Muscle Glycogen Content Modifies SR Ca^{2+} Release Rate in Elite Endurance Athletes

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ABSTRACT

GEJL, K. D., L. G. HVID, U. FRANDSEN, K. JENSEN, K. SAHLIN, AND N. ØRTENBLAD. Muscle Glycogen Content Modifies SR Ca^{2+} Release Rate in Elite Endurance Athletes. *Med. Sci. Sports Exerc.*, Vol. 46, No. 3, pp. 496–505, 2014. **Purpose**: The aim of the present study was to investigate the influence of muscle glycogen content on sarcoplasmic reticulum (SR) function and peak power output (W_{peak}) in elite endurance athletes. **Methods**: Fourteen highly trained male triathletes ($\dot{V}O_{2max}$ = 66.5 ± 1.3 mL O$_2$·kg^{-1}·min^{-1}), performed 4 h of glycogen-depleting cycling exercise (HR$_{mean}$ = 73% ± 1% of maximum). During the first 4 h of recovery, athletes received either water (H$_2$O) or carbohydrate (CHO), separating alterations in muscle glycogen content from acute changes affecting SR function and performance. Thereafter, all subjects received CHO-enriched food for the remaining 20-h recovery period. **Results**: Immediately after exercise, muscle glycogen content and SR Ca^{2+} release rate was reduced to 32% ± 4% (225 ± 28 mmol·kg^{-1} dw) and 86% ± 2% of initial levels, respectively ($P < 0.01$). Glycogen markedly recovered after 4 h of recovery with CHO (61% ± 2% of preexercise) and SR Ca^{2+} release rate returned to preexercise level. However, in the absence of CHO during the first 4 h of recovery, glycogen and SR Ca^{2+} release rate remained depressed, with the normalization of both parameters at the end of the 24 h of recovery after receiving a CHO-enriched diet. Linear regression demonstrated a significant correlation between SR Ca^{2+} release rate and muscle glycogen content ($P < 0.01$, $r^2 = 0.30$). The 4 h of cycling exercise reduced W_{peak} by 5.5%–8.9% at different cadences ($P < 0.05$), and W_{peak} was normalized after 4 h of recovery with CHO, whereas W_{peak} remained depressed ($P < 0.05$) after water provision. W_{peak} was fully recovered after 24 h in both the H$_2$O and the CHO group. **Conclusion**: In conclusion, the present results suggest that low muscle glycogen depresses muscle SR Ca^{2+} release rate, which may contribute to fatigue and delayed recovery of W_{peak} 4 h postexercise. **Key Words**: CALCIUM REGULATION, MUSCLE FUNCTION, ELITE ATHLETES, RECOVERY, FATIGUE

Muscle glycogen is the primary source of fuel in humans during prolonged muscle activity. The now well-established relationship between muscle glycogen and work capacity during prolonged exercise strongly suggests a dependency of glycogen on normal muscle function. Evidence for a strong effect of muscle glycogen on muscle function comes from several sources. For instance, research has established a close relationship between muscle glycogen and fatigue resistance through experimental alterations in preexercise muscle glycogen reserves by dietary and exercise manipulations or in the reliance on endogenous glycogen during exercise by modifying the availability of fuel sources (3,9,15). Also, the McArdle disease, with deficient glycogen phosphorylase that restricts the metabolism of glycogen, leads to the development of profound fatigue during exercise (25). Thus, there are clear indications that muscle glycogen must be available in a reasonable amount to maintain normal muscle function. However, the mechanism by which glycogen depletion leads to fatigue is still not understood.

Studies on both rodent single fibers and humans have pointed to a modulating role of glycogen availability on sarcoplasmic reticulum (SR) Ca^{2+} handling (5,8,29,33). By using mouse fiber bundles, it has been demonstrated that when recovery after glycogen reducing contractions occurs in the absence of glucose supplementation, glycogen does not recover and fiber bundles are less resistant to fatigue in a subsequent series of contractions (5). Further, studies on single muscle fibers in rat have shown that the decrease in tetanic force at fatigue corresponds to the reduction in intracellular free Ca^{2+} transients (5,13), indicating that glycogen depletion is associated with a decrease of tetanic [Ca^{2+}]$_i$. Indeed, Kabbara et al. (18) have shown that the decline of tetanic [Ca^{2+}]$_i$ in single muscle fibers of the cane toad during contractions corresponds with the decline in glycogen.

