

# PGC-1 $\alpha$ Mediated Muscle Aerobic Adaptations to Exercise, Heat and Cold Exposure

Mohammed Ihsan<sup>1,2\*</sup>, Greig Watson<sup>3</sup> and Chris R. Abbiss<sup>2</sup>

**1.**Sports Physiology Department, Singapore Sports Institute, Singapore. **2.** Centre for Exercise and Sport Science Research, School of Exercise and Health Sciences, Edith Cowan University, Perth, Australia. **3.**School of Human Life Sciences, University Of Tasmania, Launceston, Australia.

## Abstract

PGC-1 $\alpha$  is regarded as a key regulator of mitochondrial biogenesis due to its central role in regulating the activity of key transcription factors associated with encoding mitochondrial components. Additionally, PGC-1 $\alpha$  has shown to mediate adaptations that increase fat metabolism and angiogenesis, contributing to the overall oxidative phenotype of the muscle. While it is well established that exercise is a potent stimulator of PGC-1 $\alpha$ , recent evidence indicates that heat and cold exposures may also influence mitochondrial biogenesis through the up-regulation of PGC-1 $\alpha$ . This highlights the potential use of these modalities in conjunction with exercise to enhance training adaptations. As such, the purpose of this review is to describe the possible mechanisms and pathways by which exercise, as well as hot and cold exposures may influence mitochondrial biogenesis. It is clear that changes in intracellular calcium, oxidative stress and phosphorylation potential are major up-regulators of PGC-1 $\alpha$  during exercise. Moreover, there is evidence implicating calcium signalling, in addition to  $\beta$ -adrenergic activation in cold-induced mitochondrial biogenesis, while PGC-1 $\alpha$  during heat exposure is likely triggered by changes in phosphorylation potential and nitric oxide signalling. However, these mechanisms appear to change considerably when cold/heat is administered following exercise, and seem to be dependent on the experimental models used (i.e. *in vitro* vs. *in vivo*, rodent vs. human). Understanding the effects heat/cold exposure and its interaction with exercise may lead to the optimisation and development of temperature-related interventions to enhance training adaptations, or aid in the treatment of mitochondrial related diseases.

**Citation:** Ihsan M, Watson G, Abbiss CR (2014) PGC-1 $\alpha$  Mediated Muscle Aerobic Adaptations to Exercise, Heat and Cold Exposure. Cellular and Molecular Exercise Physiology 3(1): e7. doi: 10.7457/cmep.v3i1.e7

**Editor:** Adam Sharples PhD.

**Received:** Jul 7, 2014; **Accepted:** Sept 30, 2014; **Published:** Sept 30, 2014.

**Copyright:** © 2014 Ihsan M, Watson G, Abbiss CR. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and work are properly cited.

**Competing Interests:** The authors have declared that no competing interests exist.

\* **E-mail:** Ihsan\_abdullah@sport.gov.sg

**Current address:** Singapore Sports Institute, 3 Stadium Drive, Singapore 397630.

## Introduction

Improvements in the oxidative potential of skeletal muscle is associated with a number of complex structural and morphological alterations, leading to an increase in mitochondrial content (Hoppeler et al., 1973), increase in metabolic enzymes (Gollnick & Saltin, 1982), oxidative fibre-type transformations (Inbar et al., 1981), enhanced capillary density (Daussin et al., 2008) and improved conduit and microvascular function (Lash & Bohlen, 1992; Rakobowchuk et al., 2008). Although mitochondrial function, independent of content may be increased by exercise training (Conley et al., 2013); the increase in mitochondrial content (i.e. mitochondrial size and number), termed mitochondrial biogenesis is especially critical to improvements in muscle aerobic capacity and function (Holloszy & Coyle, 1984). Transmission electron microscopy is regarded as the gold standard in determining mitochondrial content. However, due to the laborious nature of this technique and its unavailability in many laboratories, measures of several mitochondrial components and/or their activities have been used as surrogate markers, with cardiolipin, citrate synthase (CS) and complex I activities being the best indicators of mitochondrial content (Larsen et al., 2012). While there are many important factors involved in the biosynthesis of the mitochondrion, the transcriptional coactivator, peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) has been identified as a key regulator involved in this highly complex process (Hood, 2009; Puigserver et al., 1998; Wu et al., 1999). Indeed, there is now

good evidence demonstrating that exercise-induced mitochondrial biogenesis is modulated via PGC-1 $\alpha$  dependant mechanisms (Calvo et al., 2008; Leick et al., 2010; Leick et al., 2008; Little et al., 2011; Safdar et al., 2011; Uguccioni and Hood, 2011; Zhang et al., 2014). Exercise capacity and performance has shown to be strongly associated with muscle mitochondrial content in both animal and human models (Daussin et al., 2008; Fitts et al., 1975) and is associated with improved risk factors for a variety of chronic diseases (Bishop-Bailey, 2013). Furthermore, mitochondrial dysfunction may be associated with the pathophysiology of cardiovascular disease (Wang et al., 1999; Wisloff et al., 2005), aging (Figueiredo et al., 2008) and muscle atrophy (Romanello et al., 2010). As such, interventions that potentiate or mimic exercise-induced PGC-1 $\alpha$  expression and mitochondrial biogenesis may serve as a potential therapeutic aid to enhance skeletal muscle aerobic function and consequently benefit both the athletic and clinical populations.

Heat and cold therapy are well recognised modalities in the treatment of acute musculoskeletal injuries (Swenson et al., 1996) and have been recently utilised as recovery strategies among athletes to improve sport/training performances (Ihsan et al., 2013a; Wilcock et al., 2006). Moreover, there is emerging evidence highlighting the potential of these modalities to enhance mitochondrial adaptations either independently or in response to training. For instance, in studies using cell culture

and animal models, mitochondrial biogenesis has shown to be up-regulated following both heat and cold exposures through PGC-1 $\alpha$  dependant mechanisms similar to that observed following exercise (Bruton et al., 2010; Liu & Brooks, 2012; Puigserver et al., 1998; Tamura et al., 2014; Wu et al., 1999). However, when administered following exercise, both heat and cold interventions have demonstrated contradictory results, with some studies showing beneficial, while others detrimental effects on PGC-1 $\alpha$  and/or muscle aerobic performance (Ihsan et al., 2014; Slivka et al., 2013; Slivka et al., 2012; Tamura et al., 2014; Yamane et al., 2006). Taken together, these studies highlight the potential of these modalities to both enhance and diminish mitochondrial adaptations to training, as well implicating PGC-1 $\alpha$  as a key target in these mechanisms. As such, a thorough examination of the primary stimuli and pathways involved in exercise, heat- and cold-induced mitochondrial biogenesis via PGC-1 $\alpha$  dependant mechanisms is warranted. Elucidating the biochemical interactions involving heat/cold exposures with exercise may assist in the development of temperature optimised interventions to enhance training adaptations, or in the treatment of mitochondrial related diseases. The purpose of this review is to: i) Give a brief overview of exercise-induced mitochondrial biogenesis; ii) describe the primary stimuli inducing/activating PGC-1 $\alpha$  and their relative importance in exercise-induced mitochondrial biogenesis; iii) describe the mechanisms involved in heat and cold-induced PGC-1 $\alpha$  expression and mitochondrial biogenesis, and; iv) describe the interactions between heat and cold exposures on exercise-induced PGC-1 $\alpha$  expression and mitochondrial biogenesis. It should be noted that considerable volume of research has examined the role of PGC-1 $\alpha$  in mitochondrial biogenesis in recent years. Due to space limitations, not all of this work could be discussed within the current manuscript.

## Exercise-Induced Mitochondrial Biogenesis

It has been well established that regular endurance exercise is a potent stimulator of mitochondrial biogenesis. An exercise-induced increase in mitochondrial size and number was first demonstrated by Holloszy et al. (1967) who observed a 60% increase in mitochondrial protein content, a 2-fold increase in cytochrome c (Cyt c) and 2-fold increase in the activities of respiratory chain proteins (Complex I-IV) in rat muscle following 12 weeks of endurance training. Associated with these changes, the authors also observed a significant improvement in running time to exhaustion when compared with an untrained control group (186 $\pm$ 18 min vs. 29 $\pm$ 3 min). Similar findings have subsequently been observed by other groups using guinea pigs (Barnard et al., 1970), rats (Gollnick & Ianuzzo, 1972) and later humans (Morgan et al., 1971). Using a single-leg cycling design, Morgan et al. (1971) observed that an increase in mitochondrial protein content was largely due to increases in mitochondrial size (i.e. surface area & volume) rather than mitochondrial number. However, Hoppeler et al. (1973) later compared mitochondrial morphometry between trained and untrained subjects and reported that both mitochondrial size and number accounted for the higher mitochondrial protein content observed in the muscles of the trained subjects. These pioneering studies provide strong evidence implicating muscle contractile activity in mitochondrial biogenesis and consequently set the stage for future studies investigating the regulatory factors involved in this process.

## Factors Involved in Mitochondrial Biogenesis

Although strong evidence for the role of exercise in mitochondrial biogenesis has been developed, the molecular mechanisms involved in this process remains to be fully elucidated. The biosynthesis of the mitochondrion is a highly

complex event that requires the coordinated and cooperative function of the nuclear and mitochondrial genomes (Goffart & Wiesner, 2003; Hood, 2009; Joseph et al., 2006). Indeed, the mitochondria proteome consists of over 1000 protein subunits (Goffart & Wiesner, 2003); of which, an estimated 90 to 95% are encoded in the nucleus and following translation in the cytosol, are subsequently transported into the mitochondrial compartments for assembly (Ljubicic et al., 2010). The remainder of mitochondrial proteins are encoded in the mitochondrion itself which possesses its own genome (mtDNA) (Ekstrand et al., 2004). The transcription and replication of mtDNA is regulated independently of the nuclear genome but seems to rely on several nuclear-encoded transcription factors, particularly the mitochondrial transcription factor A (Tfam). Once imported into the mitochondria, Tfam binds with mtDNA, regulating its transcription and replication (Ekstrand et al., 2004; Moraes, 2001).

A number of transcription factors have been implicated in coordinating the encoding of the nuclear genome. Of particular importance are the nuclear respiratory factors 1 (NRF1) and 2 (NRF2). These factors are important in the transcriptional activation of many nuclear genes (Goffart & Wiesner, 2003) including the subunits for respiratory chain complexes I-V (Scarpulla, 2002) and several transcription factors responsible for mtDNA transcription (Gleyzer et al., 2005), including Tfam (Virbasius & Scarpulla, 1994). In this regard, it is evident that the NRFs are responsible for the transcription of both nuclear and mtDNA. However, the mere binding of transcription factors to gene promoters do not activate gene transcription, nor does it imply transcriptional coordination between the nuclear and mitochondrial genomes (Spiegelman & Heinrich, 2004).

Upstream factors that coordinate and regulate the transcriptional control of NRFs and Tfam were virtually unknown until the recent discovery of the transcriptional coactivator, PGC-1 $\alpha$  by Puigserver and Colleagues (1998). In that study, it was shown that PGC-1 $\alpha$  mRNA was powerfully induced in brown fat following cold exposure, with concomitant increases in the expression of cytochrome c-oxidase subunits 2 (COX2), 4 (COX4) and ATP synthase (Puigserver et al., 1998). Moreover, when ectopically introduced into cultured myotubes or white fat cells, PGC-1 $\alpha$  increased the mRNA expression of Cyt c, COX2, COX4 and ATP synthase (Puigserver et al., 1998; Wu et al., 1999) in line with a 51% increase in mitochondrial density in the myotube preparations (Wu et al., 1999). Given that COX4 and ATP synthase are encoded in the nucleus, while Cyt c and COX2 in the mtDNA, it was speculated that PGC-1 $\alpha$  may regulate both nuclear and mitochondrial encoded components (Puigserver et al., 1998; Wu et al., 1999). This hypothesis was subsequently confirmed by Wu et al. (1999), who showed that the ectopic introduction of PGC-1 $\alpha$  into cultured myotubes resulted in increased NRF1, NRF2, and Tfam mRNA expression. In this study it was shown also that PGC-1 $\alpha$  physically interacts with NRF1, significantly increasing its transcriptional activity (Wu et al., 1999). Taken together, the findings by Puigserver et al. (1998) and Wu et al. (1999) demonstrate that PGC-1 $\alpha$  coordinates and regulates mitochondrial biogenesis by inducing the expression of transcriptional factors that encode both nuclear and mtDNA, and by regulating the transcriptional activity of mitochondrial genes by activating NRF1 and subsequently Tfam via NRF1.

