Isoinertial and Isokinetic Sprints: Muscle Signalling

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Key words
- Wingate
- sprint exercise
- muscle signalling
- leptin

Abstract

To determine if the muscle signalling response to a 30 s all-out sprint exercise is modulated by the exercise mode and the endocrine response, 27 healthy volunteers were divided in 2 groups that performed isokinetic (10 men and 5 women) and isoinertial (7 men and 5 women) Wingate tests. Blood samples and vastus lateralis muscle biopsies were taken before, immediately after, 30 and 120 min after the sprints. Groups were comparable in age, height, body weight, percentage of body fat, peak power per kg of lower extremities lean mass (Pmax) and muscle fibre types. However, the isoinertial group achieved a 25% greater mean power (Pmean). Sprint exercise elicited marked increases in the musculus vastus lateralis AMPKα, ACCβ, STAT3, STAT5 and ERK1/2 phosphorylation (all $P<0.05$). The AMPKα, STAT3, and ERK1/2 phosphorylation responses were more marked after the isoinertial than isokinetic test (interaction: $P<0.01$). The differences in muscle signalling could not be accounted for by differences in Pmax, although Pmean could explain part of the difference in AMPKα phosphorylation. The leptin, insulin, glucose, GH, IL-6, and lactate response were similar in both groups. In conclusion, the muscle signalling response to sprint exercise differs between isoinertial and isokinetic sprints.

Introduction

Although the metabolic response to isokinetic and friction-loaded (isoinertial) sprint exercise is likely similar [26,29], there may be differences in muscle signalling due to different patterns of muscle activation and fatigue development [29]. During a 30 s all-out sprint exercise on friction-loaded cycle ergometers peak (Pmax) and mean (Pmean) power output are reduced at high pedalling rates and low braking forces [2]. In contrast, during cycling sprints on an isokinetic cycle ergometer, Pmax increases linearly with pedalling rate [3]. Greater fatigue develops at high than at low isokinetic speeds [3,26], although muscle energy substrate depletion, metabolite accumulation and Pmean have been reported to be similar at 60 and 140 rpm during 30 s isokinetic sprints [26].

Sprint exercise increases the AMP/ATP ratio [11] and intracellular Ca^{++} concentration [31], and due to the high energy demand by-products of the energy metabolism accumulate very fast [15]. Consequently, muscle pH drops [35], and the redox [10] and electrolyte balance are altered [23]. These changes may trigger the activation of several signalling cascades, such as AMP-activated protein kinase (AMPK)/Acetyl-coenzyme A carboxylase β (ACCβ), MAP kinases (ERK1/2 and p38 MAPK), Signal Transducer and Activator of Transcription 3 (STAT3) and 5 (STAT5), which are implicated in the regulation of muscle metabolism and the response to cellular stress in the skeletal muscle [9,11,16,20,39]. However, muscle signalling may be also dependent on the pattern of muscle recruitment [13] and the endocrine response to exercise [8,38,41].

It remains unknown if isokinetic and isoinertial sprints elicit different endocrine responses, which could explain a potential difference in sprint exercise-induced muscle signalling. Potential endocrine candidates to explain differences in muscle signalling to sprint exercise are: insulin, leptin, growth hormone (GH) and IL-6, among other hormones and cytokines. Leptin [4] and IL-6 [42] could stimulate the janus kinase 2 (JAK2)/ STAT3 cascade. In rodent skeletal muscle, leptin also activates ERK1/2 and p38 MAPK [28] and induces AMPKα phosphorylation [30]. We have recently shown that glucose ingestion prior to a sprint exercise modulates the signalling response to the sprint exercise, most likely via

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Table 1 Physical characteristics, performance and muscle morphology (mean ± SD).

	Isokinetic (n = 15) 10M, 5W	Isoinertial (n = 12) 7M, 5W
age (years)	25.3 ± 4.4	23.9 ± 2.6
height (cm)	170.8 ± 9.7	170.4 ± 10.8
body mass (kg)	72.6 ± 14.0	69.1 ± 14.4
% body fat	22.7 ± 8.6	21.4 ± 7.1
Pmax (W)	859.9 ± 226.0	839.2 ± 246.7
Pmax (W/kg body mass)	11.8 ± 1.7	12.1 ± 2.2
Pmax (W/kg lean leg mass)	51.2 ± 1.4	51.8 ± 1.4
Pmean (W)	480.9 ± 150.1	565.2 ± 158.3
Pmean (W/kg body mass)	6.5 ± 1.2	8.1 ± 1.2$
Pmean (W/kg lean leg mass)	28.1 ± 3.2	35.0 ± 2.6$
RPM max	100 ± 0.0	117.13 ± 14.0$
RPM mean	100 ± 0.0	87.7 ± 9.8$
MHC I (%)	45.4 ± 17.6	50.0 ± 8.7
MHC IIa (%)	35.6 ± 11.5	39.5 ± 9.1
MHC IIb (%)	20.3 ± 7.2	17.9 ± 6.1
type 1 (%)[a]	49.8 ± 13.6	44.9 ± 12.0
type 2a (%)[a]	34.6 ± 9.3	35.9 ± 9.2
type 2x (%)[a]	15.8 ± 10.2	19.1 ± 6.3
CSA type 1 (μm²)[a]	3994 ± 963	4529 ± 1410
CSA type 2a (μm²)[a]	5459 ± 1775	5804 ± 2062
CSA type 2x (μm²)[a]	4653 ± 1630	4883 ± 1389

