The Role of Muscle Glycogen Content and Localization in High-Intensity Exercise Performance: A Placebo-Controlled Trial

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[1]Department of Public Health, Aarhus University, Aarhus, DENMARK; [2]Department of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, DENMARK; [3]Steno Diabetes Center Aarhus, Aarhus University, Aarhus, DENMARK; and [4]Centre of Health Science, University of the Faroe Islands, Tórshavn, FAROE ISLANDS

ABSTRACT

VIGH-LARSEN, J. F., N. ØRTENBLAD, J. NIELSEN, O. EMIL ANDERSEN, K. OVERGAARD, and M. MOHR. The Role of Muscle Glycogen Content and Localization in High-Intensity Exercise Performance: A Placebo-Controlled Trial. *Med. Sci. Sports Exerc.*, Vol. 54, No. 12, pp. 2073–2086, 2022. **Purpose:** We investigated the coupling between muscle glycogen content and localization and high-intensity exercise performance using a randomized, placebo-controlled, parallel-group design with emphasis on single-fiber subcellular glycogen concentrations and sarcoplasmic reticulum Ca^{2+} kinetics. **Methods:** Eighteen well-trained participants performed high-intensity intermittent glycogen-depleting exercise, followed by randomization to a high- (CHO; ~1 g CHO·kg^{-1}·h^{-1}; $n = 9$) or low-carbohydrate placebo diet (PLA, <0.1 g CHO·kg^{-1}·h^{-1}; $n = 9$) for a 5-h recovery period. At baseline, after exercise, and after the carbohydrate manipulation assessments of repeated sprint ability (5 × 6-s maximal cycling sprints with 24 s of rest), neuromuscular function and ratings of perceived exertion during standardized high-intensity cycling (~90% W_{max}) were performed, while muscle and blood samples were collected. **Results:** The exercise and carbohydrate manipulations led to distinct muscle glycogen concentrations in CHO and PLA at the whole-muscle (291 ± 78 vs 175 ± 100 mmol·kg^{-1} dry weight (dw), $P = 0.020$) and subcellular level in each of three local regions ($P = 0.001$–0.046). This was coupled with near-depleted glycogen concentrations in single fibers of both main fiber types in PLA, especially in the intramyofibrillar region (within the myofibrils). Furthermore, increased ratings of perceived exertion and impaired repeated sprint ability (~8% loss, $P < 0.001$) were present in PLA, with the latter correlating moderately to very strongly ($r = 0.47$–0.71, $P = 0.001$–0.049) with whole-muscle glycogen and subcellular glycogen fractions. Finally, sarcoplasmic reticulum Ca^{2+} uptake, but not release, was superior in CHO, whereas neuromuscular function, including prolonged low-frequency force depression, was unaffected by dietary manipulation. **Conclusions:** Together, these results support an important role of muscle glycogen availability for high-intensity exercise performance, which may be mediated by reductions in single-fiber levels, particularly in distinct subcellular regions, despite only moderately lowered whole-muscle glycogen concentrations. **Key Words:** FATIGUE, INTERMITTENT EXERCISE, METABOLISM, EXCITATION–CONTRACTION COUPLING, CARBOHYDRATE

The association between muscle glycogen and exercise tolerance in prolonged exercise has been extensively studied during the past century and is well established (1). In contrast, during short-duration high-intensity exercise (>100% maximal oxygen consumption ($\dot{V}O_{2max}$)), the relationship between muscle glycogen and performance is less clear. During a single bout of high-intensity exercise, whole-muscle glycogen is rapidly metabolized, leading to large declines even after short durations (e.g., ~20%–30% reductions after ~30-s all-out exercise [2]) but with impaired exercise tolerance well in advance of total muscle glycogen depletion. However, when high-intensity exercise is repeated several times, or when a single high-intensity bout has to be performed with suboptimal initial muscle glycogen levels, such as during team-sport activities or at the finishing line of a cycling road race, intramuscular glycogen concentrations may become limiting for performance. Accordingly, reduced repeated sprint ability (RSA) is apparent at the end stages of ice hockey and men's and women's football games in concert with lowered muscle glycogen content (3–5).

In investigations directly testing the importance of muscle glycogen for high-intensity exercise tolerance, some (6–8) but not all (9,10) studies support such coupling. For example, Balsom et al. (6) observed accelerated fatigue development during repeated 6-s high-intensity efforts, whereas Hargreaves et al. (10) failed to see any impairments in 30-s all-out cycling performance when muscle glycogen contents were lowered before testing. This discrepancy may relate to the muscle glycogen concentrations achieved, which were only moderately lowered to ~462 mmol·kg^{-1} dw in the study by Hargreaves et al. (10)

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compared with a low level of 180 mmol·kg^{-1} dw in the study by Balsom et al. (6). In line with this, a glycogen threshold level of 250–300 mmol·kg^{-1} dw has been suggested, below which performance is impaired, although a scarcity of studies with actual muscle sampling is available to confirm this proposition and with vastly different exercise test scenarios (11). Moreover, a lack of placebo control questions any direct associations between muscle glycogen and performance, because even the perception of carbohydrate availability has been shown to alter the capacity for intermittent exercise (12).

Providing a further perspective in addition to whole-muscle glycogen measured in homogenates, muscle glycogen displays a heterogeneous storage and depletion pattern in individual fibers during exercise (11). This may entail that a significant subgroup of fibers reach critically low glycogen levels despite nondepleted whole-muscle concentrations and could explain a potential association between reduced muscle glycogen and high-intensity performance. Furthermore, glycogen is stored and utilized heterogeneously not only between fibers but also within fibers in distinct subcellular fractions. Hence, glycogen is located beneath the sarcolemma (subsarcolemmal (SS) glycogen), between myofibrils (intermyofibrillar (IMF) glycogen) and within myofibrils close to the contractile filaments (intramyofibrillar (intra) glycogen) (13). Interestingly, these subcellular fractions may support separate energy-requiring processes and provide a direct connection between muscle glycogen and key processes of excitation-contraction coupling (14–16). Therefore, depletion of specific subcellular fractions may compose a further link between glycogen reductions and impaired exercise tolerance. Especially, the intra glycogen fraction has been associated with fatigue resistance in rodent and human experiments, as well as altered tetanic Ca^{2+} and sarcoplasmic reticulum (SR) Ca^{2+} release, suggesting a potential particular importance of this pool (16–19). Reductions in muscle glycogen in general have been linked with impaired Ca^{2+} release (7,17,20), which has been shown to induce prolonged low-frequency force depression *in vitro* (specific force depression at submaximal firing frequencies), although this is yet to be clearly elucidated in human models (21).

The aim of the present investigation was therefore to examine how lowered muscle glycogen content affects high-intensity intermittent exercise performance using a randomized, double-blind, placebo-controlled design, with special reference to subcellular glycogen localization. In addition, neuromuscular function including prolonged low-frequency force depression was assessed to study potential alterations related to impaired Ca^{2+} release. We hypothesized that lowered muscle glycogen content, especially of the intra glycogen fraction, would impair high-intensity exercise tolerance. Moreover, we hypothesized that this would be accompanied by attenuated Ca^{2+} regulation and exacerbated prolonged low-frequency force depression.

METHODS

Ethical Approval

The study adhered to the code of ethics of the Declaration of Helsinki and was approved by the Central Denmark Region Committees on Health Research (application number 1-10-72-15-20). Before entering the study, written informed consent was obtained from all participants, who were informed about potential risks and discomforts and that they were able to withdraw from the study at any time.

Participants

Twenty well-trained male participants were enrolled in the study and randomized to two different experimental groups. To minimize variations between groups, randomization was performed stratified for $\dot{V}O_{2max}$ by using a cutoff level of 55 mL·kg^{-1}·min^{-1}. During the data collection, two participants were excluded because of technical errors (one from each experimental group), resulting in a total of 18 participants included in the data processing (see Table 1 for participant characteristics). Inclusion criteria were a $\dot{V}O_{2max}$ greater than 50 mL·kg^{-1}·min^{-1} and no injuries or illnesses preventing full participation in the study. The participants engaged in regular physical exercise at recreational or competitive level 3–5 times weekly (e.g., team-sport activities, running, and cycling) and were accustomed to high-intensity exercise.

Experimental Overview

The study was designed as a randomized, double-blind, placebo-controlled, parallel-group trial. The participants visited the laboratory twice within 2 wk before a main experimental day: first visit for determination of $\dot{V}O_{2max}$ and familiarization with neuromuscular assessments (voluntary and electrically induced isometric contractions) and second visit for familiarization with a repeated sprint test, a 2-min fixed-intensity cycling test, and a cycling-based glycogen-depleting exercise protocol. On the main experimental day (Fig. 1), participants performed three periods of high-intensity intermittent glycogen-depleting cycling exercise followed by randomization to a high- (CHO, ~1 g CHO·kg^{-1}·h^{-1}) or low-carbohydrate placebo diet (PLA, <0.1 g CHO·kg^{-1}·h^{-1}) for a 5-h recovery period. This was coupled with performance assessments, muscle and blood sampling, and continuous tracking of heart rate (HR) and ratings of perceived exertion (RPE) as described in detail hereinafter. The participants refrained from intake of tobacco, alcohol, or caffeine and did not participate in any strenuous exercise in the 24-h period leading up to the experimental day.

