

Is sprint exercise a leptin signaling mimetic in human skeletal muscle?

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Departments of ¹Physical Education and ²Clinical Sciences, Molecular and Translational Endocrinology Group, University of Las Palmas de Gran Canaria; ³Endocrinology Service, Doctor Negrín Hospital, Las Palmas de Gran Canaria; ⁴Genetic Unit, Childhood Hospital-Materno Infantil de Las Palmas; and ⁵Research Unit, Hospital de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain

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Guerra B, Olmedillas H, Guadalupe-Grau A, Ponce-González JG, Morales-Alamo D, Fuentes T, Chapinal E, Fernández-Pérez L, De Pablos-Velasco P, Santana A, Calbet JA. Is sprint exercise a leptin signaling mimetic in human skeletal muscle? *J Appl Physiol* 111: 715–725, 2011. First published June 9, 2011; doi:10.1152/jappphysiol.00805.2010.—This study was designed to determine whether sprint exercise activates signaling cascades linked to leptin actions in human skeletal muscle and how this pattern of activation may be interfered by glucose ingestion. Muscle biopsies were obtained in 15 young healthy men in response to a 30-s sprint exercise (Wingate test) randomly distributed into two groups: the fasting ($n = 7$, C) and the glucose group ($n = 8$, G), who ingested 75 g of glucose 1 h before the Wingate test. Exercise elicited different patterns of JAK2, STAT3, STAT5, ERK1/2, p38 MAPK phosphorylation, and SOCS3 protein expression during the recovery period after glucose ingestion. Thirty minutes after the control sprint, STAT3 and ERK1/2 phosphorylation levels were augmented (both, $P < 0.05$). SOCS3 protein expression was increased 120 min after the control sprint but PTP1B protein expression was unaffected. Thirty and 120 min after the control sprint, STAT5 phosphorylation was augmented ($P < 0.05$). Glucose abolished the 30 min STAT3 and ERK1/2 phosphorylation and the 120 min SOCS3 protein expression increase while retarding the STAT5 phosphorylation response to sprint. Activation of these signaling cascades occurred despite a reduction of circulating leptin concentration after the sprint. Basal JAK2 and p38 MAPK phosphorylation levels were reduced and increased (both $P < 0.05$), respectively, by glucose ingestion prior to exercise. During recovery, JAK2 phosphorylation was unchanged and p38 MAPK phosphorylation was transiently reduced when the exercise was preceded by glucose ingestion. In conclusion, sprint exercise performed under fasting conditions is a leptin signaling mimetic in human skeletal muscle.

IL-6; SOCS3; STAT3; p38 MAPK

LEPTIN IS A HORMONE SECRETED primarily by adipocytes from the white adipose tissue that plays a crucial role in the regulation of appetite, body fat mass, basal metabolic rate (BMR), and gonadal function (87). However, leptin administration to obese humans has elicited small effects on fat mass, appetite, and BMR (42) due to leptin resistance (49). It has been shown that a single bout of sprint exercise activates AMP-activated protein kinase (AMPK; 38), which is also activated by leptin in skeletal muscle (58). However, little is known about the influence of a single sprint on the other signaling cascades activated

by leptin in the skeletal muscles. If exercise is able to activate the same signaling cascades as leptin, then exercise could be used as a leptin mimetic to circumvent leptin resistance. This is of particular interest since it has been shown that high-intensity intermittent exercise may be as effective, if not more effective, at reducing subcutaneous and abdominal body fat than other types of exercise (15).

Animal and cell culture studies have shown that upon binding to the long form of its receptor (OB-Rb), leptin stimulates the Janus tyrosine kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathway (6–7) and the JAK2/STAT5 pathway (35). In rodent skeletal muscle, leptin also acts through the activation of extracellular signal-regulated kinase (ERK1/2) and p38-mitogen activated protein kinase (MAPK) signaling pathways (55–56).

In vitro and rodent experiments have shown that leptin signaling may be downregulated by the protein suppressor of cytokine signaling 3 (SOCS3), which blunts JAK2/STAT3-dependent leptin signaling (8, 10) and causes leptin resistance in skeletal muscle (29, 74). The protein-tyrosine phosphatase 1B (PTP1B) is also a negative regulator of insulin and leptin sensitivity, acting to dephosphorylate the insulin receptor and the OB-Rb associated kinase JAK2 (14, 27). Sprint exercise could act as leptin mimetic by reducing the basal levels of PTP1B protein expression/activity in skeletal muscle (68, 75), but also by inducing phosphorylation and activation of AMPK, ERK, p38MAPK, STAT3, and STAT5. If this is the case, sprint exercise could be used to circumvent skeletal muscle leptin resistance in obese humans, who have reduced leptin receptors in their skeletal muscles (32).

Previous work from our group and others has shown increased AMPK phosphorylation 30 min after one bout (38) or immediately and 3 h after four bouts of sprint exercise in human skeletal muscle (33). Intriguingly, this effect is blunted by the ingestion of 75 g of glucose 60 min before exercise (38). It remains unknown if glucose ingestion also inhibits the exercise-induced activation of other leptin signaling pathways. This is important because inhibition of the exercise-induced activation of leptin signaling pathways could alter the adaptive response to sprint training. Experiments in cells have shown that hyperinsulinemia inhibits leptin receptor signaling through JAK2 dephosphorylation (47). It remains also unknown if glucose ingestion, which elicits high (but physiological) levels of insulin, may influence the protein expression of the negative leptin signaling regulators SOCS3 and PTP1B after one bout of high-intensity exercise in human skeletal muscle.

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Therefore, the main aim of the study was to determine if a 30-s sprint exercise (Wingate test) may act as leptin mimetic, by examining the response of the known leptin signaling pathways in skeletal muscle immediately after and during the following 4 h after the sprint exercise. Since other hormones and cytokines, like growth hormone (GH) and interleukin 6 (IL-6), may also stimulate some of the leptin signaling cascades in muscle, we measured GH and IL-6 serum response to sprint exercise together with STAT5 phosphorylation, which has been shown to be increased by GH (48, 62) and reduced by IL-6 (40) in rodent muscle. Another aim was to determine if glucose ingestion 1 h before the exercise blunts the activation of the signaling cascades also activated by leptin in skeletal muscle. By administering the glucose 1 h before the sprint we expected to have similar plasma glucose levels at the start of the sprint in both conditions (fed and fasted; 38), with different serum insulin levels. Finally, we determined the impact of glucose ingestion on basal leptin signaling pathways, SOCS3 and PTP1B protein expression. The main hypothesis to be tested was that an acute bout of brief (30 s) high-intensity exercise will activate signaling cascades in the active skeletal muscle known to be involved in leptin signaling and that this effect will occur independently of circulating leptin levels. We hypothesized also that glucose ingestion 1 h prior to the exercise will blunt the activation of these signaling cascades normally elicited by sprint exercise due to the interfering effects of hyperinsulinemia.