Pmax and Pmean, peak and mean power output in the Wingate test, respectively
M: men; W: women; MHC: myosin heavy chain. n = 14 and 8 for muscle fiber type and CSA (cross-sectional area) assessment with ATPase histochemistry in the isokinetic and isointertial groups, respectively. [a] By ATPase histochemistry
$P < 0.05 compared to isokinetic

changes in plasma insulin [22]. Greater GH response to sprint on friction-loaded ergometers has been reported with low compared to high braking forces [38]. Growth hormone may elicit STAT5 phosphorylation in skeletal muscle [8,41].

Therefore, the main aim of this study was to determine if the muscle signalling response to a 30 s all-out sprint exercise is modulated by the exercise mode. Our hypothesis is that muscle signalling will be enhanced in isoinertial compared to isokinetic sprints, due to the higher forces exerted during isoinertial compared to isokinetic sprints. Based on previous experiments in our laboratory, braking forces appropriate to elicit Pmax were chosen for the isoinertial tests, which were compared with isokinetic sprints performed at 100 rpm, a pedalling rate that allows to achieve Pmax. Thus exercise conditions were matched to elicit similar Pmax.

Material and Methods

Subjects

17 healthy men and 10 women agreed to participate in this study. Volunteers were divided in 2 groups: isokinetic (n = 15; 10 men and 5 women) and isoinertial (n = 12; 7 men and 5 women) (**Table 1**). Volunteers were non-smoking physical education students. All subjects were informed about the potential risks and benefits of the study and provided written consent prior to the start of the experiments. The study was performed in accordance with the Helsinki Declaration and approved by the Ethical Committee and complies with updated ethical standards in sport and exercise science research [24].

General procedures

The body composition was determined by DXA (Hologic QDR-1500, Hologic Corp., software version 7.10, Waltham, MA) [18]. Prior to the start of the experiments, subjects were familiarized with the Wingate test on 2 different days. On the experimental day, subjects arrived at the laboratory at 8.00 after an overnight fast, and an antecubital vein was catheterized. After a 10 min rest period in the supine position a 20 ml blood sample was withdrawn and used to determine serum hormonal concentrations at rest. Then a muscle biopsy was obtained from the middle portion of the vastus lateralis muscle using the Bergstrom's technique with suction. 3 min after the resting muscle biopsy and blood sample, the subjects performed a 30 s Wingate test with a braking force equivalent to 8% (women) and 10% (men) of their body weight in the isoinertial group [5,6] or with a isokinetic speed of 100 rpm (isokinetic group). The braking forces used in the isoinertial test are optimal to allow maximal peak power output during the Wingate test in physical education students [2,5]. During both sprints, i.e., isoinertial and isokinetic Wingate tests, subjects tried to pedal as fast and hard as possible (i.e., all-out) from the start to the end of the exercise. In the isoinertial condition they had to overcome a constant braking force, thus at the beginning of the test they developed high pedalling frequencies but as they fatigued their pedalling frequency declined. In contrast, during the isokinetic Wingate test the braking force is servo-controlled by the ergometer, which regulates the braking force to maintain a fixed pedalling rate of 100 rpm. The latter is possible because as subjects fatigue the ergometer automatically decreases the braking force when operating in isokinetic mode.

Peak power output was calculated as the highest work output performed during 1 s interval, and mean power output from the average work performed during the 30 s. No warm-up was allowed prior to the start of the Wingate test; and stop start Wingate tests were performed by both groups. The isoinertial test were carried out on a modified mechanically braked ergometer (Monark 818E, Monark AB, Vargerg, Sweden) equipped with a SRM power meter (Schoberer, Germany) [6]. The isokinetic Wingate tests were performed on a Lode Excalibur Sport cycle ergometer (Lode, Groningen, NL) set at a fixed pedalling rate of 100 rpm.

Immediately after the end of the Wingate tests another muscle biopsy and a blood sample were obtained. Additional muscle biopsies and blood samples were taken 30 and 120 min after the end of the Wingate test. For the last 2 biopsies a new incision was performed in the contralateral leg. To avoid injury-triggered activation of signalling cascades the muscle biopsies were obtained at least 3 cm apart, following the same procedures as those described by Guerra et al. [21]. During the recovery period the subjects rested seated and had free access to water. The muscle specimens were cleaned to remove any visible blood, fat, or connective tissue. Then the muscle tissue was immediately frozen in liquid nitrogen and stored at −80 °C for later analysis. The time needed to obtain and freeze the muscle biopsies was < 30 s. The phase of the menstrual cycle was not controlled in these experiments.