## PGC-1 $\alpha$ in Exercise-Induced Mitochondrial Biogenesis

Since the pioneering work by Puigserver et al. (1998) and Wu et al. (1999), the relative importance of PGC-1 $\alpha$  in exercise-induced mitochondrial biogenesis has been rigorously investigated. Transgenic mice over-expressing PGC-1 $\alpha$  have



shown to contain greater muscle mitochondrial content and higher basal mRNA expression of several respiratory chain components such as Cyt c and COX4 when compared with wild-type controls (Calvo et al., 2008). Moreover, PGC-1 $\alpha$  over-expressing mice have shown to possess higher maximal oxygen uptake (VO<sub>2max</sub>) and demonstrated superior exercise capacity during voluntary and forced running (Calvo et al., 2008). However, loss-of-function studies using whole-body or muscle specific PGC-1 $\alpha$  knock-out models have to date demonstrated contradictory results. Leick et al. (2008) first showed that PGC-1 $\alpha$  was not essential for exercise-induced mitochondrial biogenesis, where training-induced increases in COX1, Cyt c and  $\delta$ -aminolevulinic synthase (ALAS) were not impaired in whole-body PGC-1 $\alpha$  knock-out mice. Yet, whole-body PGC-1 $\alpha$  deficiency is not an ideal model to study exercise-induced mitochondrial biogenesis in the skeletal muscle, as it is associated with numerous systemic effects, including hypermetabolism, hyperactivity and reluctance to exercise (Chinsomboon et al., 2009; Lin et al., 2004). Consequently, muscle specific PGC-1 $\alpha$  knock-out models have been recently utilised in this line of research, but again with contradictory findings (Geng et al., 2010; Rowe et al., 2012; Uguccioni & Hood, 2011). Indeed, mitochondrial biogenesis has been shown to be impaired following exercise training or chronic electrical stimulation using a PGC-1 $\alpha$  gene knock-out model in rodent muscles and culture myotubes, respectively (Geng et al., 2010; Uguccioni & Hood, 2011). Conversely, a recent study showed that mitochondrial adaptations following exercise was not impaired in muscle specific PGC-1 $\alpha$  knock-out mice (Rowe et al., 2012). Taken together, while the regulatory role of PGC-1 $\alpha$  in the mitochondrial biogenesis program is well established, its essentiality remains to be fully elucidated.

Recent studies have identified several PGC-1 $\alpha$  isoforms (Popov et al., 2014; Tadaishi et al., 2011; Ydfors et al., 2013) which are mostly exercise sensitive and collectively contribute to the overall increase in skeletal muscle PGC-1 $\alpha$  mRNA observed following exercise (Baar et al., 2002; McGee & Hargreaves, 2004; Pilegaard et al., 2003; Russell et al., 2005). However, PGC-1 $\alpha$  protein content may (Mathai et al., 2008) or may not (McGee & Hargreaves, 2004) be up-regulated following acute exercise, indicating that initial increases in PGC-1 $\alpha$  mRNA do not necessarily lead to increased protein expression. Paradoxically, gene expression of Tfam, Cyt c and the transcriptional activity of NRFs all increased shortly after exercise (Pilegaard et al., 2003; Wright et al., 2007b) indicating that initial events in mitochondrial biogenesis are mediated by the activation/phosphorylation of existing muscle PGC-1 $\alpha$  content. Indeed, Wright et al. (2007b) showed that acute exercise in rodents resulted in the translocation of PGC-1 $\alpha$  from the cytosol to the nucleus, in line with an accompanied increase in NRF1 and NRF2 binding to mitochondrial gene promoters, and subsequent increase in mitochondrial gene transcription (Wright et al., 2007b). Similar findings were demonstrated in humans by Little et al. (2010) who showed a 54% increase in PGC-1 $\alpha$  protein in the nucleus following exercise despite no changes in whole muscle PGC-1 $\alpha$  protein content. In addition to nuclear translocation, PGC-1 $\alpha$  has also shown to localise to the mitochondria following endurance exercise, forming a complex with Tfam (Safdar et al., 2011). This implicates PGC-1 $\alpha$  as a direct co-activator of Tfam, in addition to initiating Tfam translocation through NRF1 (Wu et al., 1999). Taken together, these studies suggest that exercise-induced mitochondrial biogenesis is first mediated by a rapid increase in PGC-1 $\alpha$  activity without the need for an increase in PGC-1 $\alpha$  protein expression. It is also intriguing that PGC-1 $\alpha$ , once activated is involved in an autoregulatory loop where it regulates its own transcriptional expression (Handschin et al., 2003). Indeed, it was shown that phosphorylated PGC-1 $\alpha$  co-activates the transcription factor myocyte enhancer factor 2 (MEF2) which in

turn binds to the PGC-1 $\alpha$  promoter region, regulating its transcriptional activity (Handschin et al., 2003).

It is now well accepted that the activation and up-regulation of PGC-1 $\alpha$  mediates mitochondrial biogenesis. Therefore, it is important to identify the upstream events involved in the transcription and activation of PGC-1 $\alpha$ . Contractile activity causes numerous perturbations disrupting homeostasis, which is purported to in turn act as primary signals, activating signalling cascades inducing PGC-1 $\alpha$  expression and mitochondrial biogenesis. To date, a number of primary signals have been implicated in exercise-induced expression of PGC-1 $\alpha$ , including  $\beta$ -adrenergic stimulation, calcium signalling, changes in phosphorylation potential, reactive oxygen species and nitric oxide signalling. Some of these signalling pathways are also involved in PGC-1 $\alpha$  expression following heat/cold exposures and/or post-exercise heat/cold exposures. The possible role of these signalling mechanisms and redundancy in PGC-1 $\alpha$  expression/activation and subsequent mitochondrial biogenesis during exercise (Fig. 1), passive heat/cold exposures (Fig. 2) and post-exercise heat/cold exposures (Fig. 3) will be discussed.

### $\beta$ -adrenergic Stimulation

As mentioned, PGC-1 $\alpha$  was first found to be induced in brown fat and myotube cultures following exposure to cold and a  $\beta$ -adrenergic agonist, isoproterenol (Puigserver et al., 1998). It is suggested that adrenergic stimulation increases the intracellular content of cyclic adenosine monophosphate (cAMP), which in turn leads to the activation and subsequent phosphorylation of protein kinase A and the cAMP response element binding protein (CREB), respectively (Fig. 1) (Puigserver & Spiegelman, 2003; Puigserver et al., 1998; Wu et al., 1999). It is further hypothesised that CREB directly induces the expression of PGC-1 $\alpha$  by binding to the PGC-1 $\alpha$  promoter region (Puigserver & Spiegelman, 2003; Puigserver et al., 1998; Wu et al., 1999). At rest, treatments with selective  $\beta$ -adrenergic agonist/antagonist have shown to induce or inhibit PGC-1 $\alpha$  expression in rodents, respectively (Miura et al., 2007). Moreover in rodents, treatment with  $\beta$ -adrenergic antagonists have shown to attenuate the increase in PGC-1 $\alpha$  mRNA following acute exercise (Miura et al., 2007) and mitochondrial biogenesis following endurance training (Ji et al., 1986). These studies support the findings by Puigserver et al. (1998) and implicate adrenergic mechanisms in the regulation of PGC-1 $\alpha$  and mitochondrial biogenesis. In human models however, the evidence is less clear regarding an adrenergic regulation of PGC-1 $\alpha$  (Marsh et al., 1983; Robinson et al., 2011; Robinson et al., 2010; Sable et al., 1982; Svedenhag et al., 1984; Wolfel et al., 1986). For instance, acute treatment with  $\beta$ -adrenergic blockers has shown to attenuate post-exercise mitochondrial protein synthesis (Robinson et al., 2011), while chronic ingestion during 5 to 8 weeks of intensive endurance training significantly attenuated improvements in VO<sub>2max</sub> (Marsh et al., 1983; Sable et al., 1982) and the enzyme activities of complex-II, COX and  $\beta$ -3-hydroxyacyl-CoA dehydrogenase ( $\beta$ -HAD) (Svedenhag et al., 1984). Conversely, acute infusion of adrenergic agonist or chronic ingestion of adrenergic blockers of similar dosage to previously mentioned studies (Marsh et al., 1983; Sable et al., 1982; Svedenhag et al., 1984), has also shown to have no effect on PGC-1 $\alpha$  expression and mitochondrial protein synthesis following acute exercise (Robinson et al., 2010) or mitochondrial biogenesis following 6 weeks of endurance training (Wolfel et al., 1986). Reasons for these discrepancies are currently unclear but could be a function of experimental methods used (i.e. agonists vs. antagonists) as well as complications associated with adrenergic blockers treatments, such as; the possibility of incomplete adrenergic blockade and dissimilarities in cardiovascular responses together with exercise capacities between subjects treated with blockers and controls (Marsh et



al., 1983; Robinson et al., 2010; Sable et al., 1982). However, it is also plausible that during exercise, calcium, phosphagen or reactive oxygen species (ROS) mediated regulatory pathways may compensate for the decreased  $\beta$ -adrenergic signalling and consequently enable sufficient stimulation to enable PGC-1 $\alpha$  expression and mitochondrial biogenesis (Feng et al., 2013).

## Calcium

There is evidence to suggest that cytosolic calcium ( $\text{Ca}^{2+}$ ) concentrations play a vital role in inducing PGC-1 $\alpha$  expression in skeletal muscles (Fig. 1). Indeed, basal PGC-1 $\alpha$  promoter activity has shown to be more sensitive to resting  $\text{Ca}^{2+}$  levels compared with adenosine monophosphate-activated protein kinase, ROS or p38 signalling (Zhang et al., 2014). Moreover, mitochondrial content has been found to increase in muscle cell cultures following 2 to 5 days of treatment with  $\text{Ca}^{2+}$  ionophore or caffeine (Irrcher et al., 2003; Ojuka et al., 2003). This increase was accompanied by an increase in PGC-1 $\alpha$  protein expression (Irrcher et al., 2003; Ojuka et al., 2003) and the expression (Tfam and ALAS) and transcriptional activity (NRF1 and NRF2) of key factors associated with mitochondrial biogenesis (Freyssenet et al., 1999; Ojuka et al., 2003). In contrast, cells treated with dantrolene which inhibits caffeine mediated  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum abolishes the increase in PGC-1 $\alpha$ , Tfam and the transcriptional activities of NRF1 and NRF2 (Ojuka et al., 2003).

$\text{Ca}^{2+}$  mediated PGC-1 $\alpha$  expression is likely to be controlled through the  $\text{Ca}^{2+}$  sensitive enzymes, calcineurin and  $\text{Ca}^{2+}$ -calmodulin-dependant kinase (CaMK). Supporting this, transgenic mice over-expressing an isoform of CaMK (CaMKIV) have been found to contain higher PGC-1 $\alpha$  mRNA, Cyt c protein content and have reduced fatigability during *in vitro* stimulations when compared with wild-type controls (Wu et al., 2002). In humans however, CaMKIV is not expressed while CaMKII seems to be the dominant isoform (Rose & Hargreaves, 2003). The role of CaMK and calcineurin in  $\text{Ca}^{2+}$  mediated expression of PGC-1 $\alpha$  has also been established using pharmacological treatments and electrical stimulation models (Kusuhara et al., 2007; Ojuka et al., 2003). For instance, ionomycin (an agent that increases membrane permeability to  $\text{Ca}^{2+}$ ) or electrical stimulation-induced increases in PGC-1 $\alpha$  expression in rat muscle cell cultures has shown to be reduced or abolished when co-treated with KN-62 (CaMK inhibitor) or cyclosporin A (calcineurin inhibitor), respectively (Kusuhara et al., 2007). Similar findings were reported in the study by Ojuka et al. (2003) where co-treatment with caffeine and KN-93 (a CaMK inhibitor) abolished the caffeine mediated increases in CS activity and protein expressions of ALAS, COX1 and Cyt c. While calcineurin is suggested to induce PGC-1 $\alpha$  by activating CREB through MEF2 (Akimoto et al., 2005), it is well regarded that CaMK predominantly acts through the p38 mitogen activated protein kinase (p38 MAPK) pathway, which in turn activates the transcription factor activating transcription factor 2 (ATF2) in inducing PGC-1 $\alpha$  and mitochondrial biogenesis (Akimoto et al., 2005; Wright et al., 2007a). Indeed, increased expressions of PGC-1 $\alpha$ , COX1, ALAS and CS, along with p38 MAPK and ATF2 phosphorylation were evident in rat epitrochlearis muscles following caffeine incubation (Wright et al., 2007a). However, these adaptations were attenuated following co-incubation with a p38 or CaMKII inhibitor (Wright et al., 2007a). Collectively, these studies provide strong evidence that increases in cytosolic  $\text{Ca}^{2+}$  induces PGC-1 $\alpha$  expression and subsequent mitochondrial biogenesis via parallel pathways involving CaMK (via p38-ATF2 activation) and calcineurin (via MEF2-CREB activation).

## AMP/ATP ratio

In addition to  $\text{Ca}^{2+}$  release, the increase in cellular adenosine monophosphate (AMP): adenosine triphosphate (ATP) ratio and subsequent activation of the AMP-activated protein kinase (AMPK) is another fundamental occurrence during excitation-contraction coupling. AMPK is widely termed as the “fuel sensor” due to its central role in inhibiting and activating molecular cascades that consume and generate ATP during energy depletion, respectively (Aschenbach et al., 2004; Winder, 2001). Apart from its critical role in regulating acute changes in intracellular energy metabolism, AMPK activation has also shown to regulate the gene expression and activation of PGC-1 $\alpha$  (Fig. 1). Presumably this AMPK regulated increase in PGC-1 $\alpha$  enhances mitochondrial biogenesis, thus resulting in long term adaptations that enhance the rate of ATP production (Aschenbach et al., 2004; Coffey & Hawley, 2007).

