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Human muscle fibre type-specific regulation of AMPK and downstream targets by exercise

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Keypoints

- AMP-activated protein kinase (AMPK) is an important regulator of cellular energy status during exercise.
- Most human studies investigating skeletal muscle protein signalling have been performed in whole muscle biopsy samples, yet recent studies suggest muscle fibre type-specific AMPK expression with potential fibre type-specific regulation of AMPK during exercise.
- This study provides novel and comprehensive data on human muscle fibre type-specific expression levels of AMPK subunits and downstream targets of AMPK.
- We show a differentiated response to exercise of key metabolic signalling proteins in human type I and type II muscle fibres during interval exercise, not evident during continuous exercise. These differences between exercise types were not present in whole muscle biopsy samples.
- Our findings highlight the importance of performing fibre type-specific measurements and the increased activation of AMPK in interval *vs.* continuous exercise could be important for exercise type-specific adaptations, i.e. metabolism, insulin sensitivity and mitochondrial density in human skeletal muscle.

Abstract AMP-activated protein kinase (AMPK) is a regulator of energy homeostasis during exercise. Studies suggest muscle fibre type-specific AMPK expression. However, fibre type-specific regulation of AMPK and downstream targets during exercise has not been demonstrated. We hypothesized that AMPK subunits are expressed in a fibre type-dependent manner and that fibre type-specific activation of AMPK and downstream targets is dependent on exercise intensity. Pools of type I and II fibres were prepared from biopsies of vastus lateralis muscle from healthy men before and after two exercise trials: (1) continuous cycling (CON) for 30 min at 69 ± 1% peak rate of O_2 consumption ($\dot{V}_{O_2peak}$) or (2) interval cycling (INT) for 30 min with 6 × 1.5 min high-intensity bouts peaking at 95 ± 2% $\dot{V}_{O_2peak}$. In type I *vs.* II fibres a higher β_1 AMPK (+215%) and lower γ_3 AMPK expression (−71%) was found. α_1, α_2, β_2 and γ_1 AMPK expression was similar between fibre types. In type I *vs.* II fibres phosphoregulation after CON was similar ($AMPK^{Thr172}$, ACC^{Ser221}, $TBC1D1^{Ser231}$ and GS^{2+2a}) or lower ($TBC1D4^{Ser704}$). Following INT, phosphoregulation in type I *vs.* II fibres was lower ($AMPK^{Thr172}$, $TBC1D1^{Ser231}$, $TBC1D4^{Ser704}$ and ACC^{Ser221}) or higher (GS^{2+2a}). Exercise-induced glycogen degradation in type I *vs.* II fibres was similar (CON) or lower (INT). In conclusion, a differentiated response to exercise of metabolic signalling/effector proteins in human type I and II fibres was evident during interval exercise. This

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could be important for exercise type-specific adaptations, i.e. insulin sensitivity and mitochondrial density, and highlights the potential for new discoveries when investigating fibre type-specific signalling.

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Abbreviations ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CON, continuous cycling; Cr, creatine; GS, glycogen synthase; INT, interval cycling; LKB1, serine/threonine kinase 11; MHC, myosin heavy chain; PCr, phosphocreatine; RT, room temperature; TBC1D, TBC1 domain family member.

Introduction

AMP-activated protein kinase (AMPK) is an $\alpha\beta\gamma$ heterotrimer protein which has been proposed as a regulator of cellular energy homeostasis during exercise (for reviews see Hardie *et al.* 2012; O'Neill, 2013; Richter & Hargreaves, 2013). Since the first observations of AMPK activation during exercise in skeletal muscle from rats (Winder & Hardie, 1996) and humans (Chen *et al.* 2000; Fujii *et al.* 2000; Wojtaszewski *et al.* 2000;), the functional role of AMPK has been studied intensively. Most measurements have been performed in whole muscle biopsy samples without consideration of the influence of muscle fibre types. In fact, several rodent studies have found differences in AMPK subunit and AMPK complex expression between muscles mainly composed of slow twitch (type I) or fast twitch (type II) muscle fibres (Chen *et al.* 1999; Mahlapuu *et al.* 2004; Yu *et al.* 2004; Putman *et al.* 2007; Treebak *et al.* 2009*a*; Murphy, 2011). These studies indirectly suggest that rodent type I fibres have a higher expression of α_1, α_2 and β_1 AMPK (Putman *et al.* 2007; Murphy, 2011) while type II fibres have a higher expression of β_2, γ_2 and γ_3 AMPK as well as a higher $\alpha_2\beta_2\gamma_3$ AMPK content (Chen *et al.* 1999; Mahlapuu *et al.* 2004; Yu *et al.* 2004; Treebak *et al.* 2009*a*). However, in human muscle, these differences were not evident when comparing biopsies from soleus muscle (~70% type I fibres), gastrocnemius muscle (~50% type I fibres) and vastus lateralis muscle (~50% type I fibres) (Jensen *et al.* 2012), possibly due to the small muscle–muscle variation in fibre type composition (Jensen *et al.* 2012). In contrast, the expression of different AMPK isoforms in human muscle has been reported to be influenced by denervation, exercise training, fitness level, sex and age (Nielsen *et al.* 2003; Frøsig *et al.* 2004; Wojtaszewski *et al.* 2005; Mortensen *et al.* 2009; Kostovski *et al.* 2013). All of these conditions are also associated with variations in fibre type composition, and the studies reveal that in particular the expression of γ_3 AMPK is tightly and positively correlated to muscle fibre type myosin heavy chain (MHC) II isoform expression. However, one cannot distinguish between the direct effect of these conditions *per se* or the shift/difference in MHC composition in these subjects. Thus, in order to evaluate the consequence of different fibre types on AMPK expression and regulation in humans, muscle fibre type-specific analyses must be performed. When using immunohistochemistry, Lee-Young *et al.* (2009) found different γ_3 AMPK expression among fibre types in the order IIx > IIa > I, whereas α_1 and α_2 AMPK content was similar between fibre types. In contrast, Murphy reported a higher α_1 and β_1 AMPK expression in type I compared to type II fibres when performing Western blotting on single human muscle fibres (Murphy, 2011). Although incongruent findings, these studies suggest that the expression of at least some AMPK subunits are fibre type dependent, yet the expression of other AMPK subunits in human muscle fibres remains to be determined. Furthermore, exercise-induced phosphorylation of AMPK at Thr172 ($AMPK^{Thr172}$) is higher in type IIx compared to type I and IIa fibres measured by immunohistochemistry (Lee-Young *et al.* 2009). This observation suggests a fibre type-specific regulation of AMPK activity during exercise. However, assay linearity is difficult to control when using immunohistochemistry and the findings need to be confirmed by other methods. In fact, lack of assay linearity in immunohistochemistry could explain the different findings regarding fibre type-specific α_1 AMPK expression when using immunohistochemistry (α_1 AMPK expression similar in type I *vs.* II) (Lee-Young *et al.* 2009) compared to Western blotting (higher α_1 AMPK expression in type I *vs.* II) (Murphy, 2011).

In skeletal muscle AMPK is an upstream kinase for various targets, i.e. the acetyl-CoA carboxylase (ACC) involved in fatty acid oxidation (Winder *et al.* 1997), the GTPase-activating proteins TBC1 domain family members (TBC1D) 1 and 4 regulating cellular transport of GLUT4-containing vesicles (Kramer *et al.* 2006; Treebak *et al.* 2007, 2014; Pehmøller *et al.* 2009; Frøsig *et al.* 2010; Vichaiwong *et al.* 2010; Jessen *et al.* 2011), and the glycogen synthase (GS), a key regulator of glycogen synthesis (Carling & Hardie, 1989; Jørgensen *et al.* 2004). It is well known that phosphorylation and hence the activity of these proteins are altered with exercise (Wojtaszewski *et al.* 2002; Taylor *et al.* 2008; Pehmøller *et al.* 2009;

Rose *et al.* 2009; Frøsig *et al.* 2010; Treebak *et al.* 2010; Vichaiwong *et al.* 2010; Jessen *et al.* 2011), but whether changes are fibre type specific during exercise remains elusive.

Several studies have reported that activation of AMPK is dependent on exercise intensity (Fujii *et al.* 2000; Birk & Wojtaszewski, 2006; Treebak *et al.* 2007; Rose *et al.* 2009; Bartlett *et al.* 2012). These observations possibly relate in part to changes in energy homeostasis in the individual fibre and in part to an alteration in fibre type recruitment pattern as exercise intensity increases. The latter is referred to as the motor unit size principle, in which small motor units containing type I fibres are recruited at low levels of force production (Henneman *et al.* 1965). As force production increases larger motor units containing type II fibres are activated (Henneman *et al.* 1965). Measures of fibre type-specific glycogen content have often been used as an indirect measure of the fibre type recruitment pattern during exercise (Gollnick *et al.* 1974; Vøllestad *et al.* 1984; Vøllestad & Blom, 1985; Krustrup *et al.* 2004; Altenburg *et al.* 2007). However, no studies have combined measurements of fibre type-specific regulation of AMPK and glycogen content in response to acute exercise at different intensities.

