Experimental Physiology – *Research Paper*

Acute signalling responses to intense endurance training commenced with low or normal muscle glycogen

Wee Kian Yeo[1], Sean L. McGee[2], Andrew L. Carey[1], Carl D. Paton[3], Andrew P. Garnham[4], Mark Hargreaves[2] and John A. Hawley[1]

[1]*Exercise Metabolism Group, School of Medical Sciences, RMIT University, Bundoora, Victoria, Australia*
[2]*Department of Physiology, The University of Melbourne, Victoria, Australia*
[3]*Eastern Institute of Technology, Hawke's Bay, New Zealand*
[4]*School of Exercise and Nutrition Sciences, Deakin University, Melbourne, Victoria, Australia*

We have previously demonstrated that well-trained subjects who completed a 3 week training programme in which selected high-intensity interval training (HIT) sessions were commenced with low muscle glycogen content increased the maximal activities of several oxidative enzymes that promote endurance adaptations to a greater extent than subjects who began all training sessions with normal glycogen levels. The aim of the present study was to investigate acute skeletal muscle signalling responses to a single bout of HIT commenced with low or normal muscle glycogen stores in an attempt to elucidate potential mechanism(s) that might underlie our previous observations. Six endurance-trained cyclists/triathletes performed a 100 min ride at ∼70% peak O_2 uptake (AT) on day 1 and HIT (8 × 5 min work bouts at maximal self-selected effort with 1 min rest) 24 h later (HIGH). Another six subjects, matched for fitness and training history, performed AT on day 1 then 1–2 h later, HIT (LOW). Muscle biopsies were taken before and after HIT. Muscle glycogen concentration was higher in HIGH *versus* LOW before the HIT (390 ± 28 *versus* 256 ± 67 μmol (g dry wt)$^{-1}$). After HIT, glycogen levels were reduced in both groups ($P < 0.05$) but HIGH was elevated compared with LOW (229 ± 29 *versus* 124 ± 41 μmol (g dry wt)$^{-1}$; $P < 0.05$). Phosphorylation of 5′AMP-activated protein kinase (AMPK) increased after HIT, but the magnitude of increase was greater in LOW ($P < 0.05$). Despite the augmented AMPK response in LOW after HIT, selected downstream AMPK substrates were similar between groups. Phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) was unchanged for both groups before and after the HIT training sessions. We conclude that despite a greater activation AMPK phosphorylation when HIT was commenced with low compared with normal muscle glycogen availability, the localization and phosphorylation state of selected downstream targets of AMPK were similar in response to the two interventions.

(Received 22 June 2009; accepted after revision 21 October 2009; first published online 23 October 2009)
Corresponding author J. A. Hawley: Exercise Metabolism Group, School of Medical Sciences, RMIT University, PO Box 71, Bundoora, VIC 3083, Australia. Email: john.hawley@rmit.edu.au

Substrate availability is a potent modulator of skeletal muscle training adaptation (Coyle, 2000; Hansen *et al.* 2005; Hawley *et al.* 2006). Recently, we reported that well-trained endurance athletes who undertook a 3 week training programme in which high-intensity interval training (HIT) sessions were commenced with low muscle glycogen concentration increased selected markers of endurance training adaptation to a greater extent than athletes who began all training sessions with normal glycogen levels (Yeo *et al.* 2008*b*). These adaptations included an elevated resting muscle glycogen concentration, increases in the maximal activities of citrate synthase and β-hydroxyacyl-CoA-dehydrogenase and the total protein content of cytochrome *c* oxidase subunit IV, and enhanced rates of whole-body fat oxidation during submaximal exercise (Yeo *et al.* 2008*b*).

Chronic training adaptations are thought to be the result of the cumulative effects of repeated acute bouts

 DOI: 10.1113/expphysiol.2009.049353

Table 1. Characteristics of the subjects who participated in the HIGH and LOW groups

	Age (years)	Body mass (kg)	$\dot{V}_{O_2peak}$ (l min^{-1})	$\dot{V}_{O_2peak}$ (ml kg^{-1} min^{-1})	PPO (W)
HIGH	29.8 ± 2.7	77.5 ± 4.8	4.7 ± 0.2	61.2 ± 1.4	370.1 ± 12.9
LOW	26.3 ± 2.9	77.1 ± 4.9	4.7 ± 0.2	61.9 ± 2.2	360.9 ± 10.0

Abbreviations: $\dot{V}_{O_2peak}$, peak O_2 uptake; and PPO, peak sustained power output. Data were collected during the incremental cycling test to exhaustion prior to the commencement of the study.

of exercise (Pilegaard *et al.* 2000; Widegren *et al.* 2001; Hansen *et al.* 2005; Hawley *et al.* 2006). As such, one potential mechanism that might underlie our previous observation of a greater adaptation after training with low muscle glycogen availability (Yeo *et al.* 2008*b*) is the acute myocellular stress associated with this state, which may augment the activation of several protein kinases with roles in mitochondrial biogenesis. These include the 5′AMP-activated protein kinase (AMPK; Wojtaszewski *et al.* 2003; Steinberg *et al.* 2006) and the p38 mitogen-activated protein kinase (p38 MAPK; Chan *et al.* 2004). Accordingly, the purpose of the present study was to investigate the acute signalling responses to a single session of HIT undertaken with either low or normal muscle glycogen concentration. We hypothesized that performing intense endurance exercise in the face of low muscle glycogen concentration would result in increased activation of AMPK and p38 MAPK and some of their downstream substrates.