Muscle heavy chain composition and ATPase histochemistry

The muscle samples were immediately mounted with Tissue-Tek and frozen in isopentane cooled with liquid nitrogen, and stored at −80 °C. Muscle heavy chain composition (MHC) analyses were

performed on the muscle biopsies using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), as previously reported [17]. From each biopsy 20–40 serial cross sections (10 µm) were cut and placed in 200–500 µL of lysing buffer and heated for 3 min at 90 °C. Between 2 and 12 µL of the myosin-containing samples were loaded on SDS-PAGE gels containing 6% polyacrylamide (acrylamide: bisacrylamide, 100:1) and 30% glycerol. Gels were run at 70 V for 43 h at 4 °C. Subsequently, the gels were Coomassie stained and MHC isoform bands (I, IIa, IIx) were resolved and quantified with the image analysis software Quantity One© from Bio-Rad Laboratories (Hemel Hempstead Hertfordshire, UK). Enough muscle tissue was only available from 14 and 8 subjects of the isotonic and isoinertial groups, respectively. Serial sections (10 µm) of the muscle biopsy samples 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 [32].

Western blot analysis

Materials

The complete protease inhibitor cocktail was obtained from Roche Diagnostics (Mannheim, Germany). All the primary antibodies used were from Cell Signaling Technology (Danvers, MA, USA) except for the polyclonal rabbit antiphospho-acetyl CoA carboxylase (Ser221) antibody that was obtained from Upstate Biotechnology (Lake Placid, NY, USA). The monoclonal mouse anti-alpha-tubulin antibody was obtained from Biosigma (Madrid, Spain). The secondary HRP-conjugated goat anti-rabbit antibody was from Jackson Immuno Research (West Grove, PA, USA). The Hybond-P transfer membranes and the 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).

Muscle protein extracts were prepared as described previously [19]. Total protein content was quantified using the bicinchoninic acid assay [37]. Equal amounts (50 µg) of each sample were subjected to immunoblotting as described previously [19]. Alpha-tubulin was assessed as a housekeeping protein to control for potential loading artefacts (no significant differences were observed in alpha-tubulin levels across time points). To determine Thr^{172}-AMPKα, Ser^{221}-ACCβ, Tyr^{705}-STAT3, Tyr^{694}-STAT5, Thr^{202}/Tyr^{204}-ERK1/2 and Thr^{180}/Tyr^{182}-p38 MAPK phosphorylation levels, antibodies directed against the phosphorylated and total form of these kinases were used and processed as previously reported [14]. Total protein and their phosphorylated forms were determined on separate gels. For immunosignal quantification, band densities were normalized to the values obtained from the biopsies taken immediately before the start of the sprint. Data was represented as a percentage of immunostaining values obtained for the phosphorylated form of each kinase relative to those obtained for respectively total form. Samples pertaining to the same subject were run together in the same gel.

Blood lactate

Blood lactate concentration was determined in capillary blood obtained from the ear lobe hyperemized with Finalgon®, prior to the start of the sprint and at 3, 5, 7 and 10 min into the recovery period, using a Lactate Pro analyzer (Arkay, JA).

Leptin, IL-6 and GH assays

Serum leptin, IL-6 and GH were determined by Enzyme-Linked Immunosorbent Assay (ELISA) (ELx800 Universal Microplate Reader, Bioteck Instruments Inc, Vermont, USA), using reagent kits from Linco Research (#EZHL-80SK, Linco ResearchSt. Charles, Missouri, USA) and IBL International (#BE53061 and #DB59121, IBL International, Hamburg, Germany). The sensitivity of each ELISA assay was 0.05 $ng.mL^{-1}$ for total leptin, 0.92 $pg.mL^{-1}$ for IL-6 and 0.2 $ng.mL^{-1}$ for GH. The intra-assay coefficients of variation were 3.8, 3.4 and 4.5%, and the inter-assay coefficients of variation were 4.4, 5.2, and 6.0% for leptin, IL-6, and GH, respectively.

Insulin and serum glucose measurements

Serum insulin was measured by an electrochemiluminescence immunoassay (ECLIA) intended for use on Modular Analytics analyzer E170 using insulin kit reagents (Roche/Hitachi, Indianapolis, USA). Insulin sensitivity was 0.2 $µU.mL^{-1}$ and the corresponding intra- and inter-assay coefficients of variation were 2.0 and 2.6%, respectively. Serum glucose was measured by the hexokinase method using Gluco-quant reagents (Roche/Hitachi, 11876899216, Indianapolis, USA) with a sensitivity of 2 $mg.dL^{-1}$.

Statistical analysis

The normal distribution and equality of variances was assessed with the Shapiro-Wilks' and the Levene's test, respectively. When necessary, the analysis was done on logarithmically transformed data. A mixed-model ANOVA with repeated measures over time and one factor (type of Wingate test) with 2 levels (isokinetic vs. isoinertial) was used to compare the hormonal and signalling responses, using values normalized to the level of phosphorylation observed just before the start of the Wingate test. When there was a significant condition test by time interaction, intra-group effects were tested using a repeated-measures one-way ANOVA in each group, and pairwise comparisons were carried out using the Holm-Bonferroni method. Unpaired t-tests were used for planned comparisons to test between-group differences at specific time points, the corresponding P-values were adjusted for multiple comparisons with the Holm-Bonferroni method. The relationship between variables was determined using linear regression analysis. Values are reported as the mean ± standard error of the mean (unless otherwise stated). $P < 0.05$ was considered significant. Statistical analysis was performed using SPSS v.15.0 for Windows (SPSS Inc., Chicago, IL).