Thus the aims of the present study were to investigate the fibre type-specific expression and regulation of AMPK and its downstream targets following exercise, and in addition to study whether continuous *vs.* interval exercise elicits differences in the fibre type recruitment and hence signalling response.

Methods

Ethical approval

The study was approved by The Regional Ethics Committee for Copenhagen (H-2-2012-085) and complied with the ethical guidelines of the *Declaration of Helsinki II*. Written informed consent from all participants prior to entering the study.

Experimental protocol

Nine lean, healthy, non-smoking young men (age: 24 ± 1 years, BMI: 23.1 ± 0.6) were included in the study. Maximal oxygen uptake was determined 2 weeks (range: 1–4 weeks) prior to the first experimental day by an incremental cycle test to exhaustion on a Monark ergometer cycle (Ergomedic 839E, Vansbro, Sweden) using breath by breath measurements of $\dot{V}_{O_2peak}$ (MasterScreen CPX, IntraMedic, Gentofte, Denmark) ($\dot{V}_{O_2peak}$: 49.7 ± 0.9 ml kg^{-1} min^{-1}). The experimental protocol consisted of 2 experimental days in a randomized order separated by 1–2 weeks. Before the first experimental day, subjects were instructed to record food intake for 3 days and abstain from alcohol and strenuous physical activity 36–48 h prior to the experiment. On the first experimental day, subjects arrived at the laboratory after an overnight fast using minimal physical activity. Each experimental day consisted of a 30 min rest period followed by 30 min of cycling exercise. During the first 30 min of rest, a catheter was inserted to the antecubital vein and the first blood sample was taken. After 30 min of rest, a needle biopsy from vastus lateralis muscle was obtained under local anaesthesia (3–5 ml Xylocaine, 20 mg ml^{-1}) using the Bergström needle technique with suction (Bergström, 1962). The subjects were then randomly assigned to perform 30 min of either continuous cycling exercise (CON) at ∼70% (69 ± 1%) of $\dot{V}_{O_2peak}$ or interval cycling exercise (INT). INT consisted of six high intensity bouts of 1.5 min at a workload eliciting ∼100% (95 ± 2%) $\dot{V}_{O_2peak}$ interspersed with 2.5 min active recovery at a workload corresponding to 40% $\dot{V}_{O_2peak}$. In both protocols, the first 6 min were used as a warm-up period (INT/CON) and to adjust the load to obtain a steady state $\dot{V}_{O_2peak}$ of ∼70% $\dot{V}_{O_2peak}$ (CON). During exercise, venous antecubital blood samples were taken at 10, 18 and 29 min and expired air was measured breath by breath. Immediately after exercise, another biopsy was taken from the vastus lateralis muscle of the contralateral leg. One to two weeks after the first experimental day the subjects arrived at the laboratory to a similar experimental day except that they performed the exercise protocol they had not performed on the first trial (CON or INT) after having repeated the 3 day diet regime as in the first trial.

Analysis of blood and plasma metabolites, substrates and hormones

The blood content of glucose and lactate was measured by a blood gas analyser (ABL800 FLEX, Radiometer, Copenhagen, Denmark). Plasma noradrenaline and adrenaline were measured using a 2-CAT Plasma ELISA$^{High\ Sensitive}$ kit (Labor Diagnostika Nord GmbH & Co, Nordhorn, Germany). Plasma insulin was measured using an Insulin ELISA kit (DakoCytomation, Glostrup, Denmark) and plasma fatty acids were measured using the NEFA C ACS-ACOD method (Wako Chemicals GmbH, Neuss, Germany).

Dissection of individual muscle fibres

Muscle fibres were prepared as described previously (Murphy, 2011) but with a few modifications (Albers *et al.* 2014). Muscle tissue, 20–180 mg, was freeze-dried for 48 h before dissecting individual muscle fibres using a light microscope and fine forceps in a temperature- and humidity-controlled room (n = 2128 fibres in total). A minimum of 56 fibres were dissected from each biopsy

(length: 1.9 ± 0.2 mm, mean ± SD). Each fibre was dissolved in 5 μl ice-cooled Laemmli sample buffer (125 mM Tris-HCl, pH 6.8, 10% glycerol, 125 mM SDS, 200 mM DTT, 0.004% Bromophenol Blue).

Preparation of pooled muscle fibre samples

A small fraction (1/5) of each solubilized fibre sample was used to identify MHC expression of the fibre using Western blotting and specific antibodies against MHC I or MHC II (see section on Immunoblotting). The remnant sample of each individual fibre was pooled according to MHC expression to form a sample of pooled type I and type II fibres from each biopsy. Hybrid fibres (<1%) expressing two MHC isoforms were excluded. The mean number of type I and type II fibres per biopsy included in each pool was 23 (range 9–52) and 27 (range 12–53), respectively.

Protein content of fibre pools

The protein concentration of the fibre pools was estimated using 4–20% Mini-PROTEAN TGX stain-free gels (BioRad, Hercules, CA, USA), which allowed for gel-protein imaging following UV activation on a ChemiDoc MP imaging system (BioRad). The intensity of the protein bands (40–260 kDa) was compared to a standard curve of mixed muscle homogenates with a known protein concentration (correlation coefficient squared $(r)^2 = 0.98$, correlation not shown).

Glycogen content in muscle fibre pools

Muscle fibre type-specific glycogen content was measured as previously described using dot-blotting (Albers *et al.* 2014). A quantity of 150 ng of protein from each fibre pool was spotted onto a polyvinylidene difluoride (PVDF) membrane and incubated with an antibody against glycogen (Baba, 1993; Nakamura-Tsuruta *et al.* 2012) (see section on Immunoblotting). The intensity of each dot was compared to a standard curve ($r^2 = 0.99$, correlation not shown) of muscle homogenate with a known glycogen concentration determined biochemically (see section on Muscle glycogen).

Muscle homogenate and lysate preparation

Whole muscle homogenates and lysates were prepared from 5 mg freeze-dried muscle which was dissected free of visible fat, blood and connective tissue. The muscle samples were homogenized in a buffer containing 10% glycerol, 20 mM sodium pyrophosphate, 150 mM NaCl, 50 mM Hepes (pH 7.5), 1% NP-40, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM EDTA (pH 8.0), 1 mM EGTA (pH 8.0), 10 μg μl^{-1} aprotinin, 10 μg μl^{-1} leupeptin, 2 mM Na_3VO_4, 3 mM benzamidine, 1 mM sodium butyrate, 1 mM nicotinamide and double-distilled H_2O (ddH_2O) during 2 × 60 s in a TissueLyser (Retsch Qiagen, Hilden, Germany) followed by 2 × 30 min rotation end-over-end at 4°C interspersed by 60 s in the TissueLyser. Lysates were recovered by centrifuging the homogenates (20 min, 18,320 *g*, 4°C). Homogenate and lysate protein content were determined by the bicinchoninic acid method (Pierce Biotechnology, Inc., Rockford, IL, USA).

Muscle glycogen

Glycogen content in whole muscle biopsies was measured in homogenates (150 μg protein) as glycosyl units after acid hydrolysis determined by a fluorometric method (Lowry & Passonneau, 1972).

Muscle lactate, creatine (Cr) and phosphocreatine (PCr)

Freeze-dried muscle samples were extracted with perchloric acid (1.0 mM), neutralized with potassium hydroxide (2.0 mM) and analysed for the content of lactate, Cr and PCr as previously described (Lowry & Passonneau, 1972).

Immunoblotting

To determine the MHC isoform in single muscle fibres, an equal volume of sample was loaded onto pre-cast 7.5% gels (BioRad). To measure total and phosphorylated levels of relevant proteins in muscle fibre pools, homogenates and lysates, 6 μg of protein was separated using self-cast 9% gels. An internal standard of muscle lysate (6 μg) was loaded in both sides on each gel to be able to adjust for gel to gel variation. On one gel, a standard curve of muscle homogenate or lysate was loaded, as appropriate, validating that the quantification of each protein probed for was within the linear range of detection. Following SDS-PAGE, proteins were transferred (semidry) from multiple gels (representing all samples, standards and standard curve) onto one single PVDF membrane. The membranes were blocked in 2% skimmed milk in TBS containing 0.05% Tween 20 for 60 min at room temperature (RT), followed by incubation in primary antibody overnight at 4°C. The following primary antibodies were used: anti-ACC (streptavidin, Dako, Glostrup, Denmark); anti-phospho-ACC Ser^{221}, anti-phospho-AMPK Thr^{172} and anti-LKB1 (serine/threonine kinase 11 (STK11)) (Cell Signaling Technology, Danvers, MA, USA). Anti-β-actin (Sigma-Aldrich, St Louis, MO, USA); anti-α_1 and