Methods

Subjects and preliminary testing

Twelve endurance-trained male cyclists or triathletes participated in this study (Table 1). They gave their written consent after they were informed about the possible risks of all procedures. This study was approved by the RMIT University Human Research Ethics Committee and was performed in accordance to the standards set by the latest revision of the Declaration of Helsinki. One week prior to the commencement of the study, each subject undertook an incremental cycling test to exhaustion on an electromagnetically braked cycle ergometer (Lode, Groningen, The Netherlands). The results from this test [peak O_2 uptake ($\dot{V}_{O_2peak}$) and peak sustained power output (PPO)] were used to determine the power output corresponding to 70% of each subject's $\dot{V}_{O_2peak}$ (63% of PPO) to be used in the subsequently described experimental trials. The testing protocol (Hawley & Noakes, 1992) and the equipment used for this test have been described in detail previously (Yeo *et al.* 2008*a*,*b*). The maximal incremental cycling test and all experimental trials were conducted in standard laboratory conditions (18–22°C, 40–50% relative humidity), and subjects were fan cooled during all experimental trials.

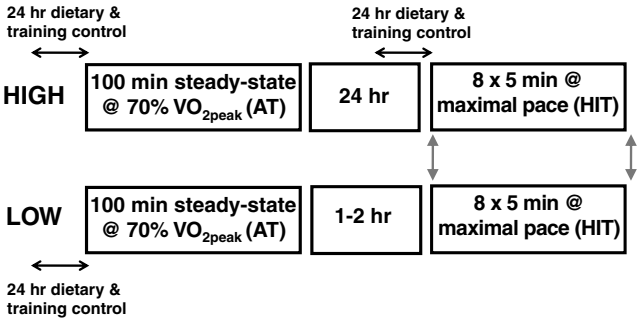

Figure 1. Overview of the study design and experimental trial
Abbreviations and symbols: AT, aerobic training; HIT, high-intensity interval training; $\dot{V}_{O_2peak}$, peak O_2 uptake; and ↕, muscle biopsy.

Experimental design

An overview of the experimental design is shown in Fig. 1. In brief, the subjects were divided into two groups matched for age, $\dot{V}_{O_2peak}$ and training history (Table 1). They then undertook two different laboratory training protocols according to the schedule of their respective groups. One group (HIGH) performed a 100 min steady-state ride (AT) at ∼70% $\dot{V}_{O_2peak}$ (63% of PPO) on the first day and high-intensity interval training (HIT; 8 × 5 min work bouts at self-selected maximal effort with 1 min recovery in between work bouts at ≤ 100 W) 24 h later. In contrast, the other group (LOW) performed both training sessions on the same day, performing the AT in the morning (08.00 h), followed by HIT after 1–2 h of rest (Fig. 1). During the time between these two training sessions, subjects in the LOW group rested in the laboratory and were given *ad libitum* access to water. The training sessions and equipment set-up have been described in detail previously (Yeo *et al.* 2008*b*). All training sessions were performed in the laboratory under the supervision of the principal investigator, and power output for all these sessions were recorded using PowerTap power meters (CycleOps, Saris Cycling Group, Madison, WI, USA). Skeletal muscle biopsies from the *vastus lateralis* were taken immediately before and within 15 s on completion of the HIT sessions (Fig. 1). The muscle biopsy procedure has been described in detail previously (Yeo *et al.* 2008*a*,*b*).

All muscle samples were rapidly frozen in liquid N_2 within ∼15 s and stored at −80°C until further analysis.

Dietary and training control

Twenty four hours prior to all training sessions, subjects' dietary intakes were 'clamped' (0.21 MJ (kg body mass)$^{-1}$; 8 g kg^{-1} day^{-1} and 65% of energy from carbohydrate; 2.0 g kg^{-1} day^{-1} protein and 1.0 g kg^{-1} day^{-1} fat). All meals and snacks were supplied, with diets being individualized for food preferences and body mass. Subjects received their food in pre-prepared packages and were required to keep a food checklist to note their compliance with the dietary instructions and their intake of any additional food or drinks. There were no restrictions on when the subjects should consume the prescribed food, so long as it was ingested outside their respective exercise schedule. Subjects were also required to finish all the food supplied to them, and empty bags and containers were brought back to the laboratory to facilitate adherence. The subjects reported to the laboratory after an overnight fast (12–14 h) to perform all the exercise sessions according to their respective schedule. They also refrained from exercise the day before the 100 min steady-state ride.

Analytical procedures

Muscle glycogen concentration. Muscle glycogen concentration was analysed as previously described (Churchley *et al.* 2007). In brief, approximately 10–15 mg of muscle was freeze dried and powdered, with all visible blood and connective tissue removed under magnification. The freeze-dried muscle sample was then extracted and glycogen concentration determined via enzymatic analyses.