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Recent findings from human studies support the findings in animal models where Ca^{2+} kinetics is influenced by glycogen levels (9,10,33). Duhamel et al. (10) examined the relationship between muscle glycogen content and SR vesicle Ca^{2+} release rate in untrained males during a prolonged fatiguing cycling session at $\approx 70\%$ of $\dot{V}O_{2peak}$. The cycling session was preceded by a glycogen-depleting exercise session and 4 d of either low or high carbohydrate (CHO) diet. Muscle glycogen content was markedly reduced at the initiation of exercise with the low CHO diet, and deteriorations in SR Ca^{2+} release occurred earlier during exercise in this condition compared with the high CHO diet. In a recent study, we demonstrated an association between muscle glycogen and SR vesicle Ca^{2+} release rate in arm muscles of elite cross-country skiers after a fatiguing cross-country skiing exercise (33). Muscle glycogen levels were manipulated in the recovery period by diet intervention where skiers were provided with either a CHO-enriched diet or water during the initial 4-h period after exercise. After 4 h of recovery with CHO, SR vesicle Ca^{2+} release rate was normalized and muscle glycogen content were markedly increased compared with postexercise, whereas both SR release and glycogen remained depressed in the group that did not receive CHO. Muscle glycogen was only reduced to a minor extent after the ski race in the leg muscles, and there was no significant change in Ca^{2+} release rate. Together, the available reports strongly indicate a mechanistic role of glycogen on SR Ca^{2+} release and muscle function. However, the association between low muscle glycogen, SR Ca^{2+} regulation, and dynamic muscle function as peak power output remains unclear. This is of particular importance in elite athletes after, for example, prolonged exercise.

On the basis of the existing human data, there is a critical level of muscle glycogen at around 250–300 mmol·kg^{-1} dw below which the SR Ca^{2+} release rate is impaired (10,33). Accordingly, minor decreases in muscle glycogen does not cause significant impairments in muscle Ca^{2+} regulation, whereas beginning exercise with a low or high muscle glycogen store accelerates or postpones the impairment of SR Ca^{2+} regulation, respectively (5,10,13). However, there are limited data and knowledge concerning the influence of training status and exercise type on the precise role of glycogen on SR function. Previous studies regarding human SR function after exercise and the interaction between SR Ca^{2+} handling and muscle glycogen concentration have used exercise protocols of 2 h or less. Training sessions and competitive events often involve exercising for a period of 3 h or more, particularly in endurance-based sports. For example, the Ironman distance triathlon is characterized by a prolonged bike segment of 180 km lasting more than 4 h. This part of the Ironman triathlon is performed at approximately 83% of maximal heart rate (HR$_{max}$), inducing enormous energy expenditure that primarily relies on endogenous glycogen (1,20). It remains unclear whether impairments in SR function are present after such long-term cycling sessions performed by elite athletes and whether

muscle glycogen modulates recovery of SR function after ultraendurance-based training sessions. Also, the association between reduced muscle glycogen, SR Ca^{2+} release rate, and functional muscle performance as peak power output is unknown at present. Furthermore, little is known about the glycogen resynthesis rate in these highly trained endurance athletes.

In the present study, we measured the muscle glycogen content, SR function, and peak power output in highly trained triathletes before and during a 24-h recovery period that followed a 4-h glycogen-depleting cycling exercise. By allowing or omitting CHO intake during the initial 4 h of the recovery period, we were able to create an experimental design where changes in muscle glycogen level were separated from acute changes affecting SR Ca^{2+} function and exercise performance. The purpose was to investigate the effects of 4 h of glycogen-diminishing cycling exercise on SR vesicle function and muscle mechanical function in leg muscles of male elite endurance athletes. A second purpose was to assess the rate of muscle glycogen resynthesis in these athletes at elite level.

METHODS

Subjects

Fourteen male elite triathletes were recruited for the study (Table 1). Six triathletes were current or former Danish National Team members, and four triathletes were recently placed in the top three at the European or World Long Distance Triathlon Championships. All subjects were fully informed of any risk associated with the experiments before verbal and written consents were obtained. The study was approved by the ethics committee of Southern Denmark (project ID S-20090140).

Experimental Overview

At least 2 d before the experimental part of the study, subjects visited the laboratory for preliminary tests and familiarization to the used peak power (W_{peak}) test. The experimental part of the study comprised 4 h of glycogen-diminishing cycling exercise performed at 73% of the

TABLE 1. Anthropometric, physiological, and training characteristics from the CHO and H_2O group as well as pooled data from both groups.