-γ_1 AMPK (Abcam, Cambridge, UK); anti-α_2 AMPK (Santa Cruz Biotechnology, Dallas, TX, USA); anti-β_1 AMPK (Arexis, Mölndal, Sweden); anti-β_2 AMPK, anti-phospho-GS Ser^{7} + Ser^{10} (GS^{2+2a}) and Ser^{640} + Ser^{644} (GS^{3a+3b}) (kindly provided by Professor D. Grahame Hardie, University of Dundee, UK); anti-γ_3 AMPK (Zymed Laboratories Inc., South San Francisco, CA, USA and also kindly provided by Professor James Hastie, University of Dundee, UK); anti-phospho-TBC1 domain family member 1 (TBC1D1) Ser^{231} (Millipore, Billerica, MA, USA); anti-phospho-TBC1D4 Ser^{704} (kindly provided by Professor Laurie Goodyear, Joslin Diabetes Centre and Harvard Medical School, USA); anti-TBC1D1 (kindly provided by Professor D. Grahame Hardie and Professor James Hastie, University of Dundee, UK); anti-TBC1D4 (Upstate, now Millipore, MA, USA); anti-MHC I and anti-MHC II (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA). Membranes were incubated for 1 h at RT with secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) which were conjugated with either horseradish peroxidase (HRP) or biotin. Membranes probed with biotin-conjugated antibody were further incubated in HRP-conjugated streptavidin (Jackson ImmunoResearch) for 45 min at RT. Protein bands were visualized using enhanced chemiluminescence (SuperSignal West Femto, Rockford, Pierce, IL, USA) and ChemiDoc MP imaging system (BioRad). Band quantification was performed using BioRad Image Lab. Membranes were re-probed with an alternative antibody after removal of the first antibody by incubation in stripping buffer (62.3 mM Tris-HCl, 69.4 mM SDS, ddH_2O and 0.08% β-mercaptoethanol pH 6.7) for 60 min at 58°C. The membranes were checked for successful removal of the primary antibody before re-probing.

AMPK activity

Isoform-specific AMPK activity was measured on 300 μg muscle lysate by sequential immunoprecipitation (IP) of the γ_3 subunit (isolating the $\alpha_2\beta_2\gamma_3$ complex) followed by IP of the α_2 subunit (isolating $\alpha_2\beta_2\gamma_1$) and lastly the α_1 subunit (isolating $\alpha_1\beta_2\gamma_1$) as described previously (Birk & Wojtaszewski, 2006). AMPK activity was measured in the presence of 200 μM AMP and 100 μM AMARA-peptide (Schafer-N, Copenhagen, Denmark) as substrate, as described previously (Birk & Wojtaszewski, 2006).

Statistics

Data are presented as means ± SEM. Data obtained in whole muscle homogenates/lysates and in blood/plasma were evaluated by a two-way repeated measures (RM) ANOVA (factors: 'exercise type' (CON *vs.* INT) and 'time' (basal *vs.* exercise)). To evaluate changes in muscle fibre pools, four two-way RM ANOVAs were used. Two tests were used to test for the factors 'fibre type' and 'intervention' (basal *vs.* exercise) in the CON and INT trial separately, and two tests were used to test for the factors 'intervention' and 'exercise type' in type I and type II fibres, separately. Significant interactions were evaluated by Tukey's *post hoc* test. Pearson correlation was used for correlated data. All statistical analyses were performed in Sigma Plot (version 11, Systat Software, Chicago, IL, USA). The level of significance was $P < 0.05$.

Results

AMPK subunit and LKB1 expression

AMPK subunit and LKB1 protein expression were unaffected by exercise intervention and trial. The expression of α_1, α_2, β_2 and γ_1 AMPK was similar between fibre types (Fig. 1*D*). In contrast, type I fibres had a higher expression of the β_1 subunit (+215%) (Fig. 1*A*) and a lower expression of the γ_3 subunit (−71%) (Fig. 1*B*) compared to type II fibres. Furthermore, type I fibres had a higher protein expression of LKB1 (+24%) compared to type II fibres (Fig. 1*C*). Actin was used as a reference protein with no difference in expression between fibre types, exercise intervention and trial (data not shown).

Metabolic characteristics during CON and INT

Despite the fact that $\dot{V}_{O_2}$ peaked between 90 and 99% $\dot{V}_{O_2peak}$ during the six high intensity exercise bouts of 1.5 min, the mean intensity for the last 24 min of the exercise session was not significantly different between CON and INT (69 ± 1 and 71 ± 2% $\dot{V}_{O_2peak}$, respectively) (Table 1). The glycogen breakdown in response to CON and INT was similar between the two trials when measured in whole muscle homogenate (Table 2). In addition, similar increases in venous plasma adrenaline and noradrenaline concentrations in CON and INT (Table 1) were evident. The mean respiratory exchange ratio during the last 24 min of the exercise session was significantly higher during INT compared to CON (Table 1). Blood lactate was significantly increased in both trials but especially after INT compared to CON (Table 1). In line with this, muscle lactate concentration was significantly increased after INT only (Table 2). Furthermore, the muscle PCr/(Cr + PCr) ratio decreased in both trials but to a greater extent after INT (−52%) compared to CON (−23%) (Table 2). Glucose and insulin concentrations in venous plasma remained unchanged during CON whereas they both increased after INT by 49% and 90%, respectively (Table 1).

Whole muscle AMPK activity

AMPK activity was measured in muscle lysates on the three main heterotrimeric complexes found in human skeletal muscle. The increase in $\alpha_2\beta_2\gamma_3$ AMPK activity with exercise was significantly higher after INT compared to CON (27- and 12-fold, respectively) (Table 2). Independently of exercise trial, $\alpha_1\beta_2\gamma_1$ AMPK activity decreased with exercise whereas $\alpha_2\beta_2\gamma_1$ AMPK activity was unchanged (Table 2).

Protein phosphorylation in whole muscle homogenate

When measured in whole muscle homogenates phosphorylation of AMPK^{Thr172}, ACC^{Ser221}, TBC1D1^{Ser231}, TBC1D4^{Ser704} and GS^{2+2a} increased during exercise to a similar extent in CON and INT (Table 3, Fig. 2). Phosphorylation of GS^{3a+3b} decreased with exercise in both trials but to a greater extent after INT (Table 3, Fig. 2).

Glycogen content in fibre pools

Basal glycogen content was lower in type I fibres compared to type II fibres (−25%) (Fig. 3*A*). This was only significant in INT, whereas a tendency towards a main effect of fibre type was found in CON ($P = 0.1$). Glycogen decreased to a similar extent in both fibre types after CON. During INT glycogen degradation was significantly smaller in type I fibres (−40%) compared to type II fibres (−73%), resulting in higher glycogen content in type I *vs.* II fibres after INT.