Nuclear fractionation and whole-cell extraction. Approximately 70–80 mg of wet muscle were cut and divided into two portions. The first portion (35–40 mg) of the wet muscle was homogenized in ice-cold buffer A [250 mM sucrose, 10 mM Tris (pH 7.5), 10 mM NaCl, 3 mM $MgCl_2$, 1 mM dithiothreitol, Protease inhibitor cocktail (PIC; 2 μl per 40 mg) and 1 mM phenylmethylsulphonyl fluoride] before being centrifuged for 5 min at 500*g* at 4°C. The supernatant was removed, and the pellets were then resuspended in ice-cold buffer B [50 mM Tris (pH 7.5), 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% Triton X-100, PIC (2 μl per 40 mg), 50 mM NaF, 50 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulphonyl fluoride and 5 mM sodium pyrophosphate] for 10 min. The lysates were then centrifuged again for 5 min at 3000*g* at 4°C and the supernatant, representing the nuclear fraction, was aliquoted and stored at −80°C until further analysis.

The second portion (35–40 mg) of wet muscle was homogenized [50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 50 mM NaF, 5 mM sodium pyrophosphate, 10% glycerol, 1% Triton X-100, 10 μg ml^{-1} trypsin inhibitor, 2 μg ml^{-1} aprotinin, 1 mM benzamidine and 1 mM phenylmethylsulphonyl fluoride] and centrifuged at 20 000*g* for 30 min at 4°C. The supernatant, representing the whole-cell lysates, was aliquoted and stored at −80°C until further analysis. Total protein concentration in both the nuclear and whole-cell lysates was determined by the bicinchoninic acid method (Pierce, IL, USA).

Western blotting. Muscle lysates containing 60 μg (p-AMPK^{Thr172}, total AMPK, p-p38 MAPK$^{Thr180/Tyr182}$ and total p38 MAPK) and 50 μg [nuclear histone deacetylase 5 (HDAC5), total HDAC5, phosphorylated cAMP-response element-binding protein (p-CREB^{Ser133}) and phosphorylated activating transcription factor-2 (p-ATF2$^{Thr69/71}$)] of total protein were electrophoresed on 10% (p-AMPK^{Thr172}, total AMPK, p-p38 MAPK$^{Thr180/Tyr182}$ and total p38 MAPK) and 5–14% (nuclear HDAC5, total HDAC5, p-CREB^{Ser133} and p-ATF2$^{Thr69/71}$) SDS-PAGE and detected by immunoblotting with antibodies specific for p-AMPK^{Thr172}, total AMPKα, (gifts from Professor Bruce Kemp, St. Vincent's Institute, Melbourne, Australia), p-p38 MAPK$^{Thr180/Tyr182}$, total p38 MAPK, nuclear HDAC5, total HDAC5, p-CREB^{Ser133} and p-ATF2$^{Thr69/71}$ (Cell Signaling, Danvers, MA, USA). An internal control (standardized human skeletal muscle sample) was used in all gels to account for variability between exposures of different membranes. The immunoreactive proteins were detected with enhanced chemiluminescence (GE Healthcare, Wauwatosa, WI, USA) on a Bio-Rad Chemidoc XRS system (Bio-Rad, Hercules, CA, USA) and quantified by densitometry (Quantity one, Bio-Rad). Phosphorylation levels of AMPK^{Thr172} and p38 MAPK$^{Thr180/Tyr182}$ were expressed relative to their respective total protein concentrations.

Statistical analysis

Treatment effects were analysed using two-factor (treatment and time) repeated-measure analysis of variance (ANOVA), and the *post hoc* analyses were performed using the Holm–Sidak method. Data were analysed using SigmaStat 3.1.1 (Systat Software, Inc., San Jose, CA, USA), and all values are expressed as means ± S.E.M., with significance reported as $P < 0.05$.

Results

Muscle glycogen concentrations

Figure 2 displays muscle glycogen content before and after the HIT session. As would be expected, muscle

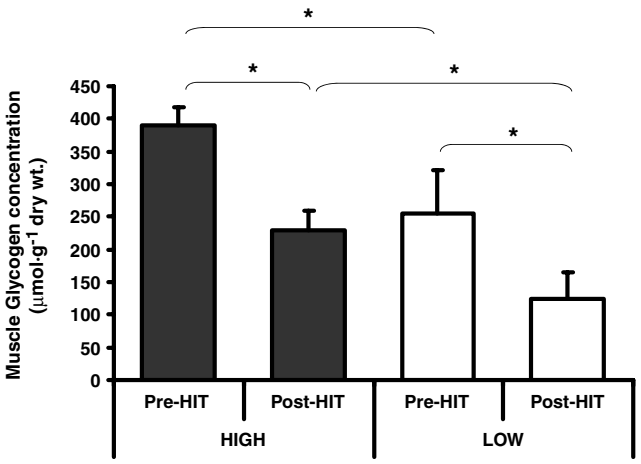

Figure 2. Muscle glycogen concentration before (Pre) and after (Post) high-intensity interval training for groups that performed HIT with normal (HIGH) *versus* low muscle glycogen concentration (LOW)
* Significantly different at $P < 0.05$.

glycogen concentration was significantly higher in HIGH compared with LOW before the HIT (390 ± 28 *versus* 256 ± 67 μmol (g dry wt)$^{-1}$). After HIT, glycogen levels were reduced in both groups ($P < 0.05$) but HIGH was still higher compared with LOW (229 ± 29 *versus* 124 ± 41 μmol (g dry wt)$^{-1}$; $P < 0.05$).

