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ORIGINAL ARTICLE

Greater basal skeletal muscle AMPK α phosphorylation in men than in women: Associations with anaerobic performance

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Abstract

Objectives: This study was designed to investigate the association of gender, fibre type composition, and anaerobic performance with the basal skeletal muscle signalling cascades regulating muscle phenotype. **Design:** Muscle biopsies were obtained from 25 men and 10 women all young and healthy. **Methods.** Protein phosphorylation of Thr¹⁷²AMPK α , Ser²²¹ACC β , Thr²⁸⁶CaMKII as well as total protein abundance of PGC-1 α , SIRT1, and CnA were measured by Western blot and anaerobic performance by the Wingate test. **Results:** Percent type I myosin heavy chain (MHC I) was lower in men (37.1 ± 10.4 vs. 58.5 ± 12.5 , $P < .01$). Total, free testosterone and free androgen index were higher in men (11.5, 36.6 and 40.6 fold, respectively, $P < .01$). AMPK α phosphorylation was 2.2-fold higher in men compared to women ($P < .01$). Total Ser²²¹ACC β and Thr²⁸⁶CaMKII fractional phosphorylation tended to be higher in men ($P = .1$). PGC1- α and SIRT1 total protein expression was similar in men and women, whereas CnA tended to be higher in men ($P = .1$). Basal AMPK α phosphorylation was linearly related to the percentage of MHC I in men ($r = 0.56$; $P < .01$), but not in women. No association was observed between anaerobic performance and basal phosphorylations in men and women, analysed separately. **Conclusion:** In summary, skeletal muscle basal AMPK α phosphorylation is higher in men compared to women, with no apparent effect on anaerobic performance.

Keywords: AMPK, muscle signalling, muscle phenotype, gender, anaerobic performance

Introduction

Human skeletal muscle is composed of different fibre types (I, IIa, and IIx) with distinctive functional and metabolic capacities. Predominantly type I fibre muscles have a higher oxidative capacity, whereas a high proportion of type II muscle fibres or myosin heavy chain (MHC) II is associated with high levels of muscle power and strength (Aagaard & Andersen, 1998). Some studies point that women have a slightly higher percentage of type I muscle fibres in their vastus lateralis than men (Richter & Ruderman, 2009). However, little is known about the molecular mechanisms that could explain a sex dimorphism in skeletal muscle phenotypes and its association with anaerobic performance in humans.

The contractile properties of muscle fibres are dependent on the variable expression of proteins involved in Ca²⁺ signalling and handling (Berchtold, Brinkmeier, & Muntener, 2000). Among these, AMP-activated protein kinase (AMPK), the transcriptional cofactor of the proliferator-activated receptor gamma (PPAR γ) coactivator 1 α (PGC-1 α), its upstream regulator silent information regulator proteins (SIRTs), calcineurin (CnA), and the Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) could explain a sexual dimorphism in muscle phenotypes.

AMPK acts as an “energy sensor” of the cell, and is activated by phosphorylation of its threonine residue 172 (Thr¹⁷²) when there is an increase in the

intracellular AMP/ATP ratio. During muscle contraction, the rate of ATP turnover increases and AMPK phosphorylation (pAMPK) can be elicited by both moderate (Roepstorff et al., 2006) and high-intensity exercises (Guerra et al., 2010; Morales-Alamo et al., 2012, 2013). AMPK also induces higher hexokinase and PGC-1 α levels, stimulating mitochondrial biogenesis and fibre type switching towards type I fibres (Richter & Ruderman, 2009; Steinberg & Jorgensen, 2007). Thus, AMPK is believed to be stimulated in the skeletal muscle by the adipocyte-derived hormone leptin (Steinberg & Jorgensen, 2007). Our laboratory has demonstrated that women have higher protein abundance of the signalling isoform of the leptin receptor in muscle than men (Guerra et al., 2008), likely due to a direct stimulation by oestrogens (Fu, Maher, Hamadeh, Ye, & Tarnopolsky, 2009). Whether women have higher basal muscle pAMPK levels compared to men remains to be elucidated.

Free intracellular Ca²⁺ concentration is another potent stimulator of signalling pathways mediating muscle fibre type plasticity. Free Ca²⁺ binds to calmodulin, which activates CnA and CaMKII. CnA dephosphorylates nuclear factor of activated T cells and regulates slow fibre gene expression (Chin, 2005). Finally, SIRT1s are NAD⁺-dependent deacetylases which are sensitive to the cellular redox state and are another “energy sensor” of the cell. SIRT1 regulates insulin sensitivity, metabolism, and mitochondrial biogenesis through deacetylation of PGC-1 α (Finkel, Deng, & Mostoslavsky, 2009). SIRT1 modulates the expression of several genes involved in lipid metabolism (Finkel et al., 2009), however, no data are available comparing skeletal muscle SIRT1 protein content in men and women.

Between-sex differences in basal signalling may account for differences in metabolic and mechanical capacities of skeletal muscle and, hence, sprint performance. Interestingly, although peak power per muscle mass is similar in men and women, men have greater anaerobic capacity (Fuentes et al., 2012). Thus, we hypothesised that basal levels of AMPK α phosphorylation, as well as the signalling components promoting increased expression of type I muscle fibres will be increased in women compared to men. We also hypothesised that basal skeletal muscle CaMKII phosphorylation would be greater in men than women.