Preliminary Testing

Maximal oxygen consumption test. A graded exercise test to exhaustion was performed on a cycling ergometer

TABLE 1. Participant characteristics.

	CHO (n = 9)	PLA (n = 9)
Age (yr)	24.0 ± 1.3	26.4 ± 2.4*
Body mass (kg)	78.2 ± 9.5	78.5 ± 8.2
Body fat (%)	8.9 ± 3.0	9.8 ± 2.1
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	56.1 ± 3.7	57.6 ± 5.4
W_{max} (W)	360 ± 31	370 ± 35
MHCI, IIa, IIx (%)	50 ± 8; 45 ± 6; 6 ± 5	51 ± 4; 45 ± 4; 4 ± 4

Data are presented as mean ± SD. *Between-group difference ($P \leq 0.05$).

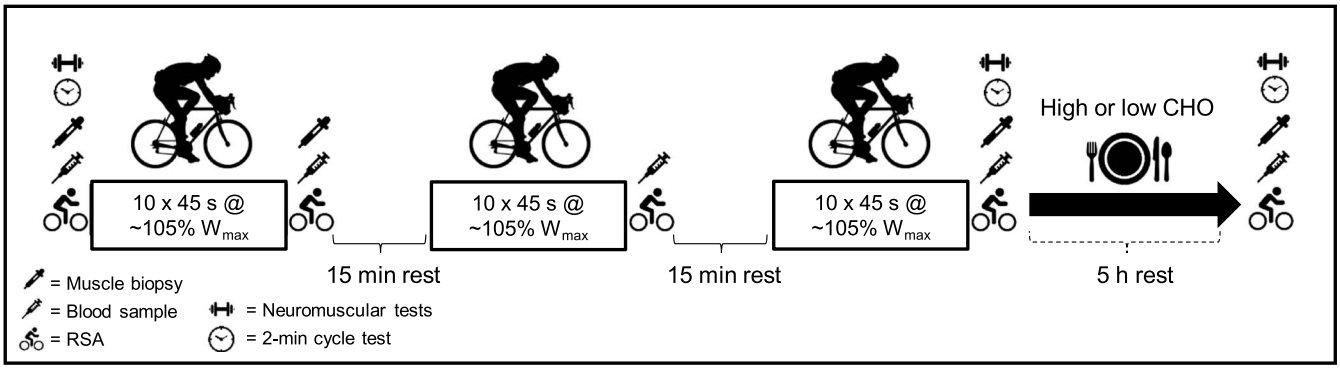

FIGURE 1—Overview of the main experimental day, including muscle and blood sampling, as well as performance assessments before and after three exercise periods of 10 × 45 s of high-intensity exercise at ~105% W_{max} interspersed by 135 s of passive recovery between bouts and 15 min between periods. After the exercise periods and performance assessments, a high-carbohydrate or low-carbohydrate (placebo) diet was administered for a 5-h recovery period before repeating all sampling and test procedures.

(Monark 894E Peakbike) to determine $\dot{V}O_{2max}$. Expired air was analyzed using an online gas analysis system (Oxigraf O2CPX; Oxigraf Inc., Sunnyvale, CA) interfaced with a computer running Innocor software (Version 8.0; Innovision ApS, Odense, Denmark). The intensity started at 120 W for a 10-min warm-up and was then increased by 25 W every minute until exhaustion. The test was performed at a fixed pedaling rate of 80 rounds per minute (RPM) and terminated when participants were unable to maintain the required rate for 5 consecutive seconds. HR was monitored continuously throughout the test to determine maximum HR, and watt max (W_{max}) was determined using the following formula:

$$W_{max} = \text{last fully completed increment } (W) + ((\text{s at final increment}/60\ \text{s}) \times 25\ \text{W})$$

Familiarization Days 1 and 2

Familiarization procedures were performed during two visits to the laboratory within 2 wk before the main experimental day. During the first visit, familiarization with the neuromuscular assessments was performed and individual customization of dynamometer settings determined, as described in detail under the Neuromuscular Assessments section. During the second visit, a repeated sprint test and a 2-min fixed-intensity test were performed similar to that during the experimental day. Finally, one period of the high-intensity intermittent glycogen-depletion protocol was performed for familiarization purposes and to test whether the predetermined load was tolerable. This load was determined from the W_{max} test, as outlined in detail hereinafter, and was intended to yield an RPE between 6 and 8 on the Borg CR10 scale (22) after the first period of exercise. Based on pilot testing, this RPE range was appropriate to be able to complete all three periods reaching maximal levels of exertion during the final period with only minor load adjustments during the protocol. If the participants reached very high RPE scores (9 or 10) during the first period, the load was decreased slightly (~5%–10%) for the experimental day, whereas the load was slightly increased if RPE did not increase greater than 5.

Experimental Day

On the experimental day, the participants arrived at the laboratory between 8 and 9 AM after consuming a standardized breakfast ~1.5 h before arrival comprising 1.8 g carbohydrate·kg^{-1} (oats, skimmed milk, sugar, raisins, juice, and water), corresponding to ~22 kJ·kg^{-1}. After a 15-min rest period, a baseline thigh muscle biopsy and a blood sample from a catheter inserted in an antecubital vein were obtained. The participants then performed a ~15-min warm-up protocol on a cycle ergometer before the first repeated sprint test. Afterward, a 5-min rest period was allowed before commencing the 2-min fixed-intensity test at 90% W_{max}. The participants then rested for a further 10 min before starting the neuromuscular test procedures, which consisted of voluntary and electrically induced contractions. After these assessments, another 5 min of cycling warm-up at a self-selected pace was performed before starting the glycogen-depletion protocol, which consisted of three periods of intermittent high-intensity cycling interspersed by two intermissions of ~15 min. After each cycling bout, RPE scores were instantly collected, whereas HR was measured continuously using a HR chest strap (Polar H10). A muscle biopsy was obtained instantly after period 1 (for analyses included in a companion article) and after the last period of glycogen-depleting exercise, whereas RSA was measured (~2.5 min after exercise) and blood samples were collected after each of the three periods. After the repeated sprint test after the last period, a 5-min rest period was allowed similar to that during the baseline condition before completing the 2-min fixed-intensity test followed by another 10-min of rest before the neuromuscular assessments. After a 5-h rest period with intake of the specific diet, all baseline measures were repeated in the same order as before the glycogen-depleting exercise, including a resting muscle biopsy and blood sample, RSA testing, the 2-min fixed-intensity test, and neuromuscular assessments.

Glycogen-Depleting Exercise

A high-intensity intermittent cycling protocol coupled with repeated sprint activities was adopted from Palmer et al. (23)

to deplete the muscle glycogen stores in both type 1 and 2 fibers. The protocol was performed on a Monark 894E cycle ergometer and consisted of three periods of 10 × 45 s of high-intensity exercise interspersed by 135 s of passive recovery between each exercise bout and ~15 min between periods. The intensity during each bout was dependent on the pedal frequency, with a fixed resistance of a magnitude corresponding to 100% W_{max} at 90 RPM with small load adjustments based on preliminary testing. Hence, each bout consisted of 10 s at 108 RPM (~120% W_{max}), 10 s at 90 RPM (~100% W_{max}), 15 s at 101 RPM (~112% W_{max}), and 10 s at 90 RPM (~100% W_{max}). The participants were continuously encouraged to achieve the prescribed pedal frequencies, but for some participants, the load was reduced slightly at certain points to avoid premature exhaustion, although at least ~90% of expected work was performed by all participants.

Diet Manipulation during the 5-h Recovery Period

The high-carbohydrate diet (CHO) consisted of both liquids and energy bars (in a 2:1 maltodextrin-to-fructose ratio; sports drink and bars, Maxim; Orklacare A/S, Ishøj, Denmark), yielding a total of ~5.2 g CHO·kg^{-1}, corresponding to ~1 g CHO·kg^{-1}·h^{-1}, with the last amount consumed 1 h before returning to the laboratory. According to the American College of Sports Medicine nutrition guidelines (24), this rate of carbohydrate provision should maximally stimulate glucose uptake. The low-carbohydrate diet (PLA) consisted of an equal amount of liquids with a similar color and a sweetened flavor, but containing no carbohydrates (sugar free orange juice, Budget; Salling Group, Brabrand, Denmark). In addition, low-carbohydrate energy bars (soft bar; EASIS A/S, Midtjylland, Denmark) were provided yielding 0.3 g CHO·kg^{-1}, whereas both diets were similarly low in fat (0.3 g·kg^{-1}) and protein (0.3 g·kg^{-1}). Thus, the diets only differed markedly in carbohydrate content (and total calories). The diets were divided into four equal-sized portions and provided for the participants instantly after exercise and 45 min, 2 h, and 3.5 h into recovery, with the last amount consumed at least 1 h before returning to the laboratory. The diets were administered in a double-blind manner, because neither the participants nor the test administrators were aware of the given diet composition, which was handled by one specific staff member who did not oversee other aspects of the experimental procedures. This double-blind condition was deemed successful, because only four of nine and five of nine in PLA and CHO, respectively, correctly guessed their prescribed diet condition after the experiment. Furthermore, of those participants who guessed correctly, only four in total stated that they were either somewhat certain or very certain of the administered condition.

