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**Published on:** 06 Jun 2008 - European Journal of Applied Physiology (Springer-Verlag)

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## Decrease in Akt/PKB signalling in human skeletal muscle by resistance exercise

Louise Deldicque · Philip Atherton · Rekha Patel · Daniel Theisen · Henri Nielens · Michael J. Rennie · Marc Francaux

Accepted: 26 May 2008 / Published online: 6 June 2008  
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**Abstract** We analysed the effects of resistance exercise upon the phosphorylation state of proteins associated with adaptive processes from the Akt/PKB (protein kinase B) and the mitogen-activated protein kinase (MAPK) pathways. Nine healthy young men ( $21.7 \pm 0.55$  year) performed 10 sets of 10 leg extensions at 80% of their 1-RM (repetition maximum). Muscle biopsies were taken from the vastus lateralis at rest, within the first 30 s after exercise and at 24 h post-exercise. Immediately post exercise, the phosphorylation states of Akt/PKB on Thr308 and Ser473 and 4E-BP1 on Thr37/46 (eukaryotic initiation factor 4E-binding protein 1) were decreased ( $-60$  to  $-90\%$ ,  $P < 0.05$ ). Conversely, the phosphorylation of p70<sup>S6k</sup> (p70 ribosomal S6 kinase) on Thr421/Ser424 was increased more than 20-fold ( $P < 0.05$ ), and this was associated with a 10- to 50-fold increase in the phosphorylation of p38 and ERK1/2 (extracellular signal-regulated kinase) ( $P < 0.05$ ). Twenty-four hours post-exercise the phosphorylation state of Akt/PKB on Thr308 was depressed, whereas the phosphorylation of p70<sup>S6k</sup> on Thr421/Ser424 and sarcoplasmic ERK1/2 were elevated. The present results indicate that high-intensity resistance exercise in the fasted state inhibits Akt/PKB and 4E-BP1 whilst concomitantly augmenting MAPK signalling and p70<sup>S6k</sup> on Thr421/Ser424.

**Keywords** Cell Signaling · Protein synthesis · Resistance exercise · MAPK · p70s6k

### Introduction

Adaptive remodelling of skeletal muscles is a feature of chronic exercise training. Recent studies in humans have identified the Akt/PKB (protein kinase B) and the MAPK (mitogen-activated protein kinase) pathways as key cascades in the regulation of skeletal muscle plasticity by exercise (Cuthbertson et al. 2006; Dreyer et al. 2006; Eliasson et al. 2006; Karlsson et al. 2004; Williamson et al. 2003). Whereas activation of the Akt/PKB pathway is associated with the modulation of translational efficiency, MAPK signalling apparently regulates sarcoplasmic and/or nuclear transcription factors (Deldicque et al. 2005; Wang et al. 1998; Widgren et al. 2001).

Research into the regulation of Akt/PKB signalling by exercise has produced contrasting results. A series of studies have demonstrated that contractile activity either positively or negatively regulates Akt/PKB activity (Blomstrand et al. 2006; Creer et al. 2005; Dreyer et al. 2006; Terzis et al. 2008), others failed to find any change (Coffey et al. 2006; Creer et al. 2005; Deshmukh et al. 2006; Eliasson et al. 2006). Such inconsistencies likely reflect exercise specificity and nutritional status, and even perhaps phosphorylation site.

Whereas the effect of exercise on Akt/PKB is not clear, food consumption is a potent, indirect regulator of its activity through associated insulin secretion (Brozinick and Birnbaum 1998; Wojtaszewski et al. 2001). The activation of Akt/PKB leads to the stimulation of mammalian target of rapamycin (mTOR) and its downstream substrates such as eukaryotic initiation factor 4E-binding protein 1 (4E-BP1)

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and p70 ribosomal S6 kinase (p70<sup>s6k</sup>), two key regulators of protein synthesis. 4E-BP1 is an inhibitor of the initiation translation factor eukaryotic initiation factor 4E (eIF4E) and when phosphorylated, eIF4E is released and can form the multi-protein eIF4F complex (Gingras et al. 1999). The assembly of this complex is necessary for cap-dependent initiation to continue. Activation of p70<sup>s6k</sup> requires a sequential series of phosphorylation events and the eIF3 translation pre-initiation complex serves an important function in organizing and coordinating these events (Holz et al. 2005). Phosphorylation of Ser/Thr residues in the autoinhibitory domain, such as at Thr 421 and Ser 424, is required for altering the conformation of p70<sup>s6k</sup> and making Thr 389 and Thr 229 available for phosphorylation, thereby fully activating p70<sup>s6k</sup> (Weng et al. 1998). In vitro, Thr 389 is phosphorylated by mTOR (Burnett et al. 1998) whereas phosphoinositide-dependent kinase 1 (PDK1) is the kinase for Thr 229.

