Effect of endurance exercise training on Ca^{2+}–calmodulin-dependent protein kinase II expression and signalling in skeletal muscle of humans

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Here the hypothesis that skeletal muscle Ca^{2+}–calmodulin-dependent kinase II (CaMKII) expression and signalling would be modified by endurance training was tested. Eight healthy, young men completed 3 weeks of one-legged endurance exercise training with muscle samples taken from both legs before training and 15 h after the last exercise bout. Along with an ∼40% increase in mitochondrial F_1-ATP synthase expression, there was an ∼1-fold increase in maximal CaMKII activity and CaMKII kinase isoform expression after training in the active leg only. Autonomous CaMKII activity and CaMKII autophosphorylation were increased to a similar extent. However, there was no change in α-CaMKII anchoring protein expression with training. Nor was there any change in expression or Thr^{17} phosphorylation of the CaMKII substrate phospholamban with training. However, another CaMKII substrate, serum response factor (SRF), had an ∼60% higher phosphorylation at Ser^{103} after training, with no change in SRF expression. There were positive correlations between the increases in CaMKII expression and SRF phosphorylation as well as F_1ATPase expression with training. After training, there was an increase in cyclic-AMP response element binding protein phosphorylation at Ser^{133}, but not expression, in muscle of both legs. Taken together, skeletal muscle CaMKII kinase isoform expression and SRF phosphorylation is higher with endurance-type exercise training, adaptations that are restricted to active muscle. This may contribute to greater Ca^{2+} mediated regulation during exercise and the altered muscle phenotype with training.

(Resubmitted 14 June 2007; accepted after revision 12 July 2007; first published online 12 July 2007)
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Skeletal muscles adapt to the functional demands placed on them (Holloszy & Booth, 1976; Baar *et al.* 2006), but the precise mechanisms of *remodelling* are unknown. It is believed that these adaptations occur through repeated stimuli by which there are coordinated changes in the specific protein degradation and synthesis which contribute to the overall change in the expression of a particular protein (Booth *et al.* 1982). In particular, there is emerging evidence that acute exercise increases the transcriptional activity of certain genes (Keller *et al.* 2001; Pilegaard *et al.* 2000, 2003) through activation of transcription factors (Irrcher & Hood, 2004; McGee *et al.* 2006; Wright *et al.* 2007) via various signalling pathways (Hawley *et al.* 2006), which has been conceptually termed *excitation–transcription coupling* (Chin, 2004). The understanding of the signalling mechanisms by which skeletal muscle becomes more fatigue resistant and has greater oxidative capacity with endurance exercise training is important as this may aid in the development of strategies to retard the development of insulin resistance (Hawley & Houmard, 2004; Kiens, 2006) as well as muscle dysfunctions (Chakkalakal *et al.* 2006).

Many signalling pathways may play a role in exercise induced skeletal muscle adaptation (for review see Hawley *et al.* 2006). Of note, AMPK expression and activity are higher after training (Frøsig *et al.* 2004), probably providing increased signalling to mitochondrial biogenesis because mice deficient in the α_2-AMPK subunit have 20–25% lower mitochondrial enzyme expression in muscle (Jørgensen *et al.* 2007). On the other hand, in these mice the exercise-training induced increase in mitochondrial biogenesis is not impaired (Jørgensen *et al.* 2005, 2007), suggesting that other contraction induced signalling molecules are important

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for this adaptive process to exercise training. One such candidate is Ca^{2+} signalling (Chin, 2004). In particular, studies of mice where skeletal muscle proteins involved in Ca^{2+} homeostasis were manipulated show that changes in Ca^{2+} handling can alter skeletal muscle phenotype (e.g. oxidative capacity and contractile properties) regardless of motor nerve innervation (Chin *et al.* 2003; Song *et al.* 2004; Racay *et al.* 2006). It has been shown that Ca^{2+} signalling may increase gene expression of myosin-IIa (Allen & Leinwand, 2002), troponin-I slow isoform (Juretić *et al.* 2007), GLUT4 (Ojuka *et al.* 2002), PGC1α (Ojuka *et al.* 2003) and mitochondrial genes (Ojuka *et al.* 2003; Freyssenet *et al.* 2004), but probably not hexokinase-II (Halseth *et al.* 2000). Furthermore, it is known that increases in intracellular Ca^{2+} can affect protein turnover in skeletal muscle (Lewis *et al.* 1982) and during exercise various Ca^{2+} signalling molecules are activated including conventional protein kinase C (Richter *et al.* 1987), Ca^{2+}–calmodulin-dependent kinases (CaMK; Rose *et al.* 2003, 2005, 2006; Liu *et al.* 2005) and protein phosphatase 2B (PP2B/calcineurin; Liu *et al.* 2001; Tothova *et al.* 2006). While PP2B is probably involved in motor nerve determined fibre type profile (Olson & Williams, 2000; Schiaffino & Serrano, 2002), there is accumulating evidence that it is not involved in many of the phenotypic modifications of skeletal muscle with repeated exercise (Parsons *et al.* 2004; Garcia-Roves *et al.* 2006; Huang *et al.* 2006).