Repeated Sprint Ability

RSA was measured using a 5 × 6-s sprint test with 24 s of recovery on an electronically braked cycle ergometer (Schoberer Rad Messtechnik, 117 GmbH, Jülich, Germany). Power measurements were recorded at a frequency of 3 Hz and transferred to an external computer with Schoberer Rad Messtechnik software (version 6.41.04) for further data extraction and analysis. All sprints were performed in the seated position with standardized strong verbal encouragement. Calibration of the cycle ergometer was performed at the beginning of each day and again after the 5-h rest period before the final sampling. At baseline and at the recovery time point, the test was preceded by an 8-min warm-up consisting of continuous exercise for 4 min at 40% W_{max}, 2 min at 50% W_{max}, and 2 min at 60% W_{max}. Subsequently, three warm-up and further familiarization attempts at the 6-s sprints were performed at ~70%, 80%, and 90% of maximal effort. The participants then passively rested for 3 min before starting the sprint test. The 3-s period with the highest average power was obtained for each of the five sprints in a repeated sprint series to avoid fluctuations in power readings at the onset and cessation of each sprint, as done previously (7). To express the total RSA, the average of all five 3-s peaks was recorded and presented in the Results section.

2-Min Fixed-Intensity Test

A fixed-intensity test of 2-min duration was performed at a workload of ~90% W_{max} to determine the RPE score during standardized exercise at the highest possible exercise intensity that was sustainable both at baseline and after the exercise and diet interventions. This test was performed using the Monark 894E cycling ergometer with a constant pedaling rate of 80 RPM.

Neuromuscular Assessments

Neuromuscular tests of isometric maximal voluntary contraction (MVC) and assessments of low- and high-frequency force development were performed. At each time point, these tests were performed after a standardized 10-min rest period after the high-intensity cycling tests. As such, the assessments performed after the glycogen-depletion protocol and subsequent cycling tests clearly underestimate the acute transient decline in muscle function immediately after cessation of exercise. However, this timing was chosen because the main aim was to assess the importance of reduced muscle glycogen content on neuromuscular function irrespective of other intracellular perturbations immediately after exercise and to ensure enough time to properly prepare the assessments.

The participants were positioned in the dynamometer (Humac Norm; CSMi, Stoughton, MA) for isometric one-leg knee extension with a hip angle of 90° and knee angle of 70°. The lateral epicondyle of the knee was aligned with the axis of rotation of the dynamometer and the rotation arm secured to the lower leg using velcro straps ~5 cm above the medial malleolus. A shoulder harness and hip strap were used to secure the participants in a firm position. All hair was removed from the specific areas of the thigh and two surface stimulation electrodes (Valutrode, 5 × 10 cm; Axelgaard Lystrup, Denmark) were placed at the proximal and distal part of the quadriceps muscle belly. The electrode positions were marked with tape to ensure consistent placement during the baseline, post, and

recovery trials. After five brief warm-up contractions at increasing intensity, two MVCs were performed interspersed by 60-s rest periods. The participants were instructed to contract as fast and forcefully as possible, with strong verbal feedback provided. Finally, low- and high-frequency force measurements were collected at 20- and 50-Hz stimulation frequencies for 0.6-s duration using a direct current stimulator (model DS7; Digitimer Electronic, England, United Kingdom). Two 50-Hz measurements with an interstimulation rest period of 30 s were initially performed after determining the individual stimulation current eliciting a force response of at least 50% MVC (140–180 mA). The same individual stimulation current was then utilized for two 20-Hz stimulations and maintained for all trials throughout the experiment. Data were analyzed using commercial software (Matlab R2018B; The MathWorks, Natick, MA).

Muscle Biopsy Sampling and Processing

Muscle biopsies were obtained from *musculus vastus lateralis* (150–200 mg wet weight (ww)) using the Bergström needle biopsy technique with suction, with the participant placed in the supine position. In preparation for the biopsies, a small incision was made through the skin under local anesthesia (~5–10 mL of 1% lidocaine). For the resting biopsies at baseline and after the recovery period, muscle biopsies were obtained immediately after making the incision. For the biopsies obtained after the first and third periods of exercise, incisions were made before each exercise period and covered with sterile Band-Aid strips and gauze. Within ~30 s after cessation of exercise, the biopsies were then obtained with the participants lying down on a bed placed next to the cycle ergometers. The biopsies were obtained in the order right leg, left leg, left leg, and right leg, with the first biopsy from each muscle performed at the most distal part and the second within 3–5 cm at a more proximal site. After sampling, the muscle tissue was dissected free of visible blood, fat, and connective tissue and divided into three difference pieces. Approximately 20 mg ww of the muscle sample was frozen in liquid nitrogen immediately after sampling and subsequently stored at −80°C for biochemical analyses. Another ~5 mg ww was fixed for transmission electron microscopy (TEM) analysis, as previously described in detail (19). In short, the segment was fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) until the next day and then rinsed four times in 0.1 M sodium cacodylate buffer. For measurements of MHC distribution and SR Ca^{2+} release and uptake rates, a third piece of muscle tissue (~20 mg ww) was homogenized in ice-cold buffer (1:10 weight/volume, 300 mM sucrose, 1 mM EDTA, 10 mM NaN3, 40 mM Tris-base, and 40 mM histidine at pH 7.8) in a glass homogenizer using a glass pestle (Kontes Glass Industry, Vineland, NJ) and subsequently snap-frozen in liquid nitrogen and stored at −80°C for later analyses.

Muscle Glycogen Assay

For biochemical assessment of whole-muscle glycogen content, muscle tissue was freeze-dried and dissected free of blood and connective tissue, and analyzed spectrophotometrically (Beckman DU 650; Beckman Instruments, Fullerton, CA) as previously described (17).

TEM Analyses

Muscle samples for TEM analyses were postfixed and stained with 1% osmium tetroxide (OsO_4) and 1.5% potassium ferrocyanide ($K_4Fe(CN)_6$) in 0.1 M sodium cacodylate buffer for 90 min at 4°C. After postfixation, the fiber segments were rinsed twice in 0.1 M sodium cacodylate buffer at 4°C and dehydrated through a graded series of alcohol at 4°C–20°C before being infiltrated with graded mixtures of propylene oxide and Epon at 20°C and embedded in 100% Epon at 30°C. Ultra-thin sections (~60 nm) were cut longitudinally using a Leica Ultracut UCT ultramicrotome and contrasted with uranyl acetate and lead citrate. The sections were photographed in a Philips CM100 electron microscope using an Olympus Veleta camera (Olympus Soft Imaging Solutions). Images were obtained at ×13.500 magnification in an unbiased systematic order (12 in the SS area (6 on each side of the fiber) and 12 in the myofibrillar area (6 in the superficial: 3 in each side of the fiber) and 6 in the central part of the fiber). Fibers were fiber-typed based on Z-disk width, as previously described (19), and 3 type 1 and three type 2 fibers included in the analysis for each biopsy sample from a total of 10 photographed and fiber-typed fibers (the 3 fibers with the widest and narrowest Z-disks included, respectively). The glycogen volume fraction (V_V) of three distinct subcellular fractions was estimated as proposed by Weibel (25), where the effect of section thickness was taken into account: $V_V = A_A - t((1/\pi)B_A - N_A((t \times H)/(t + H)))$. A_A is the glycogen area fraction, t is the section thickness (60 nm), B_A is the glycogen boundary length density, N_A is the number of particles per area, and H is the average glycogen profile diameter. Glycogen area fraction was determined by point counting and glycogen diameter by direct measurements, as previously described in detail (19). The coefficient of error for images within each fiber was 0.16, 0.17, and 0.22 for IMF, intra, and SS glycogen, respectively.

Sarcoplasmic Reticulum Vesicle Ca^{2+} Assay

Ca^{2+} release and uptake rates were measured fluorometrically in homogenates, as previously described (17). Briefly, fluorescence was converted to free Ca^{2+}, and all raw data were imported and analyzed in MATLAB version 7.0.1 (The MathWorks) using curve fitting (Curve Fitting Toolbox version 1.1.1; The MathWorks). Ca^{2+} uptake was determined as the time (τ) to reach 63% of the final Ca^{2+} uptake, whereas Ca^{2+} release rate was determined as the maximal rate measured after opening of the ryanodine receptors using previously described calculations (17). The Ca^{2+} assays for both uptake and release rates were determined in duplicate (a few as single measurements because of inadequate tissue homogenate). The protein content in the muscle homogenate was measured in triplicate using a standard kit (Pierce BCA protein reagent no. 23225).