The MAPK family is composed of four members: extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (p38, JNK) and extracellular signal-regulated kinase 5 (ERK5). Once activated, they can phosphorylate sarcoplasmic proteins or alternatively translocate to the nucleus, where they phosphorylate muscle specific transcription factors involved in muscle remodelling (Farooq and Zhou 2004). ERK1/2 and p38 have been shown to be involved in exercise-induced signalling in human skeletal muscle (Long et al. 2004; Widegren et al. 1998; Yu et al. 2001). Cycling markedly increased ERK1/2 phosphorylation, albeit only transiently, whereas similar exercise has been demonstrated to lead to a smaller but more persistent increase in p38 activation (Widegren et al. 1998). ERK1/2 and p38 are also influenced by resistance exercise (Creer et al. 2005; Karlsson et al. 2004), but a clear picture of the regulatory pattern has not yet emerged. Indeed, MAPK signalling seems to be regulated differentially according to the mode of contraction (concentric vs. eccentric), the intensity of the exercise and the training status of the subjects (Widegren et al. 2001).

Crosstalk between the MAPK pathway and p70<sup>s6k</sup> has been reported in vitro. In H4 hepatoma cells (Mukhopadhyay et al. 1992) and in cardiomyocytes (Iijima et al. 2002; Wang et al. 2001), MAPK proteins act to remove the inhibition in the autoinhibitory domain of p70<sup>s6k</sup> by phosphorylating Thr 421/Ser 424. Such events are likely to allow maximal kinase activity when in tandem with mTOR and PDK1 phosphorylation (Weng et al. 1998). Whether this is also the case in skeletal muscle has not yet been tested.

The aim of the present study was to test the hypothesis that resistance exercise performed in a fasted state increases the phosphorylation state of MAPK and p70<sup>s6k</sup> on Thr 421/Ser 424, but does not modify the phosphorylation state of Akt/PKB because feeding is required. The majority of prior studies have focused upon the signalling of exercise during

the early phase of the recovery period. However, exercise is known to alter protein metabolism for >2 days (Phillips et al. 1997). Taking a biopsy at 24 h post-exercise contributed to the understanding of exercise signalling in the late recovery period.

## Materials and methods

### Subjects

Nine healthy young men (age  $21.7 \pm 0.55$  year) partaking in no formal resistance exercise regime, were recruited. All subjects were given an oral and written account of the study before signing a consent form. The protocol was approved by the Ethic Committee of the Université catholique de Louvain, and the investigation was performed according to the principles outlined in the Declaration of Helsinki.

### Experimental protocol

In the present study, we used remaining muscle samples from the placebo group of a larger work the purpose of which was to investigate the effects of creatine supplementation on muscle signalling pathways and gene expression (Deldicque et al. 2008). To investigate the effects of resistance exercise on the variables of interest, all study participants underwent three muscle biopsies, one at rest, one immediately after exercise and one 24 h post-exercise. All biopsies were taken after a 10 h overnight fast. The procedure involved the administration of local anesthesia (1% lidocaine) and a sample extraction from the mid portion of the vastus lateralis muscle with a 4-mm Bergström biopsy needle. Blood, macroscopically visible fat and connective tissue were quickly removed, and the sample was immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

Before the experiment, subjects participated in a pre-test to determine one repetition maximum (1-RM) for each leg on a leg extension apparatus. The exercise consisted of a one-leg knee extension movement from an angle of  $90^{\circ}$ – $160^{\circ}$ . After a warm-up comprising three sets of ten repetitions at 5 kg, the load was progressively increased until the subject could not perform more than one single repetition. Subjects were allowed 2 min rest between each set and reached 1-RM within 5–6 trials.

Subjects were instructed to refrain from vigorous physical activity 2 days prior to and during the experimental phase. Food intake on the evening preceding each muscle biopsy was controlled by administering a standardised dinner (22% protein, 48% carbohydrate and 30% fat). On the first morning of the study, participants reported to the laboratory, and a first biopsy was taken at rest in a randomly chosen control leg. The exercise was then performed with

the other leg after a warm-up of three sets of ten repetitions at 5 kg. The main exercise session consisted of 10 sets of 10 repetitions at 80% of the 1-RM of the exercising leg which corresponds to a mean value of positive work of  $19,471 \pm 1,403.5$  J. The positive work of each repetition was calculated by multiplying the moved mass by “g” ( $9.81 \text{ m s}^{-2}$ ) and by the distance (height to which the mass was raised). In this calculation, we neglected the friction due to the pulleys. The subjects were instructed to lift the weight (concentric phase) with both the control and the exercising leg to a knee angle of  $160^\circ$  in 1 s and to lower the weight only with the exercising leg (eccentric phase) during the next 2 s. Each set of 10 repetitions lasted 30 s and the rest in-between sets was 2 min 30 s. A second biopsy was taken from the exercising leg within 30 s following the completion of the last repetition. A standardised breakfast was given after the exercise session (7% protein, 74% carbohydrate and 19% fat). Each participant received a standardised dinner in the evening (22% protein, 48% carbohydrate and 30% fat). A third biopsy was taken 24 h later from the exercising leg after a 10 h overnight fast at 2 cm-interval from the second one.

