

## Molecular responses to high-intensity interval exercise<sup>1</sup>

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**Abstract:** From a cell-signaling perspective, short-duration intense muscular work is typically associated with resistance training and linked to pathways that stimulate growth. However, brief repeated sessions of high-intensity interval exercise training (HIT) induce rapid phenotypic changes that resemble traditional endurance training. Given the oxidative phenotype that is rapidly upregulated by HIT, it is plausible that metabolic adaptations to this type of exercise could be mediated in part through signaling pathways normally associated with endurance training. A key controller of oxidative enzyme expression in skeletal muscle is peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ), a transcriptional coactivator that serves to coordinate mitochondrial biogenesis. Most studies of acute PGC-1 $\alpha$  regulation in humans have used very prolonged exercise interventions; however, it was recently shown that a surprisingly small dose of very intense interval exercise, equivalent to only 2 min of all-out cycling, was sufficient to increase PGC-1 $\alpha$  mRNA during recovery. Intense interval exercise has also been shown to acutely increase the activity of signaling pathways linked to PGC-1 $\alpha$  and mitochondrial biogenesis, including AMP-activated protein kinase ( $\alpha$ 1 and  $\alpha$ 2 subunits) and the p38 mitogen-activated protein kinase. In contrast, signaling pathways linked to muscle growth, including protein kinase B/Akt and downstream targets p70 ribosomal S6 kinase and 4E binding protein 1, are generally unchanged after acute interval exercise. Signaling through AMP-activated protein kinase and p38 mitogen-activated protein kinase to PGC-1 $\alpha$  may therefore explain, in part, the metabolic remodeling induced by HIT, including mitochondrial biogenesis and an increased capacity for glucose and fatty acid oxidation.

*Key words:* skeletal muscle, mitochondrial biogenesis, signal transduction.

**Résumé :** Du point de vue de la signalisation cellulaire, un travail musculaire intense de courte durée est associé à l'entraînement à la force et relié à des voies stimulant la croissance. Néanmoins, de brèves séances répétées d'un exercice de forte intensité réalisé par intervalle (HIT) suscitent de rapides modifications du phénotype ressemblant à ce qui se passe au cours de l'entraînement classique en endurance. Comme ce phénotype oxydatif présente rapidement une régulation à la hausse causée par un HIT, on peut penser que les adaptations métaboliques à ce type d'exercice sont médiées en partie par les voies de signalisation généralement associées à l'entraînement en endurance. Un régulateur important de l'expression des enzymes oxydatives est le coactivateur-1 $\alpha$  du récepteur  $\gamma$  activé de la prolifération des peroxysomes (PGC-1 $\alpha$ ), un facteur transcriptionnel servant à la coordination de la biogenèse mitochondriale. La plupart des études portant sur la régulation à court terme du PGC-1 $\alpha$  ont comporté des séances prolongées d'exercice; pourtant, on sait depuis peu qu'une petite dose d'un exercice très intense réalisé par intervalle, soit l'équivalent de seulement 2 minutes à fond de train sur un vélo, suffit pour accroître l'ARNm du PGC-1 $\alpha$  au cours de la récupération. D'autres études ont montré qu'un exercice de courte durée accroît l'activité des voies de signalisation associées au PGC-1 $\alpha$  et à la biogenèse mitochondriale incluant l'AMPK (sous-unités  $\alpha$ 1 et  $\alpha$ 2) et la protéine kinase activée par le mitogène p38. En revanche, les voies de signalisation associées à la croissance musculaire et qui incluent la protéine kinase B/Akt et des cibles en aval dont la p70 S6 kinase ribosomique et la protéine de liaison 1 à 4<sup>E</sup>, sont généralement non modifiées à la suite d'une brève séance d'exercice par intervalle. La signalisation par l'AMPK et le p38 MAPK jusqu'au PGC-1 $\alpha$  peut en partie expliquer le remodelage métabolique suscité par l'HIT y compris la biogenèse mitochondriale et l'augmentation de la capacité oxydative du glucose et des acides gras.

*Mots-clés :* muscle squelettique, biogenèse mitochondriale, transduction du signal.