Myosin Heavy-Chain Composition

Myosin heavy-chain (MHC) composition was determined from homogenate using gel electrophoresis and quantified densitometrically as previously described (19). In brief, muscle homogenate (20 μL) and sample buffer (100 μL, 10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS, 62.5 mM Tris, 0.2% bromophenolblue at pH 6.8) were mixed, boiled in water for 3 min, and loaded on an SDS-PAGE gel (6% polyacrylmide (100:1 acrylmid:bis-acrylmid), 30% glycerol, 67.5 mM Tris-base, 0.4% SDS, and 0.1 M glycine) using three different protein quantities (25–40 μg). Gels were run at 4°C at 80 V for a minimum of 42 h, and MHC bands were made visible by Coomassie staining. The MHC composition for each participant was determined as an average from two biopsies (one from each leg) from three separate lanes for each biopsy.

Blood Sampling and Analyses

Blood was drawn from a catheter placed in an antecubital vein before exercise, within a minute after each exercise period, and at the recovery time point. All blood samples were drawn in lithium–heparin and serum tubes, and distributed in 2-mL tubes for different analyses after centrifugation. Lithium–heparin samples were kept cold and centrifuged after the session, whereas serum samples were allowed to rest for 1 h at room temperature before centrifugation. Afterward, all samples were stored at −80°C. Glucose was measured directly on HemoCue using the glucose dehydrogenase method and photometric detection. Lactate was measured instantly on Biosen (EKF Diagnostics) by the enzymatic–amperometric method using chip-sensor technology. One drop (approximately 50 μL) of serum was used for lactate and glucose cuvette analyses. Plasma free fatty acid (FFA) concentration was analyzed on the Pentra 400 C using an enzymatic kit (WAKO Chemical, Neuss, Germany). Serum insulin was analyzed on the Cobas 8000, e602 (Roche), by a sandwich enzyme immunoassay and electrochemiluminescence.

STATISTICAL ANALYSES

Data were assessed for normal distribution using Q–Q plots and histogram models. A mixed-model analysis with time–group interactions was used to evaluate RSA across time points, with participant and time as random effects and time and sprint (repetitions 1–5) as fixed effects. Two-way ANOVA repeated-measure analysis was used to evaluate changes in RPE, HR, neuromuscular, blood, and muscle responses with time and group as independent factors. *Post hoc* analyses were performed using the Holm–Sidak test when appropriate. Pearson correlation coefficients were calculated and interpreted as follows: $r \leq 0.1$ (trivial), 0.1–0.3 (small/weak), 0.3–0.5 (moderate), 0.5–0.70 (large/strong), 0.7–0.9 (very large/strong), and ≥0.9 (almost perfect) (26). Correlations between subcellular glycogen fractions and RSA at the recovery time point were performed using a mean of all three type 1 and type 2 fibers for each glycogen fraction to minimize the inherent variability using single-fiber data points. Differences in glycogen volume fractions between groups and within fiber types at the recovery time point were analyzed using unpaired *t* tests with log-transformed data to achieve normal data distribution. Significance level was set at $P \leq 0.05$ with data presented as mean ± SD, except for subcellular glycogen volume fractions, which were presented as geometric means and individual values relative to the baseline geometric mean of each fraction. All statistical analyses were performed using Stata/IC16 (StataCorp, College Station, TX). Figures were created using the GraphPad Prism 4.0 software package (GraphPad Software Inc., San Diego, CA).

RESULTS

Work performed and physiological responses during the glycogen-depleting exercise. The average work load during the three periods of glycogen-depleting exercise (104% ± 5% vs 104% ± 8% W_{max}) and repeated sprints (870 ± 138 vs 912 ± 158 W) for CHO and PLA, respectively, was not different between groups (P = 0.607–0.916). This corresponded to a total work of 520 ± 70 vs 508 ± 57 kJ (excluding the repeated sprints). Likewise, the physiological responses for CHO and PLA, respectively, including mean HR during the three 10 × 45-s periods containing both work and rest intervals (79% ± 4% vs 75% ± 5% HR_{max}), mean HR during work intervals only (86% ± 3% vs 84% ± 3% HR_{max}), average blood lactate values measured immediately after each of the three exercise periods (12.5 ± 3.5 vs 12.6 ± 2.3 mmol·L^{-1}), and RPE values measured instantly after each work bout (6.5 ± 2.0 vs 6.4 ± 1.8 AU) were not different between groups (P = 0.289–0.997).

Whole-muscle glycogen content. Whole-muscle glycogen content determined biochemically was not different between groups at baseline and decreased significantly (main effect of time, $P < 0.001$) to the same extent after exercise to 80 ± 58 and 72 ± 67 mmol·kg^{-1} dw in PLA and CHO, respectively (Fig. 2). After the 5-h recovery period, including diet manipulation, muscle glycogen increased in both groups but reached significantly higher levels in CHO compared with PLA (291 ± 78 vs 175 ± 100 mmol·kg^{-1} dw, P = 0.020). Accordingly, average muscle glycogen resynthesis rates during the recovery period were 44 ± 10 and 19 ± 17 mmol·kg^{-1} dw·h^{-1} for CHO and PLA, respectively ($P < 0.001$). As can be seen from the individual data plots in Figure 2, one substantial outlier was present in the PLA group, with a muscle glycogen resynthesis rate on par with the highest rates observed in CHO. Nonetheless, it was deemed inappropriate to exclude this participant because all other metabolic assessments (FFA, lactate, and insulin levels at recovery) pointed to a response similar to that of the other participants in PLA and hence did not suggest any mistakes in the diet administration. Without the outlier value, the muscle glycogen resynthesis rate in PLA was 14 ± 9 mmol·kg^{-1} dw·h^{-1}.

Subcellular muscle glycogen volume fractions in type 1 and 2 fibers relative to baseline. Subcellular

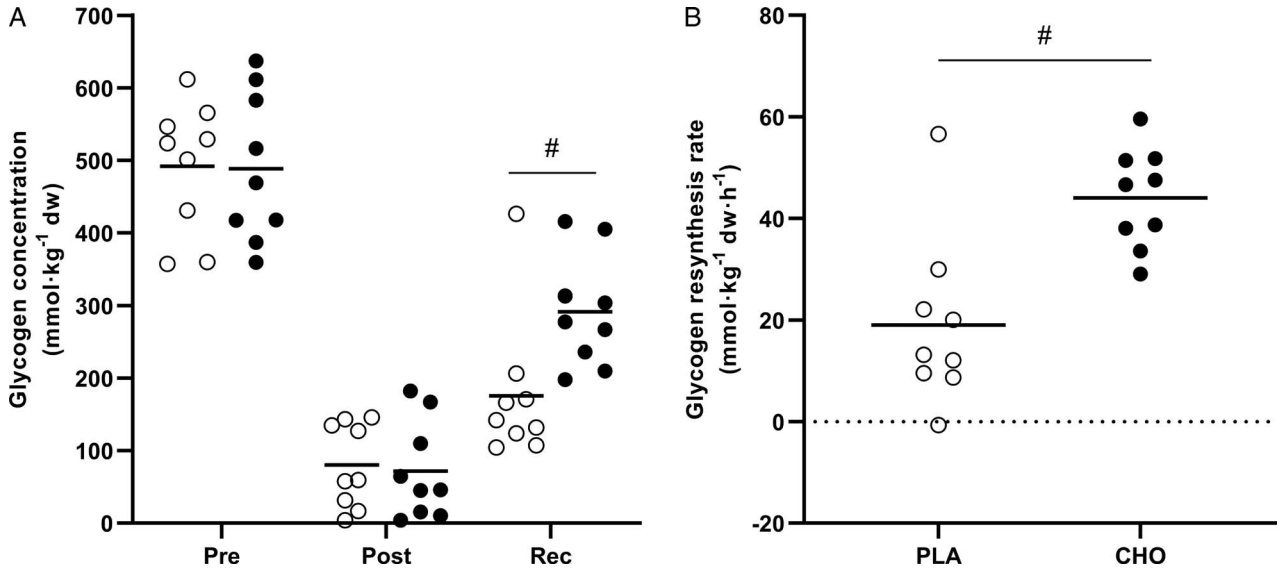

FIGURE 2—A, Whole-muscle glycogen content before exercise (pre), after exercise (post), and at the recovery time point (rec) and (B) glycogen resynthesis rate during the 5-h recovery period. # denotes significant between-group difference ($P \leq 0.05$). Open dots represent individual fibers in the low-carbohydrate placebo group (PLA), and solid dots represent individual fibers in the high-carbohydrate–supplemented group (CHO).

muscle glycogen volume fractions of IMF, intra, SS and estimated total glycogen in type 1 and 2 fibers, expressed relative to the baseline geometric mean, were significantly higher in CHO compared with PLA at the recovery time point in all three fractions in both fiber types (P = 0.001–0.046; Fig. 3). For reference, no between-group differences were present at baseline (data not shown). Furthermore, a higher amount of intra glycogen was present in type 1 compared with type 2 fibers within CHO (P = 0.018), whereas a higher amount of IMF and of total glycogen were present in type 2 compared with type 1 fibers within PLA (P = 0.009 and 0.016, respectively). Notably, in the intra fraction in PLA, 56% and 44% of the individual type 1 and 2 fibers contained less than 20% glycogen relative to the baseline geometric mean, whereas this was not the case for any individual fibers in CHO in either fiber type. In the SS fraction in PLA, 30% and 19% of the individual type 1 and 2 fibers were reduced below this same level, whereas this was only the case for 4% and 11% of the type 1 and 2 fibers in CHO. For the estimated total amount of glycogen and IMF glycogen in individual type 1 and 2 fibers, 19% and 4% were reduced below this level, whereas no individual fibers in CHO contained this low amount of glycogen.