Therefore, the main aims of this study were to determine if sex differences in skeletal muscle fibre type are associated with differences in AMPK, its downstream target acetyl-CoA carboxylase (ACC) and CaMKII phosphorylation, or to CnA, SIRT1, and PGC-1 α protein levels. A secondary aim was to

determine if AMPK, ACC, and CaMKII phosphorylation, or CnA, SIRT1, and PGC-1 α protein expression are associated with sprint performance. Such an association could indicate that the involved protein or transcription factor may play a role in the phenotypic changes associated with greater or lower anaerobic energy production.

Material and methods

Materials

The complete protease inhibitor cocktail was obtained from Roche Diagnostics (Mannheim, Germany). All the primary antibodies used were from Cell Signalling Technology (Danvers, MA, USA) except for the polyclonal rabbit antiphospho-ACC (Ser79) antibody that was purchased from Upstate Biotechnology (Lake Placid, NY, USA), the monoclonal mouse anticalcineurin α and β antibody from Sigma-Aldrich (St Louis, MO, USA), and the polyclonal rabbit antiphospho-Ca²⁺/calmodulin-dependent protein kinase II (Thr177) antibody from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The secondary horseradish peroxidase (HRP)-conjugated goat anti-rabbit and donkey anti-mouse antibodies were from Jackson Immuno Research (West Grove, PA, USA). The Hybond-P transfer membranes and the enhanced chemiluminescence (ECL) plus Western Blotting Detection System were from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK). The ChemiDoc XRS System and the image analysis software Quantity One[®] were obtained from Bio-Rad Laboratories (Hemel Hempstead, Hertfordshire, UK).

Subjects

Thirty-five physical education students agreed to participate in this investigation (Table I). Before volunteering, subjects were given full oral and written information about the procedures of the study and possible risks associated with participation. Exclusion criteria included a history of endocrine, renal, metabolic diseases, smoking, or drug abuse. None of the subjects taking part in the study had any medical condition known to affect fat metabolism or eating disorders. All women were eumenorrhic, and four of them were taking contraceptive pills. None of our volunteers accomplished exclusion criteria. The study was performed in accordance with the Helsinki Declaration of 1975, as last modified in 2000, regarding the conduct of clinical research, and was approved by the Ethical Committee of the University of Las Palmas de Gran Canaria.

Table I. Physical characteristics, anaerobic performance, hormonal concentrations, and muscle morphology (mean \pm SD).

	Men (<i>n</i> = 25)	Women (<i>n</i> = 10)
Age (year)	24.1 \pm 3.4	25.3 \pm 4.0
Height (cm)	177.6 \pm 6.2	160.7 \pm 5.5*
Body mass (kg)	78.6 \pm 8.8	57.0 \pm 6.7*
Fat mass (%)	16.1 \pm 7.0	29.1 \pm 4.2**
<i>Anaerobic performance</i>		
P_{\max} (W)	1011.6 \pm 125.9	586.4 \pm 55.6*
$P_{\max}N$ (W/kg body mass)	13.0 \pm 1.6	10.4 \pm 1.2*
$P_{\max}N$ (W/kg lean leg mass)	51.8 \pm 6.2	50.5 \pm 3.9
P_{mean} (W)	646.5 \pm 84.9	348.8 \pm 82.4*
$P_{\text{mean}}N$ (W/kg body mass)	4.1 \pm 0.6	3.0 \pm 0.6*
$P_{\text{mean}}N$ (W/kg lean leg mass)	46.4 \pm 5.8	49.3 \pm 3.3
<i>Hormonal concentrations</i>		
Leptin (ng/ml)	4.1 \pm 4.0	13.5 \pm 5.9**
FAI	69.1 \pm 24.4	1.7 \pm 2.1**
FT (ng/ml)	0.11 \pm 0.03	0.003 \pm 0.003**
TT (ng/ml)	4.6 \pm 1.3	0.4 \pm 0.2**
SHBG (nmol/l)	25.4 \pm 9.9	147.2 \pm 98.4*
β -Estradiol (pg/ml)		88.1 \pm 88.7
<i>Muscle morphology</i>		
Type I MHC (%)	42.0 \pm 10.4	60.2 \pm 12.5*
Type IIa MHC (%)	42.3 \pm 5.5	28.2 \pm 10.6*
Type IIx MHC (%)	21.8 \pm 6.6	14.4 \pm 5.2
Type I CSA (μm^2)	4399.6 \pm 1131.8	3308.8 \pm 495.7*
Type IIa CSA (μm^2)	6067.2 \pm 1571.5	3869.2 \pm 490.9*
Type IIx CSA (μm^2)	5172.8 \pm 1390.4	3439.6 \pm 658.6*
ATPase type I fibre (%)	42.4 \pm 11.5	61.9 \pm 4.1*
ATPase type IIa fibre (%)	37.9 \pm 7.6	25.9 \pm 5.2*
ATPase type IIx fibre (%)	13.5 \pm 9.7	10.9 \pm 5.0

Notes: P_{\max} , peak power output during the Wingate test; P_{mean} , mean power output during the Wingate test; $P_{\max}N$, peak power output during the Wingate test relative to kilo gram of body mass or lean leg mass; $P_{\text{mean}}N$, mean power output during the Wingate test relative to kilo gram of body mass or lean leg mass.