Self-selected training intensity during HIT

Self-selected average power output during the HIT was significantly greater in HIGH compared with LOW (308 ± 14 *versus* 270 ± 13 W, or 83 ± 2 *versus* 75 ± 3% PPO; $P < 0.05$).

Phosphorylated AMPK^{Thr172} and p-p38 MAPK$^{Thr180/Tyr182}$

Figure 3 displays p-AMPK^{Thr172} (relative to total AMPK) before and after HIT. Phosphorylated AMPK^{Thr172} was similar before the commencement of the HIT in both HIGH and LOW but increased significantly in both groups after HIT (HIGH, from 3.5 ± 0.1 to 5.4 ± 0.5 a.u. *versus* LOW, from 3.8 ± 0.3 to 7.5 ± 1.1 a.u.; $P < 0.05$) such that the phosphorylation state was higher in LOW compared with HIGH ($P < 0.05$).

Figure 4 displays p-p38$^{Thr180/Tyr182}$ (relative to total p38 MAPK) before and after HIT. There were no significant effects for treatment or time on p-p38$^{Thr180/Tyr182}$ (Fig. 4).

Histone deacetylase 5, p-CREB^{Ser133}and p-ATF2$^{Thr69/71}$

There was no significant effect of either treatment or time on total or nuclear HDAC5 (Fig. 5*A*), p-CREB^{Ser133} (Fig. 5*B*) and p-ATF2$^{Thr69/71}$ (Fig. 5*C*) with HIT.

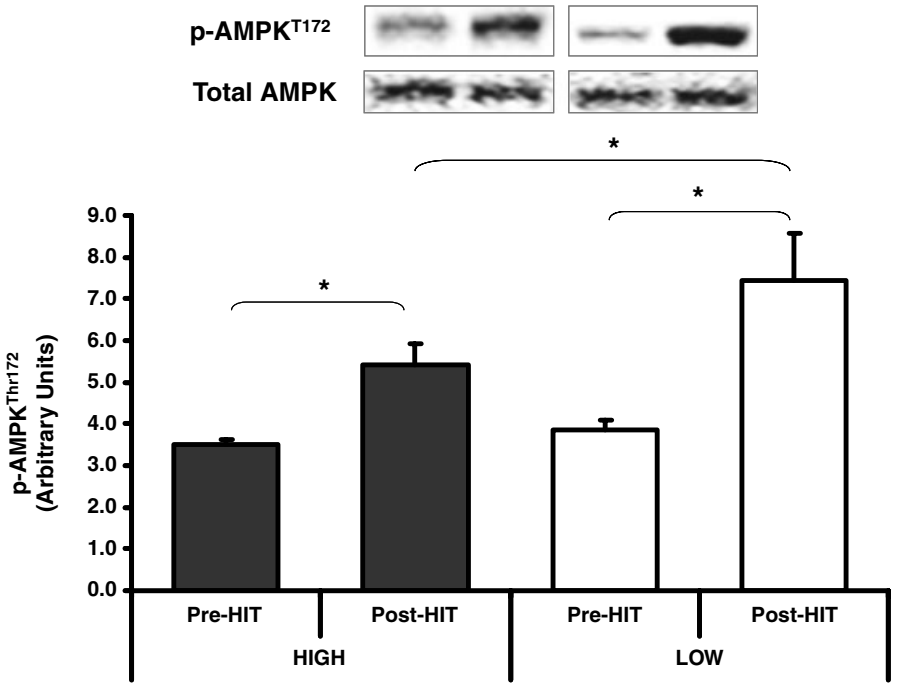

Figure 3. Phosphorylation of AMPK (relative to total AMPK) at threonine 172 (p-AMPK^{Thr172}) before (Pre) and after (Post) HIT with normal (HIGH) *versus* low muscle glycogen concentration (LOW)
* Significantly different at $P < 0.05$.

Discussion

In a recent study, we reported that athletes who participated in a 3 week training programme in which HIT sessions were commenced with low muscle glycogen concentration increased the maximal activities of several enzymes, promoting endurance adaptations to a greater extent than athletes who began all training sessions with normal glycogen levels (Yeo *et al.* 2008*b*). In this investigation, we sought to determine selected acute signalling responses to a single session of HIT commenced with either low or normal muscle glycogen concentration to try to elucidate some of the potential mechanisms that might underlie our previous observations (Yeo *et al.* 2008*b*). The main findings of the present study were that: (1) AMPK phosphorylation was greater when HIT was commenced with low compared with normal muscle glycogen availability; but (2) despite the greater AMPK activation after intense interval training with low muscle glycogen availability, the localization and phosphorylation state of selected downstream targets of AMPK (HDAC5 and CREB) were unchanged.