RSA and RPE during the 2-min test. After exercise, RSA declined similarly between CHO and PLA to ~83% of baseline levels (P < 0.001; Fig. 4) from 960 ± 121 and 1009 ± 134 W to 807 ± 129 and 840 ± 139 W for CHO and PLA, respectively. After the recovery period, RSA returned to preexercise levels in CHO (945 ± 119 W) but was still partially lowered in PLA (934 ± 158 W corresponding to an 8% ± 6% reduction, P < 0.001). It is worth noticing that the only participant in PLA with a full recovery of RSA (Fig. 4C) was the outlier participant, with a recovery of muscle glycogen content similar to the values observed in CHO. In the absence of this outlier, the reduction in RSA in PLA was 9% ± 5% (P < 0.001). Moreover, the power output in PLA was not different for each individual sprint (93% ± 6%, 92% ± 7%, 91% ± 7%, 91% ± 7%, and 94% ± 7% for sprints 1–5 at recovery compared with sprints 1–5 at baseline). RPE levels obtained after the 2-min fixed-intensity test increased similarly from baseline to postexercise (P = 0.375; Fig. 4B). After recovery, the RPE response declined to baseline levels in CHO (P = 0.912) but remained partially elevated in PLA (P < 0.001).

Correlations between RSA and whole-muscle and subcellular glycogen concentrations. Whole-muscle glycogen content at the recovery time point was strongly correlated with the relative RSA (r = 0.64, P = 0.005; Fig. 5). Further detailing of the associations between each subcellular glycogen fraction estimated using TEM and relative RSA revealed moderate (r = 0.47, P = 0.049), very strong (r = 0.71, P = 0.001), and strong (r = 0.56, P = 0.017) correlations for IMF, intra, and SS glycogen, respectively.

Blood parameters. Serum insulin and blood glucose concentrations decreased in both groups after glycogen-depleting exercise (main effect of time, P < 0.001), with no differences between groups (P = 0.950; Fig. 6). Whereas blood glucose was the same in CHO and PLA at the recovery time point, serum insulin levels were higher in the CHO condition (P = 0.033). The plasma FFA level increased similarly in both groups after exercise (main effect of time P < 0.001) and remained high in PLA (P < 0.001), whereas it decreased to baseline levels in CHO (P = 0.811).

SR Ca^{2+} release and uptake. No changes in Ca^{2+} release rate or uptake were evident after exercise (P = 0.238 and 0.125, respectively; Fig. 7). However, from postexercise to the recovery time point, the change in SR Ca^{2+} uptake differed between groups in favor of CHO (faster uptake P = 0.003)

Neuromuscular assessments. MVC and 50-Hz stimulation torque decreased similarly after exercise (10%

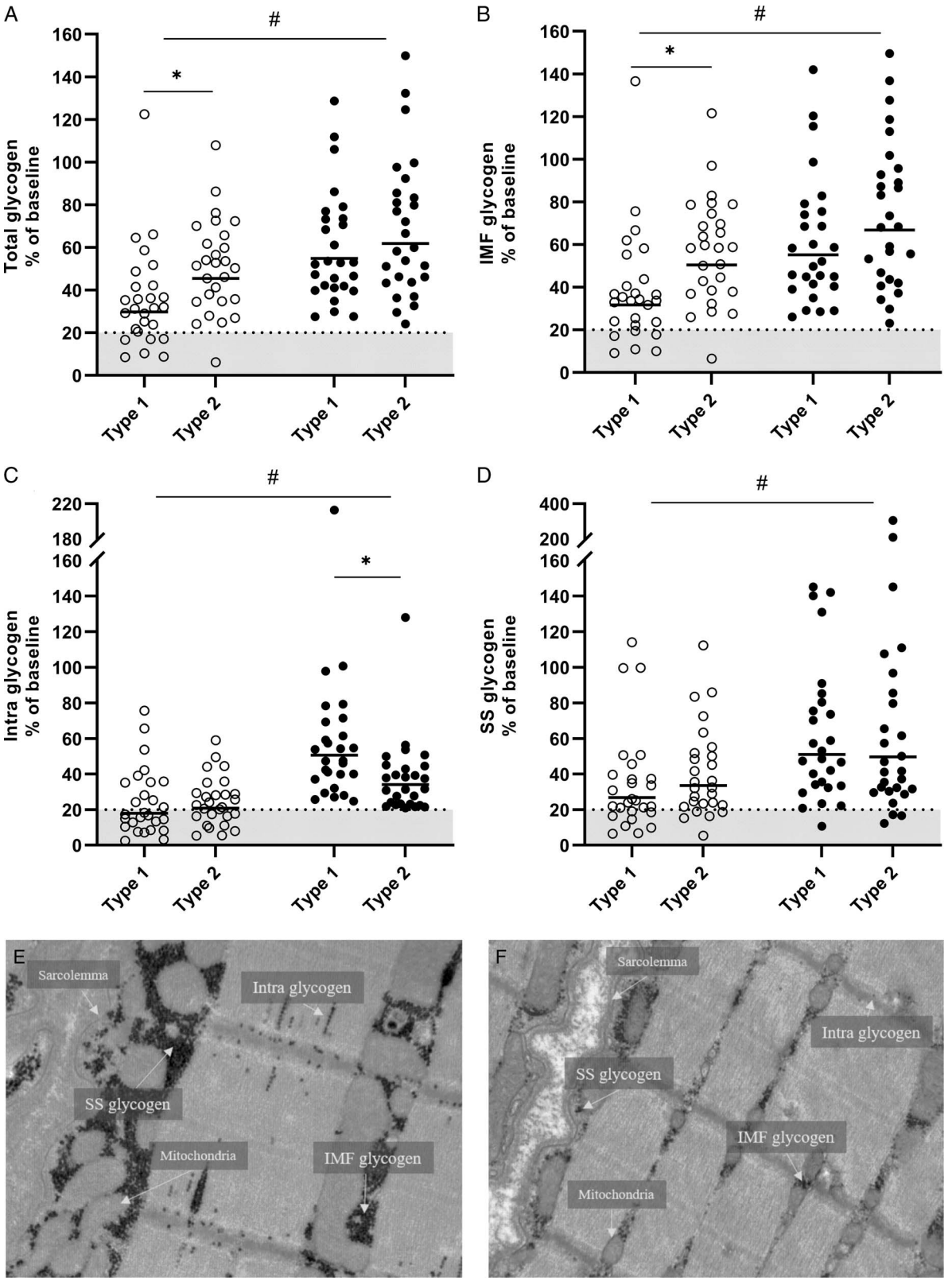

FIGURE 3—Subcellular glycogen content in type 1 and 2 single-fibers (three fibers of each type measured for each participant, total n = 108 fibers at each time point) at the recovery time point relative to the baseline geometric mean of all fibers for (A) total glycogen, (B) IMF glycogen, (C) intra glycogen, and (D) SS glycogen. Baseline geometric means for all fibers (types 1 and 2) were 23.3, 18.3, and 2.9 μm^3 μm^{-3} 10^3 for total glycogen, IMF, and intra glycogen, respectively, and 44.2 μm^3 μm^{-2} 10^3 for SS glycogen. *Open dots* represent individual fibers in the low-carbohydrate placebo group, and *solid dots* represent individual fibers in the high-carbohydrate–supplemented group. *Shaded area* below *dashed horizontal line* represents <20% of the baseline geometric mean. # denotes between-group difference for type 1 and 2 fibers; * denotes within-group difference ($P \leq 0.05$). E and F, Representative electron-microscopy images from a baseline muscle biopsy (E) and from a muscle biopsy obtained at the recovery time point after resting with a low carbohydrate intake (F).