Initial mechanistic evidence implicating AMPK in mitochondrial biogenesis came from cell culture studies. For instance, acute (18-42 h) and chronic treatment (4-9 weeks) with AMP or creatine analogues, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) and  $\beta$ -Guanadinopropionic, respectively, has shown to increase PGC-1 $\alpha$  mRNA and protein expression and associated increase in mitochondrial density (Bergeron et al., 2001; Irrcher et al., 2003; Terada et al., 2002; Winder et al., 2000; Zong et al., 2002). Moreover, electrical stimulation mimicking exercise has shown to increase AMPK activity, PGC-1 $\alpha$  protein content in C2C12 myoblasts and isolated rodent muscle preparations, respectively (Atherton et al., 2005; Irrcher et al., 2003). It has also been shown that expression of PGC-1 $\alpha$  and subsequent mitochondrial biogenesis is suppressed in transgenic mice expressing a negative mutation of AMPK (Zong et al., 2002), highlighting the importance of AMPK activation in PGC-1 $\alpha$  mediated mitochondrial biogenesis. In humans, it has been shown that endurance trained individuals possessed higher basal AMPK protein content compared with sedentary counterparts (Nielsen et al., 2003) and that regular endurance training enhances AMPK protein content and resting AMPK activity along with evidence of mitochondrial biogenesis (i.e. increase in  $\beta$ -HAD and CS activity) (Frosig et al., 2004). More recently, post-exercise AMPK phosphorylation was shown to increase (Bartlett et al., 2012; Gibala et al., 2009; Little et al., 2011; Little et al., 2010) in line with an increase in the nuclear abundance of PGC-1 $\alpha$  protein (activation) (Little et al., 2011; Little et al., 2010). These initial events were followed by a more gradual up-regulation in PGC-1 $\alpha$  mRNA and protein content between 3 h (Bartlett et al., 2012; Little et al., 2011; Little et al., 2010) and 24 h (Little et al., 2011) post-exercise, respectively. Overall, there is strong collective evidence from cell culture: rodent and human models implicating AMPK in the activation and induction of PGC-1 $\alpha$  and subsequent mitochondrial biogenesis.

Unlike other pathways where PGC-1 $\alpha$  is activated via other messengers and/or transcription factors, AMPK have shown to physically interact with PGC-1 $\alpha$ , phosphorylating it directly at distinct sites (Jager et al., 2007). Furthermore, PGC-1 $\alpha$  phosphorylation by AMPK has also shown to increase the PGC-1 $\alpha$  promoter activity and consequently its own transcriptional regulation (Jager et al., 2007). Apart from direct phosphorylation, there is evidence suggesting that AMPK might also increase PGC-1 $\alpha$  activity by activating the silent mating type information regulator 2 homolog 1 (SIRT1), which has shown to deacetylate PGC-1 $\alpha$ , consequently increasing its transcriptional activity (Suwa et al., 2008; Suwa et al., 2011). However, it must be

noted that a recent study showed no differences in skeletal muscle endurance, PGC-1 $\alpha$  expression, signalling and mitochondrial biogenesis following endurance training in SIRT1 deficient mice compared with controls, indicating that SIRT1 deacetylation of PGC-1 $\alpha$  may not be essential for exercise-induced mitochondrial biogenesis (Philp et al., 2011).

## Reactive Oxygen Species

ROS is the general term ascribed to oxygen centred radicals with one or more unpaired electrons. The primary ROS produced in biological systems is the superoxide anion which can occur at multiple sites within the muscle fibre including the mitochondria, sarcoplasmic reticulum, transverse tubules, sarcolemma and the cytosol (Jackson, 2011; Jackson et al., 2007; Powers et al., 2009). Superoxide anion rapidly dismutates to hydrogen peroxide upon production (Jackson et al., 2007). Hydrogen peroxide in addition to having a relatively long half-life, readily diffuses within or between cells, increasing the likelihood of reacting with other targets. These properties render hydrogen peroxide as an important ROS signalling molecule in skeletal muscle cells which has been shown to activate many "redox sensitive" cascades including growth, differentiation, proliferation and apoptosis (McArdle & Jackson, 2000; Powers et al., 2009; Powers et al., 2011).

The involvement of ROS in the expression of PGC-1 $\alpha$  was first demonstrated in myotube cultures, where the up-regulation of PGC-1 $\alpha$  mRNA following acute and chronic electrical stimulation, or hydrogen peroxide incubation was attenuated in the presence of antioxidants or ROS scavengers, respectively (Irrcher et al., 2009; Silveira et al., 2006). Intriguingly, ROS has shown to induce/activate PGC-1 $\alpha$  via multiple signalling pathways involving AMPK, p38 MAPK and nitric oxide signalling. For instance, ROS may initiate PGC-1 $\alpha$  transcription and activity via AMPK, as C2C12 myotubes incubated with hydrogen peroxide increased AMPK phosphorylation and subsequently PGC-1 $\alpha$  activity and mRNA expression (Irrcher et al., 2009). Further, p38 MAPK activation and endothelial nitric oxide synthase expression, both upstream targets of PGC-1 $\alpha$ , has shown to be attenuated in exercising rats administered with allopurinol (Gomez-Cabrera et al., 2005). More recently, in myotube cultures, it was shown that p38 MAPK activation as well as PGC-1 $\alpha$  activation and promoter activity following electrical stimulation was attenuated when incubated with a ROS scavenger (Zhang et al., 2014). These data consequently highlight ROS as an important primary stimulus, due to the numerous pathways (AMPK, nitric oxide and p38MAPK) it may act through in activating the PGC-1 $\alpha$  program and associated adaptations.

While these *in vitro* studies provide a sound mechanistic link between ROS and contraction-induced PGC-1 $\alpha$  expression, *in vivo* evidence regarding the role of ROS signalling in PGC-1 $\alpha$  expression is variable. For instance, the administration of vitamin C during 3 to 6 weeks of endurance training has been shown to attenuate the mRNA and protein content of PGC-1 $\alpha$ , NRF1 and Tfam, along with increases in muscle mitochondrial content in rodents (Gomez-Cabrera et al., 2008). Likewise, impaired mitochondrial biogenesis (suppressed CS activity) and reduced PGC-1 $\alpha$  mRNA and protein content has been observed in rodents fed with antioxidant rich diet during 14 weeks of endurance training (Strobel et al., 2011). Similar findings have been observed in humans, where combined vitamin C and E supplementation during 4 weeks of endurance training (5 days $\cdot$ wk $^{-1}$ ) resulted in lower basal PGC-1 $\alpha$  mRNA expression in both trained and untrained participants (Ristow et al., 2009). However, PGC-1 $\alpha$  protein content or markers of mitochondrial content were not determined in this study (Ristow et al., 2009). Collectively, these studies indicate that exercise-induced ROS

production has a functional role in the induction of PGC-1 $\alpha$  and mitochondrial biogenesis.

In contrast to the above findings, antioxidant supplementation in rodents has shown to have no effect on the expression of mitochondrial transcription factors (NRF1, NRF2 and Tfam) respiratory chain complexes (I-V), PGC-1 $\alpha$  or upstream signalling activity (p38 MAPK and AMPK) following acute and chronic endurance exercise (Higashida et al., 2011; Wadley & McConell, 2010). Likewise, research within humans has shown that Vitamin C and E supplementation during a 12-week intense endurance training program had no influence on improvements in VO $_{2\max}$ , power output at VO $_{2\max}$  and power output at lactate threshold (Yfanti et al., 2010). Moreover, muscle sample analysis within this study revealed similar increases in CS and  $\beta$ -HAD activity indicating a similar extent of local muscle aerobic adaptation (Yfanti et al., 2010). The disparities in findings within the literature are difficult to reconcile but are potentially related individual differences in response to antioxidant loading and exercise stimulus. Nevertheless, cell culture studies provide direct mechanistic evidence for ROS mediated induction and activation of PGC-1 $\alpha$  and as such cannot be discounted as a possible mechanism of mitochondrial biogenesis (Irrcher et al., 2009; Silveira et al., 2006).

## Nitric Oxide

Nitric oxide (NO) is a lipid soluble gas implicated in a wide array of biological functions such as vascular function (Kooijman et al., 2008), capillarization (Gavin, 2009), blood flow regulation (Radegran & Saltin, 1999), muscle contractile function (Kobzik et al., 1994) and cell signalling (Steensberg et al., 2007). NO is synthesised enzymatically by three recognised NO synthase (NOS) isoforms termed; neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS), with nNOS being the primary isoform in the skeletal muscle (Frandsen et al., 1996; Rudnick et al., 2004). nNOS and eNOS are primarily localised in the sarcolemma and vascular endothelium, respectively (Frandsen et al., 1996), although both isoforms also exist in the mitochondria (Frandsen et al., 1996; Kobzik et al., 1994). iNOS is not constitutively expressed in skeletal muscles but may be induced by inflammatory stress associated with exercise (Niess et al., 2000).

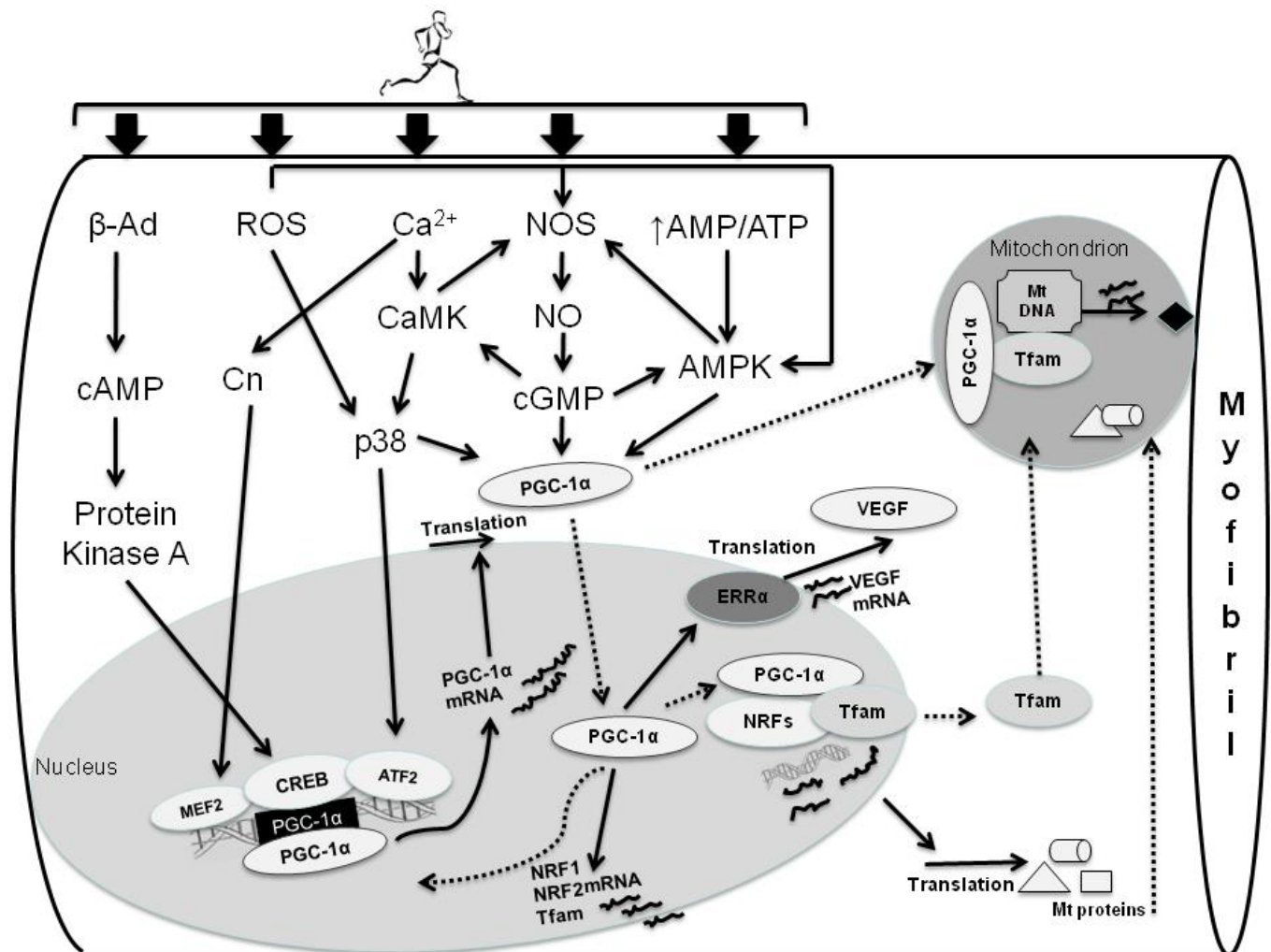
Amongst its many biological functions, recent studies have highlighted the important role of NO signalling in the expression of PGC-1 $\alpha$  and thus mitochondrial biogenesis (Fig. 1). For instance, treatment with NO donors has shown to increase the mRNA expression of PGC-1 $\alpha$ , NRF1 and Tfam and several other respiratory chain components in muscle cell cultures (Nisoli et al., 2004). In addition, eNOS deficient mice have shown to have reduced mitochondrial density when compared with wild-type controls (Nisoli et al., 2004). Guanosine monophosphate (cGMP) signalling appears to be an important pathway by which NO induces PGC-1 $\alpha$  expression. Indeed, in similar fashion to NO donors, cGMP analogues have shown to increase the mRNA expression of PGC-1 $\alpha$  and other mitochondrial transcription factors and proteomes (Nisoli et al., 2004). In addition, pharmacologically inhibiting cGMP synthesis abolished these effects, highlighting the important role of cGMP in NO induced PGC-1 $\alpha$  expression (Nisoli et al., 2004).