* $P < .05$.

** $P < .01$ compared to men.

Study design

All subjects were resting during the experimental procedures and requested to refrain from strenuous exercise, caffeine, or alcohol drinks, 24 h prior to experimental days. On the first experimental day subjects arrived to our laboratory at 8:00 am and physical performance and body composition measurements were done under fasting conditions. On a separate day, subjects reported to the laboratory at 8–9 h am after an overnight fast. Following a 10 min resting period in a supine resting position 20 ml blood samples were taken from an antecubital vein. The blood samples were allowed to clot at room temperature for 10 min in tubes containing the clot activation gel (Vacutainer[®], Becton Dickinson, France) and then were centrifuged at 3500 rpm during 15 min, and a set temperature of 4°C (Allegra 21R,

Beckman Instruments, Fullerton, CA). The serum obtained was separated and frozen at -80°C for later analysis. Next, a needle muscle biopsy specimen was obtained from the middle section of the vastus lateralis muscle under local anaesthesia with suction, and mild pressure on the lateral aspect of the thigh. The muscle samples were immediately frozen in liquid nitrogen for Western blotting and MHCs determination and stored at -80°C . The time needed to obtain and freeze the muscle biopsies was below 30 s in all cases.

Body composition and anaerobic performance

Body composition was determined by whole-body dual-energy X-ray absorptiometry measurements (Hologic QDR-1500, Hologic Corp., software version 7.10, Waltham, MA) as described elsewhere (Calbet, Dorado, Diaz-Herrera, & Rodriguez-Rodriguez, 2001). Anaerobic performance was measured by a 30 s Wingate test as reported elsewhere (Ponce-Gonzalez et al., 2012).

MHCs determination

MHC analyses were performed on the muscle biopsies using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), as reported by Larsson, Andersen, Kadi, Bjork, and Gerdlé (2002). After protein extraction using urea buffer, between 2 and 12 μl of the myosin-containing samples were loaded on SDS-PAGE. Gels were run at 70 V for 43 h at 4°C. Subsequently, the gels were Coomassie stained, and MHC isoform bands (I, IIa, and IIx) were determined based on known migration patterns and quantified with the image analysis software Quantity One from Bio-Rad Laboratories (Hemel, Hempstead, Hertfordshire, UK).

ATPase histochemistry

Serial sections (8 μm) of the muscle biopsy samples from 20 males and 7 females due to sample limitations were cut in a cryostat (-20°C), and routine ATPase histochemistry analysis was performed after preincubation at pH of 4.37, 4.60, and 10.30 as previously reported (Sanchis-Moysi et al., 2010). Fibre typing (I, IIa, and IIx) and cross sectional areas (CSA) were determined using the specialised software Visilog (Noesis, France).

Total protein extraction, electrophoresis, and Western blot analysis

Muscle protein extracts were prepared as described previously (Guerra et al., 2007), and total protein

content was quantified using the bicinchoninic acid assay (Smith et al., 1985). Equal amounts (50 µg) of each sample were subjected to immunoblotting protocol as described previously (Guerra et al., 2007). Separated gels (Bio-Rad Laboratories, Hercules, CA, USA) were loaded to detect the total and phosphorylated forms of the target molecules.

To determine Thr¹⁷²AMPK α , Ser²²¹ACC β , and Thr²⁸⁶CaMKII levels, antibodies directed against the phosphorylated and total form of these kinases were used diluted in 5% bovine serum albumin in tris-buffered saline with 0.1% Tween 20 (TBS-T) (BSA-blocking buffer). To detect PGC-1 α , CnA, and SIRT1 total protein expression, membranes were incubated with a polyclonal-specific anti human PGC-1 α , CnA, and SIRT1 antibodies (diluted in BSA-blocking buffer).

To control for differences in loading and transfer efficiency, the membranes were incubated with a monoclonal mouse anti-alpha-tubulin antibody diluted in TBS-T with 5% blotting grade blocker non-fat dry milk (blotto-blocking buffer). No significant changes were observed in alpha-tubulin protein levels during the experiments (data not shown). Antibody-specific labelling was revealed by incubation with a HRP-conjugated goat anti-rabbit antibody (1:20,000) or a HRP-conjugated donkey anti-mouse (1:10,000) antibody both diluted in blotto-blocking buffer and visualised with the ECL chemiluminescence detection kit (Amersham Biosciences). Specific bands were visualised using the ChemiDoc XRS system (Bio-Rad Laboratories, Hercules, CA, USA) and quantified by the image analysis program Quantity one[®] (Bio-Rad Laboratories, Hercules, CA, USA). The densitometry analysis was carried out immediately before saturation of the immunosignal. All proteins were measured in duplicate, and the variation coefficient was below 15%. Samples were loaded as follows: men–men–women in each gel (15 wells) to reduce inter-gel variance between groups. In addition, one muscle sample obtained from a healthy young man was loaded as an internal control on all gels to control for inter-gel variability. Overall, the variation coefficient for the control loaded in all gels was 12%. Data were represented as immunostaining values obtained for the phosphorylated form of each kinase relative to those obtained for respectively total form, or alpha-tubulin.