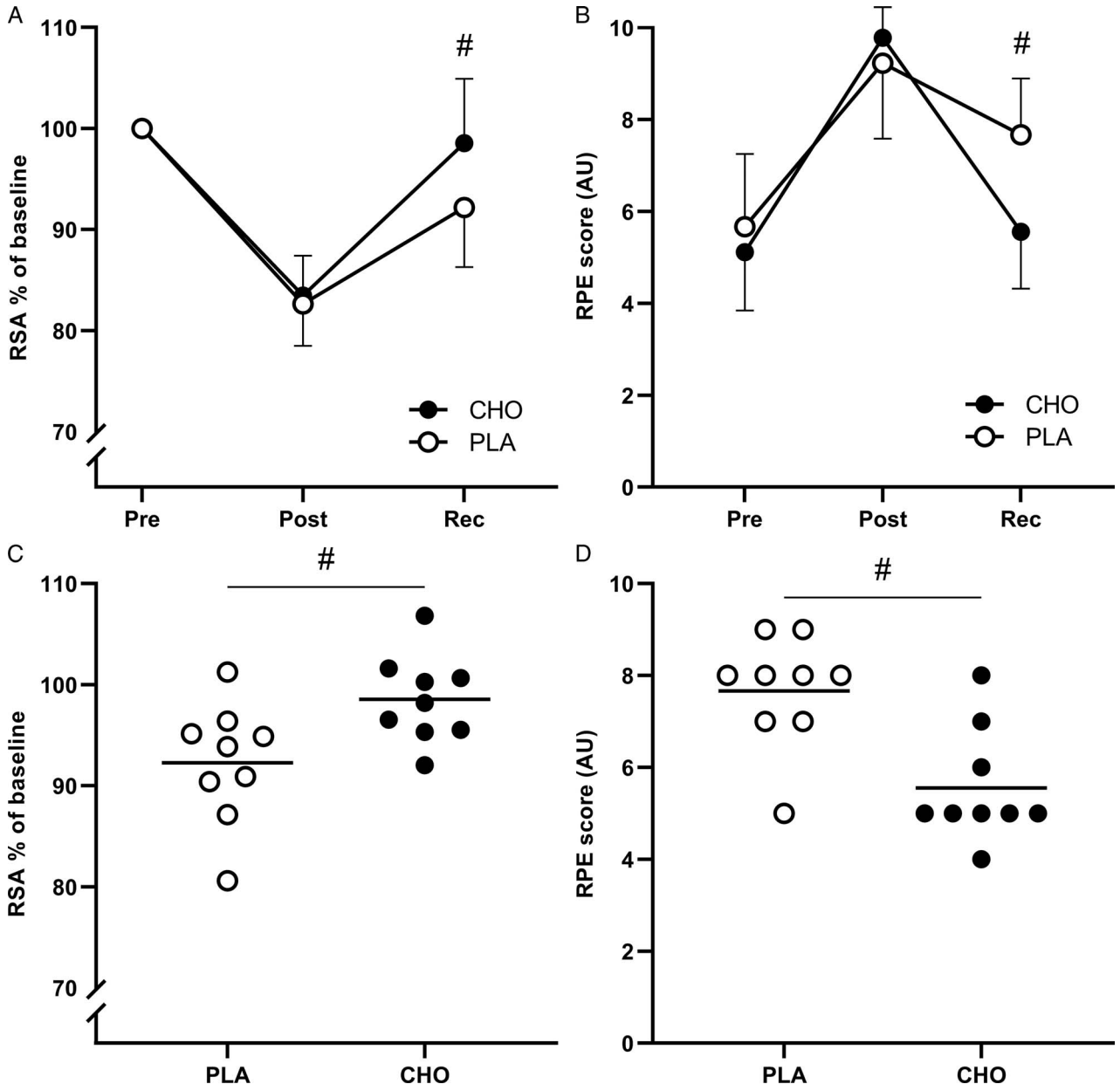

FIGURE 4—A, RSA (mean of the 3-s highest peak power of each of five 6-s sprints) relative to baseline. B, RPE values during standardized high-intensity exercise (2 min at 90% W_{max}), for the high-carbohydrate group supplemented (CHO) and low-carbohydrate placebo group (PLA). Individual values relative to baseline are shown for the recovery time point (C and D). # denotes significant between-group difference ($P \leq 0.05$).

decrease; main effect, $P < 0.001$ and $P = 0.010$, respectively), with no differences between groups (Table 2). After the recovery period, MVC was still partially lowered (~5%), with no between-group differences ($P = 0.0372$), whereas 50-Hz torque was completely restored. Torque production at 20-Hz stimulation decreased after exercise (34% decrease; main effect, $P < 0.001$, with no differences between groups). At the recovery time point, 20-Hz torque was fully recovered in PLA but still remained partially lowered in CHO (time effect, $P = 0.003$), but with no significant between-group interactions ($P = 0.179$). The 20:50 Hz ratio was reduced similarly after exercise in CHO and PLA ($P < 0.001$), with only a partial recovery after the 5-h rest period (main effect of time, $P = 0.001$), demonstrating the presence of prolonged low-frequency force depression, which still partly persisted after the recovery period.

DISCUSSION

The principal findings of the present study were that a high-intensity intermittent exercise regimen followed by reduced carbohydrate intake, leading to low muscle glycogen levels, impaired RSA and increased the RPE during subsequent high-intensity exercise. This was demonstrated using a double-blind placebo-controlled design, pointing to a direct link between muscle glycogen availability and high-intensity exercise tolerance. Moreover, these findings were coupled with near-depleted

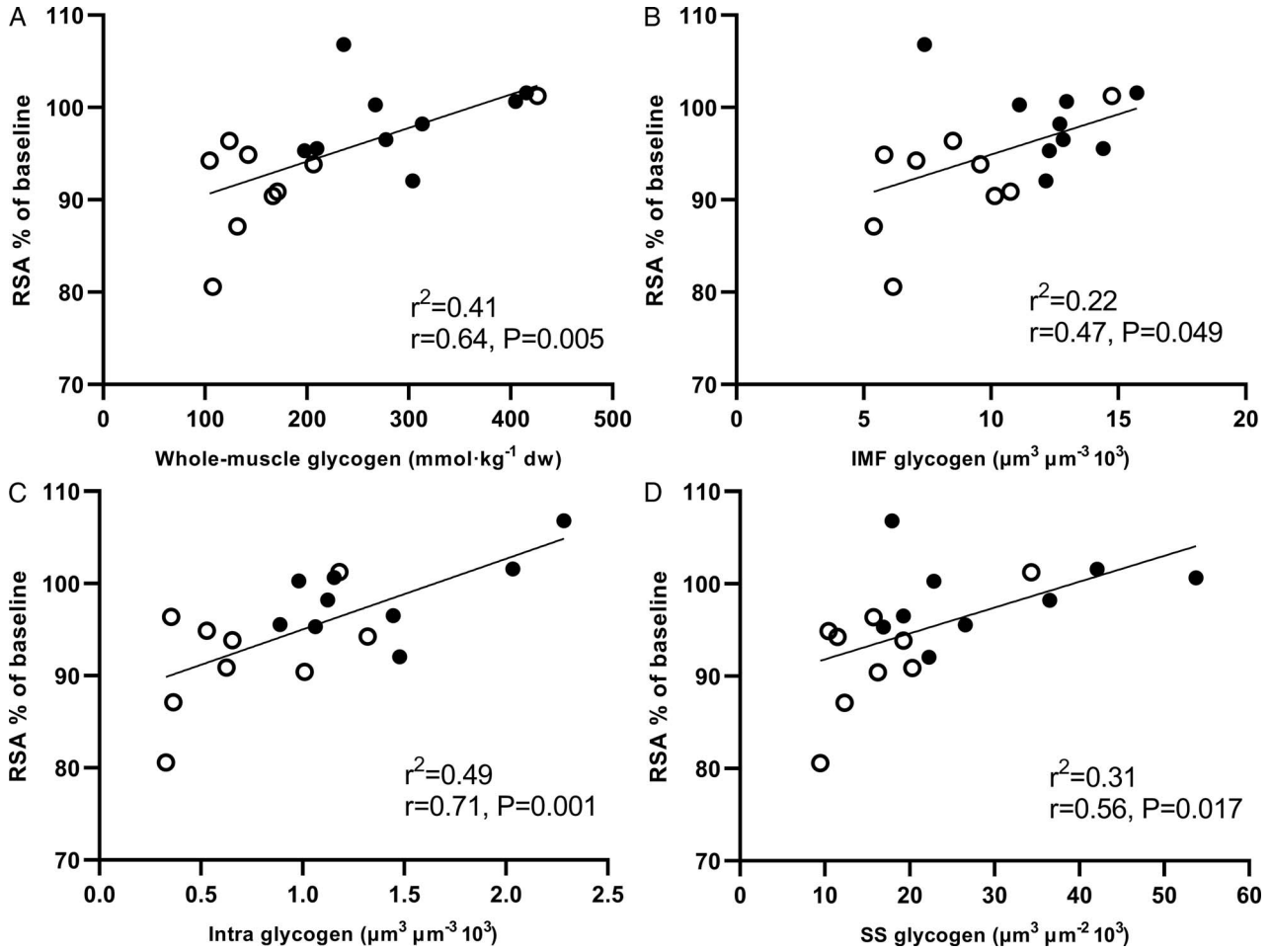

FIGURE 5—Correlations between RSA relative to baseline and absolute muscle glycogen concentrations at the recovery time point at the whole-muscle (A) and subcellular level (B–D) for the high-carbohydrate–supplemented (CHO) and low-carbohydrate placebo (PLA) groups overall (*open dots* represent the individual values for PLA, and *solid dots* the individual values for CHO).