In addition to ROS signalling, AMPK and Ca $^{2+}$  signalling pathways have also been implicated in NO mediated PGC-1 $\alpha$  expression (Fig. 1) (Lira et al., 2010; McConell et al., 2010). In a recent study, McConell et al. (2010) showed that AMPK and CaMK were activated in L6 myocytes treated with NO donors, in line with increased protein expressions of PGC-1 $\alpha$ , COX1 and COX4. Interestingly, attenuated expression of COX1 and COX4 protein was observed in myotubes co-incubated with NOS



inhibitor, N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) with either AICAR or caffeine when compared with incubation with AICAR or caffeine alone (McConnell et al., 2010). This indicates that NO signalling is both upstream and downstream of AMPK and CaMK, respectively. Similar findings were demonstrated by Lira et al. (2010), where pharmacological inhibition of AMPK attenuated PGC-1 $\alpha$  mRNA expression, despite co-treatment with NO donors. It was also shown that PGC-1 $\alpha$  mRNA expression was attenuated when muscle cells were treated with NOS inhibitor, L-NAME despite co-treatment with AICAR. Collectively, these data indicate a possible feedback loop involving NO, AMPK and CaMK in the regulation PGC-1 $\alpha$  expression, where AMPK and CaMK activates NOS, and NO production in turn activates AMPK and CaMK (Fig. 1). Despite the strong mechanistic link established by these cell culture models, existing data does not support a role for NO signalling in exercise-induced mitochondrial biogenesis. For instance, both eNOS and nNOS knock-out mice, although possessing lower

basal PGC-1 $\alpha$  expression, have been shown to have similar responses in PGC-1 $\alpha$  and NRF2 mRNA expression following acute exercise (Wadley et al., 2007). Likewise, the ingestion of the NOS inhibitor (L-NAME) has shown to have no influence on changes in PGC-1 $\alpha$  mRNA and other markers of mitochondrial biogenesis (CS, COX and  $\beta$ -HAD activities) following an acute bout of exercise in rats, despite resulting in decreased basal mRNA of several respiratory chain proteins (Wadley & McConnell, 2007). The limited evidence for NO mediated increases in PGC-1 $\alpha$  following exercise may be due redundancy of signalling cascades, whereby primary signals such as cellular Ca<sup>2+</sup> or phosphagen ratio may sufficiently induce/activate PGC-1 $\alpha$  and mitochondrial biogenesis. Regardless, NO seems to have a role in the basal regulation of mitochondrial content (McConnell & Wadley, 2008; Wadley et al., 2007; Wadley & McConnell, 2007) and hence may be crucial to long term mitochondrial adaptations in response to exercise.



**Figure 1.** Up-regulation and activation of PGC-1 $\alpha$  by exercise and the subsequent effect on the regulation of mitochondrial and nuclear encoded genes and VEGF. Exercise up-regulates PGC-1 $\alpha$  through multiple signalling pathways including  $\beta$ -adrenergic stimulation,  $\text{Ca}^{2+}$ , ROS, NO and AMPK mediated mechanisms. There seems to be regulatory feedback loop involving CaMK, AMPK with NO signalling, where NO mediated cGMP signalling activates AMPK and CaMK which in turn activates NOS. ROS appears to activate numerous downstream cascades including p38, NO and AMPK signalling. PGC-1 $\alpha$  protein resides in the cytosol and translocates to the nucleus and mitochondria upon activation. In the cytosol, it induces the expression of key transcription factors such as NRFs and Tfam. Moreover, PGC-1 $\alpha$  physically binds to and co-activates NRFs and Tfam in the nucleus and mitochondria, respectively, leading to the transcription of nuclear and mitochondria encoded genes. PGC-1 $\alpha$ , once activated, auto-regulates its own transcription through co-activating MEF2 and subsequently CREB. Encoded genes undergo translation in the cytosol and are subsequently imported into the mitochondria for assembly. PGC-1 $\alpha$  also regulates vascular adaptations by inducing the expression of VEGF through ERR $\alpha$ .



## Other PGC-1 $\alpha$ Mediated Adaptations Related to the Oxidative Phenotype:

### VEGF and Angiogenesis

In addition to PGC-1 $\alpha$  being regarded as a key regulator of mitochondrial biogenesis (Puigserver & Spiegelman, 2003; Puigserver et al., 1998; Wu et al., 1999), recent evidence has implicated PGC-1 $\alpha$  in mediating other adaptations contributing to the oxidative phenotype of the muscle. Angiogenesis refers to the formation of new capillaries from existing capillaries (Distler et al., 2003; Prior et al., 2004) and is considered one of the fundamental adaptations resulting from endurance based exercise (Brodal et al., 1977; Carrow et al., 1967; Cotter et al., 1973). The increase in muscle capillary density reduces the capillary-muscle fibre O<sub>2</sub> diffusion distance while increasing blood-capillary transit time at high cardiac outputs (Richardson et al., 1995). This evidently improves O<sub>2</sub> transport into the mitochondria, contributing to ones VO<sub>2max</sub> and endurance performance (Richardson et al., 1995).

One factor deemed critical to the angiogenic process is the vascular endothelial growth factor (VEGF) (Distler et al., 2003; Prior et al., 2004). The overall significance of VEGF to angiogenesis and resulting exercise performance was recently demonstrated by Olfert and colleagues (Olfert et al., 2009; Olfert et al., 2010). For instance, transgenic mice with muscle-specific VEGF gene knock-out displayed a 90% reduction in VEGF protein levels in the gastrocnemius muscles in line with a 48% and 39% reduction in capillary-to-fibre ratio and capillary density, respectively (Olfert et al., 2009). Not surprisingly, performance during running time to exhaustion and maximal aerobic running speed during a graded exercise test was lower in the VEGF knock-out mice by 81% and 34%, respectively (Olfert et al., 2009). In a follow up investigation, 6 weeks of endurance training increased muscle capillary density (59%) and capillary-to-fibre ratio (33%) in wild-type mice but no such adaptations were observed in mice with muscle-specific VEGF knock-out (Olfert et al., 2010). Accordingly, training-induced improvements in exercise capacity were only evident in the wild-type controls where a 13% and 20% improvement in running time to exhaustion and maximal aerobic running speed were noted (Olfert et al., 2010), implicating VEGF mediated increases in capillary density as a key adaptation necessary for performance gains.

Evidence for a role for PGC-1 $\alpha$  in skeletal muscle angiogenesis was first demonstrated by Arany et al. (2008) where C2C12 myotubes transfected with virus expressing PGC-1 $\alpha$  resulted in a 14-fold increase in VEGF mRNA expression. In addition, up-regulation of VEGF mRNA following O<sub>2</sub> deprivation treatment (Arany et al., 2008) or  $\beta$ -adrenergic stimulation (Chinsomboon et al., 2009) has shown to be significantly lower in muscle cells derived from PGC-1 $\alpha$  knock-out mice, compared with wild-type controls. The necessity of PGC-1 $\alpha$  in VEGF mediated angiogenesis has also been demonstrated using exercise models, where capillary density was shown to be significantly increased in wild-type controls compared with PGC-1 $\alpha$  muscle knock-out mice following 14 days of voluntary running (Chinsomboon et al., 2009). Collectively, these studies therefore indicate that in addition to regulating mitochondrial biogenesis, PGC-1 $\alpha$  also has a role in angiogenic adaptations through its influence on VEGF. As such, strategies that enhance the expression of PGC-1 $\alpha$  could potentially have an effect on VEGF and angiogenic processes.

### Fat Oxidation

The nuclear receptor peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) has long been identified as a receptor involved in the transcriptional regulation of genes involved in fat metabolism (Gilde & Van Bilsen, 2003). However, first evidence elucidating a role for PGC-1 $\alpha$  in fat oxidation stems from the study by Vega et al. (2000). In this study, ectopic expression of either PPAR $\alpha$  or PGC-1 $\alpha$  alone resulted in only a modest increase in key  $\beta$ -oxidation enzymes; medium and long chain acyl coenzyme A dehydrogenase (MCAD & LCAD) and carnitine palmitoyltransferase 1 (CPT1). In contrast, ectopic expression of both PPAR $\alpha$  and PGC-1 $\alpha$  resulted in a marked increase in the mRNA expression MCAD, LCAD and CPT1 (Vega et al., 2000). This indicates that PPAR $\alpha$  and PGC-1 $\alpha$  work cooperatively in regulating the expression of genes involved in  $\beta$ -oxidation.

A subsequent study by Wende et al. (2007) showed that PGC-1 $\alpha$  transgenic mice over-expressing PGC-1 $\alpha$  exhibited higher basal mRNA expression of MCAD, CPT1 and sarcolemma fatty acid transporter CD36 compared with controls. Similar findings were reported by Calvo et al. (2008), where mice over-expressing PGC-1 $\alpha$  had higher basal mRNA content of  $\beta$ -oxidation proteins (MCAD, LCAD & CD36), compared with wild-type controls. These findings highlight the pivotal role of PGC-1 $\alpha$  in the transcriptional control of proteins involved in fatty acid transport and oxidation, consequently enhancing the overall capacity for fatty acid oxidation.

PGC-1 $\alpha$  also seems to increase fat oxidation by suppressing glycolysis. Evidence from this comes from the observation that PGC-1 $\alpha$  regulates the expression of pyruvate dehydrogenase kinase 4, which inactivates the pyruvate dehydrogenase complex, an enzyme responsible for catalyzing the conversion of pyruvate to acetyl coenzyme A and hence glucose oxidation (Kolobova et al., 2001; Wende et al., 2005). Taken together, PGC-1 $\alpha$  seems to have an important role in enhancing fat oxidation capacity and may mediate classic adaptation of increased fat utilization following an exercise training regime.

## Role of Temperature in PGC-1 $\alpha$ Mediated Adaptations

### Passive Heat Exposure

The production of metabolic heat during exercise appears to have a role in muscle oxidative phenotype transformations (Harris et al., 2003; Hooper, 1999; Liu & Brooks, 2012; Naylor et al., 2011; Yamaguchi et al., 2010). This is inferred from recent studies that have demonstrated that heat stress may result in similar muscle adaptations to that observed following endurance exercise (Harris et al., 2003; Hooper, 1999; Liu & Brooks, 2012; Naylor et al., 2011; Yamaguchi et al., 2010). For instance, mild heat stress (39-40°C) has been shown to increase PGC-1 $\alpha$  activity and induce PGC-1 $\alpha$  mRNA and protein expression in a variety of muscle cell lines (Liu & Brooks, 2012; Yamaguchi et al., 2010), in addition to an increase in the mRNA content of key mitochondrial transcription factors (NRF1, NRF2 and Tfam), complex-IV subunits (COX2 and COX4) as well as glucose transporter 4 (GLUT4) mRNA (Liu & Brooks, 2012). In humans, heat exposure has shown to induce adaptations where PGC-1 $\alpha$  may potentially be involved (Michael et al., 2001; Prior et al., 2004). These adaptations include decreases in resting blood glucose concentration, improved brachial artery function and remodelling (Hooper, 1999; Naylor et al., 2011).

Although evidence suggests that elevations in muscle tissue temperature may influence muscle aerobic adaptations, much research is still needed to identify the signalling pathways involved (Harris et al., 2003; Liu & Brooks, 2012; Yamaguchi et al., 2010). Current evidence indicates that PGC-1 $\alpha$  expression/activation and associated adaptations such as mitochondrial biogenesis and GLUT4 expression following heat stress may be via mechanisms involving NOS, SIRT1, and possibly AMPK signalling (Fig. 2). For instance, endothelial cell cultures subjected to heat treatment have shown to increase both eNOS activity and expression (Harris et al., 2003). Moreover, heat treatment has also shown to increase AMPK activity and SIRT1 expression, in line with increased PGC-1 $\alpha$  and GLUT4 expression, downstream transcription factors (NRFs and Tfam) and respiratory chain complexes I-V in muscle cell cultures (Brooks et al., 1971). However, a recent study reported decreased AMPK signalling in plantaris and soleus muscles of mice subjected to acute heat exposure (Tamura et al., 2014). Chronic exposure (5 weeks) resulted in increased CS and  $\beta$ -HAD activities as well as protein content of respiratory complexes I-V, however AMPK signalling was not investigated following chronic exposure in this study (Tamura et al., 2014). This disparity in results could be a function of the experimental models used (i.e. *in vivo* vs. *in vitro*) and/or heat exposure conditions (temperature and duration) (Liu & Brooks, 2012; Tamura et al., 2014).