MATERIALS AND METHODS

The Complete protease inhibitor cocktail and the PhosSTOP phosphatase inhibitor cocktail were obtained from Roche Diagnostics (Mannheim, Germany). All the primary antibodies used were from Cell Signaling Technology (Danvers, MA) except for the polyclonal rabbit anti-human SOCS3 antibody that was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), the monoclonal mouse anti-PTP1B antibody was obtained from Calbiochem (Darmstadt, Germany), and the monoclonal mouse anti-alpha-tubulin antibody that was obtained from Biosigma (Sigma, St. Louis, MO). The secondary HRP-conjugated goat anti-rabbit and donkey anti-mouse antibodies were from Jackson ImmunoResearch (West Grove, PA). The Immuno-Blot PVDF membranes, Immun-Star western kit, ChemiDoc XRS System, and the image analysis software Quantity One were obtained from Bio-Rad Laboratories (Hemel Hempstead Hertfordshire, UK).

Subjects. Fifteen healthy male physical education students (age = 23.4 ± 0.6 yr, height = 178 ± 1.8 cm, body mass = 77.5 ± 2.1 kg, body fat = $14.7 \pm 1.9\%$) agreed to participate in this investigation (Table 1). Before volunteering, subjects were given full oral and written information about the course of the study and possible risks

associated with participation. Written consent was obtained from each subject. The study was performed in accordance with the Helsinki Declaration and approved by the Ethical Committee of the University of Las Palmas de Gran Canaria.

General procedures. The body composition of each subjects was determined by DXA (Hologic QDR-1500, Hologic, software version 7.10, Waltham, MA) as described elsewhere (4, 65). On a different day, subjects reported to the laboratory at 8:00 AM after an overnight fast, and an antecubital vein was catheterized. After 10 min rest in the supine position a 20-ml blood sample was withdrawn and used to measure serum glucose and insulin. Then a muscle biopsy was obtained from the middle portion of the vastus lateralis muscle using the Bergstrom's technique with suction, as described elsewhere (39, 64). Subjects were randomly divided into a control group (7 subjects) and the glucose group (8 subjects), matched for physical characteristics and performance (Table 1). Three minutes after the resting muscle biopsy (R) and blood sample, the control group performed a 30-s Wingate test with a braking force equivalent to 10% of their body mass as described elsewhere (19, 20). No warm up was allowed prior to the start of the Wingate test. Right after the Wingate test, another muscle biopsy and a blood sample were obtained. The time needed to obtain and freeze the muscle biopsies immediately after the Wingate test was below 30 s in all cases. To avoid injury-triggered activation of p38 MAPK or ERK1/2, the muscle biopsies were obtained at least 2–3 cm apart, following the same procedures as those described by Guerra et al. (37). During the following 4 h the subjects were fasting and sat quietly in the laboratory or in the library of our faculty. During the recovery period additional muscle biopsies and blood samples were obtained at 30, 120, and 240 min. The glucose group had two biopsies prior to the sprint exercise. The first blood sample and biopsy (baseline) was obtained under fasting conditions at 8:30 AM after subjects rested for 10 min in the supine position. Immediately following the baseline biopsy they ingested 75 g of glucose (Glucomedics-75 orange, Biomedics, Madrid, Spain) and they rested on a bed or seated on a chair for 60 min. At the end of this period, another muscle biopsy (named as "resting biopsy", R) and another blood sample were obtained. Three minutes after the muscle biopsy, subjects performed a Wingate test, also without warming up, and muscle biopsies and blood samples were obtained with the same time frame as for the control group. 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.

Total protein extraction, electrophoresis, and Western blot analysis. Muscle protein extracts were prepared as described previously (39) and total protein content was quantified using the bicinchoninic acid assay (72). Equal amounts (50 μg) of each sample were subjected to immunoblotting protocol as described previously (39). To determine Tyr⁷⁰⁵-STAT3, Tyr⁶⁹⁴-STAT5, Tyr^{1007/1008}-JAK2, Thr²⁰²/Tyr²⁰⁴-ERK1/2, and Thr¹⁸⁰/Tyr¹⁸²-p38 MAPK phosphorylation levels, antibodies directed against the phosphorylated and total form of these kinases were used all diluted in 5% bovine serum albumin in Tris-buffered saline with 0.1% Tween 20 (TBS-T; BSA-blocking buffer). To detect SOCS3 and PTP1B protein expression, membranes were incubated with a rabbit polyclonal specific anti-human SOCS3 antibody and with a mouse monoclonal specific anti-human PTP1B antibody, respectively (both diluted in BSA-blocking buffer). To control for differences in loading and transfer efficiency across membranes, 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). Antibody-specific labeling 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 visualized with the Immun-Star western kit (Bio-Rad Laboratories, Hercules, CA). Specific bands were visualized with the ECL chemiluminescence kit, using the ChemiDoc XRS system (Bio-Rad Labo-

Table 1. *Physical characteristics and performance*

| | Control Group (n = 7) | Glucose Group (n = 8) |
|-----------------------|--------------------------|--------------------------|
| Age, yr | 23 ± 2 | 23 ± 2 |
| Height, cm | 176.2 ± 9.1 | 180.1 ± 3.9 |
| Body mass, kg | 78.1 ± 10.4 | 76.9 ± 6.3 |
| %Body fat | 17.7 ± 6.6 | 12.0 ± 7.2 |
| Pmax, W | 1,018.6 ± 138.7 | 1,032.2 ± 135.2 |
| Pmax, W/kg body mass | 13.2 ± 2.1 | 13.3 ± 1.4 |
| Pmean, W | 676.9 ± 91.1 | 706.9 ± 37 |
| Pmean, W/kg body mass | 8.7 ± 1.1 | 9.2 ± 0.7 |

Values are means ± SD. All $P > 0.05$.

ratories) and analyzed with the image analysis program Quantity one© (Bio-Rad Laboratories). The densitometry analysis was carried out immediately before saturation of the immunosignal. For immunosignal quantification, band densities were normalized to the values obtained from the respective basal biopsy in each group [resting biopsy (R) taken immediately before the start of the sprint in the control group and -60 min biopsy (-60 min) taken immediately before the ingestion of 75 g of glucose and 60 min prior the start of the sprint]. Data were represented as a percentage of immunostaining values obtained for the phosphorylated form of each kinase relative to those obtained for, respectively, total form or as a percentage of immunostaining values obtained for SOCS3 or PTP1B relative to those obtained for alpha-tubulin. Alpha-tubulin content in the muscle biopsies of the two experimental groups was similar and steady after the sprint exercise (data not shown, $P > 0.05$). Western blot analysis of all proteins studied was performed in triplicate for each muscle biopsy with a variation coefficient $< 10\%$. Samples from each subject were run in the same gel.

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, IN). Insulin sensitivity was $0.20 \mu\text{U/ml}$.

Serum glucose was measured by the hexokinase method using Gluco-quant reagents (Roche/Hitachi, 11876899216) with a sensitivity of 2 mg/dl .

Assessment of insulin resistance. In each subject, the degree of insulin resistance was estimated by the Homeostasis model assessment (HOMA). In brief, fasting plasma insulin and fasting plasma glucose values were used to calculate an index of insulin resistance. The HOMA index was calculated as fasting insulin concentration ($\mu\text{U/ml}$) \times fasting glucose concentration (mmol/l)/22.5.

