraio MA. (2008) *Physiological Reviews*, **88**, 1379-1406. doi:10.1152/physrev.90100.2007.