AMPK is a member of a metabolite-sensing protein kinase family that functions as a metabolic 'fuel gauge' in skeletal muscle. During exercise, AMPK becomes activated in response to changes in cellular energy status (e.g. increased AMP/ATP ratio) in an intensity-dependent manner and serves to inhibit ATP-consuming pathways and to activate pathways involved in carbohydrate and fatty acid metabolism to restore ATP levels. AMPK may also be involved in the adaptive response of skeletal muscles to endurance exercise training (for review see Reznick & Shulman, 2006). In the present study, HIT induced a significant elevation of AMPK in all subjects, regardless of whether they commenced the session with low or normal muscle glycogen levels (Fig. 3). However, AMPK phosphorylation was increased to a greater extent when HIT was commenced with low compared with normal muscle glycogen availability (Fig. 3). The greater AMPK activation in LOW occurred despite significantly greater absolute and relative power outputs being sustained by HIGH subject, who commenced the HIT session with normal glycogen levels. In order to create conditions that were similar to our previous study (Yeo *et al.* 2008*b*) and to account for differences in the ability to train (i.e. relative intensity) with different levels of substrate availability, the subjects were deliberately instructed to cycle at their self-selected maximal effort during HIT sessions rather than 'clamping' the training intensity. Wojtaszewski *et al.* (2003) have previously reported that AMPK activity in resting human muscle and the degree of activation during subsequent cycling exercise are dependent on the fuel status of the muscle cells (i.e. AMPK activity is elevated in muscle low in glycogen). However, in that study there were larger differences in pre-exercise muscle glycogen content (909 ± 75 *versus* 163 ± 12 μmol (g dry wt)$^{-1}$), and subjects cycled at a constant, submaximal power output (i.e. 70% of $\dot{V}_{O_2peak}$). One possible explanation for our finding of a higher AMPK in the face of low muscle glycogen availability is recent evidence showing that glycogen binding to the glycogen-binding domain on the AMPK β subunit allosterically inhibits AMPK activity and phosphorylation by upstream kinases (McBride *et al.* 2009).

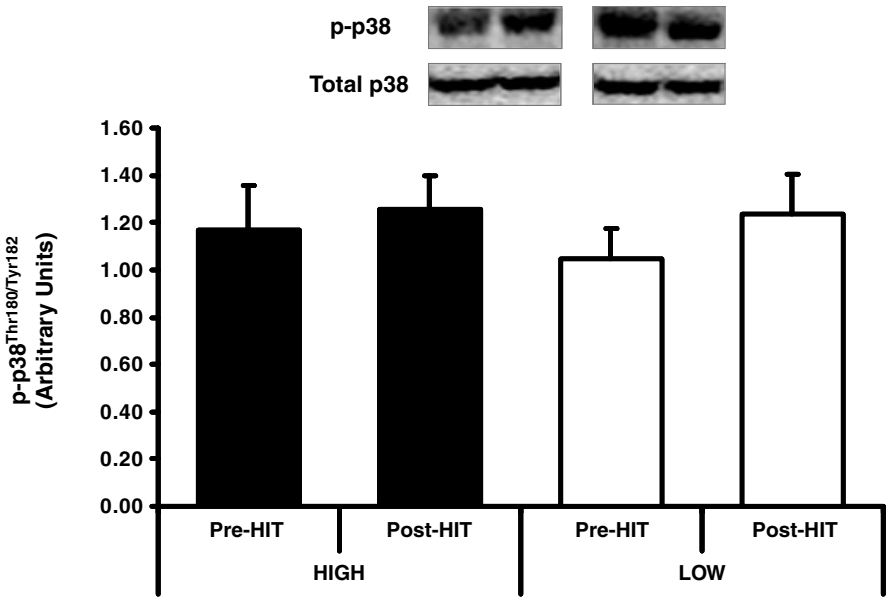

Figure 4. Phosphorylation of p38 MAPK (relative to total p38 MAPK) before (Pre) and after (Post) HIT with normal (HIGH) *versus* low muscle glycogen concentration (LOW)