#### Protein extraction and cell fractionation

About 20–30 mg of frozen muscle were ground in a mortar and homogenized in ice-cold hypotonic buffer [20 mM Hepes, 5 mM sodium fluoride, 1 mM sodium molybdate, 0.1 mM EDTA, 0.5% NP-40, protease inhibitor cocktail (Roche Applied Science)] for 5 min on ice. The homogenates were then centrifuged for 30 s at 10,000g. The supernatant, containing the sarcoplasmic proteins, was stored at  $-80^\circ\text{C}$ . The pellet was re-suspended in a buffer containing 20 mM Hepes, 5 mM sodium fluoride, 1 mM sodium molybdate, 0.1 mM EDTA, 20% glycerol, a protease inhibitor cocktail and the same volume of a saline buffer containing 20 mM Hepes, 5 mM sodium fluoride, 1 mM sodium molybdate, 0.1 mM EDTA, 20% glycerol, 0.8 M NaCl and a protease inhibitor cocktail. The solution was then homogenized on a rotary mixer for 30 min at  $4^\circ\text{C}$  and centrifuged for 10 min at 10,000g. The supernatant, containing the nuclear proteins, was stored at  $-80^\circ\text{C}$ . Sarcoplasmic and nuclear protein concentrations were determined using a protein assay kit (Bio-Rad Laboratories) with BSA as a standard. Fraction purity was verified by immunoblotting for nuclear histone 1 (anti-histone 1, 1:1,000, Santa Cruz). The nuclear fraction was positive for histone 1 whereas the sarcoplasmic was negative.

#### SDS/PAGE and immunoblotting

Cell lysates (70  $\mu\text{g}$  for sarcoplasmic proteins and 30  $\mu\text{g}$  for nuclear proteins) were combined with Laemmli sample

buffer and separated by SDS/PAGE. After electrophoretic separation at 40 mA, the proteins were transferred to a PVDF membrane at 80 V for 4 h for a western blot analysis. Membranes were then incubated in a 5% Blotto solution. Subsequently, membranes were incubated with the following antibodies (1:500) overnight at  $4^\circ\text{C}$ : phospho-Akt/PKB Ser 473 (Cell Signaling), phospho-Akt/PKB Thr 308 (Cell Signaling), total Akt/PKB (Cell Signaling), phospho-p70<sup>s6k</sup> Thr 389 (Santa Cruz), phospho-p70<sup>s6k</sup> Thr 421/Ser 424 (Santa Cruz), total p70<sup>s6k</sup> (Santa Cruz), phospho-p38 Thr 180/Tyr 182 (Cell Signaling), total p38 (Cell Signaling), phospho-ERK1/2 Thr 202/Tyr 204 (Cell Signaling), total ERK (Cell Signaling), phospho-eEF2 Thr 56 (Cell Signaling), phospho-4E-BP1 Thr 37/46 (Cell Signaling), total 4E-BP1 (Cell Signaling). Antibodies from Cell Signaling were diluted in TBST containing 1% BSA and antibodies from Santa Cruz were diluted in a 5% Blotto solution.

Membranes were washed in TBST and incubated for 1 h at room temperature in a secondary antibody conjugated to horseradish peroxidase (1:10,000, Cell Signaling). After an additional three washes, chemiluminescence detection was carried out using an Enhanced Chemiluminescent Western blotting kit (ECL Plus, Amersham Biosciences). Then, the membranes were stripped and re-probed with a total antibody to verify the relative amount of the analyzed proteins through the whole experiment. The films were then scanned on an ImageScanner using the Labscan software and quantified with the Image Master 1D Image Analysis Software (Amersham Biosciences). The results represent the phosphorylated form of the protein. A value of 1 was arbitrarily assigned to the pre-exercise conditions which were used as a reference for the post-exercise values.

#### Cell culture experiments

C2C12 murine skeletal muscle myoblasts (ATCC, USA) were seeded in 60 mm-diameter culture dishes and were grown in Dulbeccos's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco), 1% penicillin/streptomycin (5,000 U/5,000  $\mu\text{g/ml}$ ; Gibco) and 100  $\mu\text{M}$  non-essential amino acids (Gibco). When cells were 70% confluent, the proliferation medium was replaced by a differentiation medium containing 1% horse serum (Gibco), 1% penicillin/streptomycin (5,000 U/5,000  $\mu\text{g/ml}$ ) and 100  $\mu\text{M}$  non-essential amino acids. The differentiation medium was replaced each day for 3 days. At this time, cells were incubated for 30 min with either or both SB202190 (10  $\mu\text{M}$ , Sigma) and PD098059 (50  $\mu\text{M}$ , Sigma) specific inhibitors of p38 and ERK1/2, respectively. At the end of the incubation period, cells were rinsed once with PBS and harvested in a lysis buffer (pH 7.0) containing 20 mM Tris, 270 mM sucrose, 5 mM EGTA, 1 mM EDTA, 0.1% Triton X-100, 1 mM sodium orthovanadate, 50 mM sodium  $\beta$ -glycerophosphate, 5 mM

sodium pyrophosphate, 50 mM sodium fluoride, 1 mM 1,4-dithiothreitol (DTT) and a protease inhibitor cocktail (Roche Applied Science). The homogenate was immediately centrifuged at 10,000g for 10 min at 4°C. Protein concentration of the supernatant was determined using the DC protein assay kit (Bio-Rad Laboratories) with bovine serum albumin (BSA) as a standard, before immunoblotting for p70<sup>s6k</sup> (Thr 421/Ser 424) as described.