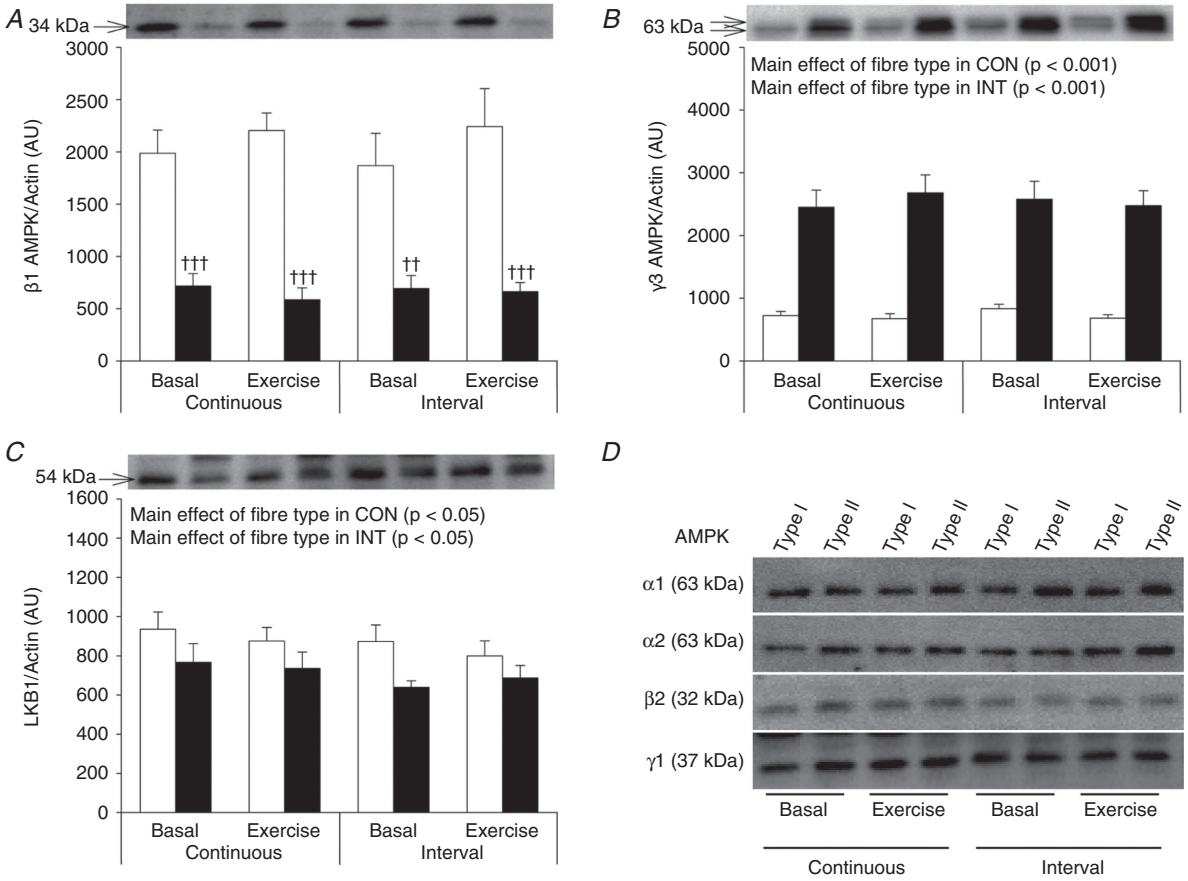

Figure 1. AMPK subunit and LKB1 protein expression in type I and type II muscle fibre pools
AMPK subunit and LKB1 protein expression in type I and type II muscle fibre pools
Muscle fibre type-specific protein expression of β_1 AMPK (*A*), γ_3 AMPK (*B*), LKB1 (*C*) and α_1, α_2, β_2 and γ_1AMPK (*D*) was evaluated by Western blotting. Two bands are apparent for γ_3 AMPK both being γ_3 AMPK. This was verified by immunoprecipitation of γ_3 AMPK followed by Western blotting (data not shown). Quantified values of each protein are expressed relative to actin content. Open bars represent type I fibres and filled bars represent type II fibres. Representative blots are shown above bars for each protein loaded in the same order as the graph below. *D* shows the representative blots of the AMPK subunits with no difference between fibre types. Data are expressed as means ± SEM. AU, arbitrary units. $^{\dagger\dagger}P < 0.01$ *vs.* type I muscle fibres, $^{\dagger\dagger\dagger}P < 0.001$ *vs.* type I muscle fibres.

Table 1. Metabolic characteristics during exercise interventions

	Continuous		Interval	
	Basal	Exercise	Basal	Exercise
Blood glucose (mmol l^{-1})	5.3 ± 0.1	5.5 ± 0.2	5.1 ± 0.4	7.6 ± 0.4$^{***\#\#\#}$
Plasma insulin (μIU ml^{-1})	3.81 ± 0.43	2.31 ± 0.33	4.43 ± 0.42	8.44 ± 1.11$^{***\#\#\#}$
Blood lactate (mmol l^{-1})	0.7 ± 0.1	4.6 ± 0.6***	0.7 ± 0.1	14.0 ± 0.8$^{***\#}$
Plasma fatty acids (μmol l^{-1})	435 ± 61	329 ± 39†	351 ± 65‡	248 ± 28†‡
Adrenaline (nmol l^{-1})	0.20 ± 0.07	2.84 ± 0.94†††	0.31 ± 0.12	2.71 ± 0.62†††
Noradrenaline (nmol l^{-1})	1.58 ± 0.40	21.48 ± 3.55†††	1.71 ± 0.38	29.86 ± 5.41†††
$\%\dot{V}_{O_2peak}$ (mean)	—	69.2 ± 0.9	—	71.1 ± 1.8
RER (mean)	—	0.91 ± 0.01	—	1.01 ± 0.01$^{\#\#\#}$

Values are means ± SEM. $^{***}P < 0.001$ *vs.* basal, $^{\#\#\#}P < 0.001$ *vs.* CON, $^{\dagger}P < 0.05$ main effect of exercise, $^{\dagger\dagger\dagger}P < 0.001$ main effect of exercise, $^{\ddagger}P < 0.05$ main effect of exercise type. RER, respiratory exchange ratio.

Table 2. Parameters of cellular energy status and whole muscle AMPK activities

	Continuous		Interval	
	Basal	Exercise	Basal	Exercise
Glycogen (nmol (mg protein)$^{-1}$)	607 ± 31	328 ± 22***	664 ± 54	278 ± 31***
Lactate (mmol (kg dry wt)$^{-1}$)	75.5 ± 3.2	88.9 ± 5.7	69.8 ± 5.9	153.6 ± 7.8$^{***\#\#\#}$
Cr (mmol (kg dry wt)$^{-1}$)	29.2 ± 4.8	45.5 ± 4.9$^{(*)}$	25.9 ± 2.6	61.8 ± 4.3$^{***\#\#}$
PCr (mmol (kg dry wt)$^{-1}$)	58.8 ± 3.9	49.3 ± 3.6††	60.1 ± 3.5	31.0 ± 3.9††
PCr/(Cr + PCr) (× 100)	67.8 ± 3.5	52.4 ± 3.0**	69.9 ± 2.6	33.4 ± 4.2$^{***\#}$
$\alpha_1\beta_2\gamma_1$ AMPK activity	1.66 ± 0.17	1.42 ± 0.15††	1.54 ± 0.18	1.25 ± 0.17††
$\alpha_2\beta_2\gamma_1$ AMPK activity	0.95 ± 0.16	1.10 ± 0.17	1.00 ± 0.09	1.20 ± 0.22
$\alpha_2\beta_2\gamma_3$ AMPK activity	0.20 ± 0.02	2.59 ± 0.48**	0.20 ± 0.04	5.70 ± 0.89$^{***\#\#\#}$

Values are means ± SEM. $^{**}P < 0.01$ *vs.* basal, $^{***}P < 0.001$ *vs.* basal, $^{\#}P < 0.05$ *vs.* CON, $^{\#\#}P < 0.01$ *vs.* CON, $^{\#\#\#}P < 0.001$ *vs.* CON, $^{\dagger\dagger}P < 0.01$ main effect of exercise, $^{(*)}P = 0.06$ *vs.* basal. Units of AMPK activity: pmol min^{-1} mg^{-1}.

Table 3. Protein phosphorylation before and after CON and INT in whole muscle homogenate (values are arbitrary units)

	Continuous		Interval	
	Basal	Exercise	Basal	Exercise
pAMPK^{Thr172}	89 ± 9	184 ± 30†††	87 ± 12	216 ± 32†††
pACC^{Ser221}	49 ± 6	315 ± 29†††	45 ± 6	392 ± 40†††
pTBC1D4^{Ser704}	231 ± 16	422 ± 89††	222 ± 18	604 ± 109††
pTBC1D1^{Ser231}	216 ± 20	308 ± 24††	214 ± 26	401 ± 46††
pGS^{2+2a}	108 ± 16	367 ± 58†††	129 ± 16	288 ± 42†††
pGS^{3a+3b}	750 ± 62	439 ± 46***	777 ± 61	326 ± 32$^{***\#}$

Values are means ± SEM. $^{***}P < 0.001$ *vs.* basal, $^{\#}P < 0.05$ *vs.* CON, $^{\dagger\dagger}P < 0.01$ main effect of exercise, $^{\dagger\dagger\dagger}P < 0.001$ main effect of exercise. Representative Western blots are shown in Fig. 2.

AMPK phosphorylation in fibre pools

In the basal state, the phosphorylation of AMPK^{Thr172} was similar between fibre types in both trials (Fig. 3*B*). After CON there was a main effect of exercise to increase phosphorylation of AMPK^{Thr172} with no difference between fibre types. In contrast, after INT the phosphorylation of AMPK^{Thr172} increased by 52% in type I fibres (not significant) and by 184% in type II fibres ($P < 0.01$).

ACC in fibre pools

In response to both CON and INT there was a main effect of exercise to increase ACC^{Ser221} phosphorylation.

Yet, a tendency towards a different fibre type response to exercise was observed after INT ($P = 0.08$) (Fig. 4*A*). When ACC^{Ser221} phosphorylation was related to total ACC protein (Fig. 4*B*) this difference became significant as phosphorylation of ACC^{Ser221}/ACC increased less in type I fibres compared with type II fibres. In both fibre types, phosphorylation of ACC^{Ser221}/ACC was increased to a higher level after INT compared to CON. ACC protein expression was not different between fibre types. A minor increase in ACC protein expression with exercise was observed after CON and in type I fibres (Fig. 4*C*). This was unexpected; however, in whole muscle homogenate no effect of exercise could be detected (data not shown).

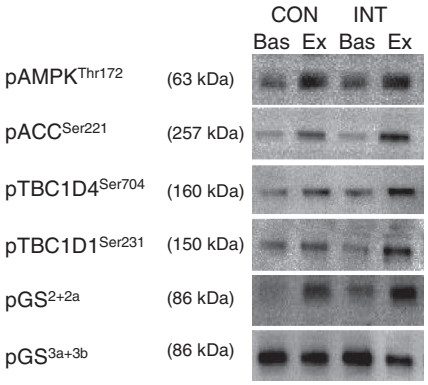

Figure 2. Representative Western blots of protein phosphorylation before and after CON and INT in whole muscle homogenate
Phosphorylation of AMPK^{Thr172}, ACC^{Ser221}, TBC1D4^{Ser704}, TBC1D1^{Ser231}, GS^{2+2a} and GS^{3a+3b} in whole muscle homogenate was evaluated by Western blotting. Quantified data are given in Table 3.