glycogen levels in a substantial number of individual fibers, especially of the intra fraction in type 1 and 2 fibers. Notably, the recovery of RSA was moderately to strongly correlated with whole-muscle glycogen content and with the glycogen stored in each subcellular fraction including a very strong correlation between the specific location of intra glycogen and RSA. Ca^{2+} kinetics remained unaltered during the experiment, except a between-group difference in Ca^{2+} uptake at the recovery time point favoring the CHO group. Finally, alterations in neuromuscular function, including prolonged low-frequency

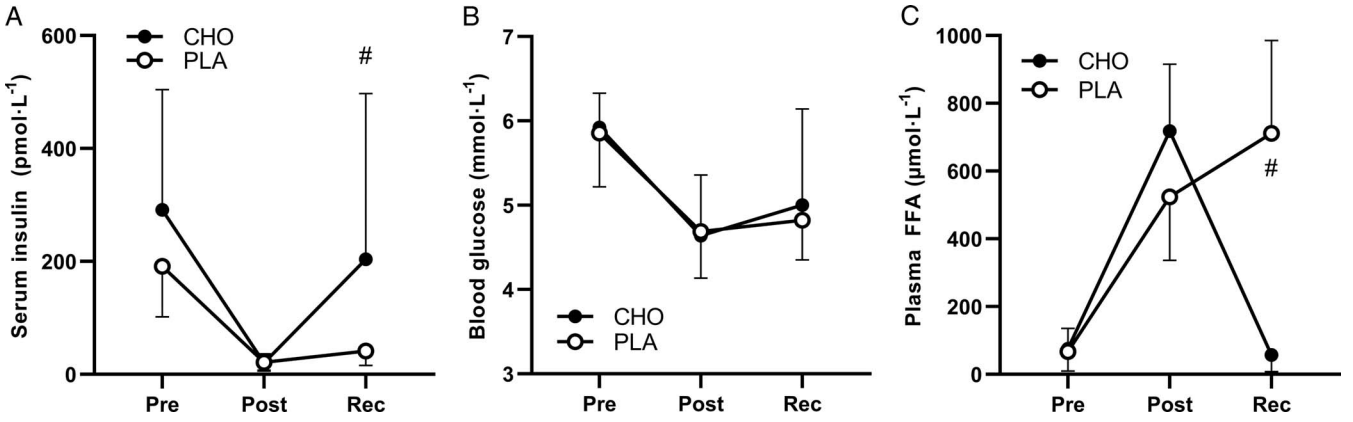

FIGURE 6—Changes in blood concentrations of insulin, glucose, and FFA before exercise (pre), after exercise (post), and at the recovery time point (rec) in the high-carbohydrate–supplemented (CHO) and placebo (PLA) groups. # denotes significant between-group difference ($P \leq 0.05$).

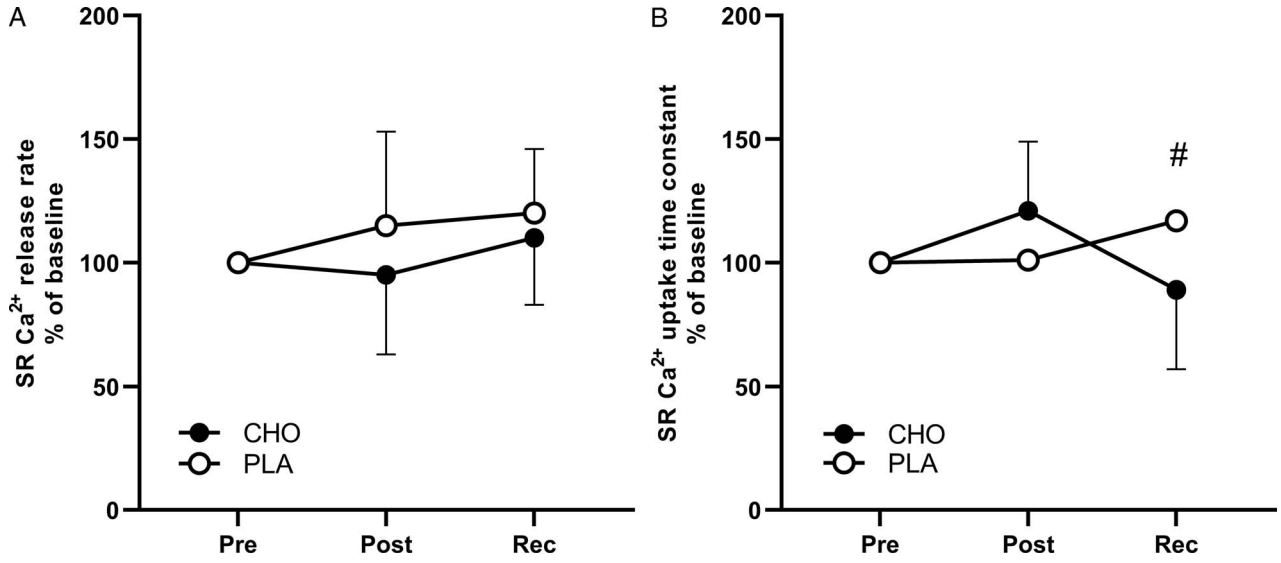

FIGURE 7—A, $SRCa^{2+}$ release rate and (B) uptake time constant (time to reach 63% of uptake) relative to baseline for the high-carbohydrate–supplemented (CHO) and low-carbohydrate placebo (PLA) groups. # denotes significant between-group difference ($P \leq 0.05$).

force depression, were present throughout the recovery period but unaffected by glycogen condition.

Our findings support an importance of muscle glycogen content for repeated high-intensity exercise performance, as has been observed in some but not all previous studies (for a review, see Vigh-Larsen et al. [11]). Herein, an ~8% performance impairment was observed with a 5 × 6-s repeated sprint protocol more closely resembling the peak demands experienced in intermittent team sports compared with all-out 30-s or continuous time-to-exhaustion tests commonly applied in the previous literature (11). This repeated sprint scenario entails maximal rates of glycogenolytic flux and should reflect potential limitations in energy supply and muscle function (27). In line with our results, Gejl et al. (7) and Balsom et al. (6) similarly reported ~5% performance decrements when applying 5- to 6-s sprint efforts in single or repeated exercise bouts. However, these studies were not blinded, and conceivably, this magnitude of change could at least partly fall within the range of possible outcomes confounded by positive and negative expectations of the administered conditions (28). Thus, the present results applying a placebo-controlled design provide more robust support for the observed outcomes being likely intervention-induced effects.

Importantly, a limited number of previous studies have measured the actual muscle glycogen concentrations achieved after the applied exercise and diet interventions. From these, a critical threshold for impaired performance below a glycogen concentration of ~250–300 mmol·kg^{-1} dw has been suggested (11). Thus, neither 75-s (10) nor 30-s (9) maximal cycling sprint performance was altered in low-glycogen conditions when values were only moderately lowered to 462 and 350 mmol·kg^{-1} dw, whereas lower glycogen levels of ~180–264 mmol·kg^{-1} dw (6–8) all provoked impaired performance during maximal cycling sprints of varying duration. In line with this, Bangsbo et al. (29) reported no reduction in intensive one-leg knee-extensor work capacity when the initial glycogen concentration was ~372 mmol·kg^{-1} dw in a low-glycogen condition. However, during a second bout when the initial glycogen concentration was ~310 mmol·kg^{-1} dw, dropping to ~223 mmol·kg^{-1} dw after exercise, performance was impaired. In our study, total muscle glycogen values were reduced to 175 mmol·kg^{-1} dw in the low-glycogen condition, well below the proposed glycogen threshold, and accordingly, performance was impaired. On the other hand, the glycogen concentrations were only partially recovered in the CHO group to 292 mmol·kg^{-1} dw (range, 198–416 mmol·kg^{-1} dw), thus overlapping the proposed threshold for performance impairment. Accordingly, performance was lowered for some individual participants, whereas at the mean level, no reduction was present. Thus, based on these results and from the evaluation of individual data points in the correlation matrix between whole-muscle glycogen content and recovery of RSA, our results seem to support a threshold for impaired performance below a glycogen concentration of ~250 mmol·kg^{-1} dw. This may be important for several intermittent and endurance-type sports where high-intensity efforts have to be performed after prior glycogen-consuming exercise and suggests that strategies (e.g., diet, training, pacing,

TABLE 2. Neuromuscular function before exercise, after exercise, and at the recovery time point.