Leptin, IL-6, and GH. Serum leptin, IL-6, and GH were determined by ELISA (ELx800 Universal Microplate Reader, Biotech Instruments) using reagent kits from Linco Research (#EZHL-80SK, Linco Research, St. Charles, MO) and IBL International (#BE53061 and #DB59121, IBL International, Hamburg, Germany). The sensitivity of each ELISA assay was 0.05 ng/ml for total leptin, 0.92 pg/ml for IL-6, and 0.2 ng/ml for GH. The intra-assay coefficients of variation were 3.8, 3.4, and 4.5%, and the interassay coefficients of variation were 4.4, 5.2, and 6.0% for leptin, IL-6, and GH, respectively.

Real-time PCR. The mRNA levels of three genes [peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α), cell division cycle 14 homolog A isoform 1 (Cdc14a), and myosin heavy polypeptide 3 (Myh3)] known to be modified by leptin administration in rodent skeletal muscle (70) were determined by real-time PCR in the muscle biopsies obtained under fasting conditions pre exercise and 30, 120, and 240 min after the sprint. These genes were selected from the most responsive genes in rodent skeletal muscle of *ob/ob* mice submitted to replacement doses of leptin twice a day during 28 days (70). The expression of PGC-1 α and Cdc14a mRNA has been reported to increase and Myh3 to decrease with leptin treatment in *ob/ob* mice skeletal muscle (70). Total RNA was isolated using a RNeasy tissue fibrous kit (Qiagen, Valencia, CA). RNA concentration was measured using spectrophotometry (Nanodrop, Thermo Scientific, Wilmington, DE); all the RNAs had a A260/A280nm ratio of 2.0 ± 0.2 . An aliquot of each RNA was used to determine RQI index (Experion, Bio-Rad Laboratories); all the RNAs had values between 5 and 8. For first strand cDNA synthesis, constant amounts of 150 ng of total RNA was reverse transcribed using oligodT primers (Transcriptor, Roche Diagnostics, Mannheim, Germany). The transcript levels of each gene were quantified by real-time PCR (Light Cycler 480, Roche Diagnostics) using primers and Taqman probes designed using the RTR assays system (Roche Diagnostics). Each cDNA was amplified at the following conditions: 95°C for 10 min followed by 45 cycles of 30 s at 95°C and 30 s at 60°C using LC 480 Probes Master Mix (Roche Diagnostics). All the results were normalized to the levels

of two housekeeping transcripts (18S rRNA and GAPDH), and relative quantification was calculated using Livak method. Relative mRNA expression was expressed as fold expression over the calibrator sample (Resting: "R"). All samples were run in duplicate, and the average values were calculated.

Statistics. 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. For between-groups comparisons, the individual responses were normalized to the level of phosphorylation observed just before the start of the Wingate test. A mixed-model ANOVA with repeated measures over time and one factor (treatment, not repeated) with two levels (control vs. glucose) was used to compare the responses with the value just before the start of the Wingate test, using values normalized to the level of phosphorylation observed just before the start of the Wingate test. When there was a significant treatment by time interaction, intra-group effects were tested using one-way ANOVA separately 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. Since we missed three muscle biopsies corresponding to the 240 min time point (two in the glucose group and one in the control group), these analyses were limited to the first 120 min. The 240 min point was compared with the pre-exercise condition using a paired *t*-test. Likewise, pre-glucose values were compared with pre-exercise values using a paired *t*-test, since we had only one comparison of this type. The relationship between variables was determined using linear regression analysis. Values are reported as the mean \pm standard error of the mean (unless otherwise stated). $P \leq 0.05$ was considered significant. Statistical analysis was performed using SPSS v.15.0 for Windows (SPSS, Chicago, IL).

RESULTS

Influence of glucose ingestion on glucose, insulin, leptin, IL-6, and GH serum concentrations at rest and after exercise. Insulin serum concentration was elevated 1 h after the ingestion of glucose by 3.8-fold, ($P < 0.05$, Table 2). Thus, compared with the control group (CG), insulin concentration was elevated by 4.5-fold in the glucose group (GG) prior to the start of the Wingate test (Table 2), whereas plasma glucose concentrations were similar in both groups (Table 2). The serum insulin responses to the Wingate test were significantly different (time by group interaction $P < 0.05$). Compared with pre-exercise values, 30 min after exercise insulin concentration was increased (by 123%, $P < 0.05$) and decreased (by 53%, $P < 0.05$) in the control and glucose groups, respectively (Table 2).

During the recovery period there was a significant group-by-time interaction ($P < 0.01$) for glucose concentration, which increased in the control group to peak at 30 min while it decreased in the glucose group to reach a nadir at 30 min (both, $P < 0.05$, compared with pre-exercise values). Thus at 30 min postexercise the plasma glucose concentration was 34% higher in the control compared with the glucose group (105.9 ± 3.1 and $79.0 \pm 6.7 \text{ mg/dl}$, respectively, $P < 0.05$; Table 2).

Leptin concentration was reduced in response to exercise by 17% and 26% (120 and 240 min after sprint exercise, $P < 0.05$, Table 2) in the CG. Glucose ingestion prior to exercise accentuated this response and leptin was reduced by 60, 69, and 65% at 30, 120, 240 min into the recovery period, respectively ($P < 0.05$; ANOVA interaction vs. CG $P = 0.06$, Table 2).

Table 2. Serum glucose, insulin and leptin concentration before and during the recovery period after sprint exercise in both groups