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## Introduction

From a cell-signaling perspective, exercise is often broadly classified as either “strength” or “endurance”, with short-duration, intense muscular work usually associated with hypertrophy, and prolonged, low-to-moderate-intensity work associated with increased mitochondrial mass and oxidative capacity (Baar 2006; Coffey and Hawley 2007). The distinct pathways that regulate either cell growth or mitochondrial biogenesis intersect at a number of points in an inhibitory fashion, resulting in a response that is largely exclusive for one type of exercise or the other (Baar 2006). Studies in animals (Atherton et al. 2005) have shown that electrical stimulation of isolated muscles with either prolonged low-frequency bursts (to mimic endurance training) or short high-frequency bursts (to simulate resistance training) selectively activates signaling cascades associated with mitochondrial biogenesis (e.g., AMP-activated protein kinase, AMPK) or muscle growth (e.g., protein kinase B/Akt; PKB), respectively. In light of these findings, Atherton et al. (2005) proposed the “AMPK-PKB switch” hypothesis as a mechanism that partially mediates specific adaptations to endurance and resistance training. However, there is considerable overlap in the signaling response to divergent contractile stimuli in human skeletal muscle (Dreyer et al. 2006; Mascher et al. 2007) and investigators have questioned the veracity of the putative AMPK-PKB switch hypothesis (Coffey et al. 2006).

## High-intensity interval exercise

High-intensity interval exercise is characterized by relatively brief, intermittent periods of muscle contraction, often performed with an “all-out” effort or at an intensity close to that which elicits peak oxygen uptake. Depending on the specific intensity, a single effort may last from a few seconds to up to several minutes, with multiple efforts separated by up to a few minutes of rest or low-intensity exercise for recovery. Although sometimes equated with strength or heavy resistance training, high-intensity interval exercise training (HIT) does not induce marked fibre hypertrophy (Ross and Leveritt 2001). Rather, there is a growing appreciation of the potential for HIT to stimulate the skeletal muscle remodeling normally associated with traditional endurance training (Gibala and McGee 2008). As few as 6 sessions of HIT over 2 weeks, totaling ~15 min of “all-out” cycle exercise (~600 kJ total work), has been shown to increase the maximal activity of mitochondrial enzymes and improve performance during tasks that rely heavily on aerobic energy provision (Burgomaster et al. 2005; Gibala et al. 2006). Other adaptations documented after several weeks of HIT include an increased muscle content of proteins associated with the transport and oxidation of glucose and fatty acids and reduced nonoxidative energy provision during matched-work exercise (Burgomaster et al. 2008).

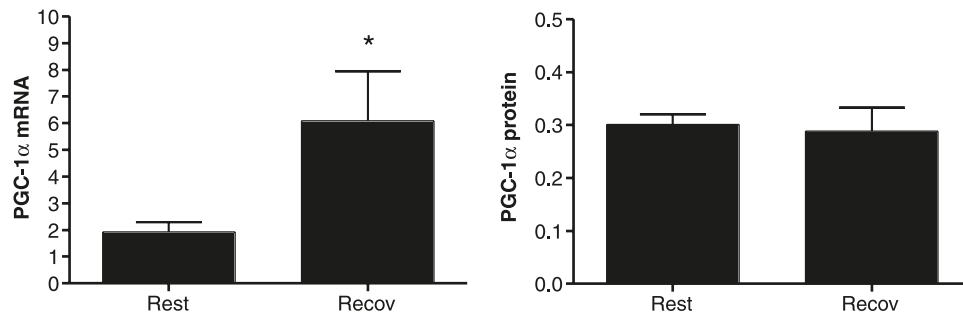
## Muscle remodeling after HIT: potential signaling mechanisms

Given the oxidative phenotype that is rapidly upregulated by HIT, it is plausible that metabolic adaptations to this type of exercise could be mediated, in part, through signaling