#### Statistical analysis

The difference between pre-exercise and post-exercise conditions was tested for significance using an analysis of variance (ANOVA) on ranks for repeated measures (Friedman test). When significant, Student–Newman–Keuls post hoc tests were applied. For the cell culture experiments, rank sum tests were used to assess the difference between control and inhibitory treatment. The significance threshold was set to  $P < 0.05$ . The results are presented as the means  $\pm$  SEM.

## Results

#### Influence of exercise on the phosphorylation state of the Akt/PKB pathway intermediates

Immediately after exercise, the phosphorylation state of Akt/PKB was decreased on both Ser 473 ( $-60\%$ ,  $P < 0.05$ , Fig. 1a) and Thr 308 ( $-70\%$ ,  $P < 0.05$ , Fig. 1b). This exercise-induced inhibition was also observed for 4E-BP1 on Thr 37/46 ( $-90\%$ ,  $P < 0.05$ , Fig. 1e), with a similar trend for p70<sup>s6k</sup> on Thr 389, although the significance threshold was not reached (Fig. 1c). Conversely, the phosphorylation state of p70<sup>s6k</sup> on Thr 421/Ser 424 was increased by more than 20-fold ( $P < 0.05$ , Fig. 1d).

Twenty-four hour post-exercise, the phosphorylation state of Akt/PKB on Ser 473 and 4E-BP1 returned to basal values, whereas the phosphorylation state of Akt/PKB on Thr 308 remained depressed ( $P < 0.05$ , Fig. 1b) and the phosphorylation state of p70<sup>s6k</sup> on Thr 421/Ser 424 remained elevated ( $P < 0.05$ , Fig. 1d). At this time, the phosphorylation state of p70<sup>s6k</sup> on Thr 389 was markedly elevated in two of the nine subjects, while it remained similar to basal values in the others.

Exercise did not modify the phosphorylation state of eEF2 on Thr 56 (Fig. 1f) and had no effect on the expression of the total form of Akt/PKB, 4E-BP1 and p70<sup>s6k</sup> (data not shown).

#### Exercise activates the p38 and the ERK1/2 MAPK pathways

Exercise increased more than tenfold the phosphorylation state of p38 at Thr 180/Tyr 182 in the sarcoplasm ( $P < 0.05$ ,

Fig. 2a) and more than 20-fold in the nucleus ( $P < 0.05$ , Fig. 2b). After 1 day, these values returned to pre-exercise levels. The phosphorylation state of ERK1/2 on Thr 202/Tyr 204 was increased immediately after exercise about 50-fold in the sarcoplasm ( $P < 0.05$ , Fig. 2c) and about 10-fold in the nucleus ( $P < 0.05$ , Fig. 2d). Twenty-four hour after exercise the phosphorylation state of ERK1/2 in the nucleus had returned to basal values, whilst remaining elevated in the sarcoplasm ( $P < 0.05$ ). The total form of p38 and ERK1/2, both in the sarcoplasm and in the nucleus, were not affected by exercise (data not shown).