Results

Body composition, Wingate test results, and muscle morphology results are summarized in **Table 1**. Both groups were comparable in age, height, body mass, percentage of body fat, and vastus lateralis MHC composition, fibre type distribution and cross-sectional areas (CSAs). Pmax (W), Pmax (W/kg of body weight), Pmax (W/kg lean leg mass) and Pmean (W) were similar between groups. However, compared to the isokinetic group, the isoinertial group achieved higher Pmean relative to the body mass, Pmean relative to the lean mass of the lower extremities and peak pedalling rate (RPMmax) ($P < 0.01$). In contrast, mean pedalling rate (RPMmean) was higher in the isokinetic than isoinertial group ($P < 0.01$) (**Table 1**). The blood lactate responses to the Wingate test were similar in both groups (**Table 2**).

Table 2 Capillary blood lactate concentration (mmol.L^{-1}) prior to and during the recovery period, isoinertial Wingate tests (mean ± SD).

Lactate (mmol.L^{-1})	R	3 min	5 min	7 min	10 min	Group by time interaction
isokinetic (n = 15)	1.1 ± 0.3	11.4 ± 1.4*	12.3 ± 1.3*	12.4 ± 1.3*	12.1 ± 1.5*	P = 0.94
isoinertial (n = 12)	1.0 ± 0.2	12.7 ± 1.4*	13.2 ± 2.0*	13.2 ± 2.0*	12.2 ± 2.0*	

*P < 0.05 vs. rest (R)

		R Mean ± SD	0 Mean ± SD	30 min Mean ± SD	120 min Mean ± SD	Group by time interaction
leptin (ng.mL^{-1})	isokinetic (n = 15)	9.0 ± 6.9	9.7 ± 7.4	8.7 ± 7.2	7.5 ± 6.7$^{£}$	P = 0.14
	isoinertial (n = 12)	5.4 ± 2.8	7 ± 5.2	6.6 ± 5.0	5.9 ± 4.6	
insulin (µU-mL^{-1})	isokinetic (n = 15)	5.3 ± 2.0	7.6 ± 3.4	13.3 ± 5.6$^{£}$	5.2 ± 2.3	P = 0.55
	isoinertial (n = 12)	6.7 ± 3.1	10.1 ± 5.5	15.0 ± 3.9$^{£}$	6.0 ± 2.2	
glucose (mg.dL^{-1})	isokinetic (n = 15)	90.2 ± 6.1	99.2 ± 6.2$^{£}$	98.4 ± 8.4$^{£}$	88.2 ± 5.8	P = 0.25
	isoinertial (n = 12)	86.7 ± 9.1	96.1 ± 8.8$^{£}$	98.0 ± 11.8$^{£}$	89.4 ± 13.5	
GH (ng.mL^{-1})	isokinetic (n = 15)	1.8 ± 3.4	3.8 ± 4.9	7.1 ± 5.6$^{£}$	0.3 ± 0.3	P = 0.93
	isoinertial (n = 12)	2.2 ± 4.3	4.2 ± 6.0	6.4 ± 6.6$^{£}$	0.4 ± 0.4	
IL-6 (pg.mL^{-1})	isokinetic (n = 8)	1.1 ± 0.9	1.4 ± 1.0	1.4 ± 0.7	1.5 ± 0.6	P = 0.86
	isoinertial (n = 7)	2.0 ± 0.3	2.0 ± 0.4	2.1 ± 0.5	2.3 ± 0.5	

$^{£}$P < 0.05 vs. values just after exercise (0)

Table 3 Leptin concentration (ng.mL^{-1}), insulin concentration (µU.mL^{-1}), glucose concentration (mg.dL^{-1}), GH (ng.mL^{-1}) and IL-6 (pg.mL^{-1}) prior to and during the recovery period after the sprint exercise in isokinetic and isoinertial group (mean ± SD).

Serum leptin, insulin, glucose, GH, and IL-6 concentrations

The hormonal responses to exercise are reported in **Table 3**. No significant between-group differences were observed in hormonal and glucose responses to the sprint exercise, except for IL-6, which was significantly increased in the isoinertial groups 2 h after the sprint. Compared to the values observed at the end of the exercise, leptin concentration was decreased 2 h after the end of the exercise (P < 0.01). Compared to pre-exercise values, insulin concentration was increased 2.4-fold 30 min after exercise, while the glucose concentration was increased by 10% immediately after the sprint and 30 min later (P < 0.01). Growth hormone concentration was 3.4-fold higher 30 min after the sprint (P < 0.01).