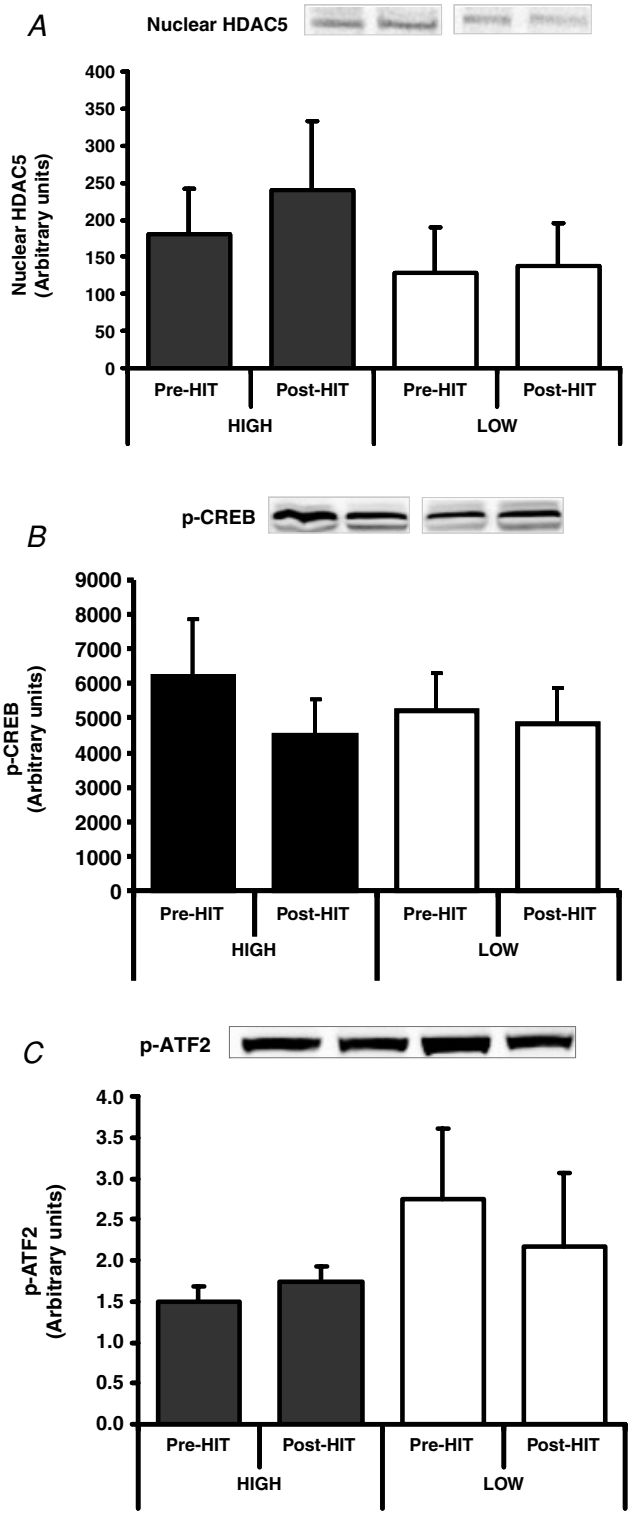

Figure 5. Putative AMPK and p38 MAPK downstream signalling
A, nuclear concentration of HDAC5; *B*, phosphorylation of cAMP response element binding protein (p-CREB); *C*, phosphorylation of activating transcription factor 2 (p-ATF2) before (Pre) and after (Post) HIT with normal (HIGH) *versus* low muscle glycogen concentration (LOW).

McBride *et al.* (2009) recently reported that AMPK is inhibited by glycogen, particularly preparations with high branching content. Moreover, these workers (McBride *et al.* 2009) also demonstrated that this inhibition of AMPK activation by several different types of carbohydrates was dependent on the glycogen-binding domain being abolished by mutation of residues required for carbohydrate binding. Collectively, these results strongly suggest that glycogen is a potent regulator of AMPK activity through its association with the glycogen-binding domain on the AMPK β subunit (McBride *et al.* 2009).

In an effort to determine whether the acute increase in AMPK activation after HIT was commenced with low muscle glycogen availability might help to explain the chronic training-induced differences in muscle adaptation observed previously (Yeo *et al.* 2008*b*), several downstream targets of AMPK were assessed. AMPK has been reported to phosphorylate and inactivate HDAC5 (McGee *et al.* 2008), which in turn leads to the removal of HDAC5 from the nucleus, allowing the myocyte enhancer factor 2 (MEF2) to bind and activate the peroxisome proliferator-activated receptor-γ coactivator 1 (PGC-1α) (Baar & McGee, 2008). In addition, PGC-1α is also known to have a cAMP-response element (CRE) in its promoter region (Branvold *et al.* 2008), while CREB has also been identified as the downstream target of AMPK (Thomson *et al.* 2008). As such it was hypothesized that the greater AMPK activation in response to intense training with low glycogen availability would result in concomitant increases in the localization and/or phosphorylation of some of these downstream targets of AMPK. This, however, was not the case; there were no differences in the localization of HDAC5 and the phosphorylation state of CREB when subjects commenced intense exercise with either low or normal muscle glycogen levels (Fig. 5). A simple explanation for these observations is that the magnitude of increase in AMPK phosphorylation was insufficient to specifically increase its activity towards HDAC5 and CREB. Alternatively, there may be other mechanisms by which chronic activation of AMPK increases mitochondrial enzyme activity, including but not limited to AMPK effects on upstream stimulatory factor-1 (USF-1; Irrcher *et al.* 2008), PGC-1α phosphorylation (Jäger *et al.* 2007), acetylation (Canto *et al.* 2009) and/or localization. Another possibility may be that the AMPK-mediated changes we have previously observed following a single exercise bout in untrained subjects (McGee & Hargreaves, 2004) are less apparent in the trained cohort used in the present study. In support of this contention, it has recently been reported that the mitochondrial content and oxidative capacity of skeletal muscle are key determinants of the activation of signalling proteins important to muscle plasticity (Ljubicic & Hood, 2009). The attenuation of kinase phosphorylation in

muscle with high mitochondrial content suggests that these proteins may require a greater stimulus input for activation to propagate these cues downstream to evoke phenotypic adaptations.