Of the multifunctional CaMKs, CaMKI and CaMKIV have been implicated as putative signalling molecules in the adaptive process to exercise (McKinsey *et al.* 2000*a*,*b*; Wu *et al.* 2002; Smith *et al.* 2007), but since neither of these are expressed in skeletal muscle (Rose & Hargreaves, 2003; Chin, 2004; Akimoto *et al.* 2005; Rose *et al.* 2006), this is probably not the case. On the other hand, CaMKII isoforms are expressed in skeletal muscle (Bayer *et al.* 1998; Damiani *et al.* 2000; Rose *et al.* 2006) and studies of non-human mammals show that CaMKII activity and expression are higher with training (Antipenko *et al.* 1999; Flück *et al.* 2000*b*). These latter observations suggest that endurance training in man might also lead to increased CaMKII expression/activity in skeletal muscle. In addition, CaMKII is enriched in skeletal muscle nuclei (Flück *et al.* 2000*a*) and can phosphorylate proteins involved in the transcriptional *machinery* such as serum response factor (Flück *et al.* 2000*a*), myogenin (Tang *et al.* 2004), histone deacytalase 4 (Liu *et al.* 2005) and possibly cAMP response element binding protein (Hook & Means, 2001). Thus, CaMKII is an attractive candidate for activity dependent skeletal muscle adaptation as also hypothesized by others (Chin, 2004, 2005; Hood *et al.* 2006).

Here we examine CaMKII activity and expression of CaMKII isoforms in skeletal muscle of humans before and after short-term endurance exercise training. Potential downstream targets such as serum response factor and cAMP response element binding protein were also examined as well as particular genes which these might activate such as the β-subunit of the mitochondrial F_1-ATP synthase.

Methods

Subjects and intervention

The specific details of subjects and the training regime have been reported (Frøsig *et al.* 2004, 2007). Eight healthy, young men (age: 25 ± 1 years, BMI: 24.6 ± 0.5 kg m^{-2}) completed 3 weeks of one-legged knee-extension endurance exercise training with muscle biopsy samples taken from the vastus lateralis of both the active and passive leg before training and 15 h after the last exercise bout. Subjects consumed a standardized diet (i.e. ∼56% carbohydrate, 29% fat, 15% protein; 13.5 ± 0.2 MJ day^{-1}) for two days prior to sampling. The exercise training consisted of dynamic knee extension exercise (70–85% peak work load; PWL) for 1–2 h per session, four to six sessions per week. Within each exercise session there was also a small bout (i.e. 5–7 min) of exercise at 100% of PWL in order to recruit all motor units of the vastus lateralis (Gollnick *et al.* 1974) and thereby train all muscle fibres. Subjects gave informed consent prior to participation and the study was carried out in accordance with the *Declaration of Helsinki*.

Tissue preparation

All materials were from Sigma-Aldrich (USA) unless stated otherwise. For skeletal muscle sample protein extraction, samples (15–20 mg) were freeze-dried and then homogenized while in an ice slurry (i.e. 0°C) in a buffer (15 μl per mg tissue, original weight) containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 50 mM sodium fluoride, 5 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 1 mM PMSF, 1 mM dithiothreitol, 1 mM benzamidine, 0.5% (v/v) protease inhibitor cocktail, and 1% (v/v) Nonidet P-40, using a polytron homogenizer (PT 1200, Kinematica) until no visible particles remained. The homogenates were mixed thoroughly by end-over-end rotation at 4°C for 30 min, and then spun at 6000 *g* for 10 min at 4°C. The clarified supernatant was taken and stored at −80°C until required. A small aliquot of each lysate was taken prior to storage for total protein concentration analysis.

Analytic techniques

Protein concentration of tissue extracts was determined in triplicate by the bicinchoninic acid (BCA) method using bovine serum albumin standards (Pierce Biotechnology

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Inc., Rockford, IL, USA) and BCA assay reagents (Pierce Biotechnology). A maximal coefficient of variance of 5% was accepted between replicates. Samples were immunoblotted for protein expression and phosphorylation according to Rose *et al.* (2005). The primary antibodies used were anti-F_1-ATPase-β (Santa Cruz Biotechnlogy Inc., Santa Cruz, CA, USA; sc-16689), anti-CaMKII (BD Biosciences–Pharmingen, USA; 612624), anti-phospho-Thr^{287}-CaMKII (Cell Signalling Technology, Inc., MA, USA; 3361), anti-phospholamban (Cyclacel, UK; 010-14), anti-phosphoThr^{17}-phospholamban (Cyclacel, UK; 010-13), anti-serum response factor (Santa Cruz Biotechnology; sc-335), anti-phospho-Ser^{103}-serum response factor (Cell Signalling Technology; 4261), anti-cAMP response element binding protein (Cell Signalling Technology; 9192) and anti-phospho-Ser^{133}-cAMP response element binding protein (Cell Signalling Technology; 9191). Secondary antibodies were from DakoCytomation (Glostrup, Denmark). Band intensity was quantified by Kodak imaging software (Kodak 1D 3.5, USA). Preliminary experiments demonstrated that the amounts of protein loaded were within the dynamic range for the conditions used and the results obtained (data not shown), and that the phosphospecific antibodies were indeed phosphospecific (data not shown; Rose *et al.* 2006). To measure kinase activity muscle extracts were analysed according to Rose & Hargreaves (2003). In brief, CaMKII activity of muscle extracts was measured in the presence (maximal activity) or absence (autonomous activity) of Ca^{2+}-calmodulin with autocamtide-2 (Upstate Biotech., USA) as the peptide substrate. This assay has been shown to be specific for CaMKII as using a specific inhibitor for CaMKII reduces the majority of phosphotransfer activity by lysate proteins in the presence or absence of calmodulin (Rose & Hargreaves, 2003).