| | -60 min | R | 0 | 30 min | 120 min | 240 min | Group by Time Interaction |
|--------------------------------|------------|--------------|--------------|---------------|--------------|-------------|---------------------------|
| <i>Control Group</i> | | | | | | | |
| Glucose, mg/dl (<i>n</i> = 7) | | 91.04 ± 9.0 | 101.2 ± 7.3* | 105.9 ± 8.3*‡ | 95.4 ± 14.8‡ | 95.0 ± 5.8‡ | <i>P</i> < 0.05 |
| Insulin, μU/ml (<i>n</i> = 7) | | 6.4 ± 3.3‡ | 10.0 ± 7.4‡ | 14.3 ± 2.73* | 5.32 ± 2.0 | 4.4 ± 2.7 | <i>P</i> < 0.05 |
| HOMA (<i>n</i> = 7) | | 1.5 ± 0.8 | | | | | |
| Leptin, ng/ml (<i>n</i> = 7) | | 4.1 ± 3.7 | 3.9 ± 3.2 | 3.8 ± 3.4 | 3.4 ± 2.9 | 3.0 ± 3.2* | <i>P</i> < 0.05 |
| IL-6, pg/ml (<i>n</i> = 7) | | 1.9 ± 0.4 | 1.9 ± 0.4 | 2.1 ± 0.5 | 2.2 ± 0.6 | 2.6 ± 0.7* | <i>P</i> = 0.94 |
| GH, ng/ml (<i>n</i> = 7) | | 0.3 ± 0.7 | 3.6 ± 5.7* | 4.8 ± 4.5* | 0.2 ± 0.1 | 0.1 ± 0.0 | <i>P</i> = 0.73 |
| <i>Glucose Group</i> | | | | | | | |
| Glucose, mg/dl (<i>n</i> = 8) | 87.2 ± 9.0 | 107.8 ± 44.4 | 97.4 ± 32.4 | 79.0 ± 18.8 | 76.4 ± 13.8 | 84.8 ± 3.9* | <i>P</i> < 0.05 |
| Insulin, μU/ml (<i>n</i> = 8) | 7.4 ± 5.8 | 28.6 ± 18.5† | 29.7 ± 21.7† | 13.5 ± 12.4* | 4.8 ± 3.4* | 2.6 ± 1.7* | <i>P</i> < 0.05 |
| HOMA (<i>n</i> = 8) | 1.6 ± 1.3 | | | | | | |
| Leptin, ng/ml (<i>n</i> = 8) | 2.3 ± 4.2* | 2.6 ± 4.6 | 2.6 ± 4.6 | 2.2 ± 4.0* | 2.0 ± 3.4* | 0.8 ± 1.0 | <i>P</i> < 0.05 |
| IL-6, pg/ml (<i>n</i> = 8) | 2.3 ± 0.2 | 2.4 ± 0.4 | 2.5 ± 0.4 | 2.4 ± 0.3 | 2.5 ± 0.3 | 3.2 ± 1.4* | <i>P</i> = 0.94 |
| GH, ng/ml (<i>n</i> = 8) | 1.4 ± 3.6 | 0.1 ± 0.4 | 1.4 ± 3.4* | 5.0 ± 4.6* | 0.7 ± 1.1 | 0.4 ± 0.3 | <i>P</i> = 0.73 |

Values are means ± SD. **P* < 0.05 vs. R (resting just prior to exercise); †*P* < 0.05 vs. -60 min. ‡*P* < 0.05 vs. glucose group. (*n* = 6 in both groups for the 240-min sample). For leptin in the glucose group, statistical analysis was performed with logarithmically transformed data.

IL-6 concentration was increased by 36% 240 min after the end of the Wingate test (*P* < 0.05) (Table 2). This response was not affected by the ingestion of glucose prior to exercise (Table 2).

Growth hormone concentration was increased by 7- and 10-fold immediately following and 30 min after the end of the Wingate test, respectively (*P* < 0.05; Table 2). This response was not affected by the ingestion of glucose prior to exercise.

Influence of glucose ingestion on JAK2/ STAT3 phosphorylation at rest and after exercise. Basal JAK2 phosphorylation was reduced 1 h after the ingestion of glucose (from 100 ± 4.8% to 53.5 ± 13.1%, *P* < 0.05; Fig. 1A). Jak2 phosphorylation did not change significantly in response to exercise in none of groups (all *P* > 0.05 vs. basal values; Fig. 1A).

Basal STAT3 phosphorylation was not affected by the ingestion of glucose (100 ± 3.7% and 80.7 ± 32.9%, baseline and pre-exercise, respectively, *P* = 0.1; Fig. 1B). Compared with pre-exercise conditions, STAT3 phosphorylation was increased 30 min after the control sprint exercise (from 100 ± 5.2% to 249 ± 72%, *P* < 0.05; Fig. 1B). Glucose ingestion blunted and delayed the 30 min STAT3 phosphorylation in response to the Wingate test. The magnitude of STAT3 phosphorylation 30 min after the Wingate was higher during the test performed under fasting conditions than after glucose ingestion (*P* < 0.05; Fig. 1B).

Influence of glucose ingestion on STAT5 phosphorylation at rest and after exercise. Basal STAT5 phosphorylation was not affected by the ingestion of glucose (100 ± 23.2% to 113 ± 35.5%, baseline and pre-exercise, respectively *P* > 0.05; Fig. 1C). Compared with pre-exercise conditions, STAT5 phosphorylation was increased 30 min (from 100 ± 6.9% to 331.1 ± 60.9%, *P* < 0.05) and 120 min (from 100 ± 6.9% to 317.8 ± 90.3%, *P* < 0.05) after the sprint exercise (Fig. 1C). Glucose ingestion retarded this response. Compared with pre-exercise values, STAT5 phosphorylation was increased 120 min (from 113.4 ± 35.5% to 1037.3 ± 443.9%, *P* < 0.05) and 240 min (from 113.4 ± 35.5% to 436.5 ± 181.1%, *P* < 0.05) after the sprint exercise in the glucose group (Fig. 1C).

Influence of glucose ingestion on ERK1/2 phosphorylation at rest and after exercise. Basal ERK1/2 phosphorylation was reduced 1 h after the ingestion of glucose (from 100 ± 8.3% to

29.2 ± 5.1%, *P* < 0.05 vs. -60 min; Fig. 2A). The magnitude of ERK1/2 phosphorylation immediately before sprint exercise (R) was higher in the control compared with the glucose group (*P* < 0.05). Compared with pre-exercise conditions, ERK1/2 phosphorylation was increased 30 min after the sprint exercise in the control group (from 100 ± 6.9% to 467 ± 168%, *P* < 0.05 vs. resting; Fig. 2A). The ingestion of glucose retarded this response (interaction, *P* < 0.05), such that 2 h after the Wingate test ERK1/2 phosphorylation was still elevated in the glucose but not in the control group (*P* < 0.05; Fig. 2A).

Influence of glucose ingestion on p38 MAPK phosphorylation at rest and after exercise. Basal p38 MAPK phosphorylation was increased approximately by 2.5-fold 1 h after the ingestion of glucose (from 100 ± 1% to 247.3 ± 40.4%, *P* < 0.05; Fig. 2B). The magnitude of p38 MAPK phosphorylation immediately before sprint exercise (R) was lower in the control compared with the glucose group (*P* < 0.05). However, p38 MAPK phosphorylation did not change significantly during the 4 h following the control Wingate test (Fig. 2B). In contrast, compared with pre-exercise levels, p38 MAPK was reduced 30 min after the Wingate test performed following glucose ingestion (from 247.3 ± 40.4% to 100.2 ± 23.7%, *P* < 0.05; Fig. 2B).

Influence of glucose ingestion on SOCS3 and PTP1B protein expression at rest and after exercise. Basal SOCS3 protein expression was unaffected 1 h after the ingestion of glucose (from 100 ± 6% to 123 ± 33.3%, *P* = 0.45; Fig. 3A). Compared with pre-exercise conditions, SOCS3 protein expression was increased 120 min after the control sprint exercise (from 100 ± 8.41% to 248.5 ± 42.5%, *P* < 0.05; Fig. 3A); however, SOCS3 protein expression was unchanged when the sprint exercise was performed after the ingestion of glucose (Fig. 3A). SOCS3 protein expression was not related to serum leptin concentration or p38 MAPK in either group.

