Neuromuscular Fatigue and Metabolism during High-Intensity Intermittent Exercise

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[1]Section of Integrative Physiology, Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, DENMARK; [2]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, ITALY; and [3]Department of Biomedical Sciences for Health, University of Milan, Milan, ITALY

ABSTRACT

FIORENZA, M., M. HOSTRUP, T. P. GUNNARSSON, Y. SHIRAI, F. SCHENA, F. M. IAIA, and J. BANGSBO. Neuromuscular Fatigue and Metabolism during High-Intensity Intermittent Exercise. *Med. Sci. Sports Exerc.*, Vol. 51, No. 8, pp. 1642–1652, 2019. **Purpose:** To examine the degree of neuromuscular fatigue development along with changes in muscle metabolism during two work-matched high-intensity intermittent exercise protocols in trained individuals. **Methods:** In a randomized, counter-balanced, crossover design, 11 endurance-trained men performed high-intensity intermittent cycle exercise protocols matched for total work and including either multiple short-duration (18×5 s; SS) or long-duration (6×20 s; LS) sprints. Neuromuscular fatigue was determined by preexercise to postexercise changes in maximal voluntary contraction force, voluntary activation level and contractile properties of the quadriceps muscle. Metabolites and pH were measured in vastus lateralis muscle biopsies taken before and after the first and last sprint of each exercise protocol. **Results:** Peak power output ($11\% \pm 2\%$ vs $16\% \pm 8\%$, $P < 0.01$), maximal voluntary contraction ($10\% \pm 5\%$ vs $25\% \pm 6\%$, $P < 0.05$), and peak twitch force ($34\% \pm 5\%$ vs $67\% \pm 5\%$, $P < 0.01$) declined to a lesser extent in SS than LS, whereas voluntary activation level decreased similarly in SS and LS ($10\% \pm 2\%$ vs $11\% \pm 4\%$). Muscle [phosphocreatine] before the last sprint was 1.5-fold lower in SS than LS ($P < 0.001$). Preexercise to postexercise intramuscular accumulation of lactate and H^+ was twofold and threefold lower, respectively, in SS than LS ($P < 0.001$), whereas muscle glycogen depletion was similar in SS and LS. Rate of muscle glycolysis was similar in SS and LS during the first sprint, but twofold higher in SS than LS during the last sprint ($P < 0.05$). **Conclusions:** These findings indicate that, in endurance-trained individuals, multiple long-sprints induce larger impairments in performance along with greater degrees of peripheral fatigue compared to work-matched multiple short-sprints, with these differences being possibly attributed to more extensive intramuscular accumulation of lactate/H^+ and to lower rates of glycolysis during multiple long-sprint exercise. **Key Words:** CENTRAL FATIGUE, PERIPHERAL FATIGUE, REPEATED SPRINTS, SPRINT INTERVAL TRAINING (SIT), ALL-OUT EXERCISE, PERFORMANCE

The capacity to conduct repeated high-intensity exercise is a key determinant of performance in several sports (e.g., football, basketball, handball, rugby, hockey, water polo, tennis, badminton, and cycling). Performance deteriorates as intense brief efforts are repeated due to neuromuscular fatigue development, which manifests as a reduction in the force-generating capacity of the skeletal muscles (1). Neuromuscular fatigue may result from an impaired muscle function due to metabolic and ionic perturbations (i.e., peripheral fatigue) (2) and/or a reduced capacity of the central nervous system to activate muscles (i.e., central fatigue) (3). Multiple-sprint exercise has been extensively used as a model to investigate the interaction between high-intensity intermittent exercise performance and neuromuscular fatigue (4). Peripheral fatigue develops early and persists throughout multiple-sprint exercise while central fatigue tends to manifest toward task-end (5,6), suggesting that the capacity to conduct repeated high-intensity exercise is limited by a combination of peripheral and central factors. However, owing to reports showing relatively small preexercise to postexercise deficits in central neural drive (5–7), peripheral fatigue appears to be the predominant cause of the decline in multiple-sprint performance.

Depletion of energy substrates and accumulation of metabolic by-products contribute to skeletal muscle fatigability during repeated intense muscle contraction (2). In accordance, reduced phosphocreatine (PCr) availability has been proposed as a critical factor for power output recovery during multiple-sprint exercise (8,9); an assumption supported by evidence of improved multiple-sprint performance after creatine supplementation (10). Furthermore, the increased intracellular inorganic

phosphate (P_i) resulting from PCr breakdown and adenosine triphosphate (ATP) hydrolysis correlates well with the decline in force during repeated maximal contractions (11).

Likewise, intramuscular accumulation of lactate and the associated lowering of intracellular pH have been suggested to be responsible for fatigue development during intense exercise (12), possibly via the detrimental effects of hydrogen ions (H^+) on glycolytic energy provision (13) and release of potassium (K^+) from the contracting skeletal muscle (14). The purported negative impact of intramuscular H^+ accumulation on intense intermittent exercise capacity is supported by the correlation observed between muscle buffer capacity and multiple-sprint performance (15). Taken together, it is conceivable that the magnitude of metabolic and ionic perturbations during multiple-sprint exercise affects fatigue development and the associated decline in performance.

Consistent with the task-dependent nature of fatigue, manipulating duration of the sprints and/or the recovery periods characterizing multiple-sprint exercise likely leads to differential metabolic and ionic disturbances within the contracting skeletal muscle. In support, we recently showed that work-matched multiple-sprint exercise protocols, differing for sprint and recovery duration, elicited different levels of metabolic stress (16), implying that the metabolic determinants of neuromuscular fatigue can be investigated by using different multiple-sprint exercise models. Moreover, although the neuromuscular and metabolic responses to multiple short-duration (≤ 10 s) sprints interspersed with relatively short recovery periods (≤ 60 s) (i.e., repeated-sprint exercise) is well documented (4,17), the association between neuromuscular fatigue development and muscle metabolism during multiple long-duration (≥ 20 s) sprints interspaced by comparatively longer rest intervals (i.e., speed endurance exercise) (18) is inadequately explored. Hence, examining the peripheral and central components of neuromuscular fatigue along with the metabolic and ionic changes occurring in skeletal muscle during either multiple short- or long-duration sprint exercise would provide new insights into the mechanisms underlying muscular performance impairments as intense brief efforts are repeated. In addition, an in-depth characterization of the metabolic profile of different multiple-sprint exercise regimes may elucidate the nature of the bioenergetic stressors underpinning skeletal muscle remodeling in response to long-term high-intensity intermittent exercise training. Lastly, given the lack of data on the physiological response to intense intermittent exercise in athletes, exploring the neuromuscular and metabolic responses to intense intermittent exercise in well-trained individuals would fill this void while providing relevant information for coaches and exercise physiologists, who regularly use high-intensity intermittent exercise drills as a mean to improve performance in athletes.

Thus, the purpose of the present study was to examine the degree of neuromuscular fatigue in concert with a comprehensive analysis of muscle and blood metabolic perturbations attained during two diverse multiple-sprint exercise protocols in trained individuals. The exercise protocols were matched for mechanical work, intensity, and exercise-to-recovery ratio, but differed in sprint and recovery duration. We hypothesized that the protocol including multiple long-sprints would induce a greater decline in performance and a higher degree of neuromuscular fatigue in association with more marked muscle metabolic perturbations compared with the protocol comprising multiple short-sprints.

METHODS

Subjects

Twelve healthy trained men were initially included, of which 11 completed the study. Subject characteristics were (mean $\pm$ SEM): age, 31.6 ± 2.6 yr; height, 177 ± 2 cm; weight, 74.7 ± 2.8 kg; and maximal oxygen consumption, ($\dot{V}O_{2max}$) 61.5 ± 2.0 mL·min^{-1}·kg^{-1}. All subjects were amateur cyclists or triathletes regularly performing cycling-based training, with a training history of at least 6 yr. Before inclusion, subjects were informed of risks and discomforts associated with the experimental procedures. Each subject gave his oral and written informed consent. Inclusion criteria were males, 18 to 40 yr of age, weekly training volume above 3 h, $\dot{V}O_{2max}$ above 50 mL·min^{-1}·kg^{-1}, body mass index below 30 kg·m^{-2}. Exclusion criteria were smoking and chronic disease. The study was approved by the regional research ethics committee of Copenhagen, Denmark (H-16000378) and adheres to the principles of the Declaration of Helsinki, except for registration in a database.