There is some preliminary evidence that other primary signals may also be involved in heat-induced muscle oxidative phenotype transformations (Fig. 2). For instance, short term heat stress (30–45 min at 40–43°C) has shown to result in increased ROS formation and Ca<sup>2+</sup> leakage from sarcoplasmic reticulum in rat diaphragm and skeletal muscle fibres, respectively (van der Poel & Stephenson, 2007; Zuo et al., 2000). However, while both ROS and Ca<sup>2+</sup> are well known primary signals responsible for the induction/activation of PGC-1 $\alpha$  and mitochondrial biogenesis, CaMKII, a downstream target of Ca<sup>2+</sup>, and; p38 MAPK, a downstream target of both ROS and Ca<sup>2+</sup> (Gomez-Cabrera et al., 2005; Irrcher et al., 2009; Zhang et al., 2014) have shown to not activate following heat treatment *in vitro* (Yamaguchi et al., 2010) and *in vivo* (Tamura et al., 2014). As such, it seems that CaMKII and p38 MAPK signalling are not involved in heat-induced PGC-1 $\alpha$  mechanisms and ROS and Ca<sup>2+</sup> might act through AMPK-NOS pathways in up-regulating PGC-1 $\alpha$  and mediating associated adaptations (Fig. 2).

### Post-exercise Heat Exposure

In contrast to passive exposure, acute post-exercise heat exposure has shown to significantly increase p38 MAPK activation compared with exercise per se (Tamura et al., 2014). Interestingly, AMPK signalling was down-regulated and no changes were evident in CaMKII signalling compared with control and exercise (Tamura et al., 2014). Moreover in this study, CS and  $\beta$ -HAD activities, along with the expression of respiratory chain complexes were significantly higher following 5 weeks of post-exercise heat treatment, compared with exercise (Tamura et al., 2014). While the signalling pathways were not investigated following chronic phase of this study, acute mechanisms suggest that mitochondrial biogenesis following post-exercise heat treatment was mediated primarily via p38 MAPK signalling (Fig. 3) (Tamura et al., 2014).

We are aware of only one study that has investigated the effect of post-exercise heat exposure on muscle oxidative adaptations in humans (Slivka et al., 2012). In this study, it was shown that passive recovery following exercise undertaken in hot ambient conditions (3 h at 33°C) resulted in reduced PGC-1 $\alpha$  mRNA expression compared with recovery undertaken in neutral (3 h at 20°C) and cold conditions (3 h at 7°C). The considerable

disparity in results between rodent and human models may be due to the duration of exposure (30 min vs. 3 h) and consequently changes in body temperature. Indeed, it's possible that while mild heat exposure may accentuate muscle oxidative adaptations to exercise, prolonged heat exposure may result in detrimental effects. Dose-response studies, investigating the relationship between increases in post-exercise body temperature and oxidative adaptations are certainly required in the future to ascertain this.

### Passive Cold Exposure

Considering that heat exposure has shown to induce PGC-1 $\alpha$  (Liu & Brooks, 2012; Yamaguchi et al., 2010), it is interesting that PGC-1 $\alpha$  was first discovered in a study investigating the regulation of uncoupling proteins in response to cold exposure (Puigserver et al., 1998). In this study, it was shown that PGC-1 $\alpha$  mRNA was significantly induced in brown fat of mice following 3 to 12 h of cold exposure at 4°C, with parallel increases in several respiratory chain subunits; COX2, COX4 and ATP synthase (Puigserver et al., 1998). Further evidence may be gathered from studies on hibernating species, where PGC-1 $\alpha$  expression has shown to be readily induced in a variety of organs including skeletal muscles following cold exposure (Eddy & Storey, 2003). It is suggested that cold exposure induces PGC-1 $\alpha$  via adrenergic stimulation and subsequently CREB binding to the PGC-1 $\alpha$  promoter region (Fig. 2), as similar up-regulation in PGC-1 $\alpha$  mRNA was evident when brown fat and muscle culture mediums were treated with a  $\beta$ -adrenergic agonist, isoproterenol (Puigserver et al., 1998).

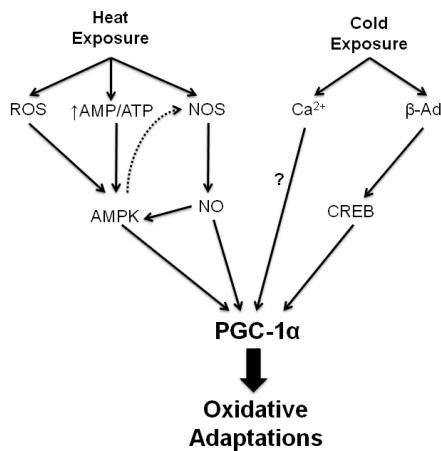
The finding that mitochondrial biogenesis may be up-regulated by cold exposure is attractive, as it is a feasible option to then formulate temperature related therapeutic/ergogenic programs in both athletic and clinical settings. In this regard, it is puzzling that there has been very little research investigating muscle oxidative adaptations in response to cold exposure using *in vivo* models (Bruton et al., 2010; Walters & Constable, 1993; Wijers et al., 2008; Wijers et al., 2011). In an early study, a 22 to 23% increase in CS activity, indicating an increase in mitochondrial content was observed in rat skeletal muscles following daily (5 days·wk<sup>-1</sup>) cold water immersion (60 min at 20°C) over a 17 to 19 wk period (Walters & Constable, 1993). However, in this study it was possible that mitochondrial biogenesis were the result of muscle contractions associated with shivering thermogenesis. However, a more recent study has observed a 50% increase in PGC-1 $\alpha$  protein expression as well as increased CS and  $\beta$ -HAD activities following cold acclimatisation (18°C for 4 wk followed by 4°C for 4–5 wk's) in mice flexor digitorum brevis muscles, which do not partake in the shivering response (Bruton et al., 2010). In addition, the decrement in force was 40% greater in the control compared with the cold acclimatised mice during stimulated repetitive contractions, indicating improved fatigue resistance following cold acclimatisation (Bruton et al., 2010). It was also found that basal muscle Ca<sup>2+</sup> content was 50% higher, while no indication of AMPK phosphorylation were evident in the cold acclimatised mice compared with controls (Bruton et al., 2010). As such, while it is possible that Ca<sup>2+</sup>, in addition to  $\beta$ -adrenergic mechanisms could be involved in cold-induced PGC-1 $\alpha$  and mitochondrial biogenesis, targets downstream of Ca<sup>2+</sup> signalling have yet to be elucidated (Fig. 2).

Very few studies have investigated the effect of passive cold exposure on muscle aerobic adaptations in humans as well (Wijers et al., 2008; Wijers et al., 2011). Studies by Wijers et al. (2008; 2011) reported no changes in CS activity or in the protein content of respiratory chain complexes (I–V) following a 48 to 82 h, 16°C non-shivering cold exposure (Wijers et al., 2008; Wijers et al., 2011). Although the exposure increased energy





expenditure, indicating an increase in non-shivering thermogenesis and slightly but significantly decreased body temperatures, these changes were probably insufficient to induce mitochondrial biogenesis, at least within the 48 to 82 h period.



**Figure 2.** Primary signals activating the PGC-1 $\alpha$  program following passive heat exposure include changes in AMP/ATP ratio, ROS and NO signalling. These primary signals seem to activate PGC-1 $\alpha$  mainly through AMPK and possibly through NO mediated cGMP signalling, with a potential feedback loop between NO and AMPK. Cold exposure activates PGC-1 $\alpha$  via  $\beta$ -adrenergic stimulation and Ca<sup>2+</sup> mediated mechanisms although the specific pathways downstream of Ca<sup>2+</sup> in cold-induced PGC-1 $\alpha$  activation have yet to be elucidated.

### Post-exercise Cold Exposure

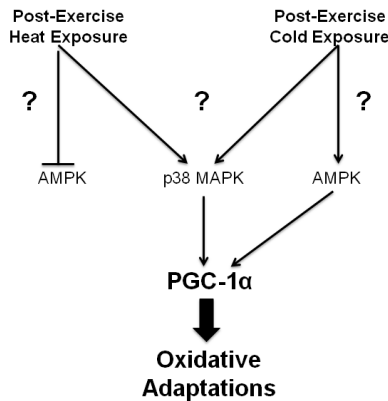
As opposed to passive cold exposure, more studies have investigated the effects of cold exposure following exercise instead (Halsen et al., 2014; Ihsan et al., 2014; Slivka et al., 2013; Slivka et al., 2012; Yamane et al., 2006). In an early study, it was shown that improvements in VO<sub>2max</sub> and cycling time to exhaustion during single leg cycling following 4 weeks of endurance training were attenuated in the leg subjected to regular post-exercise cooling (2 x 20 min at 5°C) (Yamane et al., 2006). Moreover, it was reported that resting femoral artery diameter in the control limb was significantly increased following endurance training while no such adaptation was observed in the limb subjected to regular cooling, indicating the possibility of attenuated vascular adaptations to training (Yamane et al., 2006). Indeed, cold water immersion could potentially retard vascular adaptations through its ability to decrease post-exercise muscle blood flow/perfusion (Gregson et al., 2011; Ihsan et al., 2013a; Mawhinney et al., 2013) and consequently minimising shear stress on the vessel walls, an important stimulus for vascular adaptations (Naylor et al., 2011). This is in contrast with a recent finding where some benefits in cycling performance were evident following regular whole body post-exercise cold water immersion over a 5-week training block (Halsen et al., 2014). However, it must be mentioned that in this study, performance testing was preceded by cold water immersion interventions the day before (Halsen et al., 2014). As such, it is difficult to ascertain whether performances were maintained/enhanced by the recovery effects of cold water immersion or due to enhanced adaptations per se, as day to day training performances have shown to be better maintained following cold water immersion treatment (Vaile et al., 2008).

Data at the molecular level seems conflicting as well regarding the role of post-exercise cold exposure on muscle oxidative adaptations to exercise (Ihsan et al., 2014; Slivka et al., 2013; Slivka et al., 2012). For instance, recent studies by Slivka et al.

(2013; 2012) investigated the effect of environmental temperature during post-exercise recovery and reported significant elevations in PGC-1 $\alpha$  mRNA expression when a 3 to 4 h post-exercise recovery period was undertaken in cold (7°C), compared with temperate (20°C) conditions. Interestingly, the mRNA of downstream transcription factors, namely NRF1 and estrogen related receptor  $\alpha$  (ERR $\alpha$ ) were shown to be attenuated following cold exposure (Slivka et al., 2013). This is indeed unusual, as PGC-1 $\alpha$  has been well shown to increase NRF1 expression and transcriptional activity (Wu et al., 1999) and consequently suggests other pathways besides PGC-1 $\alpha$  might regulate NRF1 expression following post-exercise cold exposure. Regardless, this study lends some support for the study by Yamane et al. (2006), as NRF1 has shown to encode a variety of mitochondrial components (Scarpulla, 2002) and PGC-1 $\alpha$  has shown to act through ERR $\alpha$  in inducing VEGF expression (Chinsomboon et al., 2009). A key consideration however, that must be accounted for is that the increase in PGC-1 $\alpha$  following cold exposure as demonstrated by Slivka et al. (2013; 2012) was accompanied by significant elevations in whole body VO<sub>2</sub> and shivering thermogenesis, which independently may increase PGC-1 $\alpha$  mRNA expression. As such, it is not possible in these studies to determine if differences in cell signalling were temperature or contraction driven. Nevertheless, recent study by Ihsan et al. (2014) has demonstrated that cold-induced shivering thermogenesis was not necessary to enhance aerobic adaptations mediated by exercise. Indeed, using a one-legged cooling design to avoid shivering, Ihsan et al. (2014) showed that 15 min of post-exercise cold water immersion (10°C) resulted in increased mRNA content of PGC-1 $\alpha$  following exercise. Moreover, unpublished data from our laboratory show increased AMPK and p38 MAPK signalling along with increased complex III and  $\beta$ -HAD protein expression following regular post-exercise cooling over 4 weeks of endurance training (Ihsan et al., 2013b, unpublished observation).

Overall, there is considerable disparity and ambiguity regarding the effects of post-exercise cold water immersion. Data from performance studies are unclear (Halsen et al., 2014; Yamane et al., 2006), but potentially indicate that training adaptations may be attenuated, as data from the study by Halsen et al. (2014) could be due to the recovery effects of cold water immersion. From the molecular studies (Ihsan et al., 2014; Slivka et al., 2013; Slivka et al., 2012), the increase in PGC-1 $\alpha$  following post-exercise cold exposure appears to be a consistent effect and seems to be via AMPK and p38 MAPK signalling (Fig. 3), although other mechanisms such as  $\beta$ -adrenergic pathways are possible as well. These results indicate that exercise adaptations may be accentuated by cold water immersions. It is thus difficult to reconcile the findings by Ihsan et al. (2014) and Slivka et al. (2013; 2012) where the molecular data is supportive of mitochondrial biogenesis, to that of Yamane et al. (2006), where indices of aerobic performance seem attenuated following post-exercise cold water immersion. One possibility is that while cold exposure might increase mitochondrial biogenesis, it may also in parallel decrease mitochondrial efficiency, through increased mitochondrial uncoupling mechanisms. Indeed, cold exposure has shown to increase, in a PGC-1 $\alpha$  dependant manner, the expression of uncoupling proteins and uncoupled respiration in cultured myotubes (Wu et al., 1999). Moreover, mitochondrial state 4 (i.e. uncoupled) respiration has shown to significantly correlate (R<sup>2</sup> = 0.65) with increases in energy expenditure during short term cold exposure (48 h) in humans (Wijers et al., 2011). Alternatively, since NRF1 and ERR $\alpha$  expressions have shown to be attenuated following post-exercise cold exposure (Slivka et al., 2013), it is possible that events downstream of PGC-1 $\alpha$  may be negatively affected by this intervention, resulting in impaired mitochondrial biogenesis. This is probably unlikely, as

previously mentioned, unpublished data from our laboratory show increased complex III and  $\beta$ -HAD protein expressions following regular post-exercise cooling over 4 weeks of endurance training (Ihsan et al., 2013b). More research investigating the effect of post-exercise cooling on mitochondrial efficiency and regulatory events downstream of PGC-1 $\alpha$  are needed to fully elucidate its effect on adaptations to exercise and training.