Skeletal muscle signalling response to sprint exercise

Sprint exercise elicited marked increases in the musculus vastus lateralis AMPKα, ACCβ, STAT3, STAT5 and ERK1/2 phosphorylation (all P < 0.05). AMPKα, STAT3 and ERK1/2 phosphorylation responses to sprint exercise were different between groups (time × type of Wingate interaction: P < 0.01). In contrast, p38 MAPK phosphorylation did not change significantly in response to exercise.

AMPKα/ACCβ pathway

Compared to pre-exercise values, right after the exercise AMPKα phosphorylation showed a trend to be increased (ANOVA, P = 0.09); 30 min after the sprint AMPKα phosphorylation was enhanced by about 6-fold and 2.6-fold after isoinertial and isokinetic sprint, respectively (both P < 0.05), being the phosphorylation elicited by the isoinertial sprint significantly greater than that evoked by the isokinetic sprint (P < 0.05) (**Fig. 1a**). This difference persisted after accounting for Pmax (normalized per kg of lower extremities lean mass), but lost its statistical significance after accounting for Pmean (normalized per kg of lower extremities lean mass) (P = 0.11). In both groups, the ACCβ phosphorylation was similarly enhanced by about 3-fold at both, the end of the sprint and 30 min into the recovery period (time × type of Wingate interaction: P = 0.26) (**Fig. 1b**). These results were similar when the statistical analysis was adjusted for either Pmax or Pmean (both normalized per kg of lower extremities lean mass).

STAT3 and STAT5

The STAT3 phosphorylation response was delayed after the isokinetic compared to the isoinertial Wingate test (Interaction: P < 0.05) (**Fig. 2a**). Compared to resting values, STAT3 phosphorylation was increased by almost 5-fold 30 min after the isoinertial Wingate test (P < 0.01), while a trend for a smaller (2.5-fold) increase was observed 2 h after the isokinetic Wingate test (P = 0.12). 30 min after exercise, STAT3 phosphorylation was about 8-fold higher in the isoinertial compared to the isokinetic group (P < 0.01). These results were similar when adjusted for either Pmax or Pmean (both normalized per kg of lower extremities lean mass).

Compared to pre-exercise values, STAT5 phosphorylation was similarly increased immediately after and during the next 120 min of the recovery period following both types of sprint (**Fig. 2b**). There was no relationship between the GH response to the Wingate test and the increase of STAT5 phosphorylation, either when each group was analyzed separately or both groups conjointly.

MAP Kinases: ERK1/2 and p38

Similarly to STAT3, the phosphorylation of ERK in response to the Wingate test was delayed in the isokinetic compared to the isoinertial group (**Fig. 3a**). Compared to pre-exercise levels, ERK1/2 phosphorylation was increased by almost 4-fold 30 min into the recovery period in the isoinertial group (P < 0.05, compared to the isokinetic group), while the increase was more moderate (2-fold) and occurred 2 h after isokinetic sprint. In contrast, p38 MAPK phosphorylation did not change significantly in response to exercise (**Fig. 3b**).