Hormonal assays

Serum leptin was determined by enzyme-linked immunosorbent assay (ELx800 Universal Microplate Reader, Biotech Instruments Inc., Vermont, USA), using reagent kits from Millipore (#EZHL-80SK, Millipore EMD, Billerica, MA, USA) and following

the manufacturer's instructions. The sensitivity of the total leptin assays was 0.05 ng/ml. The intra-assay coefficient variation was 3.8%, and the inter-assay coefficient of variation was 4.4%.

Serum total testosterone (TT) and sex hormone-binding globulin (SHBG) concentrations were measured by a fully automated two-site, solid-phase, chemiluminescent enzyme immunometric assay (Immulite 2000[®], Siemens Healthcare Diagnostics). Samples were processed interserially including different batches, six calibrations and two control levels for each sample series. The intra-assay coefficients of variation were 9.8% and 2.2%, respectively, and the inter-assay coefficients of variation were 11.0% and 5.1%. Lower end sensitivity endpoints were settled at 15 ng/dl and 0.02 nmol/l.

Free androgen index (FAI), as an estimation of bioavailable testosterone, was calculated as described by Ly and Handelsman (2005). Moreover, free testosterone (FT) concentration was estimated using other procedures (Vermeulen, Verdonck, & Kaufman, 1999). 17 β -Oestradiol was measured by a competitive electrochemiluminescence immunoassay intended for use on Modular Analytics analyzer E170 using E2 reagents (Roche/Hitachi, 03000079122, Indianapolis, IN, USA). Results were determined via a calibration curve with analytic sensitivity of 18.4 pmol/l. The intra-assay coefficient of variation was 9.5%, and the inter-assay coefficient of variation was 5.8%.

Statistical analysis

Variables were checked for normal distribution by using the Shapiro-Wilks test and for equality of variances with the Levene's test. When necessary, the analysis was done on logarithmically transformed data. Unpaired *t*-tests were used to test between-group differences. The relationship between variables was determined using linear regression analysis. Correlations were tested with the Pearson's test. Values are reported as the mean \pm standard error of the mean. *P* < 0.05 was considered significant. Statistical analysis was performed using SPSS v. 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Gender differences in hormones, anaerobic performance, and muscle morphology

Anthropometrics, body composition, hormone concentrations, anaerobic performance, and fibre typing by both MHC type distribution and ATPase staining, as well as fibre types CSA are reported in **Table 1**. Both genders were comparable in age, but

women were smaller, had lower body mass and higher percentage of body fat compared to men (all, $P < .01$, Table I). Men reached a higher peak and mean power output during the Wingate test than women ($P < .05$, Table I). However, when accounting for the lean mass of the lower extremities, both sexes achieved similar relative peak power values, but the relative mean power output was greater in men than women (Table I). Men had a higher percentage of type IIa, fibres, and lower proportion of type I fibres, measured by both ATPase staining and MHC electrophoresis, compared to women (all $P < .01$, Table I). CSAs were higher in males in all fibre types (all $P < .05$, Table I). Compared to men, women had a higher serum concentration of leptin (13.5 ± 5.9 vs. 4.1 ± 4.0 ng/ml, $P < .01$, Table I). TT, FT, and FAI were 11.5, 36.6, and 40.6 fold higher in men than women (all $P < .01$, Table I), whereas SHBG was higher in women (25.4 ± 9.9 vs. 147.2 ± 98.4 nmol/l, $P < .01$, Table I).

Gender comparisons in basal skeletal muscle signalling

The basal Thr¹⁷²AMPK α /Total AMPK α (fractional phosphorylation of AMPK α) was twofold higher in men than in women (2.3 ± 1.4 vs. 1.0 ± 0.5 a.u., respectively, $P < .01$; Figure 1, panel A). The total amount of Thr¹⁷²AMPK α protein was also higher in men compared to women (5.7 ± 0.8 vs. 2.6 ± 0.4 a.u., $P < .05$). No significant between-sex differences were observed in Ser²²¹ACC β /ACC β phosphorylation (Figure 1, panel B), although total Ser²²¹ACC β levels tended to be higher in men (0.8 ± 0.1 vs. 0.5 ± 0.1 a.u., respectively, $P = .08$). Both total Thr²⁸⁶CaMKII phosphorylation and Thr²⁸⁶CaMKII/Total CaMKII tended to be higher in men than women (0.8 ± 0.1 vs. 0.4 ± 0.1 and 2.5 ± 2.9 vs. 1.2 ± 1.3 a.u., respectively, $P = .10$; Figure 1, panel C). No significant differences between sexes were observed in the total amount of AMPK α , ACC β , and CaMKII. PGC-1 α and SIRT1 total protein expression was similar in both groups. CnA tended to be higher in men than in women ($P = .10$, Figure 2, panel A, B, and C).