TBC1D1 in fibre pools

TBC1D1 expression was 52% lower in type I fibres compared to type II fibres (Fig. 5*A*) independent of exercise *per se* and exercise trial. In response to CON, phosphorylation of TBC1D1^{Ser231}/TBC1D1 increased with no difference between fibre types. In contrast, in response to INT TBC1D1^{Ser231}/TBC1D1 phosphorylation increased in both fibre types but to a lesser extent in type I fibres (+63%) compared to type II fibres (+97%) (Fig. 5*E*). Furthermore, in type II fibres, the increase in TBC1D1^{Ser231}/TBC1D1 phosphorylation with exercise was higher after INT compared to CON ($P = 0.05$).

TBC1D4 in fibre pools

TBC1D4 expression was 21% lower in type I fibres compared to type II fibres (Fig. 5*B*). The phosphorylation of TBC1D4^{Ser704}/TBC1D4 increased in response to exercise in both CON and INT. The increase was less in type I fibres (+43% and +82% after CON and INT, respectively) compared to type II fibres

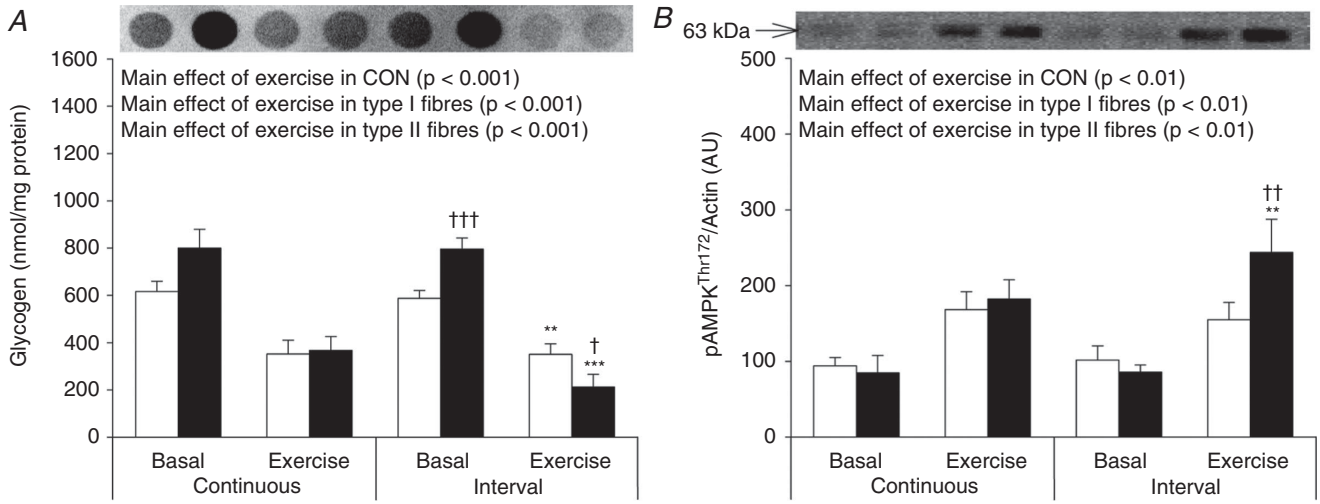

Figure 3. Glycogen content and AMPK^{Thr172} phosphorylation in type I and type II muscle fibre pools before and after CON and INT
A, muscle fibre type-specific glycogen content measured by dot-blotting. *B*, muscle fibre type-specific phosphorylation of AMPK^{Thr172} evaluated by Western blotting. Quantified values of AMPK^{Thr172} phosphorylation are expressed relative to actin content. Open bars represent type I fibres and filled bars represent type II fibres. Representative blots are shown above bars in the same order as the graph below. Data are expressed as means ± SEM. AU, arbitrary units. $^{**}P < 0.01$ *vs.* basal conditions, $^{***}P < 0.001$ *vs.* basal conditions, $^{\dagger}P < 0.05$ *vs.* type I muscle fibres, $^{\dagger\dagger}P < 0.01$ *vs.* type I muscle fibres, $^{\dagger\dagger\dagger}P < 0.001$ *vs.* type I muscle fibres. A borderline main effect of fibre type in glycogen content was observed in CON ($P = 0.1$). Furthermore, a borderline interaction between exercise and exercise type was observed in glycogen content in type II fibres ($P = 0.08$) (*A*).

(+131% and +347% after CON and INT, respectively) (Fig. 5*F*). Furthermore, in type II fibres the increase in phosphorylation of TBC1D4^{Ser704}/TBC1D4 was significantly smaller after CON (−25%) compared to INT.

Glycogen synthase in fibre pools

GS protein expression was 10% higher in type I fibres compared to type II fibres; however, this was only significant in the INT trial (main effect of fibre type $P < 0.05$ in INT, Fig. 6*A*). In CON, there was a main effect of exercise to increase GS^{2+2a} phosphorylation. Although not significant, the absolute values indicate that the increment was higher in type I compared to type II fibres. In line with this, after INT the increase in GS^{2+2a} phosphorylation was significant only in type I fibres (+76%) and not in type II fibres (+16%) (Fig. 6*B*). In type I but not in type II fibres, statistical main effects were evident for increased GS^{2+2a} phosphorylation by exercise.

After CON phosphorylation of GS^{3a+3b} decreased to a similar extent in type I (−52%) and type II fibres (−56%) (Fig. 6*C*). In contrast, after INT GS^{3a+3b} phosphorylation

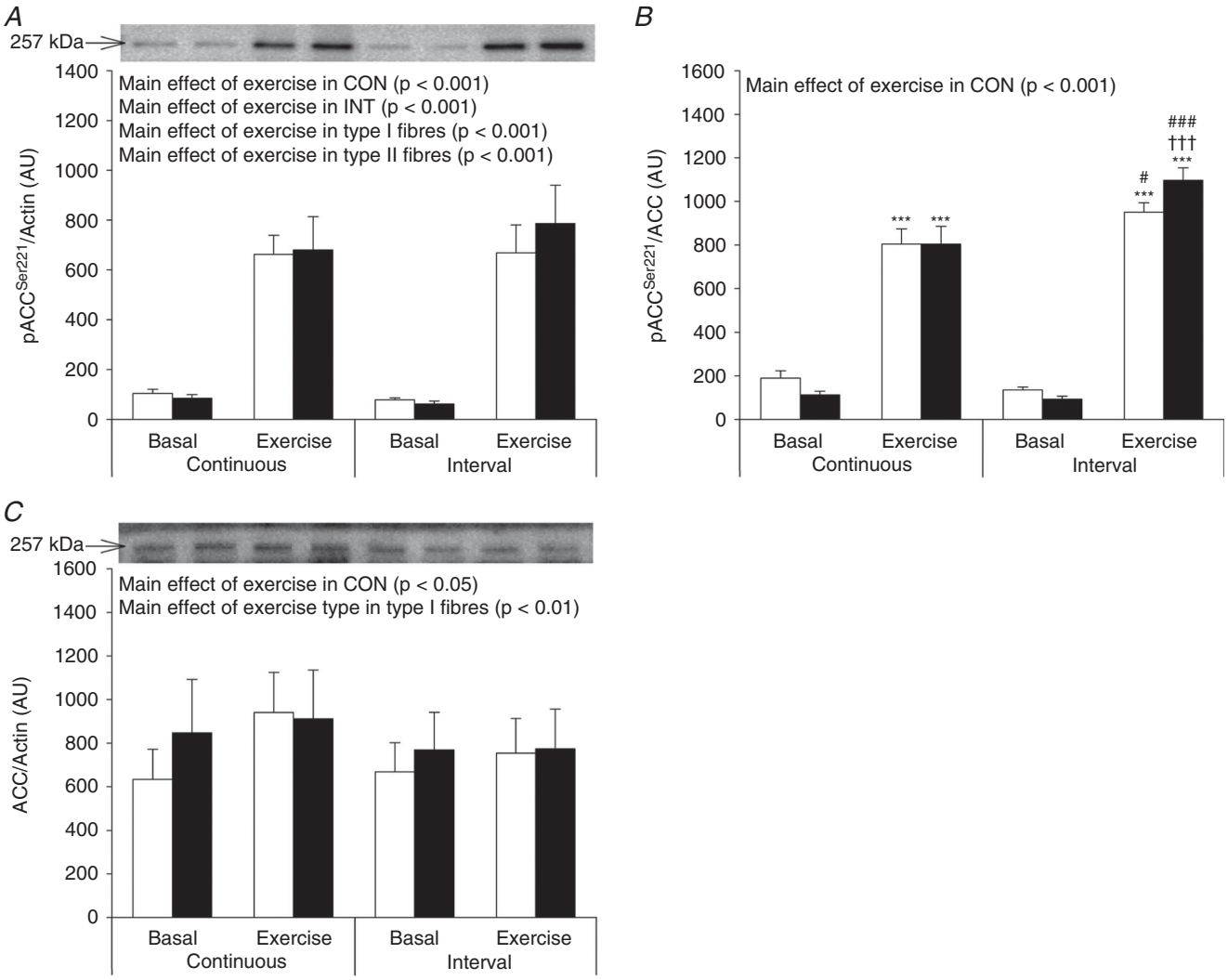

Figure 4. ACC^{Ser221} phosphorylation and ACC expression in type I and type II muscle fibre pools before and after CON and INT
Muscle fibre type-specific phosphorylation of ACC^{Ser221} (*A*) and protein expression of ACC (*C*) was evaluated by Western blotting. Quantified values are expressed relative to actin content. Phosphorylation of ACC^{Ser221} was also expressed relative to ACC content (*B*). Open bars represent type I fibres and filled bars represent type II fibres. Representative blots are shown above bars for each protein loaded in the same order as the graph below. Data are expressed as means ± SEM. AU, arbitrary units. $^{***}P < 0.001$ *vs.* basal conditions, $^{\dagger\dagger\dagger}P < 0.001$ *vs.* type I muscle fibres, $^{\#}P < 0.05$ *vs.* CON and $^{\#\#\#}P < 0.001$ *vs.* CON. A borderline interaction between exercise and fibre type was observed for ACC^{Ser221} phosphorylation after INT ($P = 0.08$) (*A*).

decreased to a lesser extent in type I fibres (−51%) compared to type II fibres (−73%) (Fig. 6*C*). Similar effects of fibre type and exercise trial were observed when phosphorylation of GS^{2+2a} and GS^{3a+3b} was related to total GS protein (Fig. 6*D* and *E*).