Study Design

This study was a part of a larger project including three experimental trials designed to investigate the acute metabolic and molecular responses to two maximal-intensity intermittent exercise regimes and a moderate-intensity continuous exercise protocol (16). For the purpose of the present study, only the two intermittent exercise regimes were considered. In a randomized, counterbalanced, crossover design, two experimental trials were performed on separate occasions interspaced by 7 to 14 d. In addition, an incremental test to exhaustion was carried out for determination of $\dot{V}O_{2max}$ before the experimental trials.

Incremental test. Subjects completed the incremental test to exhaustion on a mechanically braked cycle-ergometer (LC6; Monark Exercise AB, Vansbro, Sweden). The test protocol consisted of two submaximal 4-min bouts at 150 and 225 W followed by an incremental ramp test with increments of 25 W·min^{-1} until volitional exhaustion. Pulmonary gas exchanges were measured breath-by-breath using an online gas analysis system (Oxycon Pro, Viasys Healthcare, Hoechberg, Germany). $\dot{V}O_{2max}$ was determined as the highest pulmonary oxygen consumption ($\dot{V}O_2$) value achieved during a 30-s period. Criteria used for achievement of $\dot{V}O_{2max}$ were a plateau in $\dot{V}O_2$ despite an increase in workload and a respiratory exchange ratio above 1.10. Heart rate was monitored throughout the test (Polar Team[2]; Polar Electro Oy, Kempele, Finland)

and maximal HR (HR$_{max}$) established as the highest value achieved during the test checked for spikes. After the incremental cycling test to exhaustion, participants were accustomed to the neuromuscular function test procedures.

Experimental trials. The experimental trials included two exercise protocols consisting of either multiple short-duration (SS) or long-duration (LS) sprints (Fig. 1). The exercise protocols were matched for mechanical work (SS: 81 ± 3 kJ; LS: 80 ± 3 kJ) and exercise-to-recovery ratio (1:6). SS included 18 × 5 s "all-out" efforts interspersed with 30 s of passive recovery, whereas LS included 6 × 20 s "all-out" efforts interspersed with 120 s of passive recovery. Preliminary trials were conducted with four subjects to determine the number of 5-s sprints necessary to elicit the same mechanical work sustained during 6 × 20 s sprints, resulting in 18.3 ± 0.3 sprints. The exercise protocols were performed on a mechanically braked cycle-ergometer (894E; Monark Exercise AB, Vansbro, Sweden) interfaced with dedicated software (Monark Anaerobic Test Software 3.3; Monark Exercise AB). Saddle and handlebar heights were recorded during the first trial and the same settings were replicated during the second trial. The exercise protocols were preceded by a standardized warm-up consisting of 7 min of continuous cycling at a workload corresponding to 65% $\dot{V}O_{2max}$ (199 ± 7 W) followed by 5 min at rest. Foot straps were used to secure the feet to the pedals. The pedal right arm crank starting position was 45° forward to the vertical axis. Upon the start command, subjects began pedaling as fast as possible and continued until the stop command. During cycling, when a pedaling frequency of 100 rpm was reached, the dedicated software automatically applied the workload and started the timer. Braking forces corresponding to 0.90 and 0.75 N·kg^{-1} body mass were used for SS and LS, respectively. Subjects were verbally encouraged to maintain maximum pedaling speed throughout each sprint.

Control procedures. On the experimental days, subjects reported to the laboratory 120 min after ingesting their last meal. Subjects were instructed to refrain from caffeine, alcohol and exercise for 24 h before each trial and to keep their training load constant during the 6 d preceding each trial. Also, subjects were asked to report their food habits in a questionnaire, so that an individual diet plan was developed to standardize food intake during the 48 h preceding each experimental trial. Daily carbohydrate, protein, and fat intake in the 48 h before the experimental trials were 4.8 ± 0.2, 1.7 ± 0.1 and 0.8 ± 0.0 g·kg^{-1} body mass, respectively.

Measurements and Data Analysis

Exercise performance. Values for peak power output (PPO), mean power output (MPO) and mechanical work (kJ) for each sprint were provided by the software interfaced with the cycle ergometer. In addition, the decrement in PPO (PPO$_{dec}$) and MPO (MPO$_{dec}$) over the sprints within each exercise trial was calculated as previously described (17):

$$PO_{dec} = \left[1 - \frac{(PO_{first\ sprint} + PO_2 + PO_3 + ... + PO_{last\ sprint})}{PO_{best} \times number\ of\ sprint} \right] \times 100$$

Respiratory and HR measurements. Pulmonary gas exchanges were measured breath-by-breath at rest and during exercise using an online gas analysis system (Oxycon Pro; Viasys Healthcare). The breath-by-breath pulmonary $\dot{V}O_2$ data were initially examined to exclude errant breaths, defined as any value lying more than 4 SD away from the local mean (e.g., due to swallowing and coughing). These breath-by-breath data sets were subsequently linearly interpolated to provide 1 s values. Values of $\dot{V}O_2$ were averaged to obtain one value for each 5 s and 20 s sprint during SS and LS, respectively. Heart rate was monitored continuously during the exercise protocols (Polar Team^2, Polar Electro Oy, Kempele, Finland).

Neuromuscular function. Isometric contractions and electrical stimulations of the quadriceps muscle before and after exercise were used to measure neuromuscular fatigue. After
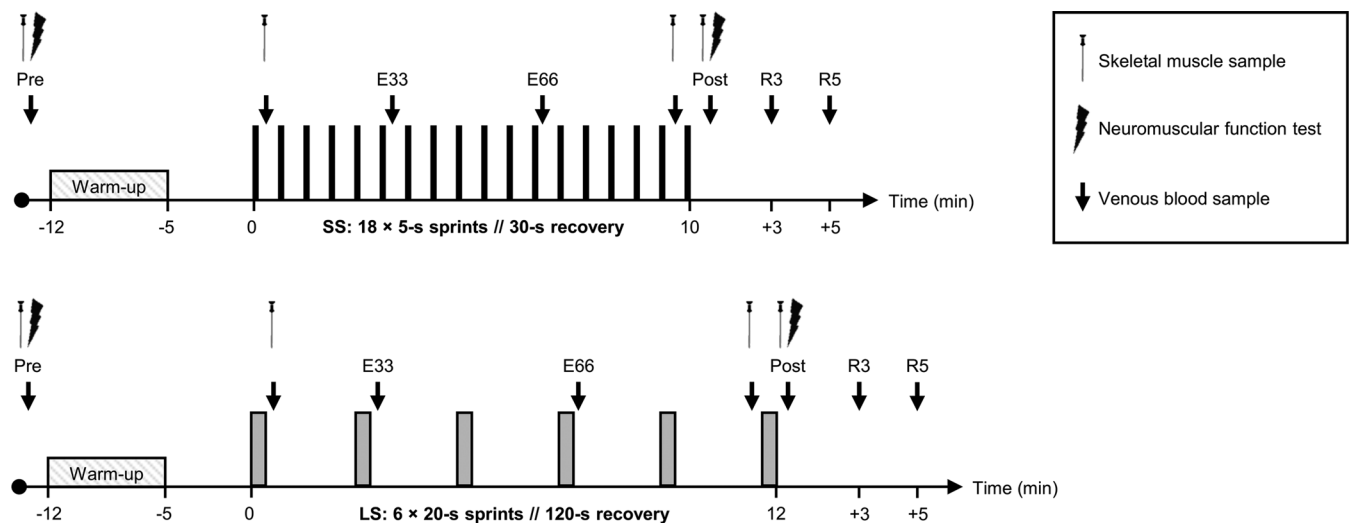

FIGURE 1—Schematic presentation of the experimental trials consisting of multiple SS and LS sprint exercise.