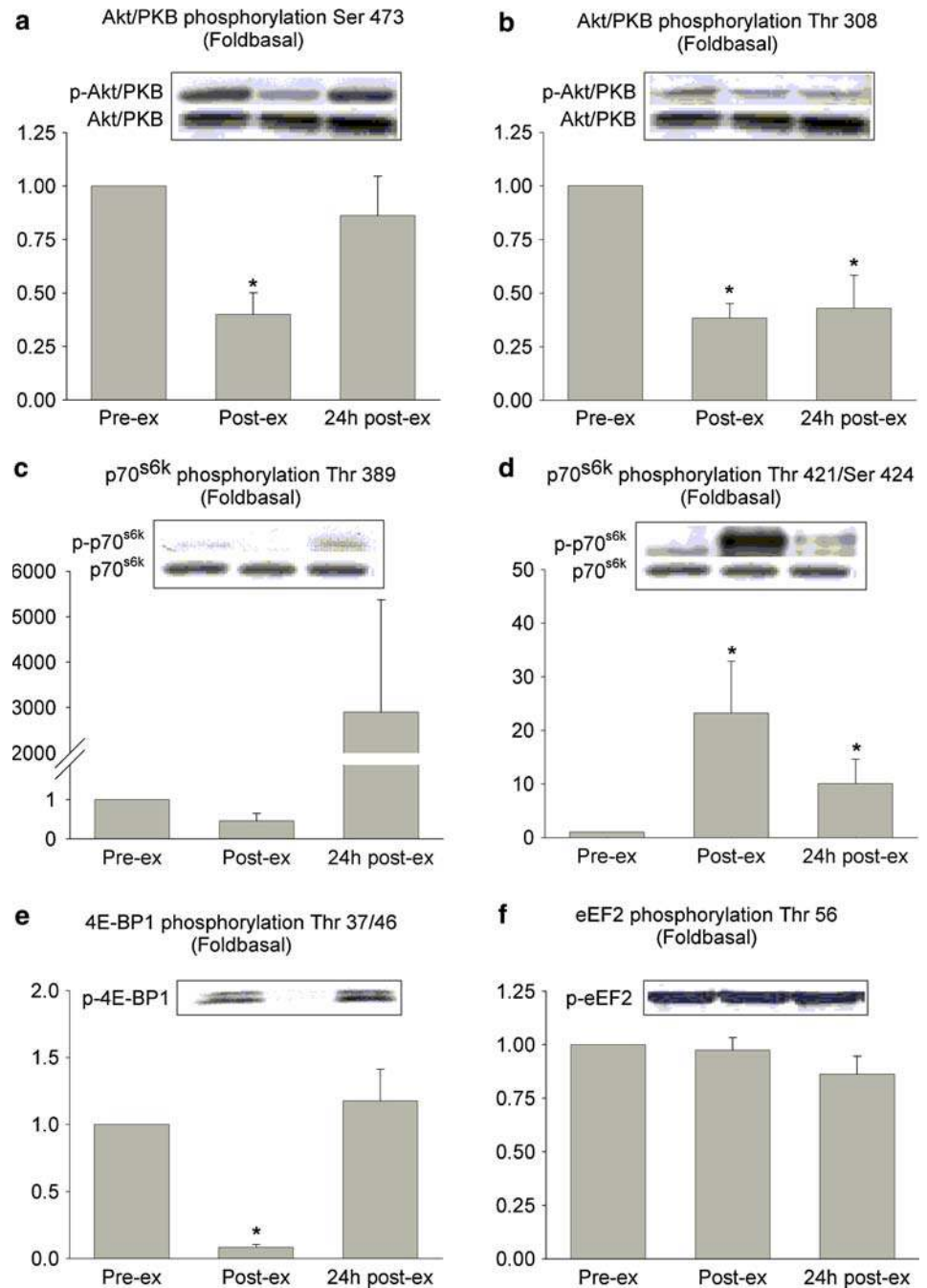
#### Inhibition of p38 and ERK1/2 by using pharmacological agents

A potential crosstalk between MAPK pathway and p70<sup>s6k</sup> was tested in myogenic C2C12 cells by using SB202190 (10  $\mu$ M), an inhibitor of p38, and PD098059 (50  $\mu$ M), an inhibitor of ERK1/2 (Fig. 3). The phosphorylation state of p70<sup>s6k</sup> on Thr 421/Ser 424 was decreased by 75% ( $P < 0.05$ ) when myotubes were incubated with SB202190 and by 20% with PD098059 ( $P < 0.05$ ). Incubating the cells with both inhibitors did not further decrease the phosphorylation state of p70<sup>s6k</sup> as compared with SB202190 alone ( $-75\%$ ,  $P < 0.05$ ).

## Discussion

The present results have partially been presented in a previous study (Deldicque et al. 2008), the purpose of which was to investigate the effect of creatine coupled to an exercise session on gene expression and Cell Signaling. The current analyses emphasize the effect of contractile activity alone and show that resistance exercise of high-intensity and comprising a large component of eccentric contraction decreases the phosphorylation state of Akt/PKB when subjects are in the fasted state. It is unlikely that the nutritional status of the subjects is responsible for the drop we observed in Akt/PKB phosphorylation. Blomstrand et al. (2006) found that immediately after resistance exercise, the phosphorylation state of Akt/PKB was decreased to the same extent in the placebo group and in the group receiving branched chain amino acids. The decrease in the Akt/PKB phosphorylation state fits well with the findings that exercise in the fasted state decreases protein synthesis and increases protein breakdown (Rennie and Tipton 2000; Wolfe 2000). However, the phosphorylation state of Akt/PKB has been found to increase after low intensity resistance exercise (Creer et al. 2005). One could postulate that high intensity (>80% 1-RM) and low intensity resistance exercise induce opposite responses on Akt/PKB. One potential candidate mediating the decrease in Akt/PKB

**Fig. 1** Effect of exercise on the phosphorylation state of Akt/PKB, p70<sup>S6k</sup>, 4E-BP1 and eEF2. Phosphorylation states of Akt/PKB on Ser 473 (a) and on Thr 308 (b), p70<sup>S6k</sup> on Thr 389 (c) and on Ser 421/Thr 424 (d), 4E-BP1 on Thr 37/46 (e) and eEF2 on Thr 56 (f) at rest, within 30 s following exercise and 24 h after exercise in the fasted state. A representative immunoblot of the phosphorylated form and the total form is shown at the top of each graph. Values of the phosphorylated form are expressed as the means  $\pm$  SEM ( $n = 9$ ). \* $P < 0.05$  post-exercise versus pre-exercise



phosphorylation is the AMP-activated protein kinase (AMPK). Indeed, AMPK activity has been shown to be increased during high intensity resistance exercise (Dreyer et al. 2006; Koopman et al. 2006) and to lead to a decrease in the phosphorylation state of Akt/PKB on Ser 473 (Bolster et al. 2002).