	Pre	Post	Rec
MVC torque (N·m)			
CHO	284 ± 48	252 ± 53*	264 ± 45*
PLA	277 ± 33	259 ± 30*	268 ± 27*
50-Hz torque (N·m)			
CHO	185 ± 35	165 ± 40*	183 ± 40
PLA	207 ± 31	191 ± 34*	217 ± 39
20-Hz torque (N·m)			
CHO	120 ± 36	72 ± 25*	90 ± 33*
PLA	121 ± 37	88 ± 34*	111 ± 26
20:50 Hz ratio (%)			
CHO	65 ± 12	43 ± 7*	48 ± 11*
PLA	58 ± 11	46 ± 13*	51 ± 9*

Post, postexercise; Pre, preexercise; Rec, recovery.
*Significant difference from pre ($P \leq 0.05$).
Data are presented as mean ± SD.

and/or substitutions) should be incorporated to avoid reductions below this level.

As such, the glycogen content achieved in the low-glycogen condition matches the end-game values (~200 mmol·kg^{-1} dw) observed in different team sports where substantial depletion of individual fibers has been reported using semiquantitative immunohistochemistry techniques (3–5). Herein, using quantitative electron microscopy imaging, we support these results by demonstrating substantially lowered single-fiber glycogen levels. Thus, despite nondepleted global levels, the glycogen content in individual fibers may become too low to support maximal glycogen phosphorylase activity. During high-intensity exercise where full recruitment of the muscle fiber pool is vital, this may become limiting for performance. Notably, the degree of depletion was even more pronounced when evaluating the pool-specific glycogen content in individual fibers. In particular, very low levels of intra glycogen in individual fibers of both main fiber types were found and, to a smaller extent, SS and IMF glycogen mainly in type 1 fibers. This means that even within a single muscle fiber with nondepleted glycogen levels, the glycogen content in specific intracellular compartments can be exhausted. Although all muscle glycogen fractions are anticipated to serve important functions, intra glycogen specifically has been linked with fatigue resistance in electrically stimulated single fibers (16) and in endurance exercise performance (19). In our study, we observed moderate to strong associations between whole-muscle as well as subcellular glycogen fractions and performance but with a very strong association present between intra glycogen and RSA. However, because these correlations are very sensitive to alterations in individual values, they should be interpreted cautiously. Thus, we can surmise that these results partly support previous links between intra glycogen and fatigue resistance or, diversely, that the relatively small disparities in the correlation coefficients point to a similar importance of all three pools in the given context.

The proposed importance of intra glycogen has been speculated to relate to energy provision for Na^+-K^+-ATPases in the diffusion-restricted triadic junctions, where access may be favored from the intra space (16,18). Furthermore, colocalization of the energy-sensitive Ca^{2+} release channels with the triadic junctions fosters another mechanism by which glycogen depletion, potentially specifically of intra glycogen, may affect contractility through perturbations in the local intracellular metabolic homeostasis. As such, a coupling between intra glycogen and SR Ca^{2+} release rate has been observed in isolated vesicles (7,17) as well as directly in single fibers during repetitive stimuli (18), whereas IMF glycogen has been linked with half-relaxation time (Ca^{2+} uptake) (16). In the present study, we measured Ca^{2+} kinetics in isolated SR vesicles, but in contrast with previous results and our initial hypothesis, no reductions in SR Ca^{2+} release rate were observed neither during exercise nor recovery, whereas a difference between intervention groups in Ca^{2+} uptake (improved in the carbohydrate-supplemented group) was evident at the recovery time point. This seemingly indicates that altered Ca^{2+} uptake could be implicated in deteriorated muscle performance in a low-glycogen condition, which is in line with findings by Duhamel et al. (20). However, no reduction in Ca^{2+} uptake was detected immediately after exercise when the glycogen concentrations were most dominantly lowered (~75 mmol·kg^{-1} dw), opposing the concept of a role of muscle glycogen for SR Ca^{2+} kinetics. Interestingly, despite several previous studies observing reductions in both SR Ca^{2+} release and uptake after exercise (7,20,30,31), some studies have reported diverse responses dependent on fiber type and exercise modality (32–36). For example, Holloway et al. (34) demonstrated reduced Ca^{2+} kinetics in oxidative rodent muscle after repetitive intense stimuli, whereas a potentiation effect in glycolytic muscle was observed for both SR Ca^{2+} release and uptake rates. Moreover, Dossett-Mercer (37) observed no impairment in SR Ca^{2+} handling after repetitive high-frequency stimulation in gastrocnemius muscle of anesthetized rats, whereas Tupling et al. (35) reported increases in Ca^{2+} ATPase activity during exercise in human skeletal muscle. Although speculative, acute exercise-induced potentiation could thus explain why we observed no effect instantly after exercise but instead in a rested state at the recovery time point where reductions in membrane bound glycogen granules may have altered SR Ca^{2+} uptake function. Finally, different methodological approaches could be implicated in the inconsistent findings, although our samples were processed in the same laboratory with the same methodological approach as in previous studies demonstrating altered Ca^{2+} release in low-glycogen conditions and after exercise (7,17). Notably, a proposed prerequisite for observing an effect of muscle glycogen on calcium cycling properties in the *in vitro* assay is a maintained coupling between the SR membrane and glycogenolytic complexes (colocalization of glycogen granules and enzymes involved in glycogenolysis) that is important for SR function (38). Moreover, because the assay is performed under apparently optimal conditions (e.g., ATP present in the solution), direct effects of changes in energy status of the muscle cell may not be captured, which, under *in vivo* conditions, are vital for Ca^{2+} release channel opening probability (21). Thus, deficits in Ca^{2+} handling properties could be present and go undetected with the present methodology.

Previous associations between muscle glycogen depletion and fatigue during prolonged exercise have, under certain conditions, been attributed to a concomitant decrease in liver glycogen resulting in hypoglycemia-induced central fatigue and/or reduced glucose delivery to the active muscles (39). However, in the present study, blood glucose did not reach hypoglycemic levels after the glycogen-depleting exercise and did not differ between intervention groups at the recovery time point, opposing a role of blood glucose in the responses observed. The carbohydrate intake, on the other hand, altered serum insulin and plasma FFA levels with higher and lower values in the carbohydrate-supplemented condition, respectively, which could alter the muscle metabolism and oxygen kinetics (40). Moreover, insulin is a known stimulator of Na^+-K^+-ATPase activity, and glucose ingestion has recently been demonstrated to alter potassium accumulation during high-intensity exercise, although with no impact on exercise tolerance and with the effects likely being minor compared with the large contraction-induced Na^+-K^+-ATPase activation (41,42). Finally, direct stimulatory effects of carbohydrate

ingestion on reward centers in the brain could also have been present, yet the ingestion of carbohydrates was terminated more than an hour before the last test procedures (43).

In the present study, not only maximal performance but also RPE values during work-matched high-intensity exercise were markedly altered in the low-glycogen condition. The mechanisms for this are not clear but could relate to metabolite perturbations sensed by muscle afferents, altered oxygen kinetics (e.g., through increased fat metabolism), and/or alterations in muscle fiber recruitment and firing frequency patterns (44). Herein we measured the development of prolonged low-frequency force depression (20:50 Hz torque ratio), which is thought to reflect an alteration in calcium release mediating a decrease in force at lower firing frequencies given the asymptotic nature of the force–Ca^{2+} relationship (21). However, despite persistent prolonged low-frequency force depression at the recovery time point, no difference was evident between groups. This is in line with findings by Cheng et al. (45) where glycogen concentrations similarly were manipulated by exercise and diet. In that study though, only a small separation between experimental groups was achieved and with low glycogen levels (<200 $mmol \cdot kg^{-1}$ dw) present in both a high- and low-carbohydrate–supplemented condition. In contrast, Young and Davies (46) observed accelerated fatigue development during repetitive 20-Hz stimuli (330 ms every second for 2 min), although not during single 20- or 50-Hz stimulations, suggesting that prolonged low-frequency force depression may be apparent during repeated contractions due to accelerated metabolite perturbations in a low-glycogen condition.

As a potential limitation of the present study, we opted for a non–calorie-matched approach to manipulate the muscle glycogen stores. This enabled us to achieve the placebo-controlled design, which would have been unattainable if larger amounts of food were provided for both groups. Simultaneously, this ensured a very low carbohydrate intake in the placebo group and limited glycogen resynthesis. The parallel-group study design was chosen to avoid potential learning effects from completing the procedures twice as in a crossover design and to reduce the risk of the participants being able to identify the prescribed diet condition if exposed to both diets. Finally, it should be noted that the subcellular glycogen distribution and associations with performance may be context-specific dependent on previous exercise and/or diet, as well as the specific recovery regimen applied.

CONCLUSIONS

In conclusion, our results support a critical role of muscle glycogen content for repeated high-intensity exercise performance, which may be associated with depletion of a substantial amount of individual fibers and subcellular glycogen fractions despite only moderately lowered whole-muscle glycogen concentrations in the low-carbohydrate–supplemented condition. In accordance, total muscle glycogen and all three subcellular glycogen fractions were significantly associated with repeated sprint performance. Finally, Ca^{2+} release and prolonged low-frequency force depression were unrelated to muscle glycogen content, whereas Ca^{2+} uptake was superior in the carbohydrate-supplemented condition after recovery, suggesting a potential role of glycogen in this aspect of calcium handling.

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