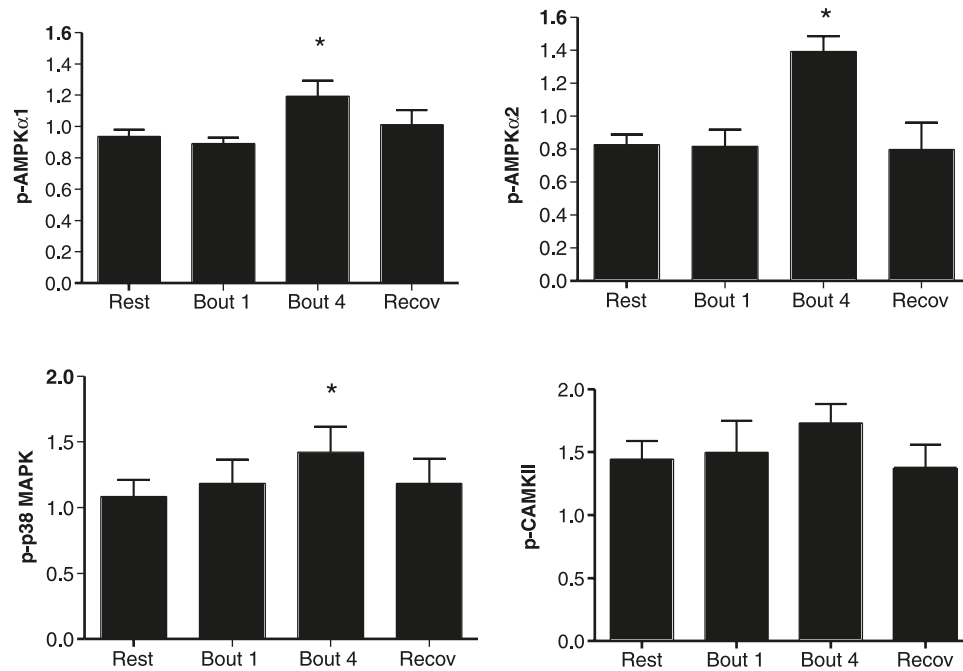
pathways normally associated with endurance training. A key regulator of oxidative enzyme expression in a number of cell types, including skeletal muscle, is peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ), a transcriptional coactivator that serves to coordinate mitochondrial biogenesis (Baar 2004). In a recent study (Gibala et al. 2009), we measured the mRNA expression and protein content of PGC-1 $\alpha$  in human skeletal muscle in response to an acute session of brief, intense interval exercise ( $4 \times 30$  s all-out Wingate tests separated by 4 min of recovery). Most studies of acute PGC-1 $\alpha$  regulation in humans have used very prolonged exercise interventions (Pilegaard et al. 2003; Watt et al. 2004) and it has been suggested that sustained contractile activity lasting ~1 h may be required before an increase in PGC-1 $\alpha$  mRNA is observed (Russell et al. 2005). However, we (Gibala et al. 2009) showed that a surprisingly small dose of very intense interval exercise — equal to only 2 min of all-out cycling — was sufficient to increase PGC-1 $\alpha$  mRNA during recovery (Fig. 1). One previous study examined the effect of interval-type exercise on PGC-1 $\alpha$  mRNA in humans (De Filippis et al. 2008); however, the volume of exercise was much greater than in a recent study from my laboratory (Gibala et al. 2009). De Filippis et al. (2008) had subjects cycle for 8 min at moderate intensity (70% maximum heart rate), then for 2 min at a higher intensity (90%), followed by 2 min with no resistance, and this was repeated 4 times. The relative increase in PGC-1 $\alpha$  mRNA reported in that study (8-fold change from rest measured after 5 h of recovery) was greater than the 2-fold increase reported after 3 h of recovery by Gibala et al. (2009). However, total exercise time in the interval study (Gibala et al. 2009) was only one twentieth of the protocol used by De Filippis et al. (2008) and total training time (including recovery periods between intervals) was less than one third.

Despite an acute increase in PGC-1 $\alpha$  mRNA, PGC-1 $\alpha$  protein content was unchanged after 4 repeated bouts of 30 s all-out cycling (Fig. 1). This finding is consistent with 2 other human studies that showed no change in PGC-1 $\alpha$  protein despite increased mRNA expression after an acute bout of moderate-intensity cycle exercise lasting 1 h (Coffey et al. 2006) or 3 h (Watt et al. 2004). In contrast, the recent study by De Filippis et al. (2008) reported that PGC-1 $\alpha$  protein content was increased by 20% and 40%, respectively, when measured 30 and 300 min after exercise. Mathai et al. (2008) also recently reported a rapid increase in PGC-1 $\alpha$  protein immediately after an acute bout of moderate cycle exercise to exhaustion, which persisted for 24 h into recovery. Both high-intensity intermittent and low-intensity prolonged exercise have been reported to acutely increase PGC-1 $\alpha$  protein content in rodent muscle (Terada et al. 2005). It is possible that, despite the acute increase in PGC-1 $\alpha$  mRNA, more than one “dose” of intense intermittent exercise is necessary to increase PGC-1 $\alpha$  protein content in human muscle. Performing repeated bouts of low-volume, intense interval exercise has been shown to increase PGC-1 $\alpha$  protein content after several weeks of training, similar to traditional endurance training (Burgomaster et al. 2008). Alternatively, Wright et al. (2007) recently showed in rodents that 2 h of endurance exercise caused a shift in PGC-1 $\alpha$  subcellular localization from the cytosol to the nucleus but did not in-

**Fig. 1.** mRNA expression and protein content of PGC-1 $\alpha$  at rest and 3 h after an acute session of high-intensity interval exercise. \*,  $p \leq 0.05$  vs. rest. Reproduced with permission from Gibala et al. (2009).