Discussion

This study provides evidence that exercise-induced regulation of AMPK, ACC, TBC1D1, TBC1D4 and GS is dependent on muscle fibre type and exercise intensity. In fact, most proteins were regulated to a similar extent

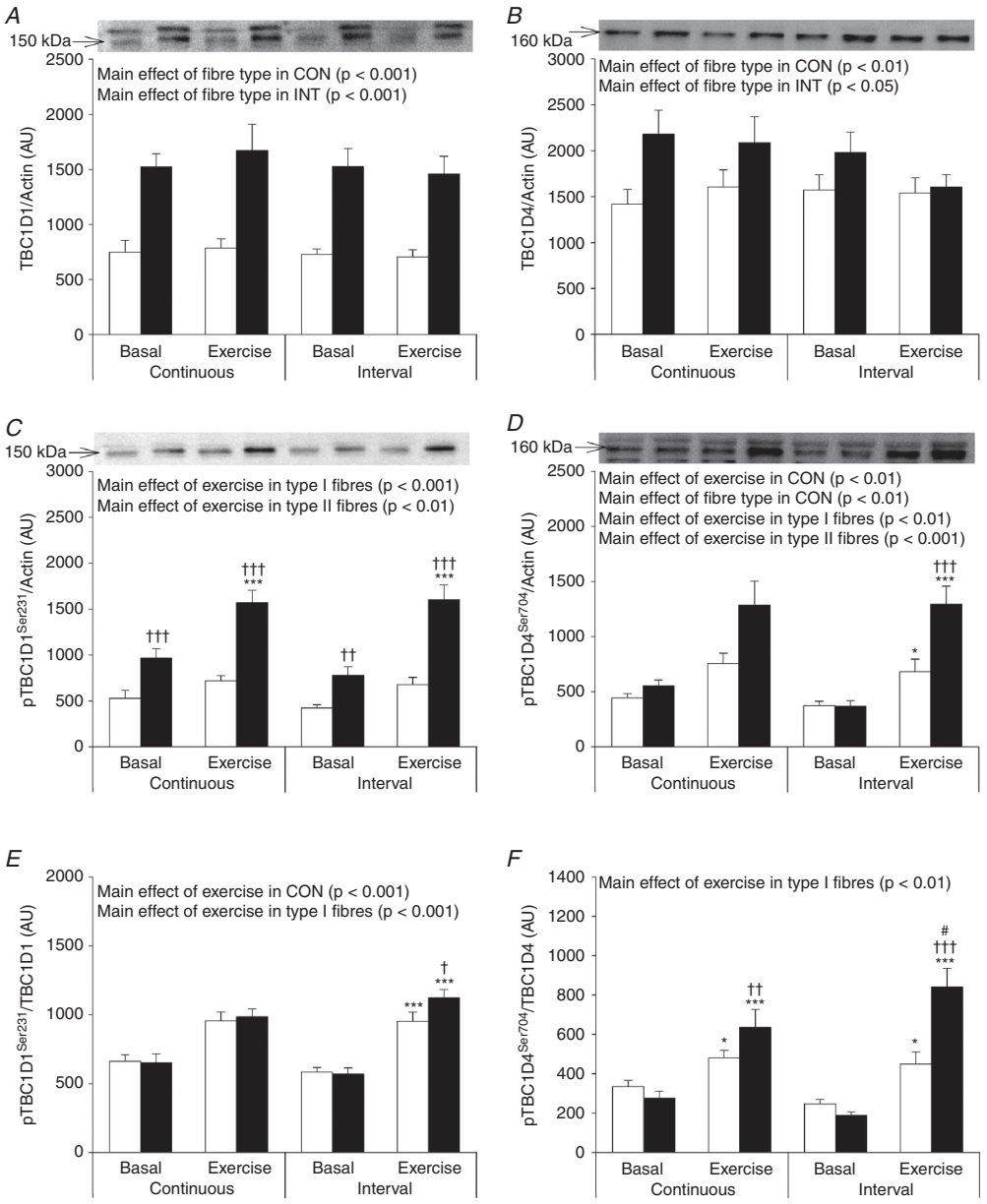

Figure 5. TBC1D1 and TBC1D4 in type I and type II muscle fibre pools before and after CON and INT
Muscle fibre type-specific protein expression of TBC1D1 (*A*) and TBC1D4 (*B*) and phosphorylation of $TBC1D1^{Ser231}$ (*C*), TBC1D4 ^{Ser704} (*D*) were evaluated by Western blotting. Quantified values are expressed relative to actin content. Phosphorylation of $TBC1D1^{Ser231}$ was expressed relative to TBC1D1 content (*E*) and phosphorylation of $TBC1D4^{Ser704}$ was expressed relative to TBC1D4 content (*F*). Open bars represent type I fibres and filled bars represent type II fibres. Representative blots are shown above bars in the same order as the graph below. Data are expressed as means ± SEM. AU, arbitrary units. $^{*}P < 0.05$ *vs.* basal conditions, $^{***}P < 0.001$ *vs.* basal conditions, $^{\dagger}P < 0.05$ *vs.* type I muscle fibres, $^{\dagger\dagger}P < 0.01$ *vs.* type I muscle fibres, $^{\dagger\dagger\dagger}P < 0.001$ *vs.* type I muscle fibres, $^{\#}P < 0.05$ *vs.* CON. Borderline interaction between exercise and fibre type in CON was observed for $TBC1D4^{Ser704}$ phosphorylation ($P = 0.05$) (*D*) and between exercise and exercise type in type II fibres for $TBC1D1^{Ser231}$ expressed relative to TBC1D1 ($P = 0.05$) (*E*).

in type I and II muscle fibres during exercise in CON, whereas exercise during INT elicited a fibre type-specific regulation. All protein-signalling responses investigated (except GS^{3a+3b} phosphorylation) were similar in the two exercise trials when measured traditionally in whole muscle homogenate as reported by others (Bartlett