Basal PTP1B protein expression was not significantly changed by either ingestion of glucose or exercise (Fig. 3B). In the glucose group, PTP1B protein expression was negatively associated with Thr²⁰²/Tyr²⁰⁴-ERK1/2 phosphorylation (*r* = -0.85, *P* < 0.05) and STAT3 phosphorylation (*r* = -0.84, *P* < 0.05).

Gene expression. The changes observed in PGC1 α , Myh3, and Cdc14a mRNA levels after the sprint in the control group are depicted in Fig. 4. PGC1 α mRNA was increased by 3.2-fold 240 min after the sprint ($P < 0.05$; Fig. 4A). In contrast, Myh3 mRNA levels were reduced by 30 and 40% 120

and 240 min after the sprint, respectively ($P < 0.05$; Fig. 4B). Cdc14a mRNA content was not affected by exercise (Fig. 4C).

DISCUSSION

The present study examined changes in leptin signaling cascades in human skeletal muscle after a single sprint exercise. Because leptin and insulin share some signaling pathways in skeletal muscle (41) and since hyperinsulinemia inhibits leptin receptor signaling (59) and AMPK α phosphorylation in human skeletal muscle (38), we have also determined the influence that glucose ingestion has on the activation of leptin signaling pathways in response to sprint exercise. In a previous work, we showed that glucose ingestion 1 h before sprint exercise facilitates the exercise-induced Akt (also known as protein kinase B) and AS160 phosphorylation and prevents the exercise-induced AMPK α phosphorylation (38). In the present study we showed that a 30-s all out cycling sprint performed under fasting conditions stimulated STAT3, STAT5, and ERK phosphorylation, increasing 3.2-fold PGC-1 α mRNA content. STAT3 and ERK phosphorylation (9, 60) and PGC-1 α (50, 70) can be induced by leptin. Likewise, the myosin heavy polypeptide 3, the mRNA expression of which is known to be downregulated in *ob/ob* mice skeletal muscle by leptin (70) was also downregulated by sprint exercise under fasting conditions. In addition, our study shows that sprint exercise can increase SOCS3 protein in skeletal muscle, which can be also induced by leptin (10). In agreement with our hypothesis, glucose ingestion, likely through the sustained elevation of insulin, blunted the exercise-induced STAT3 and ERK phosphorylation observed at 30 min post-sprint in the control condition and retarded STAT5 phosphorylation. Likewise, the elevation of SOCS3 protein expression observed 120 min after the fasting Wingate was also blunted when the sprint was preceded by the ingestion of glucose. These changes in signaling occurred with minimal changes in circulating leptin and IL-6 concentrations. Altogether, these results indicate that sprint exercise performed under fasting conditions elicits signaling events similar to those described in rodent skeletal

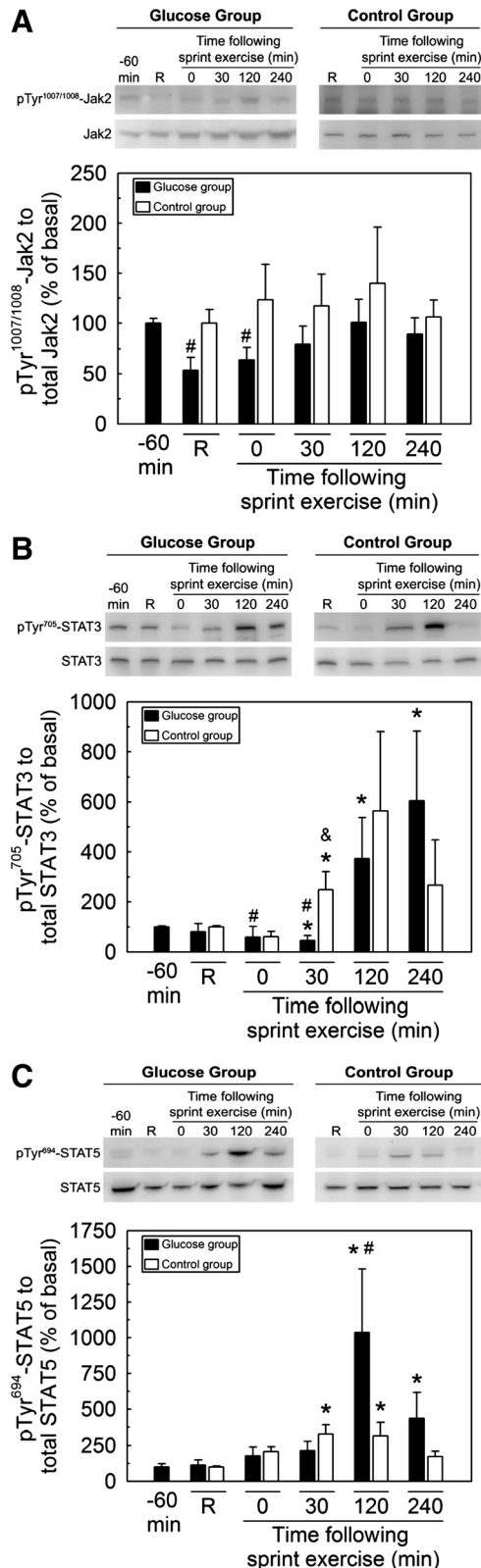


Fig. 1. Levels of JAK2 (A), STAT3 (B), and STAT5 (C) phosphorylation, before and after a Wingate test performed 60 min after the ingestion of 75 g of glucose (solid bars) or under fasted conditions (open bars). *A, top*: a representative Western blot with the anti-phospho-JAK2 antibody shows different levels of band densities obtained for both phospho-JAK2 and total JAK2 at the different time points in both experimental groups. *Bottom*: densitometric analysis of immunoblots. Values are relative to total JAK2 immunosignals. *B, top*: a representative Western blot with the anti-phospho-STAT3 antibody shows different levels of band densities obtained for both phospho-STAT3 and total STAT3 at the different time points in both experimental groups. *Bottom*: densitometric analysis of immunoblots. Values are relative to total STAT3 immunosignals. *C, top*: a representative Western blot where the anti-phospho-STAT5 antibody shows different levels of band densities obtained for both phospho-STAT5 and total STAT5 at the different time points in both experimental groups. *Bottom*: densitometric analysis of immunoblots. Values are relative to total STAT5 immunosignals. All values were normalized to the average of basal values obtained prior to the start of the Wingate test in the control group (R) and before the ingestion of glucose (-60 min; 60 min prior sprint exercise) in the glucose group, which were assigned a value of 100%. In the glucose group, $n = 8$ for all time points except 240 min for which $n = 6$. In the control group, $n = 7$ for all time points except 240 min for which $n = 6$. The statistical analysis of STAT3 and STAT5 was performed on logarithmically transformed data. [#] $P < 0.05$ vs. -60 min. ^{*} $P < 0.05$ vs. R. [&] $P < 0.05$ vs. glucose group.