Calculations and statistics

Statistical analyses were performed using SigmaStat v. 3.1 with two-way ANOVA for repeated measures used with Student–Neuman–Kuels *post hoc* testing. Correlations and regression analyses were performed using SPSS 14.0. Differences were considered to be significant when P was less than 0.05.

Results

The subject characteristics and general adaptations to training have been reported (Frøsig *et al.* 2004). In particular, similar to other studies (Dela *et al.* 1992; Kiens *et al.* 1993; Kristiansen *et al.* 2000), this training protocol resulted in significant increases in functional aerobic work capacity (~16%) and skeletal muscle mitochondrial enzymes (CS: ~37%; βHAD: ~35%) as well as increases in hexokinase-II (~300%) and glucose transporter-4 (~50%), changes which were restricted to the active leg (Frøsig *et al.* 2004; Frøsig *et al.* 2007). In addition, there was also a 42 ± 8% increase in the expression of the β-subunit of the mitochondrial F_1-ATP synthase (F_1ATPase-β) in skeletal muscle of the active leg after training (Figs 1 and 2).

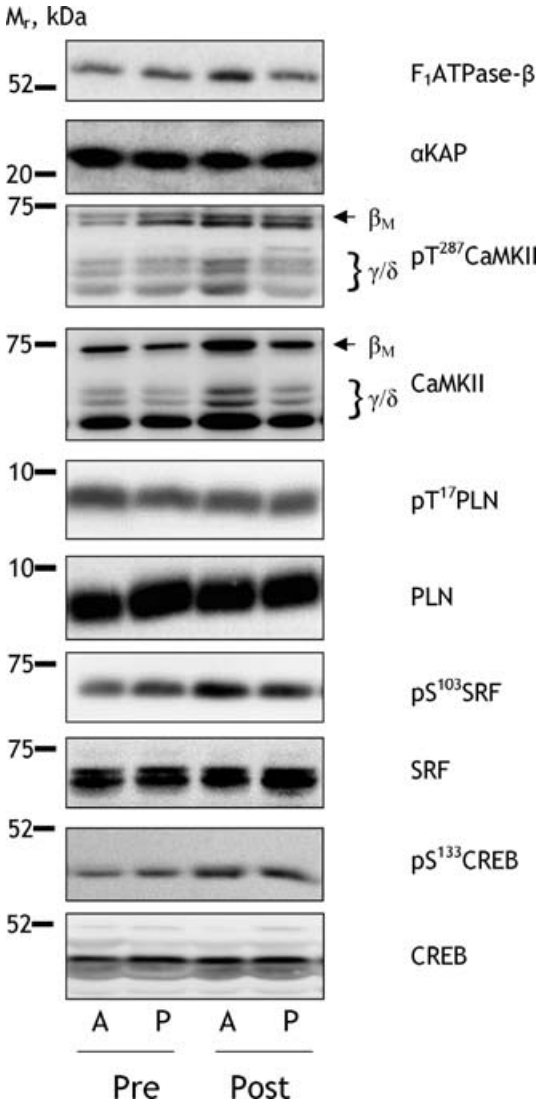

Figure 1. Representative immunoblots
Skeletal muscle samples from the vastus lateralis muscle of the passive (P) and active (A) leg before (Pre) and after (Post) 3 week of exercise training were extracted and lysates were immunoblotted for total and phosphorylated proteins as described in Methods. Isoforms of CaMKII as well as the relative electrophoretic mobility (Mr) of proteins are indicated. F_1ATPase-β: β-subunit of F_1-ATP synthase; αKAP: αCaMKII kinase anchoring protein; CaMKII: Ca^{2+}–calmodulin-dependent protein kinase II; PLN: phospholamban; SRF: serum response factor; CREB: cAMP response element binding protein.