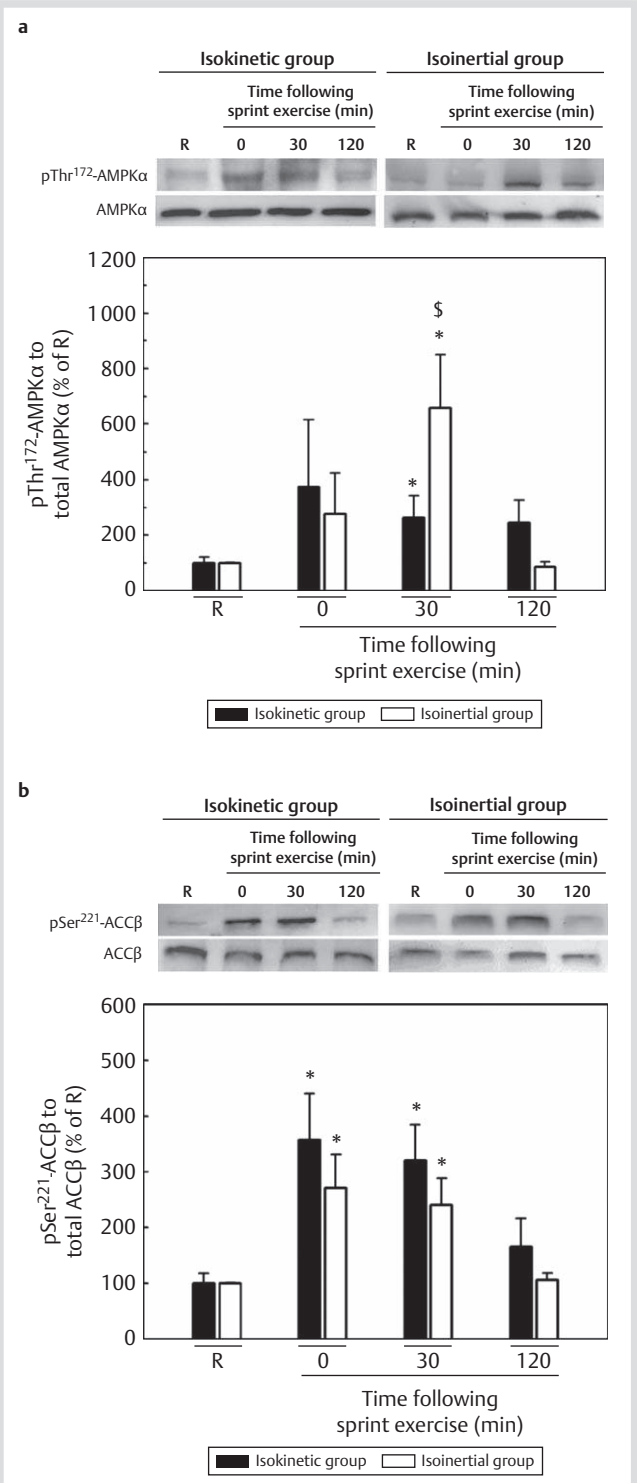

Fig. 1 Levels of Thr^{172}-AMPKα (A) and Ser^{221}-ACCβ phosphorylation before and after isokinetic (black bars) and isoinertial (white bars) Wingate tests. Values in both experimental groups were normalized to the average observed in the biopsies obtained immediately before the sprint exercise (R), which were assigned a value of 100 %. **a** Top panel: a representative Western blot with antibodies against AMPKα and phospho-AMPKα. Lower panel: AMPK phosphorylation densitometric values relative to total AMPKα. *P < 0.05 vs. rest (R). $P < 0.05 vs. isokinetic; P < 0.01, time × type of Wingate interaction. **b** Top panel: a representative Western blot with antibodies against ACCβ and phospho-ACCβ. Lower panel: ACCβ phosphorylation values relative to total ACCβ. *P < 0.05 vs. rest (R); P > 0.05, time × type of Wingate interaction. N = 15 for the isokinetic group and N = 12 for the isoinertial group.