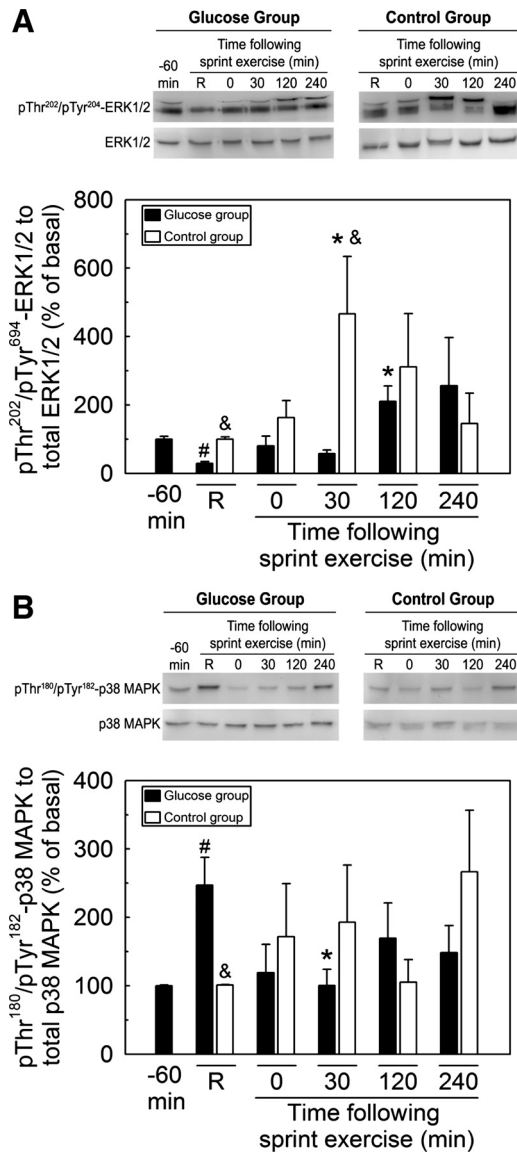


Fig. 2. Levels of ERK1/2 (A) and p38 MAPK (B) phosphorylation before and after a Wingate test performed 60 min after the ingestion of 75 g of glucose (solid bars) or under fasted conditions (open). *A*, top: a representative Western blot with the anti-phospho-ERK1/2 antibody shows different levels of band densities obtained for both phospho-Thr²⁰²/Tyr²⁰⁴-ERK1/2 and total ERK1/2 at the different time points in both experimental groups. *Bottom*: densitometric analysis of immunoblots. Values are relative to total ERK1/2 immunosignals. *B*, top: a representative Western blot with the anti-phospho-p38 MAPK antibody shows different levels of band densities obtained for both phospho-p38 MAPK and total p38 MAPK at the different time points in both experimental groups. *Bottom*: densitometric analysis of immunoblots. Values are relative to total p38 MAPK immunosignals. All values were normalized to the average of basal ones obtained prior to the start of the Wingate in the control group (R) and before the ingestion of glucose (-60 min; 60 min prior sprint exercise) in the glucose group, which were assigned a value of 100%. In the glucose group, $n = 8$ for all time points except 240 min for which $n = 6$. In the control group, $n = 7$ for all time points except 240 min for which $n = 6$. # $P < 0.05$ vs. -60 min. * $P < 0.05$ vs. R. & $P < 0.05$ vs. glucose group.

muscle after leptin injections, i.e., sprint exercise under fasting conditions acts as a leptin signaling mimetic in human skeletal muscle. However, glucose ingestion prior to the sprint exercise blunts this effect.

JAK2/STAT3/PGC-1 α response to sprint exercise and oral glucose ingestion. The JAK2/STAT3 signaling pathway is the main signaling cascade activated by leptin (60–61), eliciting mitochondrial biogenesis and lipid oxidation in skeletal muscle (50). Gibala et al. (33) showed that repeated sprints increase PGC-1 α mRNA. The present investigation shows that even a

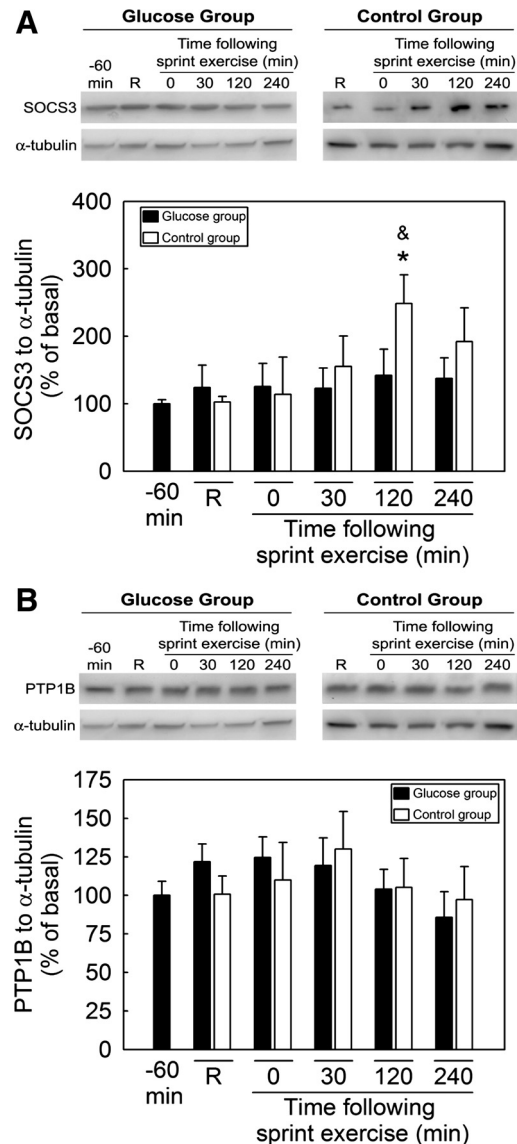


Fig. 3. Levels of SOCS3 (A) and PTP1B (B) protein expression, before and after a Wingate test performed 60 min after the ingestion of 75 g of glucose (solid bars) or under fasted conditions (open). *A*, top: a representative Western blot with the anti-SOCS3 antibody shows different levels of band densities observed at the different time points in both experimental groups. *Bottom*: densitometric analysis of immunoblots. Values are relative to alpha-tubulin immunosignals. *B*, top: a representative Western blot with the anti-PTP1B antibody shows different levels of band densities observed at the different time points in both experimental groups. *Bottom*: densitometric analysis of immunoblots. Values are relative to alpha-tubulin immunosignals. All values were normalized to the average of basal ones obtained prior to the start of the Wingate test in the control group (R) and before the ingestion of glucose (-60 min; 60 min prior sprint exercise) in the glucose group, which were assigned a value of 100%. In the glucose group, $n = 8$ for all time points except 240 min for which $n = 6$. In the control group, $n = 7$ for all time points except 240 min for which $n = 6$. * $P < 0.05$ vs. R. & $P < 0.05$ vs. glucose group.