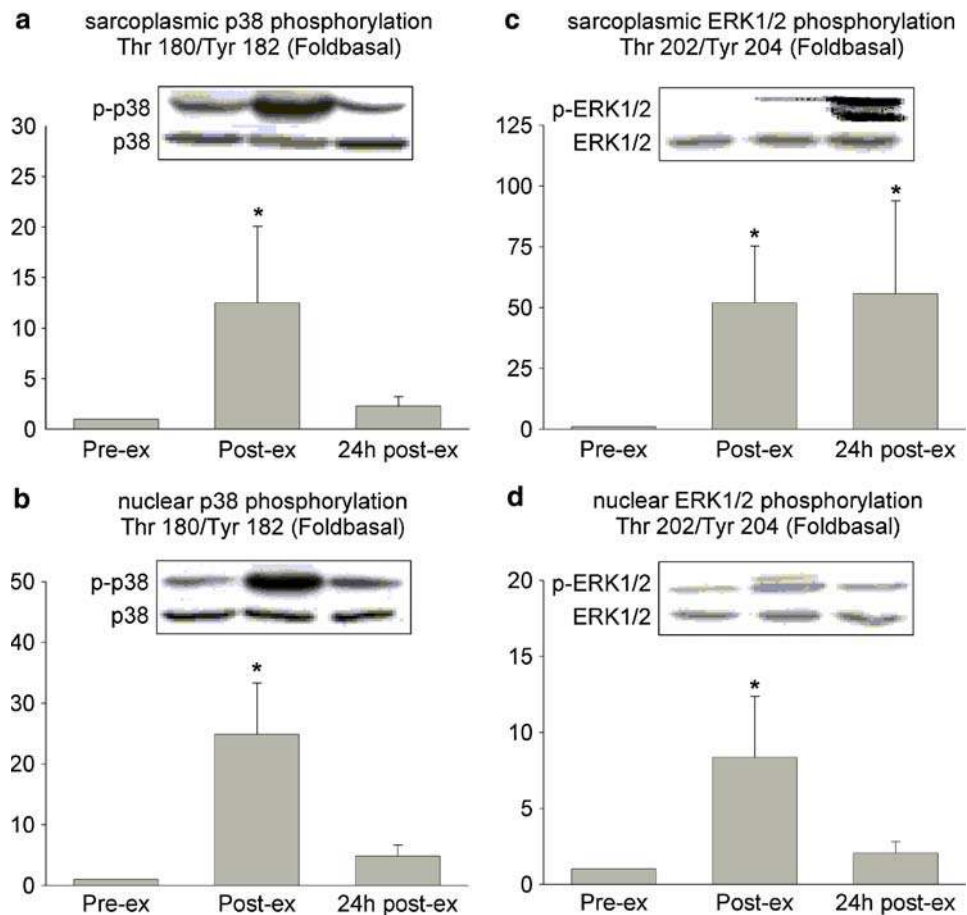
The type of contraction, concentric versus eccentric, could also be an explanation for the discrepancies observed after exercise on Akt/PKB. Eccentric exercise tends to decrease Akt/PKB phosphorylation compared to concentric exercise (Eliasson et al. 2006) and in our protocol, the

eccentric component was high. Eccentric contractions are known to induce greater proteolysis and muscle damage when compared to concentric contractions (Sorichter et al. 1995). Thus the type and the intensity of muscle contraction most likely trigger different responses at the cellular level.

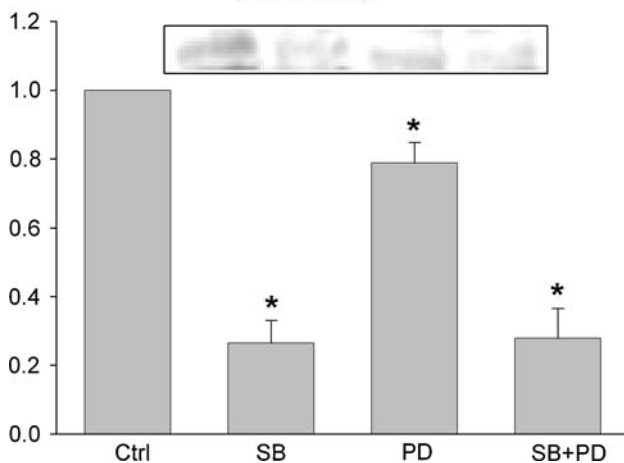
Immediately after exercise, the phosphorylation state of p70<sup>S6k</sup> on Thr 389 was slightly but not significantly depressed (Fig. 1c). This trend could have been the consequence of decreased Akt/PKB phosphorylation. On the other hand, a large increase was found in the phosphorylation at

**Fig. 2** Effect of exercise on the phosphorylation state of p38 and ERK1/2. Phosphorylation state of p38 on Thr 180/Tyr 182 and ERK1/2 on Thr 202/Tyr 204 in the cytoplasm (**a, c**) and in the nucleus (**b, d**) at rest, within the 30 s following exercise and 24 h after exercise in the fasted state. A representative immunoblot of the phosphorylated form and the total form is shown at the top of each graph. Values of the phosphorylated form are expressed as the means  $\pm$  SEM ( $n = 9$ ).