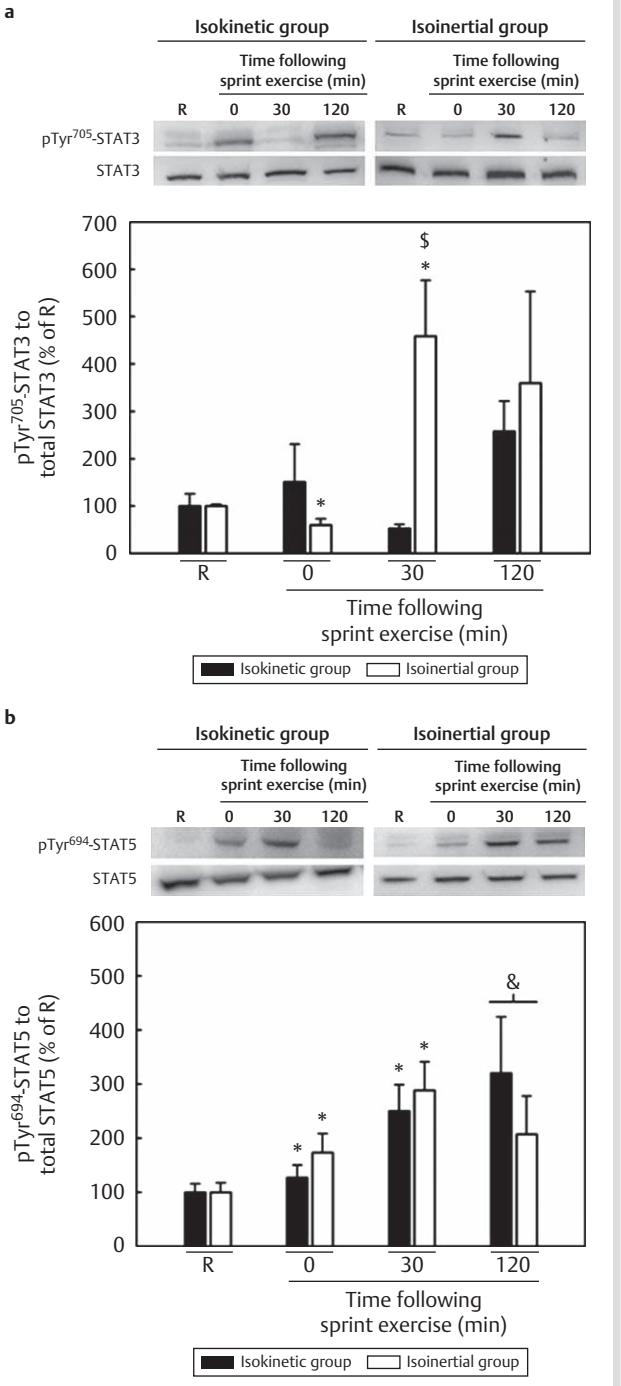

Fig. 2 Levels of Tyr^{705}-STAT3 (A) and Tyr^{694}-STAT5 (B) phosphorylation before and after isokinetic (black bars) and isoinertial (white bars) Wingate tests. Values in both experimental groups were normalized to the average observed in the biopsies obtained immediately before the sprint exercise (R), which were assigned a value of 100 %. **a** Top panel: a representative Western blot with antibodies against STAT3 and phospho-STAT3. Lower panel: STAT3 phosphorylation values relative to total STAT3. *P < 0.05 vs. R. Statistical analysis performed with logarithmically transformed data. $P < 0.05 vs. isokinetic group; P < 0.01, time × type of Wingate interaction. **b** Top panel: a representative Western blot with antibodies against SAT5 and phospho-STAT5. Lower panel: STAT5 phosphorylation values relative to total STAT5. *P < 0.05 vs. R; & P < 0.05 ANOVA R vs. 120 min. P > 0.05, time x type of Wingate interaction. N = 15 for the isokinetic group and N = 12 for the isoinertial group.

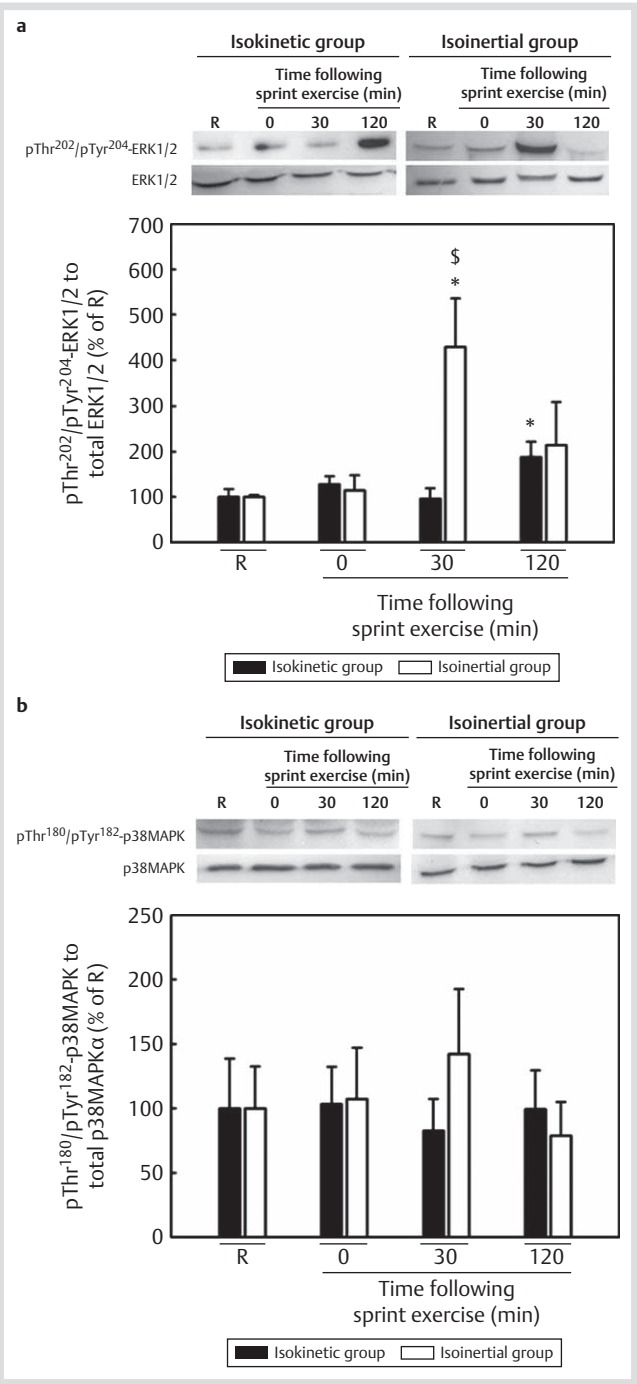

Fig. 3 Levels of Thr^{202}/Tyr^{204}-ERK1/2 and Thr^{180}/Tyr^{182}-p38MAPK phosphorylation before and after isokinetic (black bars) and isoinertial (white bars) Wingate tests. Values in both experimental groups were normalized to the average observed in the biopsies obtained immediately before the sprint exercise (R), which were assigned a value of 100%. **a** Top panel: a representative Western blot with antibodies against ERK1/2 and phospho-ERK1/2. Lower panel: ERK1/2 phosphorylation values relative to total ERK1/2. *P<0.05 vs. rest (R); $P<0.05 vs. isokinetic; P<0.01, time×type of Wingate interaction. **b** Top panel: a representative Western blot with antibodies against p38 MAPK and phospho-p38 MAPK. Lower panel: p38 MAPK phosphorylation values relative to total p38 MAPK. P>0.05, time×type of Wingate interaction. N=15 for the isokinetic group and N=12 for the isoinertial group.

Discussion

In this investigation we have shown that the muscle signalling response to sprint exercise is influenced by the muscle contraction pattern. Compared to isokinetic, isoinertial sprints elicited greater AMPKα, ERK and STAT3 phosphorylation levels after a 30 s all-out test, even when the loading conditions allowed the achievement of an almost identical Pmax. These differences in signalling cannot be explained by the observed changes in circulating insulin, glucose, leptin, GH, IL-6 and lactate concentrations, which were similar in both groups, despite greater mean power output and lower mean pedalling rate in the isoinertial group. Both sprints elicited similar ACCβ and STAT5 phosphorylation, whilst no changes were observed in p38MAPK.