exercise, given the muscle biopsy procedure and the transfer from the cycle ergometer to the dedicated experimental setup, neuromuscular function assessments were carried out 85 ± 6 s after cessation of the last sprint. For each subject, the time delay during the first trial was recorded and replicated during the second trial. Measurements were conducted on the right leg with the participants sat on a custom-made chair with their thighs parallel to the floor and knee joint angle of $90°$ of flexion. A strain gauge (Tedea-Huntleigh, UK) was strapped around the right ankle above the malleoli. To ensure that subjects remained in the same position throughout the test protocol, two Velcro strips were tied around hip and thighs. Self-adhesive electrodes were placed on the skin 25% distal from spina iliaca anterior superior and 25% proximal from patella covering vastus lateralis and rectus femoris muscle. Before exercise, after the preparation phase, subjects performed a standardized warm-up consisting of 10 isometric knee extensions (alternating 3 s of contraction and 7 s of rest) with contraction intensity increasing progressively up to maximal levels during the last three isometric contractions. Then, the optimal intensity of the electrical stimulation was determined by administering percutaneous electrical stimulations to the quadriceps muscle at rest in 50 mA step-wise increments from 50 mA until either the maximum quadriceps twitch amplitude was elicited or maximal stimulator output (999 mA; $n = 2$) was achieved, or if subjects felt pain. The determined optimal intensity was further increased by 20% to ensure a supramaximal stimulus for all subsequent stimulations. Finally, subjects performed three isometric maximal voluntary contractions (MVC) of 3 s duration, each separated by 30 s. After exercise, subjects performed only one MVC. Percutaneous electrical muscle stimulations were delivered during and ~2 s postcontraction to assess voluntary activation level and contractile properties of the quadriceps muscle, respectively. The electrical stimulation used could activate $35.0\% \pm 2.0\%$ and $35.0\% \pm 1.7\%$ of subjects' peak MVC in SS and LS, respectively. These values were of the same magnitude of those observed by using electrical and magnetic femoral nerve stimulations (19). Muscle stimulations were produced by a constant current stimulator (Digitimer DS7AH, Hertfordshire, United Kingdom) in square wave pulses of 200 µs. The isometric contraction force was recorded through the strain gauge which transmitted the signal to an amplifier connected to a laptop computer. Data were recorded at 1 kHz in LabChart 8 (ADInstruments, San Diego, CA).

The following parameters were determined: MVC as the highest force (N) recorded during the isometric voluntary contraction; potentiated peak twitch force as the highest force (N) recorded during the stimulated single twitch ~2 s after relaxation from the MVC; time to peak twitch as the time (ms) from single twitch stimulation until peak twitch force was achieved; half-relaxation time as the time (ms) from peak twitch force until force reached half of peak twitch force; voluntary activation level calculated as: $[1 - (\text{superimposed twitch force/peak twitch force})] \times 100$, where the superimposed twitch force is the additional force elicited by a single stimulation delivered on the MVC plateau (20). A correction was applied to the equation if the superimposed stimulation was delivered slightly before or after the peak MVC (21).

During measurements, subjects received strong verbal encouragements with no visual feedback. From the three MVC measurements before exercise, the greatest MVC and the related twitch were selected for data analysis.

Muscle biopsy sampling. After 15 min of rest in the supine position, two 3-mm incisions were made over the lateral portion of the left thigh under local anesthesia (2 mL lidocaine without epinephrine, 20 mg·mL^{-1} Xylocain, AstraZeneca). Muscle biopsies of the vastus lateralis muscle were collected before, during, and immediately after exercise (Fig. 1) using a percutaneous Bergstrom needle with suction. Specifically, muscle samples were obtained at rest and immediately after the first sprint from the distal incision as well as 10 s before and immediately after the last sprint from the proximal incision. Muscle samples were snap-frozen in liquid nitrogen and stored at −80°C until further analysis.

Muscle metabolite, glycogen, and pH analyses. The muscle samples were freeze dried for 48 h and dissected free of blood, fat and connective tissue. Dissection was performed under a stereo microscope with an ambient temperature of ~18°C and a relative humidity <30%. After dissection, muscle tissue was weighed and separated into different tubes for analyses.

Muscle ATP, PCr, lactate, pyruvate, and glucose-6-phosphate concentration (i.e., [ATP], [PCr], [lactate], [pyruvate], and [G-6-P]) were determined on dry weight (dw) muscle tissue (~2.0 mg). Determination was made by extraction in 3 mol·L^{-1} perchloric acid, neutralization to pH 7.0 with 2.2 M KHCO$_3$, followed by fluorometric analyses as previously described (22). Muscle glycogen concentration ([glycogen]) was determined on ~1.5 mg dw muscle tissue by hexokinase method, as previously described (22). Muscle pH was measured by a small glass electrode (Radiometer GK2801, Copenhagen, Denmark) after homogenizing ~2 mg dw in a nonbuffering solution containing 145 mM KCl, 10 mM NaCl, and 5 mM iodoacetic acid.

Blood sampling and analyses. Venous blood samples were taken from a cannula inserted into the antecubital vein. Blood samples were collected at rest before exercise, immediately after the first sprint, at 33% (E33) and 66% (E66) of exercise task completed, 10 s before and immediately after the last sprint, and after exercise at 3 min (R3) and 5 min (R5) into recovery (Fig. 1). For determination of blood metabolites and ion concentrations ([glucose], [lactate], pH, and [HCO$_3^-$]), venous blood samples were drawn in heparinized 2 mL syringes and immediately analyzed (ABL 800 Flex; Radiometer, Copenhagen, Denmark). For determination of plasma catecholamines, insulin, free fatty acids (FFA) and glycerol, venous blood samples were collected in 2 mL syringes and transferred to an Eppendorf tube containing 30 µL ethylenediaminetetraacetic acid (0.2 mol·L^{-1}), after which they were spun at 20,000g for 3 min to collect plasma, which was stored at −20°C until analysis. Plasma catecholamines were analyzed by using an enzyme immunoassay kit (2-CAT Plasma Elisa High Sensitive BA E-4500 LDN; Nordhorn, Germany). Plasma insulin was determined using an enzyme immunoassay ELISA kit (ALPCO,

Salem, NH). Measurements of FFA concentration were carried out with an enzymatic colorimetric assay (Wako NEFA C kit; Wako Chemicals Inc., Richmond VA) adapted for the Pentra C400 Horiba Medical. The kit reagents were reconstituted according to the manufacturer's instructions, and the method was calibrated with a standard solution of 1.0 mmol·L^{-1} oleic acid contained in the kit. Glycerol concentration was measured with a GPO-PAP method, using Glycerol kit (Randox laboratories, Crumlin, UK) adapted for the Pentra C400 Horiba Medical.

Calculations

Anaerobic energy production. The mean rate of anaerobic energy production (i.e., anaerobic ATP utilization rate) and the mean rate of glycogenolysis and glycolysis during the first and the last sprint were determined by the difference in muscle metabolite concentrations in the muscle sample obtained before and immediately after the sprint. The mean rate of anaerobic ATP utilization was estimated from values of ATP, PCr, lactate, and pyruvate, as previously described (23):

$$\text{anaerobic ATP utilization rate} = \frac{2(\Delta \text{ATP}) + \Delta \text{PCr} + 1.5\Delta \text{lactate} + 1.5\Delta \text{pyruvate}}{\text{sprint duration}}$$

The mean rate of glycogenolysis and glycolysis were estimated from lactate, pyruvate, G-6-P, and sprint duration:

$$\text{glycogenolytic rate} = \frac{0.5\Delta \text{lactate} + 0.5\Delta \text{pyruvate} + \Delta \text{G-6-P} + 0.33\Delta \text{G-6-P}}{\text{sprint duration}}$$

$$\text{glycolytic rate} = \frac{0.5\Delta \text{lactate} + 0.5\Delta \text{pyruvate}}{\text{sprint duration}}$$

For ATP and PCr, Δ represents net reduction during the sprint. For lactate, pyruvate, and G-6-P, Δ represents net accumulation during the sprint.

$0.33\Delta \text{G-6-P}$ was used as estimate of glucose-1-phosphate and fructose-6-phosphate accumulation, as previously described (24).

Pyruvate oxidation and lactate diffusion to the circulation were not included in the calculations of the anaerobic ATP utilization rate, glycogenolytic rate and glycolytic rate.