\* $P < 0.05$  post-exercise versus pre-exercise



p70<sup>s6k</sup> phosphorylation Thr 421/Ser 424 (Foldbasal)



**Fig. 3** Effect of the inhibition of p38 and ERK1/2 on the phosphorylation state of p70<sup>s6k</sup> in C2C12 cells. Phosphorylation state of p70<sup>s6k</sup> on Ser 421/Thr 424 after addition of the inhibitor of p38 (SB202190, 10  $\mu$ M), the inhibitor of ERK1/2 (PD098059, 50  $\mu$ M) and both inhibitors for 30 min in C2C12 cells. The experiments were carried out after 72 h of differentiation, when myotubes are formed. Values are expressed as the means  $\pm$  SEM ( $n = 4$ ). \* $P < 0.05$  treatment versus control

Thr 421/Ser 424 (Fig. 1d). p70<sup>s6k</sup> possesses many sites of phosphorylation, and to be fully activated, each site has to be phosphorylated by specific kinases in a sequential manner (Weng et al. 1998). The first step in p70<sup>s6k</sup> activation involves the phosphorylation of a cluster of (Ser/Thr) Pro sites. The latter are situated in the autoinhibitory domain in the carboxyl-terminal tail, including, amongst others, Thr 421 and Ser 424. The identity of the kinases phosphorylating these sites in vivo is currently not established. MAPK, SAPK (stress-activated protein kinase) and cdc2 (or Cdk1, cyclin dependent kinase 1) are capable of phosphorylating them in vitro (Iijima et al. 2002; Mukhopadhyay et al. 1992; Wang et al. 2001, 1998). In the present study, both p38 and ERK1/2 were increased immediately after exercise and could therefore be responsible for the enhanced phosphorylation state of p70<sup>s6k</sup> observed on Thr 421/Ser 424.

In search of support for this hypothesis, we tested if MAPK also contributed to the phosphorylation state of Thr 421/Ser 424 in myogenic cells, as already observed in H4 hepatoma (Mukhopadhyay et al. 1992) and cardiomyocytes (Iijima et al. 2002; Wang et al. 2001). We measured the phosphorylation state of p70<sup>s6k</sup> on Thr 421/Ser 424 after having incubated C2C12 cells with SB202190 and PD098059, specific inhibitors of p38 and ERK1/2,

respectively (Fig. 3). The addition of each inhibitor or both together decreased the phosphorylation state of Thr 421/Ser 424, indicating that in myogenic cells p38 and ERK1/2 contribute to the phosphorylation of these sites. Although in vitro results are not directly translatable to in vivo findings and should thus be taken with caution, the current results suggest that during exercise, p38 and ERK1/2 could remove the autoinhibition of  $p70^{S6k}$  by phosphorylating the first sites in the hierarchical activation of this kinase. As suggested in Fig. 4, this priming of  $p70^{S6k}$  possibly participates in the potentiation of protein synthesis in exercised muscles when nutrients are provided during recovery (Louis et al. 2003). Indeed, nutrients are required to achieve a positive protein balance following exercise at least in part by initiating signalling leading to the phosphorylation of Thr 389 (Cuthbertson et al. 2006) and therefore conferring full activation of  $p70^{S6k}$ .

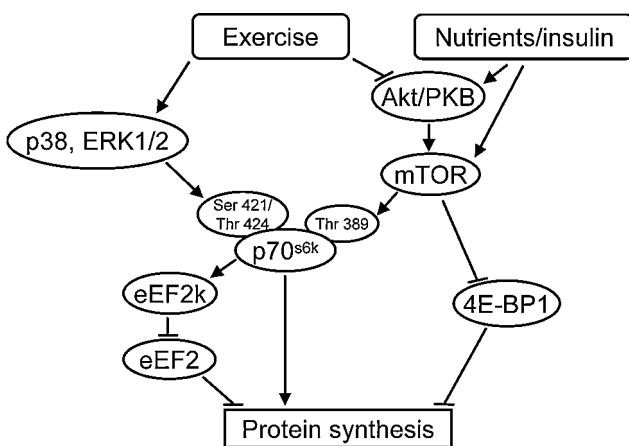
As illustrated in Fig. 1e, 4E-BP1 was dephosphorylated immediately after exercise. Recently, two studies have demonstrated similar findings (Dreyer et al. 2006; Koopman et al. 2006). This is in line with our results on the phosphorylation of Akt/PKB and the fact that protein synthesis is decreased immediately after exercise.