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Effect of training on CaMKII activity and expression

Representative immunoblots are shown in Fig. 1. In response to 3 weeks of training, there was an ∼1-fold increase in the expression of CaMKII kinase isoforms (i.e. β_M, γ and δ) but not the α isoform (αKAP; Fig. 3*A* and *C*). Similar to CaMKII kinase isoform expression, there was an ∼90% increase in maximal CaMKII activity (Fig. 3*D*). Furthermore, there was an increase in autonomous (i.e. Ca^{2+}–CaM independent) CaMKII activity and CaMKII phosphorylation at Thr^{287} (Fig. 3*B* and *E*). There were strong positive correlations between the increases in total CaMKII kinase isoform expression and maximal ($r^2 = 0.86$, $P < 0.01$) and autonomous ($r^2 = 0.79$, $P < 0.05$) CaMKII activity as well as pT^{287}-CaMKII ($r^2 = 0.66$, $P < 0.05$) with training (Fig. 3*F*), indicating that these changes were attributable to the increase in CaMKII expression. Importantly, the changes that were observed were restricted to skeletal muscle of the active leg.

Effect of contraction on putative CaMKII substrates

Representative immunoblots are shown in Fig. 1. There were no effects of training on phospholamban expression or phosphorylation at Thr^{17} (Fig. 4*A* and *B*). Serum response factor (SRF) phosphorylation at Ser^{103} was higher in skeletal muscle of the active leg with training, without changes in SRF expression (Fig. 4*C* and *D*). cAMP response element binding protein (CREB) phosphorylation at Ser^{133} was higher in skeletal muscle of both legs after training, with no changes in CREB expression (Fig. 4*E* and *F*). There was a positive correlation between the increases in total CaMKII kinase isoform expression and SRF phosphorylation ($r^2 = 0.88$; $P < 0.01$) and F_1ATPase-β expression ($r^2 = 0.80$; $P < 0.05$; Fig. 5*A*), but not CREB phosphorylation ($r^2 = 0.01$; $P > 0.5$; data not shown). There was a strong positive correlation between the increases in skeletal muscle SRF phosphorylation and F_1ATPase-β expression with training ($r^2 = 0.92$, $P < 0.01$; Fig. 5*B*). There was no relationship between changes in CREB phosphorylation and F_1ATPase-β expression with training ($r^2 = 0.03$; $P > 0.5$; data not shown).

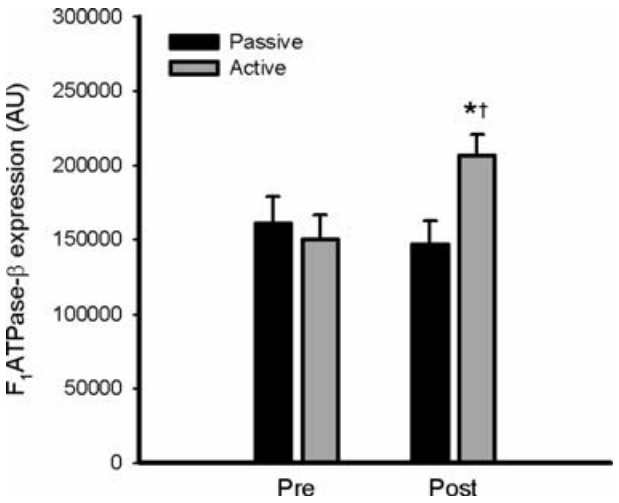

Figure 2. Endurance exercise training increases skeletal muscle F_1ATPase-β expression
Skeletal muscle samples from the vastus lateralis muscle of the passive and active leg before (Pre) and after (Post) 3 weeks of exercise training were extracted and lysates were immunoblotted for β-subunit of F_1-ATP synthase expression. Data are means ± S.E.M., $n = 8$; *different from Pre, $P < 0.01$; †different from Passive, $P < 0.01$.

Discussion

The main finding of this study is that skeletal CaMKII expression and maximal activity (Fig. 3) are increased by short-term endurance training in humans. The increase in maximal CaMKII activity was probably due to higher CaMKII expression as these variables covaried closely (Fig. 3*F*). Furthermore, autonomous (i.e. Ca^{2+}–CaM independent) CaMKII activity and phosphorylation at Thr^{287} were increased to a similar extent (Fig. 3). The increases in CaMKII autonomous activity and phosphorylation were also closely related to changes in CaMKII expression indicating that these changes were caused primarily by higher CaMKII expression with training (Fig. 3*F*). Importantly, it has been previously reported that AMP activated protein kinase (AMPK) expression and activity are also increased in trained muscle at rest (Frøsig *et al.* 2004). Thus, together with the present data, it is apparent that effects of physical training may not only be evoked during the training periods themselves during which AMPK and CaMKII are activated (Rose *et al.* 2006; Wojtaszewski *et al.* 2000), but enhanced AMPK and CaMKII expression/activity in the rest periods between training bouts may also affect muscle gene expression.