Statistics

Between-trial differences at each sampling time were determined with a linear mixed model including trial and sampling time as fixed factors and subjects as random factor. Within-trial differences were determined by using a linear mixed model for each trial, with sampling time as a fixed factor and subjects as random factor. In addition, to estimate between-trial differences in the exercise-induced changes across sampling time a linear mixed model was used with trial-time interaction as fixed factor, subjects as random factor, and baseline value of the outcome variable as covariate. Model checking was based on Shapiro–Wilk's test and Q–Q plots. In case of heteroscedasticity (i.e., unequal variance), log-transformation was applied before analysis. Model-based *t*-tests with no multiplicity adjustments were used in pairwise comparisons to identify between- and within-trial differences. Linear regression analysis was used to determine the Pearson's correlation coefficient (r) between the rates of preexercise to postexercise change of muscle metabolic and neuromuscular variables. Intertrial reliability of neuromuscular function measurements at baseline was estimated using the intraclass correlation coefficient (ICC) with 95% confidence intervals (95% CI). The level of significance for all analyses was defined as $P < 0.05$. Statistical analyses were carried out with R ver. 3.4.1 and the extension packages *lme4* and *multcomp*. Data are presented as mean ± SEM.

RESULTS

Exercise performance. Peak power output was similar in SS and LS during the first sprint, whereas it was higher in SS than LS during the last sprint ($P = 0.001$) (Fig. 2A). From the first to the last sprint, PPO decreased by 195 ± 66 W ($-16\% \pm 5\%$) in SS ($P < 0.001$) and by 313 ± 51 W ($-29\% \pm 4\%$) in LS ($P < 0.001$), with a smaller decrease in SS compared with LS ($P = 0.041$). The decrement in PPO (PPO$_{dec}$) was $11\% \pm 2\%$ and $16\% \pm 8\%$ in SS and LS, respectively, and was smaller in SS than LS ($P = 0.003$). Mean power output was higher ($P < 0.001$) in SS than LS during both the first and the last sprint. From the first to the last sprint, MPO decreased by 142 ± 57 W ($-13\% \pm 5\%$) in SS ($P < 0.001$) and by 202 ± 34 W ($-25\% \pm 4\%$) in LS ($P < 0.001$). The decrement in MPO (MPO$_{dec}$) was $10\% \pm 2\%$ and $14\% \pm 2\%$ in SS and LS, respectively, with no difference between SS and LS ($P = 0.052$).

Systemic response to exercise. Mean pulmonary $\dot{V}O_2$ during the entire exercise period (i.e., exercise and recovery intervals included) was 38.3 ± 1.5 mL·min^{-1}·kg^{-1} in SS and 27.4 ± 0.6 mL·min^{-1}·kg^{-1} in LS, corresponding to $63\% \pm 2\%$ and $45\% \pm 1\%$ of $\dot{V}O_{2max}$, respectively, and being higher in SS than LS ($P < 0.001$). Mean HR during the exercise period was $82\% \pm 1\%$ and $77\% \pm 1\%$ of HR$_{max}$ in SS and LS, respectively, and was higher in SS than LS ($P < 0.001$).

Neuromuscular function. Intertrial reliability (intraclass correlation coefficient (95% CI)) for neuromuscular function measurements at baseline was 0.93 (0.74–0.98), 0.87 (0.53–0.97), and 0.95 (0.81–0.99) for MVC force, voluntary activation level and peak twitch force, respectively. MVC after exercise was greater in SS than LS ($P < 0.001$). MVC decreased by $10\% \pm 5\%$ and $25\% \pm 6\%$ with exercise in SS ($P = 0.042$) and LS ($P < 0.001$), respectively, with a smaller exercise-induced decrease in SS than LS ($P = 0.046$) (Fig. 2B). Voluntary activation level after exercise was not different between SS and LS and decreased with exercise in both SS ($P < 0.001$) and LS ($P = 0.012$) (Fig. 2C). Peak twitch force after exercise was greater in SS than LS ($P < 0.001$), and decreased by $33\% \pm 5\%$ and $67\% \pm 5\%$ in SS and LS ($P < 0.001$), respectively (Fig. 2D), with a smaller exercise-induced decrease in SS than LS ($P = 0.002$). Time-to-peak twitch after exercise was lower in SS than LS ($P = 0.037$). Neither time-to-peak twitch nor twitch half relaxation time were affected by exercise (Figs. 2E and F).

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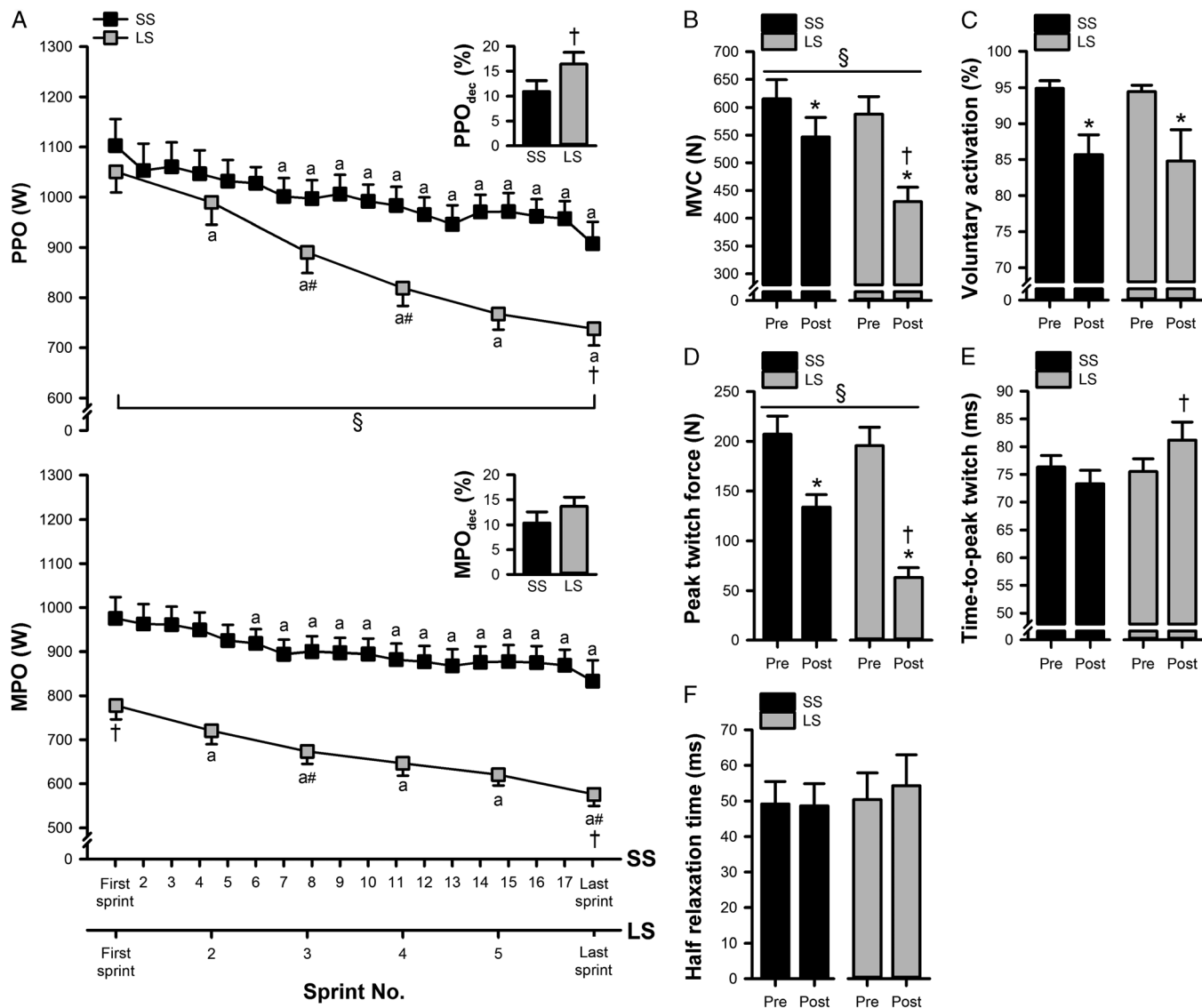
FIGURE 2—Exercise performance and neuromuscular fatigue. The PPO and MPO during each sprint of multiple SS and LS sprints (A). Neuromuscular function before (Pre) and immediately after (Post) multiple SS and LS sprints (B–F). Values are presented as means ± SEM ($n = 11$). [a]Significantly different from first sprint ($P < 0.05$). [#]Significantly different from previous sprint ($P < 0.05$). [†]Significantly different from SS ($P < 0.05$). [§]Significant difference in the change between SS and LS ($P < 0.05$). [*]Significantly different from Pre ($P < 0.05$).