**Figure 3.** Primary signals activating PGC-1 $\alpha$  and associated adaptations following post-exercise heat and cold exposures appear to be poorly characterised, although p38 signalling seems to be an important pathway in both conditions. AMPK signalling is also involved in PGC-1 $\alpha$  activation following post-exercise cold exposure while post-exercise heat exposure seems to inhibit AMPK signalling.

## Summary and Conclusions

In summary, mitochondrial biogenesis has substantial implications in exercise performance and in the reduction of risk factors related to a variety of chronic diseases. Exercise is a well-known intervention to increase muscle mitochondrial protein content and subsequently improve aerobic function. It is well accepted that the transcriptional coactivator PGC-1 $\alpha$  is a key regulator of mitochondrial biogenesis. PGC-1 $\alpha$  regulates mitochondrial biogenesis by inducing the gene expression and controlling the transcriptional activity of key factors such as NRF1, NRF2 and Tfam. Apart from its regulatory role in mitochondrial biogenesis, PGC-1 $\alpha$  is also involved in other aerobic phenotypic adaptations such as angiogenesis and fatty acid oxidation through its interactions with VEGF and PPAR $\alpha$ , respectively. To date, a number of primary signals have been identified to trigger signalling cascades inducing/activating PGC-1 $\alpha$ . These signals include  $\beta$ -adrenergic stimulation, increase in AMP:ATP ratio, increase in cellular levels of Ca<sup>2+</sup>, ROS and NO.

## References

- Akimoto T, Pohnert SC, Li P, Zhang M, Gumbs C, Rosenberg PB, Williams RS, Yan Z (2005). Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. *J Biol Chem.* **280**, 19587-19593.
- Arany Z, Foo S-Y, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, Cooper M, Laznik D, Chinsomboon J, Rangwala SM, Baek KH, Rosenzweig A, Spiegelman BM (2008). HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. *Nature.* **451**, 1008-1012.
- Aschenbach WG, Sakamoto K, Goodyear LJ (2004). 5' adenosine monophosphate-activated protein kinase, metabolism and exercise. *Sports Med.* **34**, 91-9103.
- Atherton PJ, Babraj J, Smith K, Singh J, Rennie MJ, Wackerhage H (2005). Selective activation of AMPK-PGC-1alpha or PKB-TSC2-mTOR signaling can explain specific adaptive responses to endurance or resistance training-like electrical muscle stimulation. *FASEB J.* **19**, 786-788.
- Baer K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, Holloszy JO (2002). Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J.* **16**, 1879-1886.
- Barnard RJ, Edgerton VR, Peter JB (1970). Effect of exercise on skeletal muscle. II. Contractile properties. *J Appl Physiol.* **28**, 767-770.

Changes in AMP:ATP ratio and increases in cellular levels of Ca<sup>2+</sup> and ROS appear to be the major signals triggering the PGC-1 $\alpha$  program and associated adaptations to exercise. Major second messengers downstream of these signals include AMP:ATP ratio sensitive AMPK, which activates PGC-1 $\alpha$  directly and the Ca<sup>2+</sup> sensitive CaMK and calcineurin, which acts through p38 MAPK in inducing/activating PGC-1 $\alpha$ . ROS has shown to activate multiple cascades including AMPK, p38 MAPK and NOS, rendering itself an important signalling molecule for oxidative adaptations. There is emerging evidence indicating that PGC-1 $\alpha$  may be induced by exposure to heat and/or cold exposure. This effectively renders heat and cold treatment as possible modalities to induce mitochondrial biogenesis and other PGC-1 $\alpha$  involved adaptations such as angiogenesis and increased capacity for fatty acid oxidation, either in conjunction with or independently of exercise. p38 MAPK does not seem to be involved, while AMPK, SIRT1 and NO signalling seem to be the main mechanisms in heat-induced PGC-1 $\alpha$  expression. There are considerable differences in response to post-exercise heat exposure in rodent compared with human models. For instance, mitochondrial biogenesis was found to be up-regulated through p38 pathways in rodents, while PGC-1 $\alpha$  expression was attenuated in humans following post-exercise heat exposure. More studies using human models are needed to verify the effect of post-exercise heating on muscle oxidative adaptations. Evidence from cell culture and rodent models indicate that PGC-1 $\alpha$  and mitochondrial biogenesis are strongly up-regulated following passive cooling, seemingly through  $\beta$ -adrenergic and Ca<sup>2+</sup> triggered cascades. Considerable disparity is however present regarding post-exercise cooling, where studies have demonstrated an increase in PGC-1 $\alpha$  expression and indices of mitochondrial biogenesis as well as impaired aerobic performance following post-exercise cooling. Studies investigating events downstream of PGC-1 $\alpha$  as well indices of mitochondrial efficiency following post-exercise are needed to further understand the effects of this treatment modality. Understanding the effects and mechanisms of heat/cold exposure, both in isolation and in combination with exercise will be invaluable in the delivery and optimisation of temperature related treatments both the athletic and clinical populations.

## Acknowledgments

At the time this manuscript was prepared, M. Ihsan was supported by the International Postgraduate Research Scholarship and Edith Cowan University. No source of funding was declared for the rest of the authors. G.Watson and CR Abbiss assisted in the preparation of the manuscript and CR Abbiss contributed to revising it critically for important intellectual content.

- Bartlett JD, Hwa Joo C, Jeong T-S, Louhelainen J, Cochran AJ, Gibala MJ, Gregson W, Close GL, Drust B, Morton JP (2012). Matched work high-intensity interval and continuous running induce similar increases in PGC-1 $\alpha$  mRNA, AMPK, p38, and p53 phosphorylation in human skeletal muscle. *J Appl Physiol* (1985). **112**, 1135-1143.
- Bergeron R, Ren JM, Cadman KS, Moore IK, Perret P, Pypaert M, Young LH, Semenkovich CF, Shulman GI (2001). Chronic activation of AMP kinase results in NRF-1 activation and mitochondrial biogenesis. *Am J Physiol Endocrinol Metab.* **281**, 1340-1346.
- Bishop-Bailey D (2013). Mechanisms governing the health and performance benefits of exercise. *British journal of pharmacology.* **170**, 1153-1166.
- Brodal P, Ingjer F, Hermansen L (1977). Capillary supply of skeletal muscle fibers in untrained and endurance-trained men. *Am J Physiol.* **232**, 705-712.
- Brooks GA, Hittelman KJ, Faulkner JA, Beyer RE (1971). Temperature, skeletal muscle mitochondrial functions, and oxygen debt. *Am J Physiol.* **220**, 1053-1059.
- Bruton JD, Aydin J, Yamada T, Shabalina IG, Ivarsson N, Zhang S-J, Wada M, Tavi P, Nedergaard J, Katz A, Westerblad H (2010). Increased fatigue resistance linked to Ca<sup>2+</sup>-stimulated mitochondrial biogenesis in muscle fibres of cold-acclimated mice. *J Physiol.* **588**, 4275-4288.