**Fig. 2.** Phosphorylated AMPK $\alpha$ 1, AMPK $\alpha$ 2, p38 MAPK, and CaMKII at rest, after 1 and 4 bouts of 30 s all-out cycling exercise and after 3 h of recovery. \*,  $p \leq 0.05$  vs. rest. Reproduced with permission from Gibala et al. (2009).



crease whole-muscle PGC-1 $\alpha$  protein content. The increase in nuclear abundance of PGC-1 $\alpha$  was accompanied by increased transcription factor docking to mitochondrial gene promoters and an increase in mitochondrial gene transcription. This rapid shift in PGC-1 $\alpha$  subcellular location could precipitate a rapid upregulation of mitochondrial gene transcription, without the need for increases in total PGC-1 $\alpha$  protein content (Wright et al. 2007). It is also possible that regulation of PGC-1 $\alpha$  and mitochondrial gene transcription in response to exercise is dependent on training status, and an increase in nuclear PGC-1 $\alpha$  may be a more dominant mechanism of regulation in the trained state when PGC-1 $\alpha$  total protein is already upregulated (Burgomaster et al. 2008).

Several signaling pathways have been linked to exercise-induced activation of PGC-1 $\alpha$  and mitochondrial biogenesis, including AMPK, the mitogen-activated protein kinases (MAPKs), and calcium/calmodulin-dependent protein kinase (CaMK) (Coffey and Hawley 2007). Exercise or stimulated muscle contraction in rodents is generally associated with activation of both  $\alpha$ 1 and  $\alpha$ 2 AMPK catalytic subunits,

whereas  $\alpha$ 2 appears more sensitive to exercise in humans (Jørgensen et al. 2007). This may be related to both fibre type recruitment and relative work intensity, with higher workloads associated with more pronounced changes in muscle phosphorylation potential. Consistent with the work of Chen et al. (2000), who measured AMPK  $\alpha$ 1 and  $\alpha$ 2 activity, we (2009) recently showed that intense interval exercise increased the phosphorylation of both AMPK subunits (Fig. 2). However, other evidence suggests that  $\alpha$ 2/ $\beta$ 2/ $\gamma$ 3 AMPK heterotrimer are preferentially activated during short, intense exercise and that activation of this heterotrimer is not reflected by an increase in total AMPK  $\alpha$  phosphorylation and activity (Birk and Wojtaszewski 2006). With respect to other signaling pathways, recent data from studies on isolated muscle preparations (Wright et al. 2007) suggest that p38 MAPK is downstream of CaMK II in a signaling pathway by which increases in cytosolic calcium lead to increases in PGC-1 $\alpha$ , and inhibition of p38 MAPK prevents the calcium-induced increase in mitochondrial biogenesis. The activity and (or) phosphorylation state of both p38 MAPK and CaMK II have been shown to increase after pro-

longed moderate-intensity exercise in human skeletal muscle (Rose and Hargreaves 2003; Yu et al. 2001). The study by Gibala et al. (2009) was the first to show that an acute bout of intense, low-volume interval exercise stimulates signaling through p38 MAPK; however, we did not detect a significant change in the phosphorylation state of CaMK II (Fig. 2). This could be because of temporal factors related to the timing of the muscle biopsies, insufficient statistical power due to the relatively small number of subjects, or the possibility that p38 MAPK activation after repeated sprint exercise is mediated by pathways other than CaMK II. For example, MAPK kinase (MKK) 3 and MKK6 are classical activators of p38 MAPK in response to numerous stress stimuli (Kyriakis and Avruch 2001) and AMPK has been suggested to be an activator of MKK3 and p38 (Xi et al. 2001). Finally, although high-intensity interval exercise is sometimes equated with strength or resistance exercise, it does not appear to have a major impact on signaling proteins implicated in the regulation of cell growth. We (Gibala et al. 2009) and others (Treebak et al. 2007) found little change in phosphorylation of PKB or downstream targets linked to protein synthesis, including p70 ribosomal S6 kinase and 4E binding protein 1, after intense sprint cycling in human skeletal muscle.

## Conclusion

High-intensity interval exercise represents a unique and understudied model for examining the molecular regulation of skeletal muscle remodeling. Like strength or resistance training, interval exercise is characterized by brief intermittent bouts of relatively intense muscle contraction. However, interval exercise training induces phenotypic changes that resemble those elicited after traditional endurance training. Preliminary evidence suggests that signaling through AMPK and p38 MAPK to PGC-1 $\alpha$  may explain, in part, the metabolic adaptations induced by HIT, including mitochondrial biogenesis and an increased capacity for glucose and fatty acid oxidation.

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