Muscle metabolites, glycogen, and pH. Muscle [ATP] after the last sprint was higher in SS than LS ($P = 0.045$) and decreased during the first sprint in both SS ($P = 0.018$) and LS ($P < 0.001$) and during the last sprint in LS only ($P = 0.001$) (Fig. 3A). Muscle [PCr] was higher in SS than LS after the first sprint ($P < 0.001$), whereas it was lower in SS than LS before the last sprint ($P < 0.001$) (Fig. 3B). Muscle [PCr] decreased during the first and the last sprint in both SS ($P < 0.001$) and LS ($P < 0.001$), with PCr breakdown being smaller in SS than LS during both the first ($P = 0.009$) and the last sprint ($P < 0.001$).

Muscle [lactate] after the first sprint as well as before and after the last sprint was lower in SS than LS ($P < 0.001$) (Fig. 3C). Muscle [lactate] increased during the first sprint in both SS ($P = 0.010$) and LS ($P < 0.001$) and during the last sprint in LS only ($P = 0.046$), with the increase from before

to after the first sprint and from before to after exercise being smaller in SS than LS ($P < 0.001$) (Fig. 3D). Muscle [pyruvate] after the first sprint as well as before and after the last sprint was lower in SS than LS ($P < 0.001$). Muscle [pyruvate] increased during the first sprint in both SS ($P = 0.003$) and LS ($P < 0.001$) and during the last sprint in SS only ($P < 0.001$), with the increase during the first sprint being smaller in SS than LS ($P = 0.001$). Muscle [G-6-P] after the first sprint as well as before and after the last sprint was lower in SS than LS ($P < 0.001$) (Fig. 3E). Muscle [G-6-P] increased during the first sprint in both SS and LS ($P < 0.001$), and during the last sprint in LS only ($P = 0.010$), with the increase from before to after the first sprint and from before to after exercise being smaller in SS than LS ($P < 0.001$).

Muscle [glycogen] was not different between SS and LS at any time point (Fig. 3F). Muscle [glycogen] decreased during
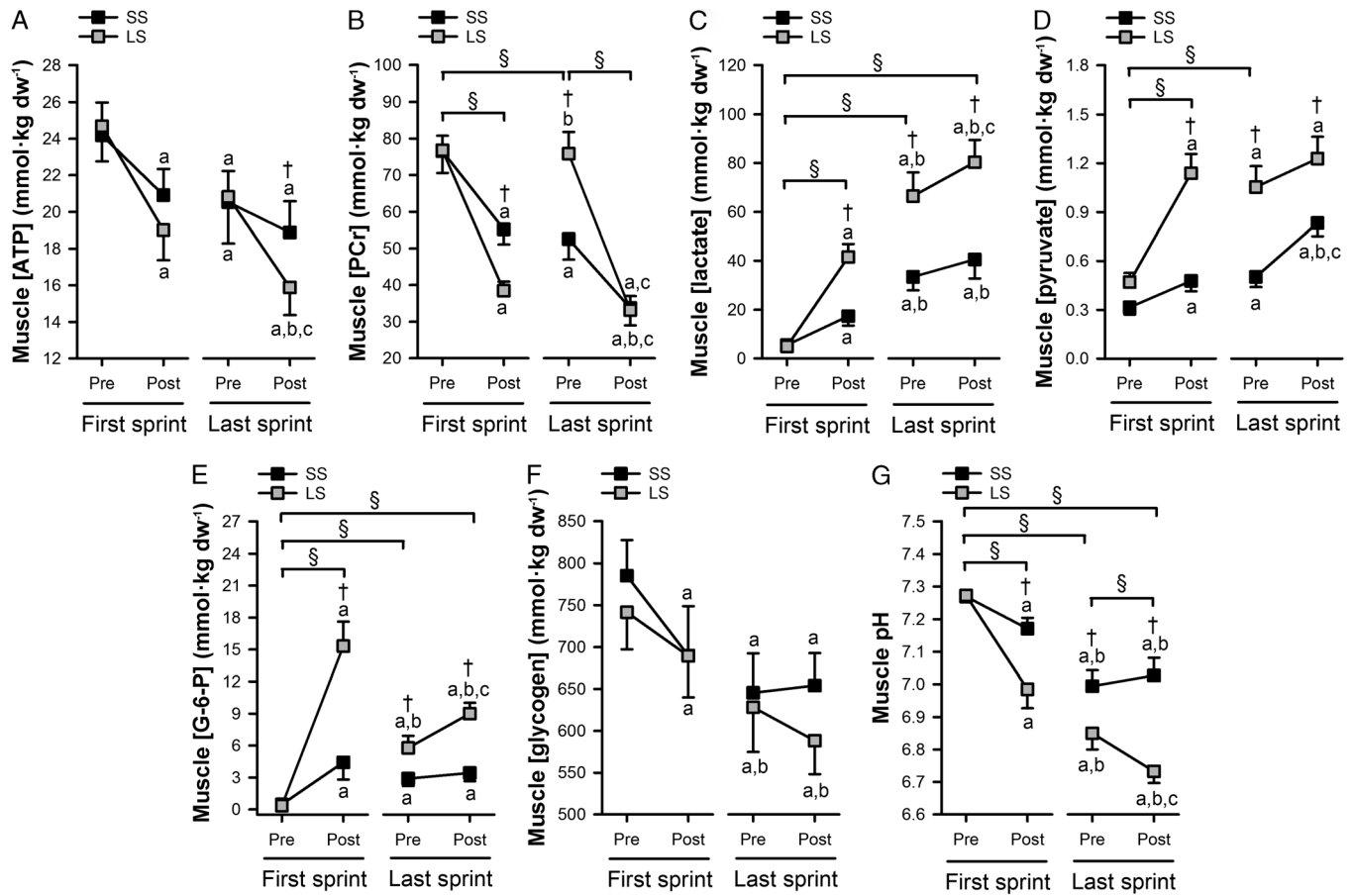
FIGURE 3—Muscle metabolites (A–E), glycogen (F) and pH (G) before (Pre) and immediately after (Post) the first and the last sprint of multiple SS and LS sprints. Values are means ± SEM ($n = 11$). [a]Significantly different from Pre first sprint ($P < 0.05$). [b]Significantly different from Post first sprint ($P < 0.05$). [c]Significantly different from Pre last sprint ($P < 0.05$). [†]Significant difference between SS and LS ($P < 0.05$). [§]Significant difference in the change between SS and LS ($P < 0.05$).

the first sprint in both SS ($P = 0.002$) and LS ($P = 0.021$), and with exercise in both trials ($P < 0.001$).

Muscle pH after the first sprint as well as before and after the last sprint was higher in SS than LS ($P < 0.001$, $P = 0.002$ and $P < 0.001$) (Fig. 3G). Muscle pH decreased during the first sprint in both SS ($P = 0.022$) and LS ($P < 0.001$), and during the last sprint in LS only ($P = 0.023$), with a smaller drop in muscle pH occurring during the first and the last sprint in SS compared with LS ($P = 0.003$ and $P = 0.021$).

Relationships between change in muscle metabolic and neuromuscular variables. Exercise-induced changes in MVC and VA were not related to preexercise to postexercise changes in muscle metabolic variables in either SS or LS. The exercise-induced decline in peak twitch force was related to preexercise to postexercise change in muscle [PCr] in SS ($r = 0.72$, $P = 0.013$), but not in LS ($r = 0.60$, $P = 0.051$). Also, the exercise-induced decline in peak twitch force was related to preexercise to postexercise changes in muscle [ATP], [lactate] and pH in LS ($r = 0.65$, $P = 0.031$; $r = 0.64$, $P = 0.034$; $r = 0.78$, $P = 0.004$), but not in SS ($r = 0.43$, $P = 0.191$; $r = 0.08$, $P = 0.804$; $r = 0.33$, $P = 0.316$).