- Calvo JA, Daniels TG, Wang X, Paul A, Lin J, Spiegelman BM, Stevenson SC, Rangwala SM (2008). Muscle-specific expression of PPARgamma coactivator-1alpha improves exercise performance and increases peak oxygen uptake. *J Appl Physiol* (1985). **104**, 1304-1312.
- Carrow RE, Brown RE, Van Huss WD (1967). Fiber sizes and capillary to fiber ratios in skeletal muscle of exercised rats. *Anat Rec*. **159**, 33-39.
- Chinsomboon J, Ruas J, Gupta RK, Thom R, Shoag J, Rowe GC, Sawada N, Raghuram S, Arany Z (2009). The transcriptional coactivator PGC-1alpha mediates exercise-induced angiogenesis in skeletal muscle. *Proc Natl Acad Sci U S A*. **106**, 21401-21406.
- Coffey VG, Hawley JA (2007). The molecular bases of training adaptation. *Sports Med*. **37**, 737-763.
- Conley KE, Amara CE, Bajpeyi S, Costford SR, Murray K, Jubrias SA, Arakaki L, Marcinek DJ, Smith SR (2013). Higher mitochondrial respiration and uncoupling with reduced electron transport chain content in vivo in muscle of sedentary versus active subjects. *J Clin Endocrinol Metab*. **98**, 129-136.
- Cotter M, Hudlicka O, Vrbova G (1973). Growth of capillaries during long-term activity in skeletal muscle. *Bibl Anat*. **11**, 395-398.
- Daussin FN, Zoll J, Dufour SP, Ponsot E, Lonsdorfer-Wolf E, Doutreleau S, Mettauer B, Piquard F, Geny B, Richard R (2008). Effect of interval versus continuous training on cardiorespiratory and mitochondrial functions: relationship to aerobic performance improvements in sedentary subjects. *Am J Physiol Regul Integr Comp Physiol*. **295**, 264-272.
- Distler JHW, Hirth A, Kurowska-Stolarska M, Gay RE, Gay S, Distler O (2003). Angiogenic and angiostatic factors in the molecular control of angiogenesis. *Q J Nucl Med*. **47**, 149-161.
- Eddy SF, Storey KB (2003). Differential expression of Akt, PPARgamma, and PGC-1 during hibernation in bats. *Biochem Cell Biol*. **81**, 269-274.
- Ekstrand MI, Falkenberg M, Rantanen A, Park CB, Gaspari M, Hulthen K, Rustin P, Gustafsson CM, Larsson N-G (2004). Mitochondrial transcription factor A regulates mtDNA copy number in mammals. *Hum Mol Genet*. **13**, 935-944.
- Feng H, Kang C, Dickman JR, Koenig R, Awoyinka I, Zhang Y, Ji LL (2013). Training-induced mitochondrial adaptation: role of peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 $\alpha$ , nuclear factor- $\kappa$ B and  $\beta$ -blockade. *Exp Physiol*. **98**, 784-795.
- Figueiredo PA, Mota MP, Appell HJ, Duarte JA (2008). The role of mitochondria in aging of skeletal muscle. *Biogerontology*. **9**, 67-84.
- Fitts RH, Booth FW, Winder WW, Holloszy JO (1975). Skeletal muscle respiratory capacity, endurance, and glycogen utilization. *Am J Physiol*. **228**, 1029-1033.
- Frandsen U, Lopez-Figueroa M, Hellsten Y (1996). Localization of nitric oxide synthase in human skeletal muscle. *Biochem Biophys Res Commun*. **227**, 88-93.
- Freyssenet D, Di Carlo M, Hood DA (1999). Calcium-dependent regulation of cytochrome c gene expression in skeletal muscle cells. Identification of a protein kinase c-dependent pathway. *J Biol Chem*. **274**, 9305-9311.
- Frosig C, Jorgensen SB, Hardie DG, Richter EA, Wojtaszewski JFP (2004). 5'-AMP-activated protein kinase activity and protein expression are regulated by endurance training in human skeletal muscle. *Am J Physiol Endocrinol Metab*. **286**, 411-417.
- Gavin TP (2009). Basal and exercise-induced regulation of skeletal muscle capillarization. *Exerc Sport Sci Rev*. **37**, 86-92.
- Geng T, Li P, Okutsu M, Yin X, Kwek J, Zhang M, Yan Z (2010). PGC-1alpha plays a functional role in exercise-induced mitochondrial biogenesis and angiogenesis but not fiber-type transformation in mouse skeletal muscle. *Am J Physiol Cell Physiol*. **298**, 572-579.
- Gibala MJ, McGee SL, Garnham AP, Howlett KF, Snow RJ, Hargreaves M (2009). Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. *J Appl Physiol* (1985). **106**, 929-934.
- Gilde AJ, Van Bilsen M (2003). Peroxisome proliferator-activated receptors (PPARs): regulators of gene expression in heart and skeletal muscle. *Acta Physiol Scand*. **178**, 425-434.
- Gleyzer N, Vercauteren K, Scarpulla RC (2005). Control of mitochondrial transcription specificity factors (TFB1M and TFB2M) by nuclear respiratory factors (NRF-1 and NRF-2) and PGC-1 family coactivators. *Mol Cell Biol*. **25**, 1354-1366.
- Goffart S, Wiesner RJ (2003). Regulation and co-ordination of nuclear gene expression during mitochondrial biogenesis. *Exp Physiol*. **88**, 33-40.
- Gollnick PD, Ianuzzo CD (1972). Hormonal deficiencies and the metabolic adaptations of rats to training. *Am J Physiol*. **223**, 278-282.
- Gollnick PD, Saltin B (1982). Significance of skeletal muscle oxidative enzyme enhancement with endurance training. *Clin Physiol*. **2**, 1-12.
- Gomez-Cabrera M-C, Borrás C, Pallardo FV, Sastre J, Ji LL, Vina J (2005). Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. *J Physiol*. **567**, 113-120.
- Gomez-Cabrera M-C, Domenech E, Romagnoli M, Arduini A, Borrás C, Pallardo FV, Sastre J, Vina J (2008). Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. *Am J Clin Nutr*. **87**, 142-149.
- Gregson W, Black MA, Jones H, Milson J, Morton J, Dawson B, Atkinson G, Green DJ (2011). Influence of cold water immersion on limb and cutaneous blood flow at rest. *Am J Sports Med*. **39**, 1316-1323.
- Halsom SL, Bartram J, West N, Stephens J, Argus CK, Driller MW, Sargent C, Lastella M, Hopkins WG, Martin DT (2014). Does hydrotherapy help or hinder adaptation to training in competitive cyclists? *Med Sci Sports Exerc*. **46**, 1631-1639.
- Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM (2003). An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle. *Proc Natl Acad Sci U S A*. **100**, 7111-7116.
- Harris MB, Blackstone MA, Ju H, Venema VJ, Venema RC (2003). Heat-induced increases in endothelial NO synthase expression and activity and endothelial NO release. *Am J Physiol Heart Circ Physiol*. **285**, 333-340.
- Higashida K, Kim SH, Higuchi M, Holloszy JO, Han D-H (2011). Normal adaptations to exercise despite protection against oxidative stress. *Am J Physiol Endocrinol Metab*. **301**, 779-784.
- Holloszy JO (1967). Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem*. **242**, 2278-2282.
- Holloszy JO, Coyle EF (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol Respir Environ Exerc Physiol*. **56**, 831-838.
- Hood DA (2009). Mechanisms of exercise-induced mitochondrial biogenesis in skeletal muscle. *Appl Physiol Nutr Metab*. **34**, 465-472.
- Hooper PL (1999). Hot-tub therapy for type 2 diabetes mellitus. *N Engl J Med*. **341**, 924-925.
- Hoppeler H, Luthi P, Claassen H, Weibel ER, Howald H (1973). The ultrastructure of the normal human skeletal muscle. A morphometric analysis on untrained men, women and well-trained orienteers. *Pflügers Arch*. **344**, 217-232.
- Ihsan M, Watson G, Lipski M, Abbiss CR (2013a). Influence of postexercise cooling on muscle oxygenation and blood volume changes. *Med Sci Sports Exerc*. **45**, 876-882.
- Ihsan M, Watson G, Choo HC, Lewandowski P, Papazzo A, Cameron-Smith D, Abbiss CR (2014). Postexercise Muscle Cooling Enhances Gene Expression of PGC-1 $\alpha$ . *Med Sci Sports Exerc*. **46**, 1900-1907.
- Inbar O, Kaiser P, Tesch P (1981). Relationships between leg muscle fiber type distribution and leg exercise performance. *Int J Sports Med*. **2**, 154-159.
- Irrcher I, Adhihetty PJ, Sheehan T, Joseph A-M, Hood DA (2003). PPARgamma coactivator-1alpha expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. *Am J Physiol Cell Physiol*. **284**, 1669-1677.
- Irrcher I, Ljubic V, Hood DA (2009). Interactions between ROS and AMP kinase activity in the regulation of PGC-1alpha transcription in skeletal muscle cells. *Am J Physiol Cell Physiol*. **296**, 116-123.
- Jackson MJ (2011). Control of reactive oxygen species production in contracting skeletal muscle. *Antioxid Redox Signal*. **15**, 2477-2486.
- Jackson MJ, Pye D, Palomero J (2007). The production of reactive oxygen and nitrogen species by skeletal muscle. *J Appl Physiol* (1985). **102**, 1664-1670.
- Jager S, Handschin C, St-Pierre J, Spiegelman BM (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A*. **104**, 12017-12022.
- Ji LL, Lennon DL, Kochan RG, Nagle FJ, Lardy HA (1986). Enzymatic adaptation to physical training under beta-blockade in the rat. Evidence of a beta 2-adrenergic mechanism in skeletal muscle. *J Clin Invest*. **78**, 771-778.
- Joseph A-M, Pilegaard H, Litvinov A, Leick L, Hood DA (2006). Control of gene expression and mitochondrial biogenesis in the muscular adaptation to endurance exercise. *Essays Biochem*. **42**, 13-29.
- Kobzik L, Reid MB, Bredt DS, Stamler JS (1994). Nitric oxide in skeletal muscle. *Nature*. **372**, 546-548.
- Kolobova E, Tuganova A, Boulatnikov I, Popov KM (2001). Regulation of pyruvate dehydrogenase activity through phosphorylation at multiple sites. *Biochem J*. **358**, 69-77.
- Kooijman M, Thijssen DHJ, de Groot PCE, Bleeker MWP, van Kuppevelt HJM, Green DJ, Rongen GA, Smits P, Hopman MTE (2008). Flow-mediated dilatation in the superficial femoral artery is nitric oxide mediated in humans. *J Physiol*. **586**, 1137-1145.
- Kusuhara K, Madsen K, Jensen L, Hellsten Y, Pilegaard H (2007). Calcium signalling in the regulation of PGC-1alpha, PDK4 and HKII mRNA expression. *Biol Chem*. **388**, 481-488.
- Larsen S, Nielsen J, Hansen CN, Nielsen LB, Wibrand F, Stride N, Schroder HD, Boushel R, Helge JW, Dela F, Hey-Mogensen M (2012). Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. *J Physiol*. **590**, 3349-3360.
- Lash JM, Bohlen HG (1992). Functional adaptations of rat skeletal muscle arterioles to aerobic exercise training. *J Appl Physiol* (1985). **72**, 2052-2062.
- Leick L, Fentz J, Bienso RS, Knudsen JG, Jeppesen J, Kiens B, Wojtaszewski JFP, Pilegaard H (2010). PGC-1{alpha} is required for AICAR-induced expression of GLUT4 and mitochondrial proteins in mouse skeletal muscle. *Am J Physiol Endocrinol Metab*. **299**, 456-465.
- Leick L, Wojtaszewski JFP, Johansen ST, Kilerich K, Comes G, Hellsten Y, Hidalgo J, Pilegaard H (2008). PGC-1alpha is not mandatory for exercise- and training-induced adaptive gene responses in mouse skeletal muscle. *Am J Physiol Endocrinol Metab*. **294**, 463-474.
- Lin J, Wu P-H, Tarr PT, Lindenberg KS, St-Pierre J, Zhang C-Y, Mootha VK, Jager S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D, Spiegelman BM (2004). Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell*. **119**, 121-135.
- Lira VA, Brown DL, Lira AK, Kavazis AN, Soltow QA, Zeannah EH, Criswell DS (2010). Nitric oxide and AMPK cooperatively regulate PGC-1 in skeletal muscle cells. *J Physiol*. **588**, 3551-3566.
- Little JP, Safdar A, Bishop D, Tarnopolsky MA, Gibala MJ (2011). An acute bout of high-intensity interval training increases the nuclear abundance of PGC-1 $\alpha$  and activates mitochondrial biogenesis in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*. **300**, 1303-1310.
- Little JP, Safdar A, Cermak N, Tarnopolsky MA, Gibala MJ (2010). Acute endurance exercise increases the nuclear abundance of PGC-1alpha in trained human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*. **298**, 912-917.
- Liu C-T, Brooks GA (2012). Mild heat stress induces mitochondrial biogenesis in C2C12 myotubes. *J Appl Physiol* (1985). **112**, 354-361.