The increase in kinase isoform expression and activity of CaMKII was perhaps expected as other studies have shown that regular running in rats (Flück *et al.* 2000*b*) and chronic low frequency motor nerve stimulation of rabbits (Antipenko *et al.* 1999) resulted in increases in CaMKII activity of fast-twitch muscles. Other work from the present laboratory has shown that CaMKIIβ_M expression was higher in gastrocnemius muscle of mice after 4 weeks of voluntary wheel running (J. T. Treebak, A. J. Rose, M. Hargreaves, J. F. P. Wojtaszewski & E. A. Richter, unpublished observations). On the other hand, studies show that denervation of rat soleus muscle resulted in higher CaMKIIγ expression (Chin, 2004) and skeletal muscle CaMKIIδ expression is higher during muscle regeneration (Abraham & Shaw, 2006). These increases may be a compensatory adaptation to counter muscle

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atrophy (Chin, 2004). In any case, isoform expression appears to be sensitive to differing levels of muscle activity.

In contrast to the expression of kinase isoforms of CaMKII, there was no difference in α-CaMKII kinase anchoring protein (αKAP) with training (Fig. 3*C*). αKAP is a truncated non-kinase splice variant of the CaMKIIα isoform expressed in neuronal cells which is expressed at relatively high levels in skeletal muscle (Bayer *et al.* 1998). αKAP is proposed to anchor CaMKII holoenzymes to surface membranes of intracellular organelles such as nuclei and sarcoplasmic reticulum (SR; Bayer *et al.* 1998; Nori *et al.* 2003) and it has been hypothesized that this results in directing CaMKII to its specific substrates in

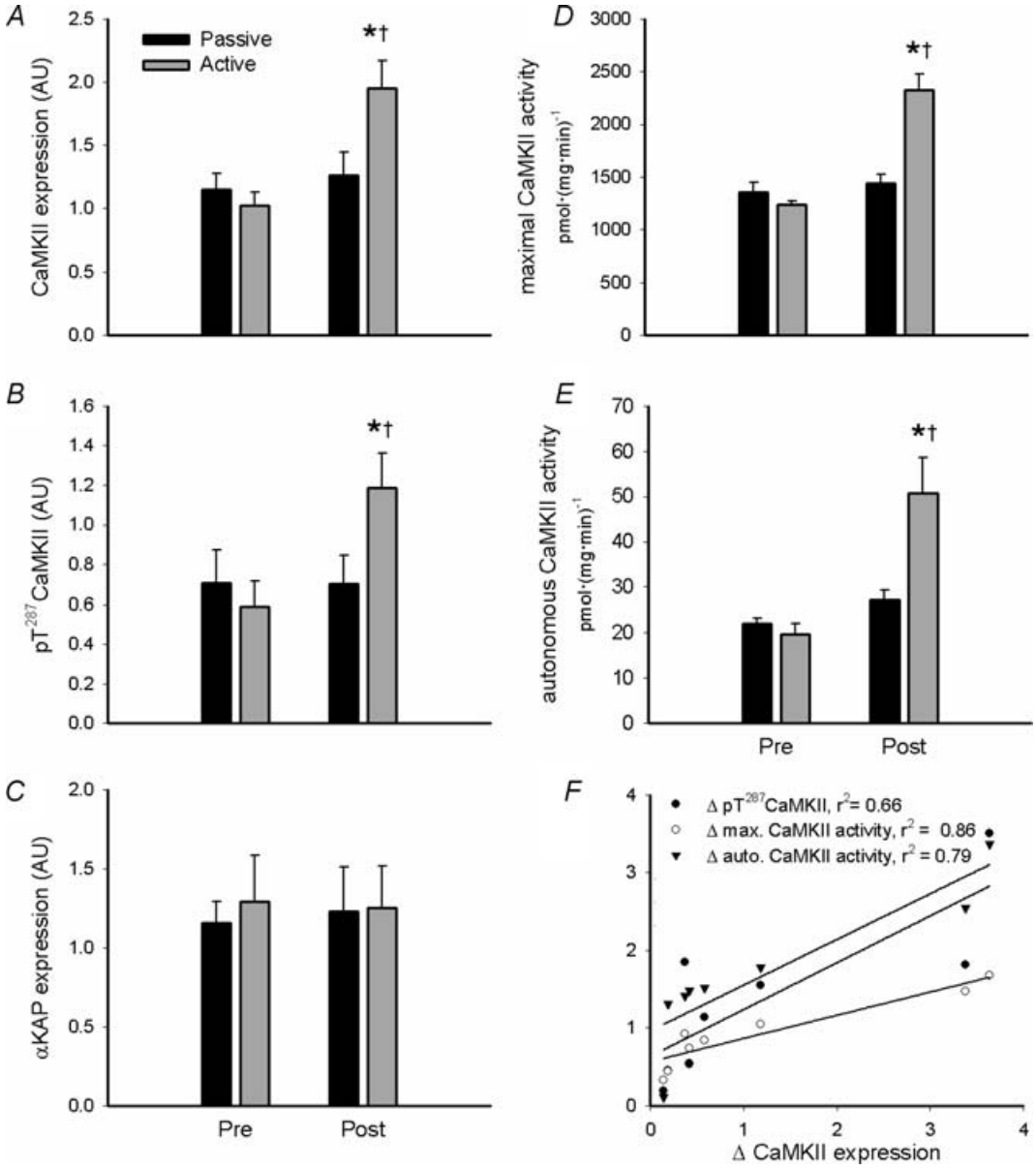

Figure 3. Endurance exercise training increases skeletal muscle CaMKII kinase isoform expression and activity