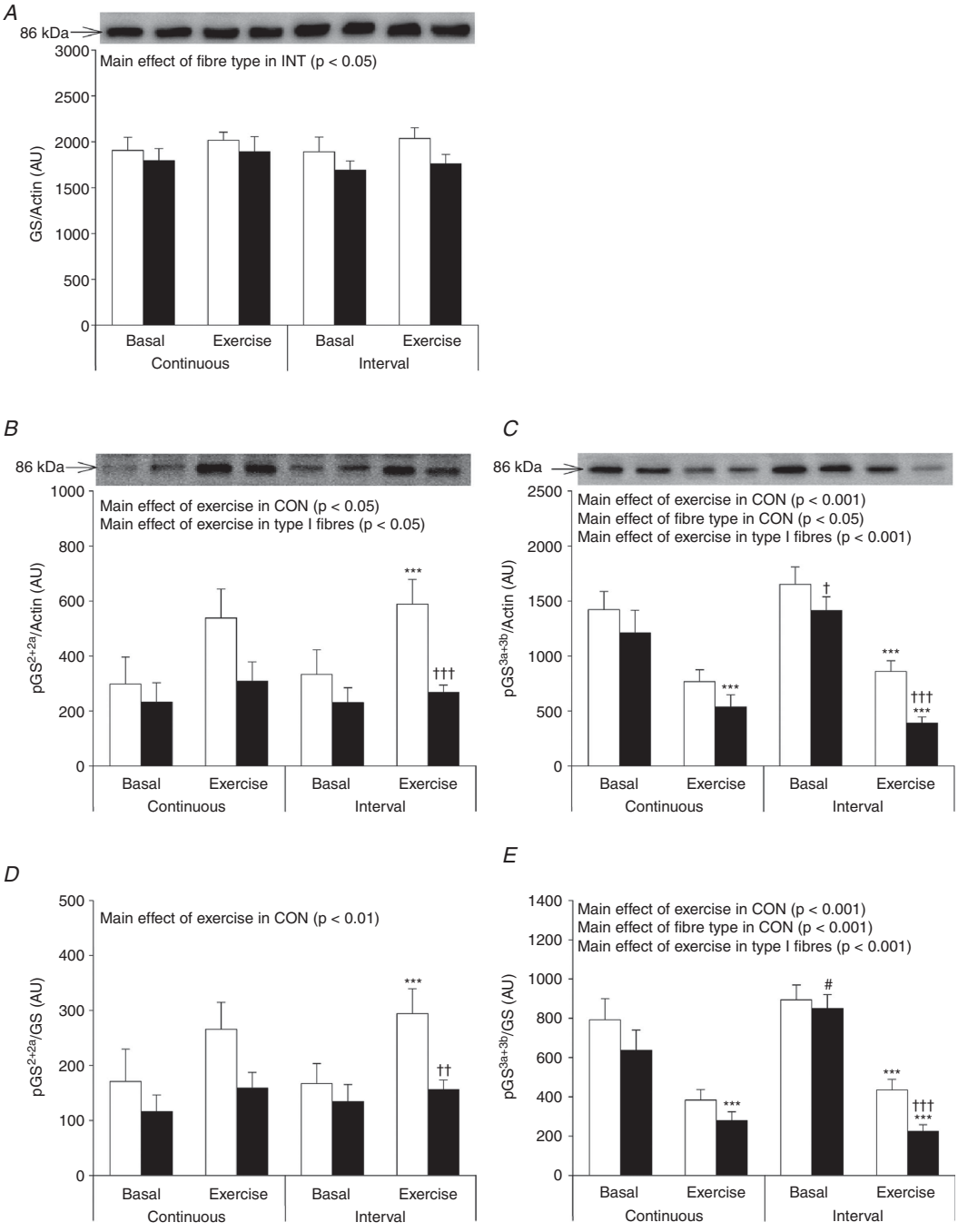

Figure 6. Glycogen synthase in type I and type II muscle fibre pools before and after CON and INT
Muscle fibre type-specific protein expression of GS (*A*) and phosphorylation of GS^{2+2a} (*B*) and $^{3a+3b}$ (*C*) were evaluated by Western blotting. Quantified values are expressed relative to actin content. Open bars represent type I fibres and filled bars represent type II fibres. Representative blots are shown above bars for each protein loaded in the same order as the graphs below. Data are expressed as means ± SEM. AU, arbitrary units. $^{***}P < 0.001$ *vs.* basal conditions, $^{\dagger}P < 0.05$ *vs.* type I muscle fibres, $^{\dagger\dagger}P < 0.01$ *vs.* type I muscle fibres, $^{\dagger\dagger\dagger}P < 0.001$ *vs.* type I muscle fibres, $^{\#}P < 0.05$ *vs.* CON.

et al. 2012). These findings underscore the relevance of measuring exercise-induced protein signalling on a muscle fibre type-specific level.

The ∼70% lower γ_3 AMPK protein content observed in type I *vs.* II muscle fibres is more pronounced than previously reported (Lee-Young *et al.* 2009). Immunohistochemical detection of γ_3 AMPK in muscle cryosections showed that the expression level of γ_3 AMPK was highest in type IIx > IIa > I muscle fibres (approximately 14% lower in type I *vs.* IIa muscle fibres and 33% lower in type I *vs.* IIx muscle fibres) (Lee-Young *et al.* 2009). In human skeletal muscle γ_3 selectively associates with α_2 and β_2 (Wojtaszewski *et al.* 2005) and thus our findings indicate a lower expression level of the $\alpha_2\beta_2\gamma_3$ AMPK complex in human type I *vs.* II muscle fibres. This is also in agreement with observations in mice, in which the abundance of $\alpha_2\beta_2\gamma_3$ AMPK complexes constitutes less than 2% of total AMPK in type I/IIa-abundant soleus muscle and approximately 20% in the type II-fibre-rich extensor digitorum longus muscle (Treebak *et al.* 2009*a*). γ_3 AMPK protein in muscle is decreased by exercise training and increased by detraining (muscle denervation) and associations with MHC expression have been reported in both conditions (Nielsen *et al.* 2003; Frøsig *et al.* 2004; Wojtaszewski *et al.* 2005; Mortensen *et al.* 2009; Kostovski *et al.* 2013). Our data may allow us to speculate that prioritization of an expression programme for MHC type I may at the same time decrease expression of γ_3 AMPK protein. However, this needs to be verified.

Confirming previous observations (Birk & Wojtaszewski, 2006; Treebak *et al.* 2007), $\alpha_2\beta_2\gamma_3$ AMPK was the only complex activated during exercise in whole muscle biopsies, in the present study. As the glycogen degradation pattern suggests a lower activation/recruitment of type I *vs.* type II muscle fibres during exercise in INT, we expected a lower $\alpha_2\beta_2\gamma_3$ AMPK activity in type I compared to type II fibres during exercise in INT. Fibre type-specific AMPK$^{\mathrm{Thr172}}$ phosphorylation during exercise supports this assumption. However, since the $\alpha_2\beta_2\gamma_3$ AMPK complex accounts for only ∼20% of all AMPK complexes in human skeletal muscle (Birk & Wojtaszewski, 2006), phosphorylation of AMPK$^{\mathrm{Thr172}}$ is not necessarily a precise measure of AMPK complex activation. Thus, we cannot exclude the possibility of a differentiated AMPK activation in type I and II muscle fibres during exercise in CON based on this measurement alone.

Since AMPK activity is also regulated allosterically, and to further delineate AMPK activation in type I and II muscle fibres during exercise, we measured site-specific phosphorylation of several confirmed AMPK targets: ACC$^{\mathrm{Ser221}}$, TBC1D1$^{\mathrm{Ser231}}$, TBC1D4$^{\mathrm{Ser704}}$ and GS^{2+2a}. In skeletal muscle, these targets have been verified using pharmacological and exercise-induced AMPK activation in various transgenic animal models in which AMPK expression has been ablated (Carling & Hardie, 1989; Ha *et al.* 1994; Jørgensen *et al.* 2004; Pehmøller *et al.* 2009; Treebak *et al.* 2010; Frøsig *et al.* 2010). In line with this, phosphorylation of ACC$^{\mathrm{Ser221}}$, TBC1D1$^{\mathrm{Ser231}}$ and TBC1D4$^{\mathrm{Ser704}}$ were closely associated with $\alpha_2\beta_2\gamma_3$ AMPK activity measured in whole muscle lysate in the present study (Fig. 7*A*–*C*).

Furthermore, these three estimates of endogenous AMPK activity showed no fibre type-specific regulation during exercise in CON whereas all depicted this during exercise in INT in agreement with the phosphoregulation of AMPK$^{\mathrm{Thr172}}$. Altogether, regulation of these AMPK downstream targets suggests that interval exercise elicits a fibre type-specific response and we propose that activation of $\alpha_2\beta_2\gamma_3$ AMPK complexes is a major contributor to this response. Our study allows us only to speculate on the consequences of this fibre type-specific regulation. TBC1D1 is proposed to regulate exercise-induced glucose uptake (An *et al.* 2010), and one interpretation of our findings is that glucose uptake by this mechanism is prioritized in type II *vs.* type I muscle fibres during interval exercise. Also, studies in rodents (Funai *et al.* 2009) and humans (Treebak *et al.* 2009*b*; Vendelbo *et al.* 2014) suggest that TBC1D4 phosphorylation in skeletal muscle is maintained for a prolonged period during recovery from exercise and may be important for post-exercise insulin sensitivity. If this is the case, our data would then suggest that type II muscle fibres contribute mostly to post-exercise insulin sensitivity – an adaptation facilitating glycogen re-synthesis in those fibres in which glycogen utilization seemingly has been largest. As discussed below such prioritization of glycogen synthesis may also be linked to the fibre type-specific regulation of GS.

AMPK is suggested to be a GS site 2 kinase (Jørgensen *et al.* 2004). Our data only support this in type I and not in type II fibres as we did not observe any changes in GS^{2+2a} phosphorylation in type II fibres after INT despite increased AMPK$^{\mathrm{Thr172}}$ phosphorylation in type II fibres. GS^{3a+3b} are the other major phosphorylation sites regulating GS activity. These sites were dephosphorylated by exercise in both fibre types, and to a lesser degree in type I *vs.* II fibres during exercise in INT. Although we do not know the resulting effect of these phosphorylation events on GS activity, our data allow us to speculate that GS activation is considerably less in type I compared to type II muscle fibres. This could be important immediately post exercise and would suggest a lower glycogen re-synthesis rate in type I compared to II muscle fibres at the time of exercise cessation.