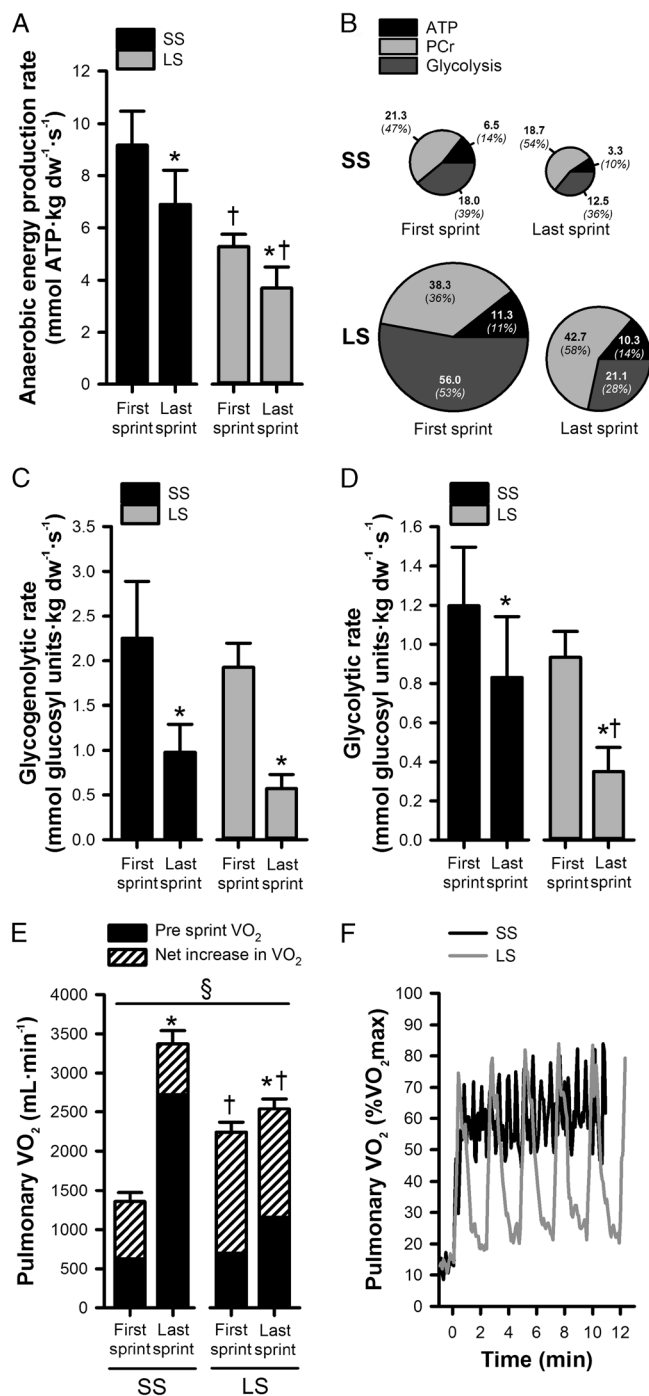**Anaerobic energy production and pulmonary V̇O₂.** The mean rate of anaerobic energy production was higher in

SS than LS during both the first ($P < 0.001$) and the last sprint ($P = 0.002$), and decreased from the first to the last sprint in both SS ($P = 0.005$) and LS ($P = 0.047$) (Fig. 4A). The rate of glycogenolysis was similar in SS and LS during the first and the last sprint, and decreased from the first to the last sprint in both SS and LS ($P < 0.001$) (Fig. 4C). The rate of glycolysis was higher in SS than LS during the last sprint ($P = 0.049$) and decreased from the first to the last sprint in both SS ($P = 0.001$) and LS ($P < 0.001$) (Fig. 4D).

Mean pulmonary V̇O₂ during the first sprint was lower in SS than LS ($P < 0.001$), whereas it was higher in SS than LS during the last sprint ($P < 0.001$). Mean pulmonary V̇O₂ increased from the first to the last sprint in both SS ($P < 0.001$) and LS ($P = 0.023$), with the increase being greater in SS than LS ($P < 0.001$) (Fig. 4E).

Blood metabolites, pH, and bicarbonate ions. Blood metabolite, pH, and bicarbonate ion concentrations are presented as Supplemental Digital Content (see Figure, Supplemental Digital Content 1, Blood metabolites, pH and bicarbonate ions, http://links.lww.com/MSS/B548).

Plasma hormones, FFA, and glycerol. Plasma hormone, FFA, and glycerol levels are reported as Supplemental Digital Content (see Table, Supplemental Digital Content 2,

FIGURE 4—Anaerobic energy production and pulmonary V̇O₂. Mean rate of anaerobic energy production (A), anaerobic ATP sources (B; note that the area of each *circle* represents total anaerobic ATP turnover, bold and italic numbers indicate absolute [mmol ATP·kg dw⁻¹] and relative [%] contribution to total anaerobic ATP turnover, respectively), mean rate of glycogenolysis (C) and glycolysis (D), and mean pulmonary V̇O₂ (E) during the first and the last sprint of multiple SS and LS sprints. Values are means ± SEM ($n = 11$). *Significantly different from first sprint ($P < 0.05$). †Significantly different from SS ($P < 0.05$). §Significant difference in the change from the first to the last sprint between SS and LS ($P < 0.05$). Pulmonary V̇O₂ during multiple SS and LS exercise in a representative subject (F).

Plasma catecholamines, insulin, FFA, and glycerol concentration before (Pre) and immediately after (Post) multiple short- (SS) and long-duration sprint (LS) exercise, http://links.lww.com/MSS/B549).

DISCUSSION

The major findings of the present study were that the greater impairments in performance attained during multiple long-sprint (LS) exercise were associated with a greater preexercise to postexercise decline in MVC and peak twitch force compared with multiple short-sprint (SS) exercise, whereas voluntary activation level decreased similarly in SS and LS. In addition, PCr utilization during a single sprint was smaller in SS than LS, but PCr availability before the last sprint was lower in SS than LS. The accumulation of muscle lactate and H⁺ was lower in SS than LS, whereas the mean rate of anaerobic energy production during the first and the last sprint was higher in SS than LS and decreased from the first to the last sprint in both trials. Lastly, the mean rate of glycolysis was similar in SS and LS during the first sprint but was higher in SS than LS during the last sprint.

Performance and neuromuscular fatigue development. The greater decline in PPO observed in LS than SS indicates that, despite the fourfold longer recovery periods characterizing LS, the capacity to reproduce maximal power outputs and exert maximal dynamic force over multiple sprints was compromised to a greater extent as prolonged sprints were repeated. In accordance, the capacity to produce voluntary force was impaired to a greater extent after LS than SS, with the decline in MVC being 2.5-fold greater in LS.

These alterations in maximal power and force were likely due to the development of neuromuscular fatigue, which is characterized by the interdependency of central and peripheral factors. In the present study, central fatigue was represented by the reduced ability of the central nervous system to activate the contracting skeletal muscle as measured by the interpolated twitch technique (20), whereas peripheral fatigue was evaluated as the decrease in contractile properties evoked by supramaximal electrical stimulation of the exercising muscle (25). The multiple-sprint protocols elicited similar exercise-induced changes in voluntary activation level, implying that the magnitude of central fatigue was independent of sprint duration. This finding coincides with other studies showing no apparent differences in voluntary activation level after repeated short-sprints interspersed with recovery intervals of diverse duration (5,7). On the other hand, the observation that LS elicited a greater decline in peak twitch force and a prolongation of time to peak twitch force compared with SS suggests that a greater degree of peripheral fatigue was attained in response to multiple long-sprints versus short-sprints. In contrast to the present findings, no differences in the contractile properties of the quadriceps muscle were observed between multiple short-sprint protocols differing for exercise-to-recovery ratio, with reports of either a ~40% or ~50% decline in peak twitch force when 10-s sprints were interspersed with either

10 versus 30 s of recovery (7) or 30 versus 180 s of recovery (5), respectively. Taken together, it can be argued that markedly different exercise-induced metabolic disturbances are necessary to detect differential deficits in contractile properties of the fatigued muscle.