Skeletal muscle samples from the vastus lateralis muscle of the passive and active leg before (Pre) and after (Post) 3 weeks of exercise training were extracted and lysates were immunoblotted for Ca^{2+}–calmodulin-dependent protein kinase II expression (CaMKII; *A* and *C*) and phospho-Thr^{287}CaMKII (*B*). Skeletal muscle extracts were assayed *in vitro* for Ca^{2+}–calmodulin-dependent protein kinase II (CaMKII) activity in the presence (i.e. maximal activity; *D*) or absence (i.e. autonomous activity; middle panel; *E*) of Ca^{2+} and calmodulin. Shown in *F* are correlations between the changes in maximal CaMKII activity and CaMKII phosphorylation and activities of skeletal muscle of the active leg. Data are means ± S.E.M., $n = 8$; *different from Pre, $P < 0.05$; †different from Passive, $P < 0.05$.

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skeletal muscle (Bayer & Schulman, 2001). This suggests that while there may be an increase in the expression of kinase isoforms of CaMKII in the present study, this increase may not be localized to intracellular organelles such as nuclei and SR.

Importantly, the changes that were observed were restricted to the active leg, indicating that the mechanisms behind these increases were attributable to factors arising within the active muscle. Conceptually, the increase in CaMKII expression could be due to either increased synthesis or lower degradation. Using microarray analyses, Mahoney *et al.* (2005) were not able to detect changes in CaMKII isoform mRNA levels 3 and 48 h after exhaustive endurance exercise. However, mice that over-express the calpain inhibitor calpastatin have 3-fold higher CaMKII expression in gastrocnemius muscle (Otani *et al.* 2007) indicating that CaMKII expression is at least partially regulated by proteolysis. Clearly, further studies are warranted to examine the mechanisms behind the higher CaMKII kinase isoform expression with exercise training.

Perhaps a more important matter to arise from this study is the functional consequences of higher CaMKII expression and activity in skeletal muscle with endurance exercise training. In particular, the higher CaMKII expression may result in greater sensitivity of Ca^{2+}–CaM signalling through CaMKII substrates and functional effects during exercise as well as at rest. When CaMKII substrates were examined, there was no effect on phospholamban (PLN) expression or phosphorylation at Thr^{17} (Fig. 4*A* and *B*), which is a known CaMKII substrate and is phosphorylated during contractions (Rose *et al.* 2006). This suggests that the higher CaMKII expression did not result in higher CaMKII signalling towards PLN *in vivo*. However, it may be that with the higher CaMKII expression there is greater regulation of PLN phosphorylation during exercise, and given that PLN phosphorylation enhances SERCA activity (Simmerman & Jones, 1998) this may result in better Ca^{2+} homeostasis in trained muscle, as has been observed in rats (Inashima *et al.* 2003). However, it should be noted that there are no studies that have examined the effects of training on skeletal muscle Ca^{2+} regulation with exercise in humans or Ca^{2+} kinetics in isolated muscle fibres. Clearly this is an important area for further work given that endurance exercise training results in better muscle performance (Holloszy & Booth, 1976) which may be related to improved Ca^{2+} homeostasis