The MAPK signal transduction cascade has been identified as a candidate system that converts contraction-induced biochemical perturbations in skeletal muscle into appropriate intracellular responses. The MAPK pathways include the stress-activated protein kinase, p38 MAPK, which has been reported to be involved in mediating skeletal muscle adaptive responses to endurance training (Fan *et al.* 2004; Akimoto *et al.* 2005). Despite the higher AMPK phosphorylation in subjects who commenced HIT with low muscle glycogen concentrations, the phosphorylation state of p38 MAPK was similar between groups before and after HIT (Fig. 4). In contrast, Yu *et al.* (2003) have previously reported a significant increase in p38 MAPK after intense exercise in subjects with a similar training profile to those recruited for the present investigation. Differences between results from the experiments of Yu *et al.* (2003) and the present study are difficult to explain, although training intensity was 'clamped' in the former investigation and was markedly higher than the 'self-selected' intensity chosen by the subjects in the present study. Furthermore, Yu *et al.* (2003) observed a main effect for time (pre *versus* post) but no interaction with training status. Not surprisingly, given the lack of differences in p38 MAPK signalling in the present study, the phosphorylation state of the selected downstream target of p38 MAPK (ATF2) was not different between groups (Fig. 5*C*).

The present study has focused on the early signalling responses to a single bout of HIT commenced with low or normal muscle glycogen. While the findings in the present study strongly suggest that resting muscle substrate availability plays an important regulatory role in AMPK activation during subsequent exercise, one should not discount other factors as being important in modulating this response. For example, catecholamine levels when exercise is undertaken with low muscle glycogen are higher than when the same intensity exercise is performed with normal muscle glycogen stores (Hansen *et al.* 2005), demonstrating that a higher stress response is elicited when training is commenced with low glycogen availability. Arterial plasma free fatty acid concentrations are also markedly elevated during exercise and subsequent recovery in well-trained subjects who perform steady-state exercise with low glycogen levels (Wojtaszewski *et al.* 2003). This finding may be particularly relevant, because raising plasma free fatty acid concentration in the absence of exercise has been shown to induce mitochondrial biogenesis in skeletal muscle (Garcia-Roves *et al.* 2007). Finally, the effect of twice-a-day training might provide an added stimulus for promoting endurance adaptations compared with training once a day. Clearly, the interaction of muscle fuel stores and the concomitant hormonal milieu during and after exercise play major roles in modulating the training response and subsequent adaptation, and this is an area for future research.

In summary, the present study shows that high-intensity interval training resulted in a significant increase in the activation of AMPK, with the magnitude of this increase being greater when training was commenced with low compared to normal muscle glycogen availability. Despite the greater AMPK activation after intense exercise in the face of low muscle glycogen availability, the localization and phosphorylation state of selected downstream targets of AMPK (HDAC5 and CREB) were unchanged. Further studies are needed to identify the precise mechanism(s) responsible for the amplified training response observed when well-trained subjects commence intense cycling with low muscle glycogen availability.

References

Akimoto T, Pohnert SC, Li P, Zhang M, Gumbs C, Rosenberg PB, Williams RS & Yan Z (2005). Exercise stimulates *Pgc-1α* transcription in skeletal muscle through activation of the p38 MAPK pathway. *J Biol Chem* **280**, 19587–19593.

Baar K & McGee SL (2008). Optimizing training adaptations by manipulating glycogen. *Eur J Sport Sci* **8**, 97–106.

Branvold DJ, Allred DR, Beckstead DJ, Kim HJ, Fillmore N, Condon BM, Brown JD, Sudweeks SN, Thomson DM & Winder WW (2008). Thyroid hormone effects on LKB1, MO25, phospho-AMPK, phospho-CREB, and PGC-1α in rat muscle. *J Appl Physiol* **105**, 1218–1227.

Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P & Auwerx J (2009). AMPK regulates energy expenditure by modulating NAD^+ metabolism and SIRT1 activity. *Nature* **458**, 1056–1060.

Chan MHS, McGee SL, Watt MJ, Hargreaves M & Febbraio MA (2004). Altering dietary nutrient intake that reduces glycogen content leads to phosphorylation of nuclear p38 MAP kinase in human skeletal muscle: association with IL-6 gene transcription during contraction. *FASEB J* **18**, 1785–1787.

Churchley EG, Coffey VG, Pedersen DJ, Shield A, Carey KA, Cameron-Smith D & Hawley JA (2007). Influence of preexercise muscle glycogen content on transcriptional activity of metabolic and myogenic genes in well-trained humans. *J Appl Physiol* **102**, 1604–1611.

Coyle EF (2000). Physical activity as a metabolic stressor. *Am J Clin Nutr* **72**, 512S–520S.