It is worth noting that other factors than metabolic disturbances may have contributed to the observed degrees of fatigue development. First, in view of the purported influence of the resistive load on the rate of decline in power output during multiple-sprint exercise (26), the observed differences in power output and fatigue between SS and LS may be partly attributed to the different resistive loads (i.e., breaking forces) used during the two trials. Second, as neuromuscular function recovery occurs within the first 2 min after exercise (27,28), and considering that neuromuscular function was assessed between the first and the second minute after exercise in the current study, we cannot exclude that the extent of central and peripheral fatigue would have been more marked immediately after exercise.

Interplay between muscle metabolic perturbations and peripheral fatigue. Given the critical role of reduced substrate availability and increased metabolic by-products accumulation in limiting the ability to reproduce performance over subsequent sprints, the multiple-sprint exercise models employed in the present study were designed to elicit marked, but differential, perturbations within both the phosphagen and glycolytic energy system. The lower muscle [PCr] observed before the last sprint in SS than LS suggests that limitations in PCr availability may have played a more prominent role in peripheral fatigue development during the multiple short- than the long-sprint protocol. This was further supported by the relationship between postexercise muscle [PCr] and peak twitch force observed in SS, but not in LS. Also, consistent with the different patterns of ATP hydrolysis and PCr breakdown observed between SS and LS, and given that changes in [PCr] generally resemble those in inorganic phosphate (P_i) (23), increased levels of P_i may have contributed to peripheral fatigue development by interacting with the sarcoplasmic reticulum calcium (Ca^{2+}) release (29). Thus, even though intracellular [P_i] was not measured in the present study, it is conceivable that P_i-dependent alterations in sarcoplasmic reticulum Ca^{2+} cycling contributed to the larger degree of peripheral fatigue observed in LS. Indeed, we recently demonstrated that phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), an indicator of alterations in myoplasmic [Ca^{2+}], was greater after multiple long-sprint exercise compared with short-sprint exercise (16), suggesting considerable differences in Ca^{2+} flux during SS and LS.

The lower rise in [lactate] and [H^+] observed during SS than LS may have contributed to the differential level of peripheral fatigue during the trials. Although the role played by lactate and H^+ in muscle fatigue development remains controversial (2), there is evidence that intense contraction-induced intracellular acidosis is linked to an increased K^+ accumulation in muscle interstitium (14) as well as to a reduced Ca^{2+} sensitivity of the contractile proteins (30), thus, possibly contributing to impairments in muscle function. In support, elevations in [H^+] have been shown to act synergistically with P_i to directly inhibit myosin's force-generating capacity during muscle fatigue (31). In addition, the lower mean glycolytic rate observed during the last sprint in LS compared with SS may have been caused by a H^+-mediated downregulation of glycogenolytic/glycolytic enzyme activity (13), possibly resulting in a greater degree of peripheral fatigue due to limitations in ATP supply.

Muscle glycogen breakdown may contribute to peripheral fatigue by causing decreased sarcoplasmic reticulum Ca^{2+} release (2). However, the current finding that muscle glycogen was depleted to a similar extent during SS and LS suggests that glycogen availability was not among the major contributors to the differential degree of peripheral fatigue between SS and LS.

Muscle metabolic response to multiple-sprint exercise in trained individuals. A purpose of the present study was to characterize the metabolic profile of the two high-intensity intermittent exercise regimes and to provide novel data with regards to the muscle metabolic responses to multiple-sprint exercise in well-trained individuals. The observation that intramuscular [PCr] decreased to a greater extent during prolonged sprints is not surprising. Likewise, the finding that, in SS, muscle [PCr] after the first sprint was similar to that measured before the last sprint (i.e., ~70% of baseline value) is in accordance with prior evidence indicating that PCr stores may only be partially restored during multiple short-sprint exercise including recovery periods shorter than 60 s (32,33). Instead, the finding that, in LS, muscle [PCr] before the last sprint was not different from muscle [PCr] at baseline indicates that 2 min of recovery were sufficient to allow for a complete replenishment of intramuscular PCr stores, even after multiple sprints. This finding contrasts with data in recreationally active individuals exhibiting incomplete resynthesis of muscle PCr (i.e., ~80% of baseline value) 2 and 4 min into recovery from either a single 20-s or 30-s sprint, respectively (9,23,34). However, it is likely that the elevated oxidative capacity associated with the higher training status of the subjects involved in the present study promoted a faster PCr resynthesis rate (35).

With regards to the magnitude and pattern of muscle lactate accumulation, the current results are in line with reports showing that, while lactate gradually accumulates in the exercising muscles as short sprints are repeated (33), a single long sprint induces an approximate 20-fold rise in muscle lactate levels, (9,23,34). Consistently, the observation that the decrease in muscle pH was greater during LS than SS is in agreement with the more marked drop in intramuscular pH observed in response to two 30-s sprints interspersed with 4 min of recovery (pH drop ~0.4 unit; (9)) than that found after repeated 6-s sprints interspersed with 30 s of recovery (pH drop ~0.2 unit; (8,36)). Notably, the trained individuals involved in the current study displayed lower lactate accumulation rates during a single sprint compared with those reported in recreationally active individuals (9,23,33,34), likely due to training status-dependent differences in muscle lactate production and/or removal; an assumption consistent with the greater muscle buffer capacity documented in trained compared with sedentary

individuals (37). A further explanation might be linked to the higher rate at which trained muscle $\dot{V}O_2$ increases at the onset of a sprint, resulting in a lower O_2 deficit and, hence, in lower lactate accumulation (38).

The observation that the mean anaerobic energy production rate (i.e., anaerobic ATP utilization rate) was lower during the first and the last sprint in LS than SS is in line with prior data showing a substantial decrease (~50%) in anaerobic ATP utilization rate during the second compared to the first half of a 20-s sprint (23). In addition, the lower anaerobic energy production rate observed during long-duration sprints may have contributed to the substantially lower mean power elicited during each single sprint in LS than SS. However, given the complex interplay between the metabolic pathways providing energy during exercise bouts of different duration, caution is needed in interpreting differences in anaerobic energy turnover rates between 5- and 20-s sprints.

Consistent with Gaitanos et al. (33), the relative decline in the anaerobic ATP utilization rate occurring from the first to the last sprint in SS was twofold larger than the relative decrease in MPO. In contrast, in LS, the anaerobic ATP utilization rate decreased by 30% from the first to the last sprint against a 25% fall in MPO. This discrepancy might be explained by a differential aerobic ATP provision as multiple-sprint exercise progresses, as suggested by the greater increase in pulmonary $\dot{V}O_2$ occurring from the first to the last sprint in SS compared with LS. Indeed, the 2.5-fold higher pulmonary $\dot{V}O_2$ observed during the last compared with the first sprint in SS may indicate a considerable rise in the aerobic ATP supply and is in line with the progressive increase in pulmonary oxygen uptake observed by McGawley and Bishop (39) during repeated short-sprint exercise. By contrary, only a slight increase in pulmonary $\dot{V}O_2$ occurred from the first to the last sprint in LS, which partly agrees with the documented lack of difference in the aerobic-derived ATP during two 20 s sprints interspaced with 2 min of recovery (23).

Notably, the observed mean rates of PCr breakdown, lactate accumulation and glycogenolysis/glycolysis during the first and the last sprint were markedly lower than those documented during sprints of similar duration in other studies (9,23,33). Such differences might be related to the higher training-status of the subjects involved in the current study, as training-induced enhancements in mitochondrial volume/function have been proposed to result in lower rates of PCr and glycogen breakdown (40). Likewise, the decline in the contribution of glycolysis to anaerobic ATP turnover occurring from the first to the last short sprint was modest compared to that reported by Gaitanos et al. (33), likely due to the lower reliance on glycolysis characterizing the endurance-trained population involved in the present study. Lastly, it should be acknowledged that the metabolic responses to high-intensity intermittent exercise might differ between individuals with different training backgrounds, thus it might not be possible to extrapolate the present findings to sprint or team sport athletes.