Correlations

When all subjects were pooled together ($n = 35$), the percentage of MHC IIa fibres was positively associated to basal Thr¹⁷²AMPK/Total AMPK α ($r = 0.39$, $P < .05$, Figure 3, panel B), as well as Thr²⁸⁶CaMKII/Total CaMKII ($r = 0.34$, $P < .05$). Thr¹⁷²AMPK/Total AMPK α and Thr²⁸⁶CaMKII/Total CaMKII α were also positively associated ($r = 0.65$, $P < .01$). Moreover, CnA was positively

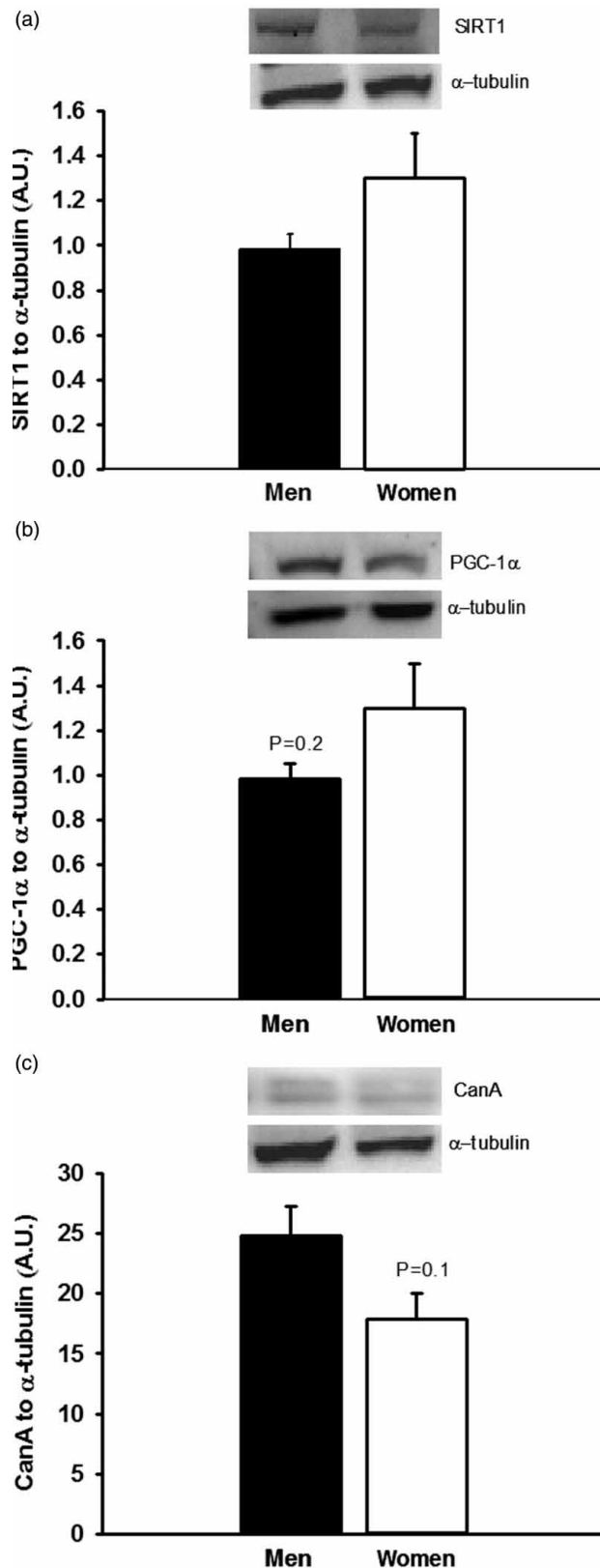


Figure 1. (a) Levels of basal Thr¹⁷²AMPK α phosphorylation to total AMPK α (a.u.). (b) Levels of basal Ser²²¹ACC β phosphorylation to total ACC β (a.u.). (c) Levels of basal Thr²⁸⁶CaMKII phosphorylation to total CaMKII (a.u.). Men (black bar) and women (white bar). Data are means \pm SD; * $P < .01$.