- Ljubicic V, Joseph A-M, Saleem A, Uguccioni G, Collu-Marchese M, Lai RYJ, Nguyen LMD, Hood DA (2010). Transcriptional and post-transcriptional regulation of mitochondrial biogenesis in skeletal muscle: effects of exercise and aging. *Biochim Biophys Acta*. **1800**, 223-234.
- Marsh RC, Hiatt WR, Brammell HL, Horwitz LD (1983). Attenuation of exercise conditioning by low dose beta-adrenergic receptor blockade. *J Am Coll Cardiol*. **2**, 551-556.
- Mathai AS, Bonen A, Benton CR, Robinson DL, Graham TE (2008). Rapid exercise-induced changes in PGC-1 $\alpha$  mRNA and protein in human skeletal muscle. *J Appl Physiol* (1985). **105**, 1098-1105.
- Mawhinney C, Jones H, Joo CH, Low DA, Green DJ, Gregson W (2013). Influence of cold-water immersion on limb and cutaneous blood flow after exercise. *Med Sci Sports Exerc*. **45**, 2277-2285.
- McArdle A, Jackson MJ (2000). Exercise, oxidative stress and ageing. *J Anat*. **197 Pt 4**, 539-541.
- McConnell GK, Ng GPY, Phillips M, Ruan Z, Macaulay SL, Wadley GD (2010). Central role of nitric oxide synthase in AICAR and caffeine-induced mitochondrial biogenesis in L6 myocytes. *J Appl Physiol* (1985). **108**, 589-595.
- McConnell GK, Wadley GD (2008). Potential role of nitric oxide in contraction-stimulated glucose uptake and mitochondrial biogenesis in skeletal muscle. *Clin Exp Pharmacol Physiol*. **35**, 1488-1492.
- McGee SL, Hargreaves M (2004). Exercise and myocyte enhancer factor 2 regulation in human skeletal muscle. *Diabetes*. **53**, 1208-1214.
- Michael LF, Wu Z, Cheatham RB, Puigserver P, Adelman G, Lehman JJ, Kelly DP, Spiegelman BM (2001). Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. *Proc Natl Acad Sci U S A*. **98**, 3820-3825.
- Miura S, Kawanaka K, Kai Y, Tamura M, Goto M, Shiuchi T, Minokoshi Y, Ezaki O (2007). An increase in murine skeletal muscle peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) mRNA in response to exercise is mediated by beta-adrenergic receptor activation. *Endocrinology*. **148**, 3441-3448.
- Moraes CT (2001). What regulates mitochondrial DNA copy number in animal cells? *Trends Genet*. **17**, 199-205.
- Morgan TE, Cobb LA, Short FA, Ross R, Gunn DR (1971). Effects of Long-Term Exercise on Human Muscle Mitochondria. In *Muscle Metabolism During Exercise*. (B Pernow, B Saltin, eds): Springer US, pp. 87-95.
- Naylor LH, Carter H, FitzSimons MG, Cable NT, Thijssen DHJ, Green DJ (2011). Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans. *Am J Physiol Heart Circ Physiol*. **300**, 664-669.
- Nielsen JN, Mustard KJW, Graham DA, Yu H, MacDonald CS, Pilegaard H, Goodyear LJ, Hardie DG, Richter EA, Wojtaszewski JFP (2003). 5'-AMP-activated protein kinase activity and subunit expression in exercise-trained human skeletal muscle. *J Appl Physiol* (1985). **94**, 631-641.
- Niess AM, Sommer M, Schlotz E, Northoff H, Dickhuth HH, Fehrenbach E (2000). Expression of the inducible nitric oxide synthase (iNOS) in human leukocytes: responses to running exercise. *Med Sci Sports Exerc*. **32**, 1220-1225.
- Nisoli E, Falcone S, Tonello C, Cozzi V, Palomba L, Fiorani M, Pisconti A, Brunelli S, Cardile A, Francolini M, Cantoni O, Carruba MO, Moncada S, Clementi E (2004). Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals. *Proc Natl Acad Sci U S A*. **101**, 16507-16512.
- Ojuka EO, Jones TE, Han DH, Chen M, Holloszy JO (2003). Raising Ca<sup>2+</sup> in L6 myotubes mimics effects of exercise on mitochondrial biogenesis in muscle. *Faseb j*. **17**, 675-681.
- Olfert IM, Howlett RA, Tang K, Dalton ND, Gu Y, Peterson KL, Wagner PD, Breen EC (2009). Muscle-specific VEGF deficiency greatly reduces exercise endurance in mice. *J Physiol*. **587**, 1755-1767.
- Olfert IM, Howlett RA, Wagner PD, Breen EC (2010). Myocyte vascular endothelial growth factor is required for exercise-induced skeletal muscle angiogenesis. *Am J Physiol Regul Integr Comp Physiol*. **299**, 1059-1067.
- Philp A, Chen A, Lan D, Meyer GA, Murphy AN, Knapp AE, Olfert IM, McCurdy CE, Marcotte GR, Hogan MC, Baar K, Schenk S (2011). Sirtuin 1 (SIRT1) deacetylase activity is not required for mitochondrial biogenesis or peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) deacetylation following endurance exercise. *J Biol Chem*. **286**, 30561-30570.
- Pilegaard H, Saltin B, Neufer PD (2003). Exercise induces transient transcriptional activation of the PGC-1 $\alpha$  gene in human skeletal muscle. *J Physiol*. **546**, 851-858.
- Popov DV, Bachinin AV, Lysenko EA, Miller TF, Vinogradova OL (2014). Exercise-induced expression of peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 $\alpha$  isoforms in skeletal muscle of endurance-trained males. *J Physiol Sci*. **64**, 317-323.
- Powers SK, Duarte J, Kavazis AN, Talbert EE (2010). Reactive oxygen species are signalling molecules for skeletal muscle adaptation. *Exp Physiol*. **95**, 1-9.
- Powers SK, Ji LL, Kavazis AN, Jackson MJ (2011). Reactive oxygen species: impact on skeletal muscle. *Compr Physiol*. **1**, 941-969.
- Prior BM, Yang HT, Terjung RL (2004). What makes vessels grow with exercise training? *J Appl Physiol* (1985). **97**, 1119-1128.
- Puigserver P, Spiegelman BM (2003). Peroxisome proliferator-activated receptor- $\gamma$  coactivator 1  $\alpha$  (PGC-1  $\alpha$ ): transcriptional coactivator and metabolic regulator. *Endocr Rev*. **24**, 78-90.
- Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell*. **92**, 829-839.
- Radegran G, Saltin B (1999). Nitric oxide in the regulation of vasomotor tone in human skeletal muscle. *Am J Physiol*. **276**, 1951-1960.
- Rakobowchuk M, Tanguay S, Burgomaster KA, Howarth KR, Gibala MJ, MacDonald MJ (2008). Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans. *Am J Physiol Regul Integr Comp Physiol*. **295**, 236-242.
- Richardson RS, Knight DR, Poole DC, Kurdak SS, Hogan MC, Grassi B, Wagner PD (1995). Determinants of maximal exercise VO<sub>2</sub> during single leg knee-extensor exercise in humans. *Am J Physiol*. **268**, 1453-1461.
- Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR, Bluher M (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A*. **106**, 8665-8670.
- Robinson MM, Bell C, Peelor FF, Miller BF (2011).  $\beta$ -Adrenergic receptor blockade blunts postexercise skeletal muscle mitochondrial protein synthesis rates in humans. *Am J Physiol Regul Integr Comp Physiol*. **301**, 327-334.
- Robinson MM, Richards JC, Hickey MS, Moore DR, Phillips SM, Bell C, Miller BF (2010). Acute {beta}-adrenergic stimulation does not alter mitochondrial protein synthesis or markers of mitochondrial biogenesis in adult men. *Am J Physiol Regul Integr Comp Physiol*. **298**, 25-33.
- Romanello V, Guadagnin E, Gomes L, Roder I, Sandri C, Petersen Y, Milan G, Masiero E, Del Piccolo P, Foretz M, Scorrano L, Rudolf R, Sandri M (2010). Mitochondrial fission and remodelling contributes to muscle atrophy. *EMBO J*. **29**, 1774-1785.
- Rose AJ, Hargreaves M (2003). Exercise increases Ca<sup>2+</sup>-calmodulin-dependent protein kinase II activity in human skeletal muscle. *J Physiol*. **553**, 303-309.
- Rowe GC, El-Khoury R, Patten IS, Rustin P, Arany Z (2012). PGC-1 $\alpha$  is dispensable for exercise-induced mitochondrial biogenesis in skeletal muscle. *PLoS One*. **7**.
- Rudnick J, Puttmann B, Tesch PA, Alkner B, Schoser BGH, Kalanova M, Kirsch K, Gunga H-C, Schiffl G, Luck G, Blotner D (2004). Differential expression of nitric oxide synthases (NOS 1-3) in human skeletal muscle following exercise countermeasure during 12 weeks of bed rest. *FASEB J*. **18**, 1228-1230.
- Russell AP, Hesselink MKC, Lo SK, Schrauwen P (2005). Regulation of metabolic transcriptional co-activators and transcription factors with acute exercise. *FASEB J*. **19**, 986-988.
- Sable DL, Brammell HL, Sheehan MW, Nies AS, Gerber J, Horwitz LD (1982). Attenuation of exercise conditioning by beta-adrenergic blockade. *Circulation*. **65**, 679-684.
- Safdar A, Little JP, Stokl AJ, Hettling BP, Akhtar M, Tarnopolsky MA (2011). Exercise increases mitochondrial PGC-1 $\alpha$  content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *J Biol Chem*. **286**, 10605-10617.
- Scarpulla RC (2002). Transcriptional activators and coactivators in the nuclear control of mitochondrial function in mammalian cells. *Gene*. **286**, 81-89.
- Silveira LR, Pilegaard H, Kusuhabara K, Curi R, Hellsten Y (2006). The contraction induced increase in gene expression of peroxisome proliferator-activated receptor (PPAR)- $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ), mitochondrial uncoupling protein 3 (UCP3) and hexokinase II (HKII) in primary rat skeletal muscle cells is dependent on reactive oxygen species. *Biochim Biophys Acta*. **1763**, 969-976.
- Slivka D, Heesch M, Dumke C, Cuddy J, Hailes W, Ruby B (2013). Effects of post-exercise recovery in a cold environment on muscle glycogen, PGC-1 $\alpha$ , and downstream transcription factors. *Cryobiology*. **66**, 250-255.
- Slivka DR, Dumke CL, Tucker TJ, Cuddy JS, Ruby B (2012). Human mRNA response to exercise and temperature. *Int J Sports Med*. **33**, 94-9100.
- Spiegelman BM, Heinrich R (2004). Biological control through regulated transcriptional coactivators. *Cell*. **119**, 157-167.
- Steensberg A, Keller C, Hillig T, Froisig C, Wojtaszewski JFP, Pedersen BK, Pilegaard H, Sander M (2007). Nitric oxide production is a proximal signaling event controlling exercise-induced mRNA expression in human skeletal muscle. *FASEB J*. **21**, 2683-2694.
- Strobel NA, Peake JM, Matsumoto A, Marsh SA, Coombes JS, Wadley GD (2011). Antioxidant supplementation reduces skeletal muscle mitochondrial biogenesis. *Med Sci Sports Exerc*. **43**, 1017-1024.
- Suwa M, Nakano H, Radak Z, Kumagai S (2008). Endurance exercise increases the SIRT1 and peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 $\alpha$  protein expressions in rat skeletal muscle. *Metabolism*. **57**, 986-998.
- Suwa M, Nakano H, Radak Z, Kumagai S (2011). Short-term adenosine monophosphate-activated protein kinase activator 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside treatment increases the sirtuin 1 protein expression in skeletal muscle. *Metabolism*. **60**, 394-403.
- Svedenhag J, Henriksson J, Juhlin-Dannfelt A (1984). Beta-adrenergic blockade and training in human subjects: effects on muscle metabolic capacity. *Am J Physiol*. **247**, 305-311.
- Swenson C, Sward L, Karlsson J (1996). Cryotherapy in sports medicine. *Scand J Med Sci Sports*. **6**, 193-200.
- Tadaishi M, Miura S, Kai Y, Kano Y, Oishi Y, Ezaki O (2011). Skeletal muscle-specific expression of PGC-1 $\alpha$ -b, an exercise-responsive isoform, increases exercise capacity and peak oxygen uptake. *PLoS One*. **6**.
- Tamura Y, Matsunaga Y, Masuda H, Takahashi Y, Takahashi Y, Terada S, Hoshino D, Hatta H (2014). Postexercise whole body heat stress additively enhances endurance training-induced mitochondrial adaptations in mouse skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*. **307**, 931-943.
- Terada S, Goto M, Kawanaka K, Shimokawa T, Tabata I (2002). Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. *Biochem Biophys Res Commun*. **296**, 350-354.
- Uguccioni G, Hood DA (2011). The importance of PGC-1 $\alpha$  in contractile activity-induced mitochondrial adaptations. *Am J Physiol Endocrinol Metab*. **300**, 361-371.
- Vaile J, Halson S, Gill N, Dawson B (2008). Effect of hydrotherapy on recovery from fatigue. *Int J Sports Med*. **29**, 539-544.

- van der Poel C , Stephenson DG (2007). Effects of elevated physiological temperatures on sarcoplasmic reticulum function in mechanically skinned muscle fibers of the rat. *Am J Physiol Cell Physiol.* **293**, C133-141.
- Vega RB, Huss JM , Kelly DP (2000). The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol.* **20**, 1868-1876.
- Virbasius JV , Scarpulla RC (1994). Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Natl Acad Sci U S A.* **91**, 1309-1313.
- Wadley GD, Choate J , McConnell GK (2007). NOS isoform-specific regulation of basal but not exercise-induced mitochondrial biogenesis in mouse skeletal muscle. *J Physiol.* **585**, 253-262.
- Wadley GD , McConnell GK (2007). Effect of nitric oxide synthase inhibition on mitochondrial biogenesis in rat skeletal muscle. *J Appl Physiol (1985).* **102**, 314-320.
- Wadley GD , McConnell GK (2010). High-dose antioxidant vitamin C supplementation does not prevent acute exercise-induced increases in markers of skeletal muscle mitochondrial biogenesis in rats. *J Appl Physiol (1985).* **108**, 1719-1726.
- Walters TJ , Constable SH (1993). Intermittent cold exposure causes a muscle-specific shift in the fiber type composition in rats. *J Appl Physiol (1985).* **75**, 264-267.
- Wang H, Hiatt WR, Barstow TJ , Brass EP (1999). Relationships between muscle mitochondrial DNA content, mitochondrial enzyme activity and oxidative capacity in man: alterations with disease. *Eur J Appl Physiol Occup Physiol.* **80**, 22-27.
- Wende AR, Huss JM, Schaeffer PJ, Giguere V , Kelly DP (2005). PGC-1alpha coactivates PDK4 gene expression via the orphan nuclear receptor ERRalpha: a mechanism for transcriptional control of muscle glucose metabolism. *Mol Cell Biol.* **25**, 10684-10694.
- Wende AR, Schaeffer PJ, Parker GJ, Zechner C, Han D-H, Chen MM, Hancock CR, Lehman JJ, Huss JM, McClain DA, Holloszy JO , Kelly DP (2007). A role for the transcriptional coactivator PGC-1alpha in muscle refueling. *J Biol Chem.* **282**, 36642-36651.
- Wijers SLJ, Schrauwen P, Saris WHM , van Marken Lichtenbelt WD (2008). Human skeletal muscle mitochondrial uncoupling is associated with cold induced adaptive thermogenesis. *PLoS One.* **3**.
- Wijers SLJ, Schrauwen P, van Baak MA, Saris WHM , van Marken Lichtenbelt WD (2011). Beta-adrenergic receptor blockade does not inhibit cold-induced thermogenesis in humans: possible involvement of brown adipose tissue. *J Clin Endocrinol Metab.* **96**, 598-605.
- Wilcock IM, Cronin JB , Hing WA (2006). Physiological response to water immersion: a method for sport recovery? *Sports Med.* **36**, 747-765.
- Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, Fernstrom M, Rezaei K, Lee SJ, Koch LG , Britton SL (2005). Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science.* **307**, 418-420.
- Wolfel EE, Hiatt WR, Brammell HL, Carry MR, Ringel SP, Travis V , Horwitz LD (1986a). Effects of selective and nonselective beta-adrenergic blockade on mechanisms of exercise conditioning. *Circulation.* **74**, 664-674.
- Wright DC, Geiger PC, Han D-H, Jones TE , Holloszy JO (2007a). Calcium induces increases in peroxisome proliferator-activated receptor gamma coactivator-1alpha and mitochondrial biogenesis by a pathway leading to p38 mitogen-activated protein kinase activation. *J Biol Chem.* **282**, 18793-18799.
- Wright DC, Han DH, Garcia-Roves PM, Geiger PC, Jones TE , Holloszy JO (2007b). Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1alpha expression. *J Biol Chem.* **282**, 194-199.
- Wu H, Kanatous SB, Thurmond FA, Gallardo T, Isotani E, Bassel-Duby R , Williams RS (2002). Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science.* **296**, 349-352.
- Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC , Spiegelman BM (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell.* **98**, 115-124.
- Yamaguchi T, Suzuki T, Arai H, Tanabe S , Atomi Y (2010). Continuous mild heat stress induces differentiation of mammalian myoblasts, shifting fiber type from fast to slow. *Am J Physiol Cell Physiol.* **298**, 140-148.
- Yamane M, Teruya H, Nakano M, Ogai R, Ohnishi N , Kosaka M (2006). Post-exercise leg and forearm flexor muscle cooling in humans attenuates endurance and resistance training effects on muscle performance and on circulatory adaptation. *Eur J Appl Physiol.* **96**, 572-580.
- Ydfors M, Fischer H, Mascher H, Blomstrand E, Norrbom J , Gustafsson T (2013). The truncated splice variants, NT-PGC-1a and PGC-1a4, increase with both endurance and resistance exercise in human skeletal muscle. *Physiol Rep.* **1**.
- Yfanti C, Akerstrom T, Nielsen S, Nielsen AR, Mounier R, Mortensen OH, Lykkesfeldt J, Rose AJ, Fischer CP , Pedersen BK (2010). Antioxidant supplementation does not alter endurance training adaptation. *Med Sci Sports Exerc.* **42**, 1388-1395.
- Zhang Y, Uguccioni G, Ljubicic V, Irrcher I, Iqbal S, Singh K, Ding S , Hood DA (2014). Multiple signaling pathways regulate contractile activity-mediated PGC-1a gene expression and activity in skeletal muscle cells. *Physiol Rep.* **2**.
- Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ , Shulman GI (2002). AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. *Proc Natl Acad Sci U S A.* **99**, 15983-15987.
- Zuo L, Christofi FL, Wright VP, Liu CY, Merola AJ, Berliner LJ , Clanton TL (2000). Intra- and extracellular measurement of reactive oxygen species produced during heat stress in diaphragm muscle. *Am J Physiol Cell Physiol.* **279**, 1058-1066.
- Winder WW (2001). Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. *J Appl Physiol (1985).* **91**, 1017-1028.
- Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M , Holloszy JO (2000). Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol (1985).* **88**, 2219-2226.