Fan M, Rhee J, St-Pierre J, Handschin C, Puigserver P, Lin J, Jäeger S, Erdjument-Bromage H, Tempst P & Spiegelman BM (2004). Suppression of mitochondrial respiration through recruitment of p160 myb binding protein to PGC-1α: modulation by p38 MAPK. *Genes Dev* **18**, 278–289.

Garcia-Roves P, Huss JM, Han DH, Hancock CR, Iglesias-Gutierrez E, Chen M & Holloszy JO (2007). Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. *Proc Natl Acad Sci USA* **104**, 10709–10713.

Hansen AK, Fischer CP, Plomgaard P, Andersen JL, Saltin B & Pedersen BK (2005). Skeletal muscle adaptation: training twice every second day *vs.* training once daily. *J Appl Physiol* **98**, 93–99.

Hawley JA & Noakes TD (1992). Peak power output predicts maximal oxygen uptake and performance time in trained cyclists. *Eur J Appl Physiol Occup Physiol* **65**, 79–83.

Hawley JA, Tipton KD & Millard-Stafford ML (2006). Promoting training adaptations through nutritional interventions. *J Sports Sci* **24**, 709–721.

Irrcher I, Ljubicic V, Kirwan AF & Hood DA (2008). AMP-activated protein kinase-regulated activation of the PGC-1α promoter in skeletal muscle cells. *PLoS ONE* **3**, e3614.

Jäger S, Handschin C, St-Pierre J & Spiegelman BM (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1α. *Proc Natl Acad Sci USA* **104**, 12017–12022.

Ljubicic V & Hood DA (2009). Specific attenuation of protein kinase phosphorylation in muscle with a high mitochondrial content. *Am J Physiol Endocrinol Metab* **297**, E749–E758.

McBride A, Ghilagaber S, Nikolaev A & Hardie DG (2009). The glycogen-binding domain on the AMPK β subunit allows the kinase to act as a glycogen sensor. *Cell Metab* **9**, 23–34.

McGee SL & Hargreaves M (2004). Exercise and myocyte enhancer factor 2 regulation in human skeletal muscle. *Diabetes* **53**, 1208–1214.

McGee SL, van Denderen BJW, Howlett KF, Mollica J, Schertzer JD, Kemp BE & Hargreaves M (2008). AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes* **57**, 860–867.

Pilegaard H, Ordway GA, Saltin B & Neufer PD (2000). Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. *Am J Physiol Endocrinol Metab* **279**, E806–E814.

Reznick RM & Shulman GI (2006). The role of AMP-activated protein kinase in mitochondrial biogenesis. *J Physiol* **574**, 33–39.

Steinberg GR, Watt MJ, McGee SL, Chan S, Hargreaves M, Febbraio MA, Stapleton D & Kemp BE (2006). Reduced glycogen availability is associated with increased AMPKα2 activity, nuclear AMPKα2 protein abundance, and GLUT4 mRNA expression in contracting human skeletal muscle. *Appl Physiol Nutr Metab* **31**, 302–312.

Thomson DM, Herway ST, Fillmore N, Kim H, Brown JD, Barrow JR & Winder WW (2008). AMP-activated protein kinase phosphorylates transcription factors of the CREB family. *J Appl Physiol* **104**, 429–438.

Widegren U, Ryder JW & Zierath JR (2001). Mitogen-activated protein kinase signal transduction in skeletal muscle: effects of exercise and muscle contraction. *Acta Physiol Scand* **172**, 227–238.

Wojtaszewski JFP, MacDonald C, Nielsen JN, Hellsten Y, Hardie DG, Kemp BE, Kiens B & Richter EA (2003). Regulation of 5′AMP-activated protein kinase activity and substrate utilization in exercising human skeletal muscle. *Am J Physiol Endocrinol Metab* **284**, E813–E822.

Yeo WK, Lessard SJ, Chen Z-P, Garnham AP, Burke LM, Rivas DA, Kemp BE & Hawley JA (2008*a*). Fat adaptation followed by carbohydrate restoration increases AMPK activity in skeletal muscle from trained humans. *J Appl Physiol* **105**, 1519–1526.

Yeo WK, Paton CD, Garnham AP, Burke LM, Carey AL & Hawley JA (2008*b*). Skeletal muscle adaptation and performance responses to once a day *versus* twice every second day endurance training regimens. *J Appl Physiol* **105**, 1462–1470.

Yu M, Stepto NK, Chibalin AV, Fryer LGD, Carling D, Krook A, Hawley JA & Zierath JR (2003). Metabolic and mitogenic signal transduction in human skeletal muscle after intense cycling exercise. *J Physiol* **546**, 327–335.

Acknowledgements

This study was supported by a research grant from Glaxo SmithKline (UK) to J.A.H., the Australian Sports Commission (J.A.H.) and the National Sports Council of Malaysia (W.K.Y.). A.L.C. and S.L.M. are supported by Peter Doherty post doctoral fellowships from the National Health and Medical Research Council. The authors would like to thank Professor Bruce Kemp for his kind gift of the AMPK antibodies and Professor Louise Burke for help with nutritional interventions.