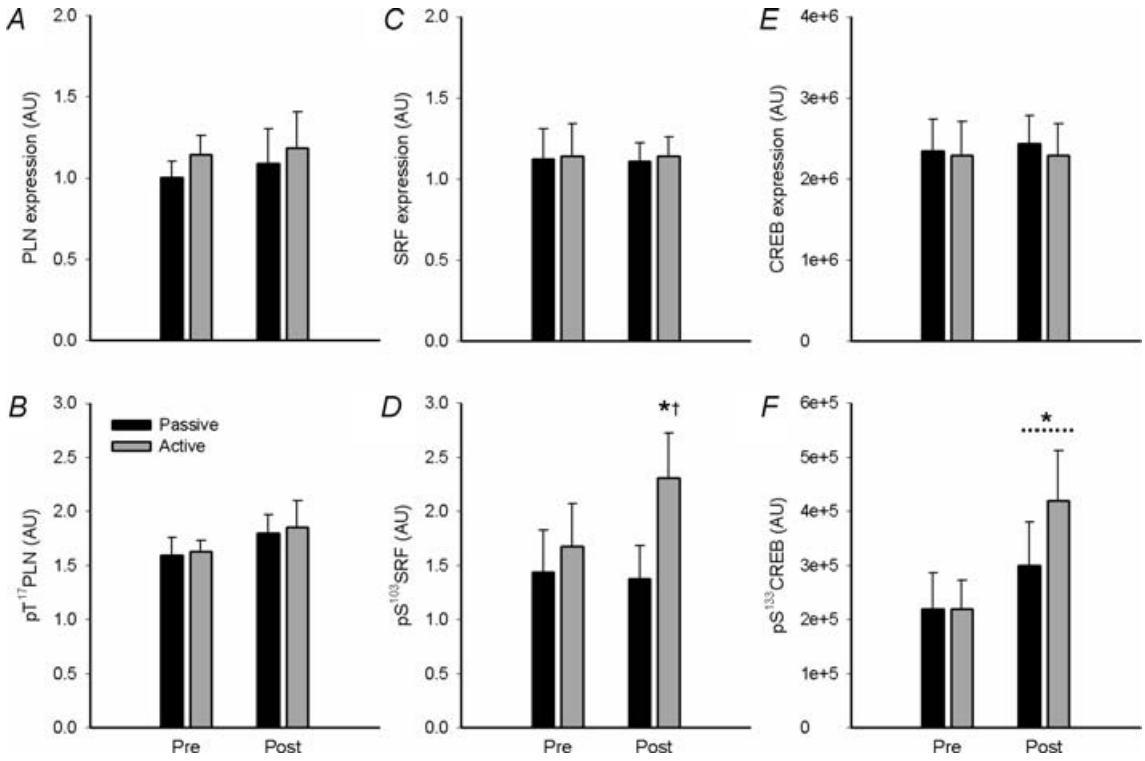

Figure 4. Effects of endurance-type exercise on phosphorylation and expression of putative skeletal muscle CaMKII substrates

Skeletal muscle samples from the vastus lateralis muscle of the passive and active leg before (Pre) and after (Post) 3 week of exercise training were extracted and lysates were immunoblotted for phospholamban (PLN) expression (*A*) and Thr^{17} phosphorylation (*B*); serum response factor (SRF) expression (*C*) and Ser^{103} phosphorylation (*D*); and cAMP-response element binding protein (CREB) expression (*E*) and phospho-Ser^{133}CREB (*F*). Data are mean ± S.E.M., $n = 8$; * different from *Pre*, $P < 0.05$; † different from *Passive*, $P < 0.05$.

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in skeletal muscle during exercise (Allen & Westerblad, 2002).

Another important function of CaMKII in skeletal muscle is likely to be regulation of gene expression. Indeed, CaMKII is enriched in skeletal muscle nuclei (Flück *et al.* 2000*a*; Nori *et al.* 2002), and nuclear CaMKII is activated with contractions (Liu *et al.* 2005). The transcription factor serum response factor (SRF) is a CaMKII substrate (Flück *et al.* 2000*a*) and the phosphorylation of SRF, but not expression, was higher in trained muscle (Fig. 4*C* and *D*), indicative of higher basal CaMKII signalling to this substrate with training. However, it should be noted that SRF may be phosphorylated by other kinases potentially activated by muscle activity (Heidenreich *et al.* 1999; Lange *et al.* 2005). Importantly, while the regulation of SRF activity is complex (Pipes *et al.* 2006), SRF phosphorylation increases its DNA binding activity (Rivera *et al.* 1993) and thus may result in higher expression of genes that it activates such as the mitochondrial F_1-ATP synthase-β (Nelson *et al.* 1995) and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α; Esterbauer *et al.* 1999), the latter of which may be responsible for coordinated changes in oxidative capacity with endurance training (Baar, 2004). Indeed, similar to SRF phosphorylation, mitochondrial enzymes were up-regulated in the active leg after training (Frøsig *et al.* 2004), including the β subunit of the F_1-ATP synthase (Fig. 2), an expected result since chronic low frequency electrical stimulation of rabbit fast-twitch muscle increases F_1ATPase-β mRNA expression (Williams *et al.* 1987). Indeed, the magnitude of increase in SRF phosphorylation and F_1ATPase-β expression after training covaried between subjects (Fig. 5-*B*). Given that long-term Ca^{2+}-ionophore treatment of muscle cells to raise intracellular Ca^{2+} can also increase F_1ATPase-β expression (Freyssenet *et al.* 2004), and that the F_1ATPase-β gene promoter contains serum response elements (Nelson *et al.* 1995), this suggests that chronic muscle activity may act via a CaMKII–SRF pathway to increase expression of some nuclear encoded mitochondrial proteins.

The main role for SRF in skeletal muscle is probably growth and maturation of myofibers (Li *et al.* 2005), and thus perhaps hypertrophy, and skeletal muscle SRF expression has been shown to be up-regulated by load-induced hypertrophy (Flück *et al.* 2000*b*). Hence, further studies are warranted to investigate the effect of resistance type exercise on SRF and CaMKII and their roles therein. These studies are important, as CaMKII expression/activity have been shown to be altered in dystrophic skeletal muscle (Damiani *et al.* 1996; Abraham & Shaw, 2006) and Ca^{2+}–CaM and CaMKII signalling has been proposed to be a potential pathway by which degenerative muscular diseases can be retarded (Abraham & Shaw, 2006; Chakkalakal *et al.* 2006).