The majority of prior studies have focused upon the signalling of exercise during the early phase of the recovery period. However, exercise is known to alter protein metabolism for up to 2 days (Phillips et al. 1997), which motivated us to take a biopsy from our subjects at 24 h post-exercise. To the best of our knowledge, there is only one paper that has analysed the phosphorylation state of the Akt/PKB pathway 24 h after exercise (Cuthbertson et al. 2006). In that study, phosphorylation of Akt/PKB on Ser

473 was increased two- to threefold, and, at the same time, muscle protein synthesis was elevated. However, since the subjects were not in a fasted state when the biopsies were taken, these observations might not be directly attributable to exercise. Our protocol made it possible to highlight the specific effect of exercise after 24 h since the biopsies were taken after an overnight fast. The phosphorylation states of intermediates of the MAPK and Akt/PKB pathways were largely returned to pre-exercise values, except for Akt/PKB on Thr 308,  $p70^{S6k}$  on Thr 421/Ser 424 and the sarcoplasmic form of ERK1/2. These results are compatible with the hypothesis that ERK1/2 is upstream, and a potential kinase of  $p70^{S6k}$  on Thr 421/Ser 424, since both ERK1/2 and  $p70^{S6k}$  on Thr 421/Ser 424 followed the same phosphorylation time-course.

By phosphorylating eukaryotic elongation factor 2 kinase (eEF2k),  $p70^{S6k}$  renders it less active and raises the inhibition exerted by eEF2k on eEF2 (Fig. 4). Therefore, a decrease in eEF2 phosphorylation leads to its activation and an enhanced elongation. In agreement with our observation on the phosphorylation state of  $p70^{S6k}$  on Thr 389, which best reflects its activity, eEF2 phosphorylation was not modulated by exercise (Fig. 1f). eEF2 has already been found to be unaltered immediately after resistance exercise but eEF2 was dephosphorylated at 1 and 2 h after (Dreyer et al. 2006). It seems that exercise only transiently affects eEF2 during the recovery period since after 24 h, the phosphorylation state was similar to pre-exercise levels (Fig. 1f). On the other hand, eEF2 phosphorylation was increased during continuous endurance exercise and the authors suggested a role of calcium in the control of eEF2 (Rose et al. 2005). In addition to  $p70^{S6k}$  and calcium, AMP-activated protein kinase (AMPK) has been shown to regulate eEF2 phosphorylation via eEF2 kinase in vitro (Horman et al. 2002), although, it has not been confirmed during exercise in vivo (Rose et al. 2005). Further investigation is needed to clarify the role of this elongation factor in the control of protein synthesis in human skeletal muscle after exercise.

Because MAPK can phosphorylate different sarcoplasmic targets or translocate to the nucleus, we measured the phosphorylation states of p38 and ERK1/2 in both compartments (Fig. 2). Only two studies have analysed the sarcoplasmic and the nuclear forms of p38 in human biopsies after endurance exercise (Chan et al. 2004; McGee and Hargreaves 2004) and have demonstrated only moderate (two- to five-fold) increases in the phosphorylation state of nuclear (Chan et al. 2004) or both total and nuclear p38 (McGee and Hargreaves 2004). In comparison with those studies, we measured much larger increases in the phosphorylation state of p38 and ERK1/2 (10- to 50-fold) both in the sarcoplasm and in the nucleus. The greater response in our subjects is likely due to the different type of exercise. The regulation of



**Fig. 4** Proposed model for exercise signalling in human skeletal muscle. *ERK1/2* extracellular signal-regulated kinases 1 and 2, *Akt/PKB* protein kinase B, *mTOR* mammalian target of rapamycin,  $p70^{S6k}$  p70 ribosomal S6 kinase, *4E-BP1* eukaryotic initiation factor 4E-binding protein 1, *eEF2k* eukaryotic elongation factor 2 kinase, *eEF2* eukaryotic elongation factor 2



MAPK is indeed dependent on the mode and the intensity of contractions (Widegren et al. 2001). It seems that for co-activation of p38 and ERK1/2, high-intensity eccentric contractions are required (Wretman et al. 2001). A session of knee extensor resistance exercise consisting of 29 contractions at approximately 70% of the 1-RM was associated with an increase in ERK1/2 phosphorylation with no effect on p38 (Williamson et al. 2003). On the other hand, 40 repetitions of leg press exercise at 80% of the 1-RM induced an 8-fold increase in the phosphorylation state of ERK1/2 and a 5-fold increase in the phosphorylation state of p38 (Karlsson et al. 2004). Our exercise bout consisted in 100 repetitions at 80% of the 1-RM for the eccentric component, which could explain the large amplitude of the response to exercise of ERK1/2 and p38.

In summary, we report an inhibition of the Akt/PKB pathway by resistance exercise performed in the fasted state. Moreover our results show that p38 and ERK1/2 are activated and suggest that there is a crosstalk between p38 and ERK1/2 and p70<sup>S6k</sup> on Thr 421/Ser 424. This priming of p70<sup>S6k</sup> via MAPK could be an important step in potentiating protein synthesis with feeding during the recovery period from exercise. However, the present data do not give a mechanism for Akt/PKB inhibition and p38 and ERK1/2 stimulation immediately after exercise. Further study is warranted to describe these molecular phenomena.

**Acknowledgments** This work was supported by grants to MJ Rennie from UK Biotechnology and Biological Sciences Research Council (BB/X510697/1 and BB/C516779/1), US National Institute of Health AR 49869, and the EC EXEGENESIS program and to M Francaux from the Fonds de la Recherche Scientifique Medicale (3.4574.03).

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