Although AMPKα phosphorylation was significantly increased only 30 min after the end of the sprint and that this response was more intense after the isoinertial sprint, ACCβ phosphorylation was already increased immediately after the sprint in both groups, as previously reported [20,22]. Most likely, sprint exercise-induced ACCβ phosphorylation is, at least in part, independent of AMPKα activation, as observed in other experimental conditions [27].

The increase in STAT3 phosphorylation after the sprints concurs with our previous studies [22]. As a novelty we have shown that this increase was more marked after the isoinertial sprints, i.e., in the group that achieved the highest Pmean. Increases in STAT3 phosphorylation have been also reported 2 h after resistance exercise (leg extension: 3×12RM) [40]. The results from the present study combined with those of Trenerry et al. [40] could indicate that stronger activation of STAT3 may be elicited by more intense exercise. This agrees also with the recent observation of increased IL-6 response to exhaustive resistance exercise [25]. This increase in STAT3 phosphorylation appears a locally-elicited muscle response, since STAT3 phosphorylation was not preceded by an increase of known circulating activators of STAT3 phosphorylation, like leptin or IL-6. STAT3 phosphorylation is critical for satellite-cell mediated muscle hypertrophy [36].

It has been reported that the GH response to a 30 s sprint exercise depends on the mean pedal cadence [38]. In the present investigation the mean pedalling rate was 14% higher during the isokinetic than the isoinertial test, however GH increased similarly in response to sprint exercise in both groups. In accordance, STAT5 phosphorylation, which is elicited by JAK2 upon GH binding to its receptor, was also similar in both groups. Consitt et al. reported increased JAK2 and STAT5 phosphorylation after cycling at 70% VO_2max during half an hour [8]. The increase of STAT5 phosphorylation was associated to the GH increase [8]. STAT5 phosphorylation and translocation to the nucleus may induce the expression, among other genes, of insulin-like growth factor I (IGF-I) [8,34], which is implicated in the hypertrophic response to exercise. It remains, however, to be determined to what extent circulating GH is necessary to elicit muscle STAT5 phosphorylation in response to sprint exercise.

ERK1/2 was phosphorylated in response to both type of sprints, but the response was more intense and occurred earlier in the isoinertial group. In agreement, it has been reported that ERK1/2 phosphorylation increases with exercise intensity [33] and after resistance exercise [12]. Although p38MAPK phosphorylation is increased by endurance [1], resistance [12] and high-intensity intermittent exercise [7], no significant changes were observed in the present investigation. In agreement with our results, Gibala et al. did not observe changes in p38MAPK phosphoryla-

tion immediately after a single isoinertial Wingate test [16]. Our study, confirms these findings in a greater sample of subjects, including men and women. In addition, our results indicate that p38MAPK phosphorylation remains unchanged during the following 2 h after the sprint. However, after repeated sprints p38MAPK phosphorylation may be increased [16].

In theory, part of the differences in muscle signalling reported in the present investigation between the isokinetic and isoinertial groups could have been caused by the almost 25 % greater mean power output per kg of lower extremities lean mass developed during the isoinertial test. However, when we used mean power (per kg of lean body mass) as a covariate the differences observed in STAT3 and ERK1/2 phosphorylation were still statistically significant. In contrast, the greater AMPKα phosphorylation during the isoinertial compared to the isokinetic Wingate test could be in part due to the higher Pmean during the isoinertial sprint. A higher Pmean is compatible with greater metabolic stress after the isoinertial than the isokinetic sprint. Although the recovery blood lactate response was similar after both sprints, this does not rule out the possibility of greater intramuscular lactate accumulation and higher AMP/ATP ratio during the isoinertial sprints.

Limitations

This study has 2 main limitations. First, we used a 2-group design rather than a single group design; however both groups were matched for maximal peak power. Second, the potential effects on downstream indicators of the signalling pathways (e. g., mRNA expression of target genes, such as PGC1α) was not assessed. It is uncertain whether a 6-fold vs. a 2.4-fold difference in the activation of AMPK between conditions at a single time point may lead to different training adaptations.

In conclusion, sprint exercise elicits marked increases in the musculus vastus lateralis of AMPKα, ACCβ, STAT3, STAT5, and ERK phosphorylation. The AMPKα, STAT3, and ERK phosphorylation responses depend on the contraction mode, being more marked after an isoinertial than after an isokinetic 30 s all-out test (Wingate test). The differences in muscle signalling cannot be accounted for by differences in Pmax, although Pmean could explain part of the difference in AMPKα phosphorylation. The contraction mode-specific signalling revealed in this study cannot be attributed to differences in the circulating levels of leptin, insulin, glucose, GH and lactate, since these variables responded similarly after both types of sprint. New studies should determine if subtle differences in metabolic or oxidative stress between isokinetic and isoinertial sprints could explain our findings.

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