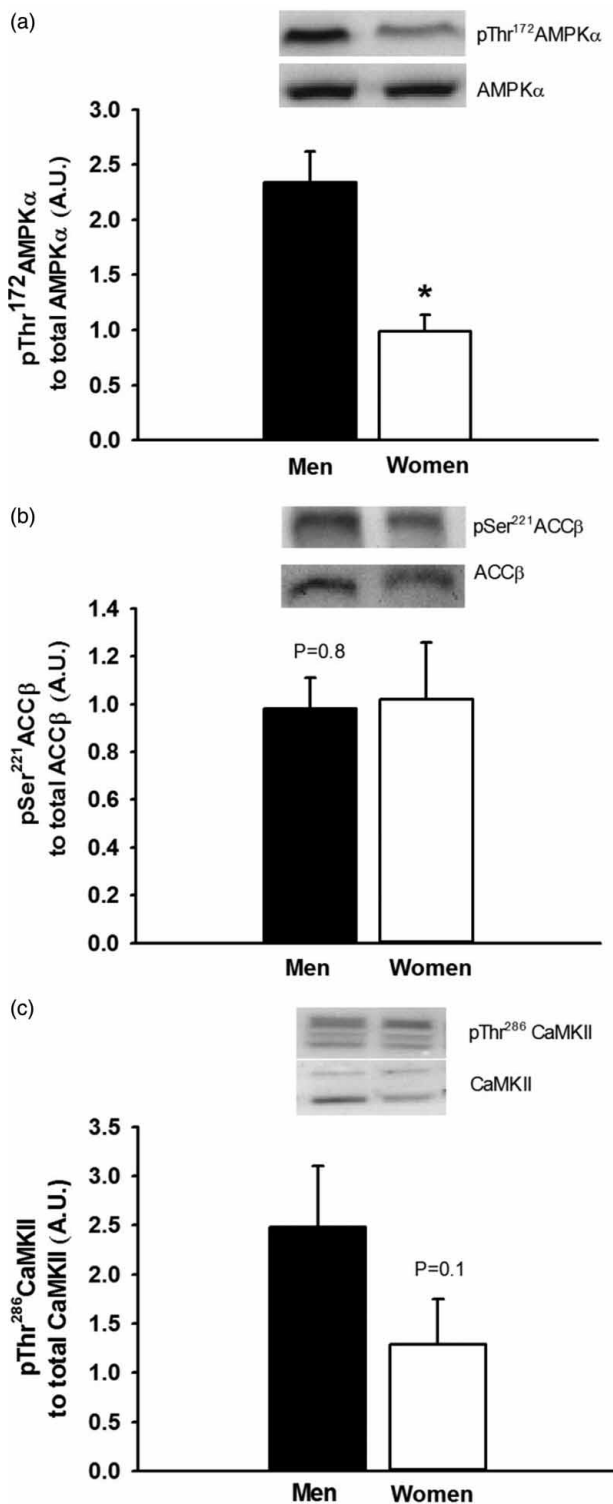


Figure 2. (a) Levels of basal SIRT1 to alpha-tubulin total protein content (a.u.). (b) Levels of basal PGC1- α to tubulin-alpha total protein content (a.u.). (c) Levels of basal CanA to alpha-tubulin total protein content (a.u.). Men (black bar) and women (white bar). Data are means \pm SD; * $P < .01$.

associated with MHC IIx ($r = 0.62$, $n = 22$, $P < .01$). Thr¹⁷²AMPK/Total AMPK α was negatively associated with SIRT1 protein expression ($r = -0.39$,

$P < .05$). SIRT1 was positively associated with PGC-1 α protein expression ($r = 0.64$, $P < .01$), and both SIRT1 and PGC-1 α total protein expression were negatively associated with MHC IIa ($r = -0.45$ and -0.47 , respectively; $P < .05$).

Absolute peak power during the Wingate tests was positively associated with basal Thr¹⁷²AMPK/Total AMPK α (Figure 3, panel C), total body lean mass, lower extremities lean mass, the percentage of MHC IIa and IIx, CSA I, IIa, and IIx, TT, and FAI ($r = 0.48$, 0.85 , 0.87 , 0.81 , 0.57 , 0.55 , 0.70 , 0.61 , and 0.90 , respectively, all $P < .05$). Absolute peak power during the Wingate tests was also negatively associated with PGC-1 α , SIRT1, MHC I, serum leptin, and SHBG ($r = -0.58$, -0.75 , -0.75 , -0.49 , -0.84 , all $P < .05$). These relationships were maintained when peak power was normalised by the lean mass of the lower extremities, except for basal Thr¹⁷²AMPK/Total AMPK α (all $P < .05$, Figure 3, panel D).

When only men were taken into consideration for the correlation analysis, the percentage of MHC I was positively associated with basal Thr¹⁷²AMPK/Total AMPK α ($r = 0.47$, $n = 25$, $P < .05$, Figure 3, panel A), as well as Thr²⁸⁶CaMKII/Total CaMKII ($r = 0.50$, $n = 25$, $P < .01$). Thr¹⁷²AMPK/Total AMPK α and Thr²⁸⁶CaMKII/Total CaMKII were also positively associated ($r = 0.56$, $n = 25$, $P < .01$). Thr¹⁷²AMPK/Total AMPK α was negatively associated with SIRT1 protein expression ($r = -0.64$, $n = 25$, $P < .01$). SIRT1 tended to be positively correlated with PGC-1 α protein expression ($r = 0.35$, $n = 25$, $P = .08$).

Absolute peak power during the Wingate tests was positively associated with lower extremities lean mass, and tended to be associated with the percentage of MHC IIx ($r = 0.45$ and 0.44 , $P < .05$ and $P = .08$, $n = 25$ and $n = 18$, respectively). This relationship reached significance when peak power was normalised by the lean mass of the lower extremities ($r = 0.68$, $n = 25$, $P < .01$). No associations were observed between either P_{\max} or $P_{\max}N$ and basal AMPK (Figure 3, panels C and D), ACC and CaMKII fractional phosphorylations. No association was observed between the lean mass of the lower extremities and AMPK, ACC, and CaMKII fractional phosphorylations. A tendency was observed towards a positive association between $P_{\max}N$ with FT and FAI ($r = 0.30$ and 0.31 respectively, $P = .1$).

Leptin was strongly correlated with the percentage of body fat ($r = 0.91$, $P < .01$, $n = 25$). Both FAI and FT tended to be positively associated with Ser²²¹ACC β /Total ACC β ($r = 0.36$, $P = .07$, and $r = 0.35$, $P = .08$ respectively, $n = 25$), whereas only FAI was significantly associated with absolute peak power during the Wingate test ($r = 0.44$, $P < .05$, $n = 25$).