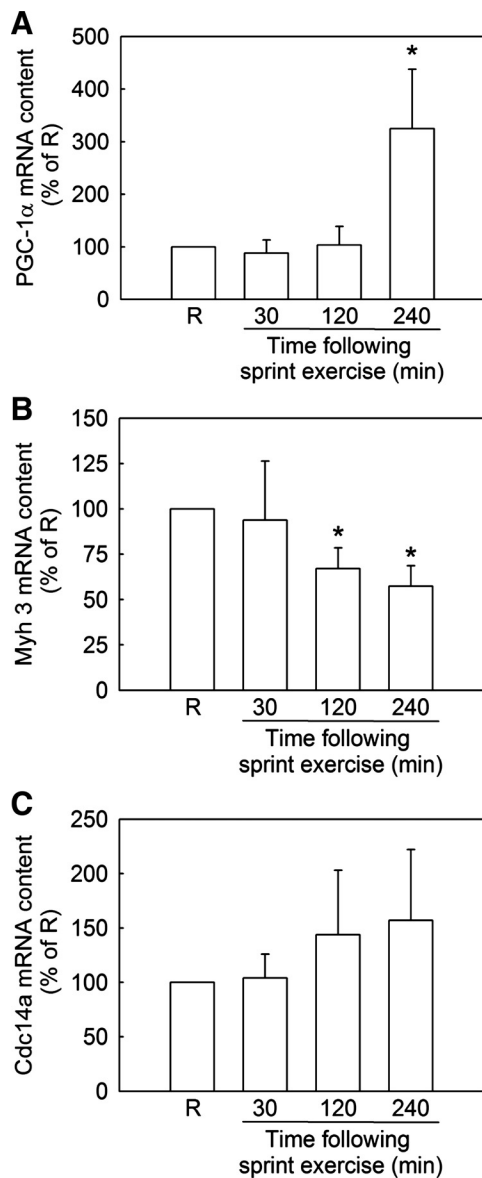


Fig. 4. PGC-1 α (A), Myh3 (B), and Cdc14a (C) gene expression levels (mRNA) in muscle biopsies obtained before and after a Wingate test performed under fasting conditions. After total RNA isolation, specific mRNA was quantified using Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and Taqman probes. The threshold cycle (Ct) was converted to a relative gene expression by the use of Livak analysis. The expression of the target gene mRNA was normalized with GAPDH and 18S-RNA mRNA content as housekeeping transcripts; $n = 7$ for all time points except 240 min for which $n = 6$. * $P < 0.05$ vs. R.

single 30-s all-out effort is enough to elicit a marked increase in PGC-1 α mRNA. Experiments with *ob/ob* mice have shown that intact leptin signaling is required for mitochondrial biogenesis and PGC-1 α deacetylation in skeletal muscle by physical activity (50) and that leptin administration increases deacetylation of PGC-1 α (70). The present investigation shows that, like leptin administration in rodents, sprint exercise activates the STAT3/PGC-1 α signaling pathway, despite a small reduction in circulating leptin levels. In vitro experiments have shown that leptin-induced lipid oxidation is abolished by treatment with a JAK2 inhibitor or STAT3 siRNA (1). The repeated

activation of the STAT3/PGC-1 α signaling pathway may be critical for the improvement in $\dot{V}O_{2\max}$ (18) and fat oxidation observed with sprint exercise (17). It remains to be determined if this type of response would be also observed in obese humans with leptin resistance.

Administration of insulin at physiological dose elicits JAK2 phosphorylation in rat skeletal muscle (69). In contrast, we observed reduced JAK2 phosphorylation 1 h after the oral ingestion of glucose (resting state) and immediately after the sprint exercise ($t = 0$), coinciding with elevated insulin compared with the control sprint. This result is in agreement with previous data indicating that hyperinsulinemia contributes to the pathogenesis of leptin resistance through a downregulation of JAK2 phosphorylation in human embryonic kidney fibroblast 293 cells (HEK 293 cells; 47). In turn, reduced JAK2 phosphorylation may blunt OB-Rb phosphorylation and, hence, leptin signaling via the leptin interaction with its main receptor. Altogether, these results suggest that JAK2 is not activated by a single bout of sprint exercise in human skeletal muscle. The progressive recovery of JAK2 phosphorylation from its nadir immediately after the Wingate test preceded by glucose ingestion could be explained by the gradual decrease of insulin levels from its peak immediately after the sprint to its lowest value 4 h after the Wingate test. JAK2 is the upstream kinase for both STAT3 (6, 7) and STAT5 (35) and, hence, the reduction of JAK2 phosphorylation after the ingestion of glucose could explain the subsequent retardation of the sprint-induced STAT3 and 5 phosphorylation.

In agreement with our results, it has been reported that STAT3 phosphorylation increases 2 h after a single bout of intense resistance exercise (78, 79). However, STAT3 phosphorylation remained unchanged after 90 min of one-legged cycling at 60% of $\dot{V}O_{2\max}$ (13). Thus exercise-induced STAT3 phosphorylation is more dependent on the intensity than the duration of the exercise. STAT3 phosphorylation is essential for the formation of homo- or heterodimers, nuclear translocation, and subsequent STAT3-dependent increases in the transcriptional activity of responsive genes (25), including genes modulated by leptin (7). This is likely the reason why the increase of PGC-1 α mRNA is delayed 4 h into the recovery period after the sprint exercise.

In addition, of leptin signaling, STAT3 is the signal transducer of numerous stimuli (76) and is involved in the regulation, among other processes, of cellular proliferation, differentiation, remodeling, repair, adaptation and regeneration of skeletal muscle, programmed cell death, inflammation, muscle hypertrophy, and the immune response (3, 45, 78, 79). Exercise increases the muscle expression and plasma concentration of IL-6 depending on exercise intensity and duration (31, 51, 53, 63). However, in agreement with our results, no changes in IL-6 plasma concentrations have been reported after sprint exercise (16). Thus STAT3-mediated signaling induced by sprint exercise does not seem explainable by changes in circulating IL-6, since the IL-6 produced by the muscle is released to the circulation and IL-6 serum concentration was only slightly increased 240 min after sprint exercise, i.e., 210 min after the increase of STAT3 phosphorylation. In addition, much higher increases in circulating IL-6 than observed in the present investigation are needed to induce STAT3 phosphorylation in human skeletal muscle (83). Moreover, glucose ingestion blunted and delayed the STAT3 phosphorylation while

the IL-6 response to sprint was similar in both conditions, implying that circulating IL-6 could hardly explain the observed responses in skeletal muscle STAT3 phosphorylation.