Another substrate of CaMKs (Hook & Means, 2001) which has been hypothesized to be involved in mitochondrial biogenesis with exercise (Akimoto *et al.* 2005) downstream of Ca^{2+} signalling (Carrasco & Hidalgo, 2006), namely cAMP response element binding protein (CREB), was also examined. There were no changes in the expression of CREB with training, but there was an increase CREB Ser^{133} phosphorylation in muscle of the active and inactive leg after training (Fig. 4*E* and *F*). CREB phosphorylation at Ser^{133} increases DNA binding activity and activates the transcription of several genes (Carrusco & Hidalgo, 2006). There are few other studies on skeletal muscle CREB with exercise; however, Widegren

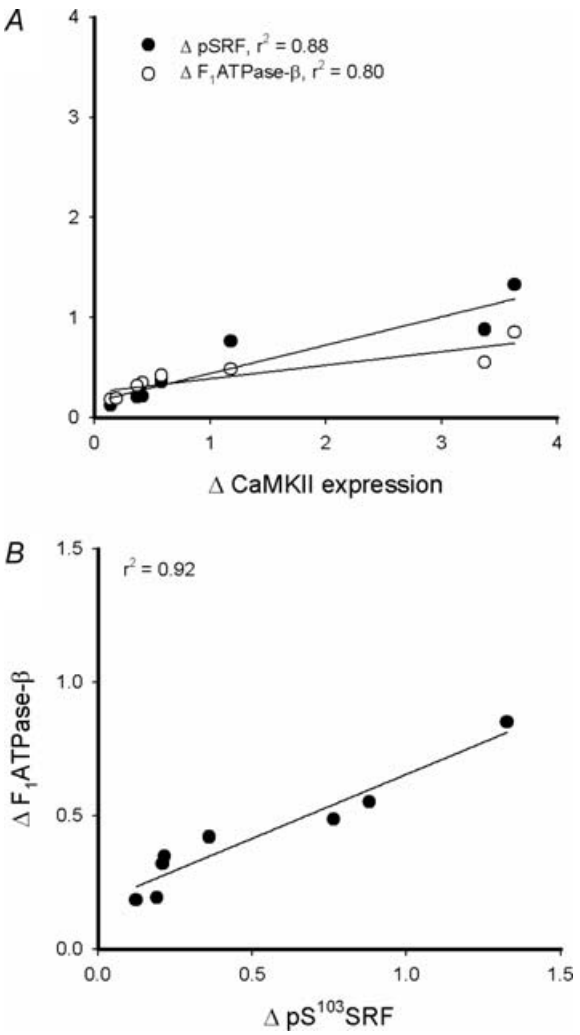

Figure 5. Changes in CaMKII kinase isoform expression positively correlate with changes in SRF phosphorylation and F_1ATPase-β expression with endurance exercise training
Shown are correlations between the changes in Ca^{2+}–calmodulin-dependent kinase II (CaMKII) expression and serum response factor (SRF) Ser^{103} phosphorylation and mitochondrial F_1-ATP synthase β-subunit expression of skeletal muscle of the active leg with endurance exercise training (*A*). Shown in *B* is a correlation between changes in SRF Ser^{103} phosphorylation and F_1ATPase-β expression.

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et al. (1998) showed that CREB phosphorylation was higher in inactive muscle only after exercise and concluded that this may be due to stimulation via humoral factors. Indeed, CREB phosphorylation/activity can be induced by hormones (Viguerie *et al.* 2006; Zheng *et al.* 2004) and metabolites (Hashimoto *et al.* 2007) that increase with acute exercise and thus the general increase in CREB phosphorylation after training may be mediated by humoral stimuli. In any case, in contrast to other adaptations (Frøsig *et al.* 2004 and Results) CREB phosphorylation was not restricted to active muscle and did not correlate with changes in CaMKII or F_1ATPase-β expression (see Results), which suggests that CREB may not be important for the adaptive process to repeated muscle contraction, at least downstream of CaMKII and AMPK.

In summary, the expression of kinase isoforms CaMKII and SRF phosphorylation in skeletal muscle are higher at rest after endurance-type exercise training. Thus, the effects of physical training may not only be evoked during acute exercise, but the enhanced CaMKII activity in the rest periods between training bouts may also affect skeletal muscle gene expression. Thus, along with other signalling pathways such as MAPK, AMPK and calcineurin and their downstream transcription factors (Hawley *et al.* 2006; Hood *et al.* 2006), the increase in CaMKII expression and signalling may contribute to the altered skeletal muscle phenotype with training.

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Acknowledgements

Betina Bolmgren is acknowledged for technical assistance. Financial support is acknowledged from the Copenhagen

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Muscle Research Centre, the Danish Medical and Natural Science Research Councils, the Danish Diabetes Association, the European Union (an Integrated Project, contract number LSHM-CT-2004-005272), as well as the Novo-Nordisk Research and Lundbeck Foundations. A.J.R. was supported by a post-doctoral fellowship from the Carlsberg Foundation and from the European Union. B.K. acknowledges the financial support of the Danish Ministry of Food, Agriculture and Fisheries, as well as the Danish Ministry of Family and Consumer Affairs. J.F.P.W. was supported by a Hallas Møller fellowship from the Novo Nordisk Foundation.

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