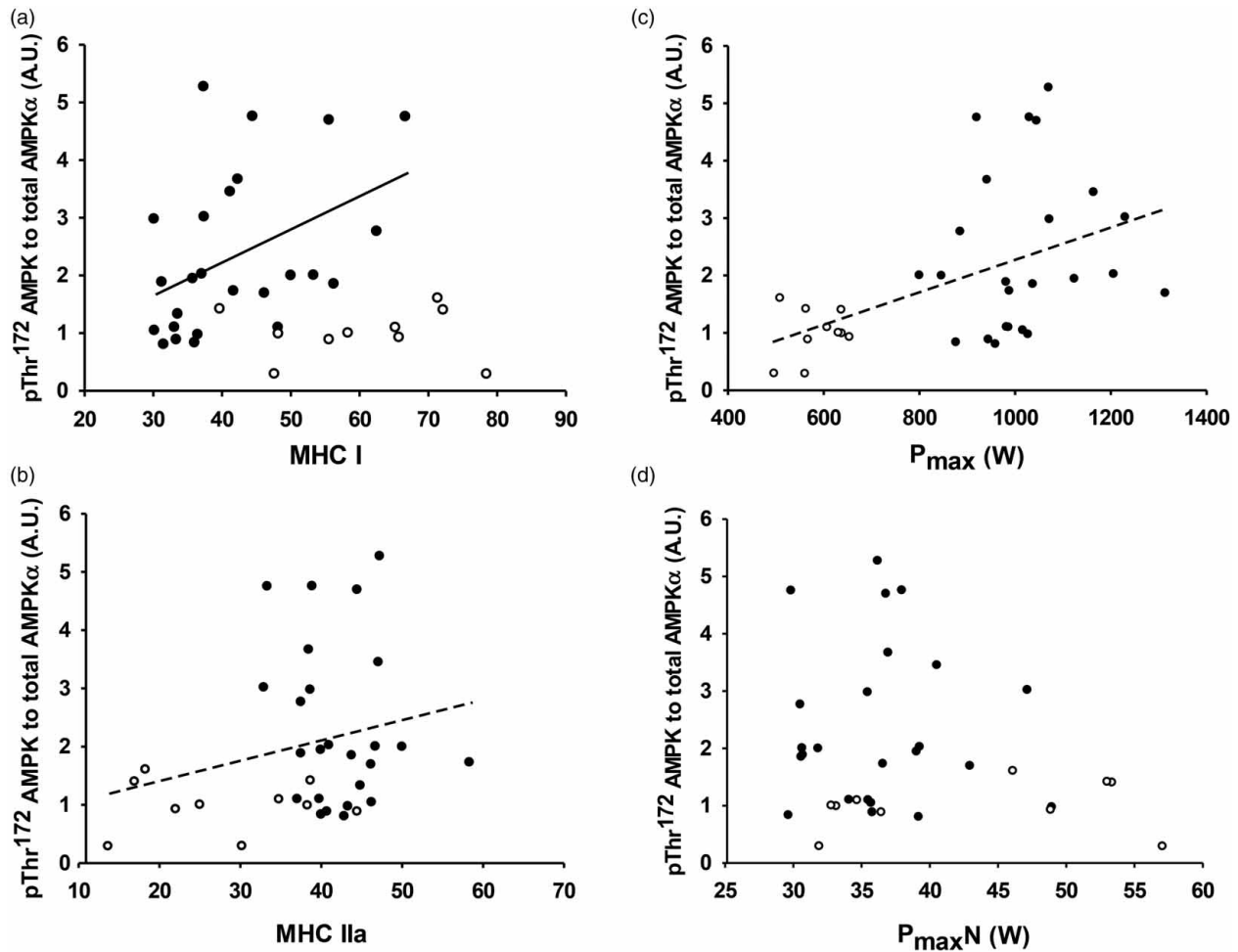


Figure 3. (a) Relationships between the levels of basal Thr¹⁷²AMPKα phosphorylation to total AMPKα (a.u.) and (a) MHC I; (b) MHC IIa; (c) P_{\max} (W); and (D) P_{\max} (W/kg lean leg mass). In men (black dots) and women (white dots); significant regression lines ($P < .05$) are presented as dashed lines for the whole population, and solid lines for men.

Discussion

In contrast to our hypothesis, the present study shows that basal skeletal muscle Thr¹⁷²AMPKα phosphorylation was twofold higher in men than in women. This difference is remarkable if compared to the two-to-six fold increase in pAMPK observed after 30 s sprints (Fuentes et al., 2012; Guerra et al., 2010; Morales-Alamo et al., 2013). Since there was a positive association between Thr²⁸⁶CaMKII phosphorylation and Thr¹⁷²AMPKα phosphorylation, and a tendency for a higher basal Thr²⁸⁶CaMKII values in men compared to women, basal differences in calcium-mediated signalling could be the mechanisms explaining this sexual dimorphism in Thr¹⁷²AMPK phosphorylation.