We used exercise-induced glycogen degradation to evaluate fibre type recruitment during exercise in CON and INT. The similar glycogen degradation in type I fibres between CON and INT suggests similar type I fibre recruitment between the two trials. Although there is no

statistical difference in glycogen levels after exercise in type II fibres in CON and INT, the glycogen degradation was statistically larger in type II fibres compared to type I fibres in INT but not in CON. We interpret this to indicate a larger recruitment of type II fibres during exercise in INT compared to CON. In line with this, we found increased lactate concentration and a greater decrease in PCr/(Cr + PCr) ratio in muscle after INT compared to CON, suggesting periods of increased metabolic stress during exercise in INT. Whether protein signalling/activity measurements mainly reflect the averaged exercise intensity or a more acute regulation at the end of exercise is unknown. To support an acute regulation, Birk & Wojtaszewski (2006) showed that activity of the AMPK $\alpha_2\beta_2\gamma_3$ complex increased after 30 s of an all-out sprint. In addition, ACC phosphorylation has been shown to be acutely (<30 s) regulated during an all-out sprint (Birk & Wojtaszewski, 2006; Gibala *et al.* 2009). Thus, a higher increase in $\alpha_2\beta_2\gamma_3$ AMPK activity in whole muscle samples as well as ACC^{Ser221} phosphorylation in fibre pools during exercise in INT compared to CON in the present study is probably influenced by the last interval exercise bout.

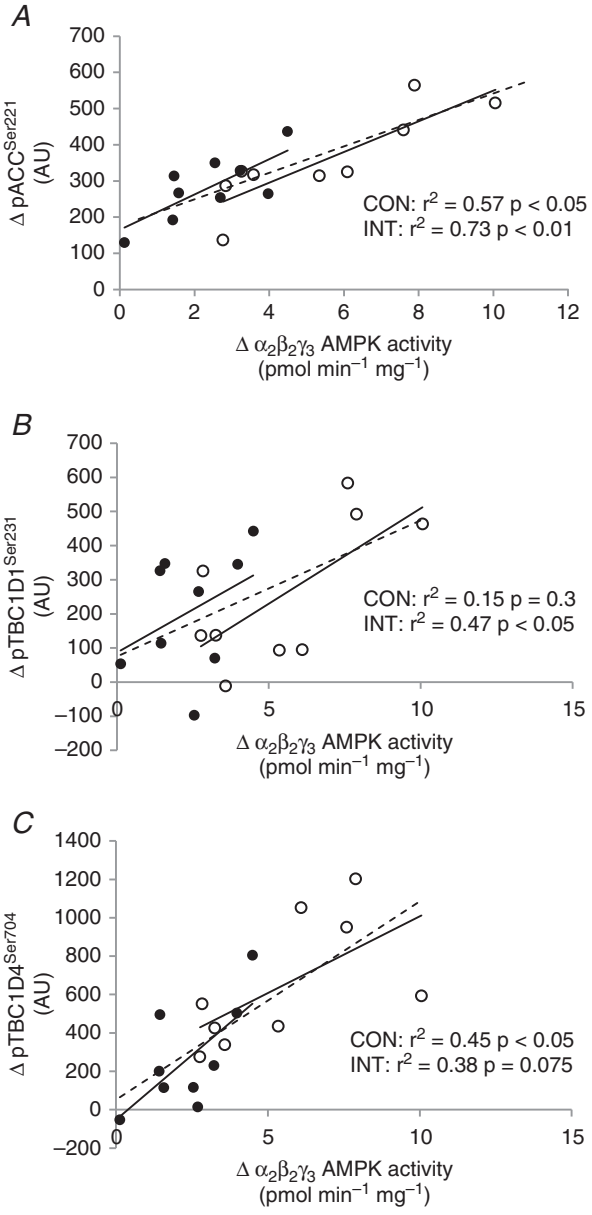

Figure 7. Correlations between AMPK activity and phosphorylation of downstream targets of AMPK in whole muscle lysate
Correlations between $\Delta\alpha_2\beta_2\gamma_3$ AMPK activity and Δphosphorylation of ACC^{Ser221} (*A*), Δphosphorylation of $TBC1D1^{Ser231}$ (*B*) and Δphosphorylation of $TBC1D4^{Ser704}$ (*C*) in whole muscle lysate after CON (filled circles) and INT (open circles). Phosphorylation of ACC^{Ser221}, $TBC1D1^{Ser231}$ and $TBC1D4^{Ser704}$ was evaluated by Western blotting. The dashed line represents both trials together (*A*: $r^2 = 0.67$, $P < 0.001$; *B*: $r^2 = 0.29$, $P < 0.05$; *C*: $r^2 = 0.56$, $P < 0.001$). Δ = change with exercise (exercise − basal).

Studies in mouse muscle predominantly expressing one fibre type have revealed that TBC1D1 and TBC1D4 has a remarkable fibre type-specific expression pattern, i.e. TBC1D1 is highly expressed in muscle rich in type II fibres whereas expression in muscle rich in type I fibres is very low (Taylor *et al.* 2008). The expression profile of TBC1D4 is reported to be exactly the opposite (Taylor *et al.* 2008). However, it is unknown whether these observations were associated with the difference in MHC expression or activity pattern between the muscles. Here we provide evidence that in young healthy men both TBC1D1 and TBC1D4 expression are lower in type I *vs.* type II muscle fibres. This is in line with what we have previously reported in middle-aged subjects (Albers *et al.* 2014). However, the differences between fibre types in humans are much smaller than those reported in mice. Our findings of a substantial amount of TBC1D1 and TBC1D4 in both fibre types is an important observation as we now, to a greater extent than previously thought, can expect findings on TBC1D1 and TBC1D4 from rodent muscles also to be of importance in human muscle. A limitation to this interpretation, however, is that our data do not allow generalization to other human muscles as we do not know whether type I and type II muscle fibres from vastus lateralis muscle resemble type I and type II fibres from other muscles (Daugaard & Richter, 2004). It has been shown in untrained subjects ($\dot{V}_{O_2peak}$ 44.8 ml kg^{-1} min^{-1}) that training-induced increases in GLUT4 content mainly occur in type I fibres (Daugaard *et al.* 2000). Thus, the training status of the subjects in the present study ($\dot{V}_{O_2peak}$ 49.7 ± 0.9 ml kg^{-1} min^{-1}) could potentially influence the differences in protein expression between muscle fibres. Another limitation to the present study is the lack of distinction between type IIa and IIx fibres in the pools of type II fibres. Yet in vastus lateralis muscle of young, healthy, active men the expression of type IIx fibres is relatively small compared to the proportion of type IIa

and type I fibres (Hvid *et al.* 2011; Jensen *et al.* 2012; Prats *et al.* 2013) whereby the proportion of type IIx fibres in pools of type II fibres is thought to be limited. Thus, the differences observed between type I and type II fibres are probably influenced only to a minor degree by the differences in protein expression/regulation between type IIa and IIx fibres.

In conclusion, this study provides evidence for a differentiated response to exercise of key metabolic signalling/effector proteins in human type I and II fibres during interval exercise, emphasizing the potential for new discoveries when investigating muscle fibre type-specific signalling. Although the Western blotting procedure used is semi-quantitative, the use of a calibration curve to ensure assay linearity in fact ensures that changes/differences are detectable (Murphy & Lamb, 2013). AMPK is proposed as a mediator of at least some health benefits of physical activity (Richter & Ruderman, 2009). Increased AMPK activation during exercise in INT *vs.* CON could then be important for exercise type-specific adaptations, i.e. insulin sensitivity and mitochondrial density.

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Additional information

Competing interests

None declared.

Author contributions

D.E.K., P.H.A. and J.F.P.W. contributed to the conception and design of the experiments. D.E.K., P.H.A. and J.F.P.W. performed clinical experiments.: D.E.K., P.H.A. and J.B.B. performed analyses. D.E.K., P.H.A., J.B.B. and J.F.P.W. interpreted the results. D.E.K., P.H.A. and J.F.P.W. drafted the article. All authors edited and revised the article critically for important intellectual content and approved the final version. Human experiments, as well as biochemical measurements, were performed in the laboratories of the Section of Molecular Physiology, August Krogh Centre, Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark.

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Translational perspective

Human skeletal muscle consists of different fibre types with molecular and functional differences. Thus, studies of skeletal muscle tissue could be confounded by the heterogeneous nature of human muscles as the proportion of the different fibre types is influenced by various factors such as training status, sex, age, health status and genetics. AMP-activated protein kinase (AMPK) is proposed as a key regulator of cellular energy homeostasis. AMPK is activated during exercise and may mediate both acute and more chronic adaptations in the skeletal muscle fibres. However, most measurements on AMPK activation in humans have been performed in whole muscle biopsy samples without consideration of the influence of muscle fibre types. In the present study, we hypothesized that the regulation of AMPK and downstream targets of AMPK occurs in a fibre type-specific manner dependent on exercise intensity. Here we show that exercise-induced regulation of AMPK and several downstream targets of AMPK is dependent on muscle fibre type and exercise intensity. Yet, when measured traditionally in whole muscle homogenate only one of several measures displayed a dependency on exercise intensity. These findings underscore the relevance of measuring exercise-induced protein signalling on a muscle fibre type-specific level. Furthermore, high intensity exercise activates AMPK predominantly in the fast twitch glycolytic fibres (type II). This might be important for the capacity of these fibres to take up and store substrates during and after exercise as AMPK is suggested to promote the ability of insulin to stimulate glucose uptake and storage.