MAPK: ERK1/2 and p38 MAPK in response to sprint exercise and oral glucose ingestion. ERK1/2 and p38 MAPK are involved in the upregulation of enzymes important for the antioxidant defense and muscle growth (34, 54), while p38 MAPK is a known upstream regulator of PGC-1 α expression in skeletal muscle (2). Sprint exercise, like leptin (58, 71), activates both AMPK (38) and p38 MAPK, and both directly phosphorylate PGC-1 α , enhancing the transcriptional activation of the PGC-1 α promoter (43, 66). p38 MAPK phosphorylation may be elicited by both resistance (26, 46) and endurance exercise (28, 86). A recent study by Gibala et al. (33) reported no significant changes in p38 MAPK phosphorylation immediately after a 30-s sprint exercise. However, Gibala's subjects were studied 2–3 h after a light meal of their own choosing and it is difficult to compare these results to ours, where p38 MAPK phosphorylation did not increase when the Wingate test was performed under fasting conditions. Our study shows that when the sprint exercise is performed 1 h after the ingestion of glucose, when p38 MAPK phosphorylation is significantly increased prior to the start of exercise, then p38 MAPK phosphorylation is reduced right after the Wingate test. Since glucose administration elicited an increase of p38 MAPK phosphorylation, the subsequent decrease of p38 MAPK phosphorylation when the sprint exercise was preceded by the ingestion of glucose could be a consequence of the gradual decrease of insulin concentrations. This response does not seem to be caused by changes in leptin, since leptin concentration was not acutely affected by sprint exercise. Thus the effect of exercise on p38 MAPK phosphorylation depends on the basal level of p38 MAPK phosphorylation prior to the start of exercise and may be modulated by insulin levels. Although no response was observed after a single 30-s sprint by Gibala et al. (33), after four 30-s sprints interspaced with 4 min of rest p38 MAPK phosphorylation was moderately increased (1.4-fold; Ref. 33). Thus it seems that p38 MAPK phosphorylation is also more dependent on the metabolic effects of exercise, which accumulate by repeated sprint exercise, and exercise duration than on exercise intensity or muscle tension (28).

ERK1/2 may be phosphorylated in skeletal muscle by leptin via phosphorylation of the Tyr985 in the OB-Rb (6, 52). However, ERK1/2 may be also phosphorylated directly by JAK2, i.e., by an OB-Rb-independent mechanism (6). Both resistance (23, 26) and endurance exercise can induce ERK1/2 phosphorylation (5, 36, 85). The effects of endurance exercise are increased with exercise duration and intensity (67, 81, 82). The high tensions achieved during sprint exercise (57) and the elevated exercise intensity achieved during the Wingate test may have facilitated ERK1/2 phosphorylation in the present study. However, this response was delayed and observed into the recovery period, particularly after the ingestion of glucose, suggesting that ERK1/2 phosphorylation in skeletal muscle is more dependent on metabolic factors than on tension.

SOCS3 and PTP1B in response to sprint exercise and oral glucose ingestion. Although glucose administration was accompanied by a small increase of serum leptin, this effect was not enough to elicit an increase in STAT3 phosphorylation and, hence, SOCS3 remained also unaffected before exercise. Little is known about the potential effect of exercise on SOCS3.

Endurance exercise induces SOCS3 mRNA in rodent skeletal muscle (73) and resistance exercise increases SOCS3 protein expression 2 h after cessation of exercise in young men (79). In the present study, SOCS3 protein expression was increased 2 h after the sprint exercise only when the exercise was performed under fasting conditions. This was preceded by an increase of STAT3 phosphorylation, which remained remarkably elevated from 30 to 120 min into the recovery period. This strong activation of STAT3 may have induced its negative regulator SOCS3 (24). The elevation of SOCS3 120 min after the control sprint may have partly inhibited STAT3 phosphorylation by JAK2 and, thereby the reduction observed in STAT3 phosphorylation at 240 min time point (8, 10). After the sprint preceded by glucose ingestion, STAT3 phosphorylation was delayed to the 120 min time point, however, no increase was observed in SOCS3 at 240 min, implying that JAK2 phosphorylation of STAT3 was limited by other mechanism(s). Our results also imply that increased JAK2 phosphorylation is not mandatory for exercise-induced STAT3 phosphorylation. Expression of SOCS3 may be also induced by GH (62). In agreement, SOCS3 expression was increased 90 min after the exercise-induced peak in serum GH in the present investigation. However, glucose administration may have counteracted the stimulating effect of GH on SOCS3 expression by a mechanism that remains to be determined. It has been reported that activation of p38 MAPK is involved in SOCS3 induction by proinflammatory mediators such as TNF- α (12) or by other cytokines such as IL-6 (11) and IL-4 (21) by facilitating the stabilization of SOCS3 mRNA (30). However, in the present study, p38 MAPK does not seem to be involved in the increased SOCS3 expression 120 min after the control Wingate test.

Although in the present study no significant changes were observed in PTP1B protein expression, a previous study has shown that in rodent skeletal muscle with increased PTP1B levels due to diet-induced obesity, a single bout of prolonged endurance exercise reduced both PTP1B protein expression and activity (68). Further studies are needed to determine if in humans with elevated PTP1B protein/activity a single bout of sprint exercise may be sufficient to restore the normal situation.

Growth hormone and STAT5 phosphorylation. In agreement with previous studies a marked increase in serum GH concentration was observed after both sprints (77). The predominant GH signal transduction cascade comprises activation of the GH receptor (GHR) dimer, phosphorylation of JAK2, and subsequently activation of STAT5. Although in some tissues GH may elicit ERK1/2 phosphorylation (84), this is not the case in human skeletal muscle (62), implying that the ERK responses observed after the sprint exercise could hardly be explained by the elevation of GH.

Increased skeletal muscle STAT5 phosphorylation was previously reported in response to aerobic exercise (22, 80). This is the first study to report STAT5 phosphorylation in response to a sprint exercise in human skeletal muscle. STAT5 phosphorylation may have been caused by GH (44, 48), which was similarly increased immediately (0 min) and 30 min after the sprint in both conditions. Glucose ingestion prior to exercise delayed the exercise-induced STAT5 phosphorylation to 120 min postexercise despite the fact that GH was similarly increased after sprint exercise in both experimental groups. However, the sole ingestion of glucose at rest did not elicit

changes in STAT5 phosphorylation, when at this time point serum insulin levels were significantly augmented in the glucose compared with the control group. In agreement, insulin per se does not induce STAT5 phosphorylation and does not alter GH-induced STAT5 phosphorylation in human skeletal muscle (62). Although leptin can induce STAT5 phosphorylation in cultured cells (35), there is no evidence for leptin induction of STAT5 phosphorylation in skeletal muscle. STAT5 phosphorylation is reduced by IL-6 in rodent skeletal muscle (40), implying that the small increase of IL-6 4 h after the sprint was likely insufficient to impede STAT5 phosphorylation. Thus it remains to be determined by which mechanisms glucose ingestion delays STAT5 phosphorylation response to sprint exercise.

In conclusion, this study shows that most of the signaling pathways activated by leptin in rodent skeletal muscle are also activated by sprint exercise in human skeletal muscle, despite a small reduction of leptin serum concentration after the sprint exercise. These findings imply that sprint exercise behave as a leptin mimetic and could be used to stimulate the leptin signaling pathways in human skeletal muscle. This opens the possibility of using sprint exercise to circumvent leptin resistance in obese humans and may lead to increased leptin sensitivity. We provide some evidence to support that the effects of sprint exercise on ERK, STAT3, STAT5, and SOCS3 are not mediated by changes in either serum leptin or IL-6 concentrations, while the expression of SOCS3 and the phosphorylation of STAT5 may have been induced by GH. Importantly, we showed that glucose ingestion 1 h prior to the sprint exercise abolishes or delays some of the exercise-elicited signaling responses, implying that the adaptative responses to sprint exercise training may be modulated by the postabsorptive state.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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