SUMMARY

The present study provides new insights into the mechanisms limiting high-intensity intermittent exercise capacity in endurance-trained individuals, showing that the capacity to repeat all-out efforts during multiple-sprint exercise is compromised by the occurrence of fatigue of both central (neural) and peripheral (muscular) origin, with longer sprint intervals resulting in larger impairments in performance along with a greater development of peripheral fatigue but with similar degrees of central fatigue compared with short sprint intervals. Among a range of metabolic perturbations possibly impairing muscle function, intramuscular accumulation of lactate and the associated alterations in acid–base homeostasis may have contributed to the greater degree of peripheral fatigue elicited by multiple long-sprint exercise compared with short-sprint exercise.

REFERENCES

1. Green HJ. Mechanisms of muscle fatigue in intense exercise. *J Sports Sci*. 1997;15(3):247–56.
2. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev*. 2008;88(1):287–332.
3. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev*. 2001;81(4):1725–89.
4. Collins BW, Pearcey GEP, Buckle NCM, Power KE, Button DC. Neuromuscular fatigue during repeated sprint exercise: underlying physiology and methodological considerations. *Appl Physiol Nutr Metab*. 2018;43(11):1166–75.
5. Hureau TJ, Ducrocq GP, Blain GM. Peripheral and central fatigue development during all-out repeated cycling sprints. *Med Sci Sports Exerc*. 2016;48(3):391–401.
6. Pearcey GE, Murphy JR, Behm DG, Hay DC, Power KE, Button DC. Neuromuscular fatigue of the knee extensors during repeated maximal intensity intermittent-sprints on a cycle ergometer. *Muscle Nerve*. 2015;51(4):569–79.
7. Monks MR, Compton CT, Yetman JD, Power KE, Button DC. Repeated sprint ability but not neuromuscular fatigue is dependent on short versus long duration recovery time between sprints in healthy males. *J Sci Med Sport*. 2017;20(6):600–5.
8. Mendez-Villanueva A, Edge J, Suriano R, Hamer P, Bishop D. The recovery of repeated-sprint exercise is associated with PCr resynthesis, while muscle pH and EMG amplitude remain depressed. *PLoS One*. 2012;7(12):e51977.
9. Bogdanis GC, Nevill ME, Boobis LH, Lakomy HK. Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. *J Appl Physiol*. 1996;80(3):876–84.
10. Preen D, Dawson B, Goodman C, Lawrence S, Beilby J, Ching S. Effect of creatine loading on long-term sprint exercise performance and metabolism. *Med Sci Sports Exerc*. 2001;33(5):814–21.
11. Lanza IR, Wigmore DM, Befroy DE, Kent-Braun JA. In vivo ATP production during free-flow and ischaemic muscle contractions in humans. *J Physiol*. 2006;577(Pt 1):353–67.

BASIC SCIENCES

12. Bangsbo J, Juel C. Counterpoint: lactic acid accumulation is a disadvantage during muscle activity. *J Appl Physiol (1985)*. 2006;100(4):1412–3; discussion 3–4.

13. Spriet LL, Lindinger MI, McKelvie RS, Heigenhauser GJ, Jones NL. Muscle glycogenolysis and H+ concentration during maximal intermittent cycling. *J Appl Physiol (1985)*. 1989;66(1):8–13.

14. Street D, Nielsen JJ, Bangsbo J, Juel C. Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle interstitium. *J Physiol*. 2005;566(Pt 2):481–9.

15. Bishop D, Edge J, Goodman C. Muscle buffer capacity and aerobic fitness are associated with repeated-sprint ability in women. *Eur J Appl Physiol*. 2004;92(4–5):540–7.

16. Fiorenza M, Gunnarsson TP, Hostrup M, et al. Metabolic stress-dependent regulation of the mitochondrial biogenic molecular response to high-intensity exercise in human skeletal muscle. *J Physiol*. 2018;596(14):2823–40.

17. Girard O, Mendez-Villanueva A, Bishop D. Repeated-sprint ability—part I: factors contributing to fatigue. *Sports Med*. 2011;41(8):673–94.

18. Iaia FM, Bangsbo J. Speed endurance training is a powerful stimulus for physiological adaptations and performance improvements of athletes. *Scand J Med Sci Sports*. 2010;20(2 Suppl):11–23.

19. Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. *J Appl Physiol (1985)*. 2009;106(2):701–10.

20. Merton PA. Voluntary strength and fatigue. *J Physiol*. 1954;123(3):553–64.

21. Strojnik V, Komi PV. Neuromuscular fatigue after maximal stretch-shortening cycle exercise. *J Appl Physiol (1985)*. 1998;84(1):344–50.

22. Lowry OH, Passonneau JV. *A Flexible System of Enzymatic Analysis [by] Oliver H. Lowry [and] Janet V. Passonneau*. New York: Academic Press; 1972. xii, 291 p. p.

23. Bogdanis GC, Nevill ME, Lakomy HK, Boobis LH. Power output and muscle metabolism during and following recovery from 10 and 20 s of maximal sprint exercise in humans. *Acta Physiol Scand*. 1998;163(3):261–72.

24. Hultman E, Sjoholm H. Energy metabolism and contraction force of human skeletal muscle in situ during electrical stimulation. *J Physiol*. 1983;345:525–32.

25. Place N, Maffiuletti NA, Martin A, Lepers R. Assessment of the reliability of central and peripheral fatigue after sustained maximal voluntary contraction of the quadriceps muscle. *Muscle Nerve*. 2007;35(4):486–95.

26. Bogdanis GC, Papaspyrou A, Theos A, Maridaki M. Influence of resistive load on power output and fatigue during intermittent sprint cycling exercise in children. *Eur J Appl Physiol*. 2007;101(3):313–20.

27. Froyd C, Millet GY, Noakes TD. The development of peripheral fatigue and short-term recovery during self-paced high-intensity exercise. *J Physiol*. 2013;591(5):1339–46.

28. Mira J, Lapole T, Souron R, Messonnier L, Millet GY, Rupp T. Cortical voluntary activation testing methodology impacts central fatigue. *Eur J Appl Physiol*. 2017;117(9):1845–57.

29. Allen DG, Clugston E, Petersen Y, Roder IV, Chapman B, Rudolf R. Interactions between intracellular calcium and phosphate in intact mouse muscle during fatigue. *J Appl Physiol (1985)*. 2011;111(2):358–66.

30. Chin ER, Allen DG. The contribution of pH-dependent mechanisms to fatigue at different intensities in mammalian single muscle fibres. *J Physiol*. 1998;512(Pt 3):831–40.

31. Woodward M, Debold EP. Acidosis and phosphate directly reduce myosin's force-generating capacity through distinct molecular mechanisms. *Front Physiol*. 2018;9:862.

32. Dawson B, Goodman C, Lawrence S, et al. Muscle phosphocreatine repletion following single and repeated short sprint efforts. *Scand J Med Sci Sports*. 1997;7(4):206–13.

33. Gaitanos GC, Williams C, Boobis LH, Brooks S. Human muscle metabolism during intermittent maximal exercise. *J Appl Physiol*. 1993;75(2):712–9.

34. Parolin ML, Chesley A, Matsos MP, Spriet LL, Jones NL, Heigenhauser GJ. Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. *Am J Phys*. 1999;277(5 Pt 1):E890–900.

35. McMahon S, Jenkins D. Factors affecting the rate of phosphocreatine resynthesis following intense exercise. *Sports Med*. 2002;32(12):761–84.

36. Bishop D, Edge J, Davis C, Goodman C. Induced metabolic alkalosis affects muscle metabolism and repeated-sprint ability. *Med Sci Sports Exerc*. 2004;36(5):807–13.

37. Sahlin K, Henriksson J. Buffer capacity and lactate accumulation in skeletal muscle of trained and untrained men. *Acta Physiol Scand*. 1984;122(3):331–9.

38. Poole DC, Barstow TJ, McDonough P, Jones AM. Control of oxygen uptake during exercise. *Med Sci Sports Exerc*. 2008;40(3):462–74.

39. McGawley K, Bishop DJ. Oxygen uptake during repeated-sprint exercise. *J Sci Med Sport*. 2015;18(2):214–8.

40. Katz A, Westerblad H. Regulation of glycogen breakdown and its consequences for skeletal muscle function after training. *Mamm Genome*. 2014;25(9–10):464–72.