There is some CaMKII activity at rest, which is increased in response to oxidative stress, and conditions increasing cytosolic Ca²⁺, like muscle contraction (Morales-Alamo et al., 2012, 2013). Given the relatively smaller muscle mass and the higher

mechanical load, Ca²⁺ transients are expected to be of higher amplitude and frequency in women. However, increased Ca²⁺ transients could have been elicited in men muscles if they have been more physically active than women during their daily life, and this issue has not been addressed in the present investigation. Another mechanism that could explain a greater Thr²⁸⁶CaMKII phosphorylation in men is the known antioxidant action of oestrogens (Vina & Borras, 2010). In agreement with our results, CaMKII activity is significantly greater in the heart of male rodents compared with females (Konhilas et al., 2004). However, as we only observed a tendency for a higher pCaMKII and CnA levels in men, more studies are needed to determine a final significance.

Mortensen et al. (2009) examined AMPK activity and expression in 91 young and older twins. In concordance with our results, men had higher muscle α2 and γ3 AMPK mRNA subunits expression, and

AMPK γ 3 activity compared with women, under resting conditions. The influence of sex on AMPK α phosphorylation has been also studied in response to submaximal endurance exercise, where α 2 associated AMPK activation was higher in men (Roepstorff et al., 2006). In contrast, after 30-s sprint exercise, Thr¹⁷²AMPK α phosphorylation was increased similarly in men and women (Fuentes et al., 2012).

In agreement with previous studies, men had a higher percent of type IIa fibres and MHC IIa (Richter & Ruderman, 2009; Roepstorff et al., 2006). Greater basal AMPK α Thr¹⁷² phosphorylation has been reported in type IIa muscle fibres from the vastus lateralis in five men using immunofluorescence (Lee-Young, Canny, Myers, & McConnell, 2009). Thus, in agreement with the immunohistochemistry-based study of Lee-Young et al. (2009), men had greater basal Thr¹⁷²AMPK phosphorylation than women, partly because men had a higher proportion of type IIa fibres. However, when only men were included in our statistical analysis, basal AMPK phosphorylation was positively associated with MHC I, which is reasonable, due to the fact that muscle AMPK signalling governs fibre switching to a more oxidative phenotype, in conjunction with PGC-1 α , PPAR δ , and SIRT1, among other transcription factors (Steinberg & Jorgensen, 2007). In accordance, pharmacologically activation of AMPK has been associated with a shift from fibre type b to IIa/x in rodents (Narkar et al., 2008). This view is further supported by the fact that caloric restriction increases basal Thr¹⁷²AMPK α phosphorylation and fast to slow muscle fibre type transitions potentially acting through SIRT1 and PGC-1 α (Speakman & Mitchell, 2011). The observed linear positive association between Thr¹⁷²AMPK α phosphorylation and MHC I in the present investigation may seem at odds with the findings of Lee-Young et al. (2009). This discrepancy may be due to methodological differences (5 men and immunohistochemistry in Lee-Young et al. study and 25 men and Western blot in the present study). Moreover, Mortensen et al. (2009) observed significant between-sex differences in AMPK γ 3 mRNA, protein levels, and activity after adjustment for MHC mRNA proportions, indicating that sex differences in AMPK γ 3 expression and activity are, at least in part, independent of skeletal muscle fibre type (Mortensen et al., 2009). It should be highlighted that most AMPK heterotrimers contain the γ 3 isoform in human skeletal muscle, implying that the increased AMPK γ 3 activity reported by this investigation should account for most of the AMPK α activity.

Mortensen et al. (2009) did not find any relationship between AMPK α 1 or α 2 protein expression and mRNA levels codifying the MHC isoforms. The difference with the present investigation can be explained because we measured MHC I, IIa, and IIx protein expression, and not mRNA levels. The linear relationships between MHC I, CaMKII, and Thr¹⁷²AMPK α phosphorylation levels in men support the idea that the AMP/ATP ratio, free Ca²⁺, and reactive oxygen and nitrogen species-mediated signalling pathways are involved in the regulation of muscle phenotype at the basal state under fasting conditions.

In this investigation, we observed a linear relationship between absolute peak power during the Wingate test and lower extremities lean mass; however, no associations were observed between anaerobic performance and basal AMPK phosphorylation, and the levels of serum testosterone, FT, and FAI in men. Thus, it seems that despite the inhibitory effect of AMPK phosphorylation on protein synthesis (Bolster, Crozier, Kimball, & Jefferson, 2002), increased levels of basal AMPK phosphorylation do not appear to have a negative influence on lean mass or sprint performance.

Although basal Thr¹⁷²AMPK α phosphorylation was associated with Wingate test peak power output, this association was only statistically significant when the whole group of subjects was analysed conjointly. This positive correlation could be due to the fact that men had greater Thr¹⁷²AMPK α phosphorylation than women, and not to AMPK-determined skeletal muscle properties. In agreement, despite the increased Thr¹⁷²AMPK α phosphorylation in men, no between-sex differences were observed in SIRT1 and PGC-1 α protein content, two of the final molecular targets mediating muscle fibre shifts.

Conclusion

A marked twofold increase in basal AMPK α Thr¹⁷² phosphorylation is observed in men's skeletal muscle compared to women with no apparent association with anaerobic performance. This study also indicates that basal AMPK and CaMKII phosphorylation levels may be playing a role in the regulation of skeletal muscle MHC I protein levels.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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