	CHO + H2O	CHO	H2O
n	14	7	7
Age (yr)	27.2 ± 0.9	27.4 ± 1.0	27.1 ± 1.5
Height (cm)	183 ± 2	182 ± 2	184 ± 2
Body mass (kg)	75.3 ± 1.4	75.0 ± 1.6	77.7 ± 2.5
History as elite athlete (yr)	4.8 ± 0.7	4.9 ± 1.0	4.7 ± 1.8
Training volume (h·wk^{-1})	16.4 ± 0.9	17.6 ± 1.3	15.3 ± 1.2
$\dot{V}O_{2max}$ (L·min^{-1})	5.0 ± 0.1	5.1 ± 0.2	4.9 ± 0.2
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	66.5 ± 1.3	68.3 ± 1.4*	63.5 ± 1.8
$\dot{V}O_{2AT}$ (mL·kg^{-1}·min^{-1})	52.0 ± 1.6	54.4 ± 1.6	48.7 ± 2.4
Watt$_{max}$ (W)	384 ± 13	399 ± 18	371 ± 17

Watt$_{max}$ defines the mean power output during the 5-min maximal open mode cycling protocol and $\dot{V}O_{2AT}$ the relative $\dot{V}O_2$ at 4 mmol lac·L^{-1} blood.
* Significantly different from H_2O, $P < 0.05$.

maximum heart rate (HR_{max}). During the first 4-h recovery period after the exercise, the subjects were randomized to receive either water (H_2O group) or a CHO-enriched diet (CHO group), which allowed discrimination between muscle glycogen levels without the influence of acute effects of exercise on muscle function. After the initial 4 h of recovery, all subjects received a CHO-enriched energy intake for the remaining 20 h of the 24-h recovery period; thus, both groups received the same total food and beverages after 24 h. Muscle biopsies were obtained from the vastus lateralis muscle before (Pre), immediately after (Post), and 4 and 24 h after exercise. After obtainment of each muscle biopsy, W_{peak} was determined and blood samples were extracted. All procedures were conducted in laboratories at the Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense.

Preliminary Measurements

Quantifications of W_{peak}, mean power output (W_{max}), aerobic capacity ($\dot{V}O_{2AT}$), and aerobic power ($\dot{V}O_{2max}$) were achieved using the same electromagnetic bicycle ergometer (Schoberer Rad Messtechnik [SRM], Julich, Germany). The stationary SRM ergometer was adjusted to mirror individual bike settings and mounted with personal pedals. Power measurements from the SRM ergometer were provided to an adjacent personal computer at a sampling frequency of 3 Hz. Further analyses were executed using the SRM software (version 6.41.04). Calibrations of the ergometer were conducted every morning of all experimental days.

Peak power output. After 12 min of moderate warm-up on the cycling ergometer, interspersed with two short accelerations, subjects performed a protocol consisting of 4×5 s of maximal sprints at the following incremental pedaling frequencies: 100, 110, 120, and 130 rpm. All sprints were separated by 55 s of recovery and conducted with a standardized forceful verbal encouragement from the test leader to reach maximal performance. Because of small fluctuations in the data sampling, W_{peak} was defined as the highest mean power during a 3-s period at each pedaling frequency. Similar protocols have previously been used, validated, and have demonstrated high reproducibility (7).

Respiratory measures. Aerobic capacity was determined on the cycle ergometer using a progressive submaximal endurance protocol. Initial workload was dependent on body mass and was, on average, 190 ± 5.1 W for the first 5 min of the protocol. Hereafter, power output was electronically increased by 30 W every 5 min until blood lactate concentration exceeded 4 $mmol \cdot L^{-1}$ blood. Blood lactate was determined from capillary blood fingertip samples collected over the last 30 s of each 5-min step using the YSI Model 1500 Sport (YSI, Inc., Yellow Springs, OH). The YSI was calibrated before each test by two standardized solutions containing 5 and 15 mmol lactate. $\dot{V}O_2$ was calculated continuously throughout the aerobic capacity test on the basis of ventilation and expired gas concentrations using

a mixing chamber system (AMIS 2001; Innovision, Odense, Denmark). Before each test, the gas analyzer was calibrated by a known gas solution and the ventilation sensors by manual calibration with a 3-L syringe. Aerobic capacity was defined as oxygen consumption (mL $O_2 \cdot kg^{-1} \cdot min^{-1}$) at 4 mmol $lactate \cdot L^{-1} \cdot blood$.

Maximal aerobic capacity ($\dot{V}O_{2max}$) was determined from a 5-min all-out cycling protocol. Subjects were instructed to produce the highest possible mean power output (W_{max}) throughout the test. $\dot{V}O_{2max}$ was defined as the highest mean $\dot{V}O_2$ obtained during a 15-s period. Heart rate and power output were sampled throughout the test to determine HR_{max} and W_{max}, respectively.

Prolonged Cycling Exercise

Workload during the bike segment of Ironman triathlon is approximately 83% of HR_{max} and lasts more than 4 h (1,20). However, requiring subjects in the current study to maintain a mean workload of 83% of HR_{max} was not feasible considering that no CHO was supplied during exercise. Thus, mean heart rate (HR_{mean}) during exercise was intended to be 75% of HR_{max} with a self-selected pedaling frequency. However, if a subject considered that maintaining 75% of HR_{max} for 4 h was not feasible after 2 h of exercise, the load was downregulated to the highest achievable HR_{mean}. By use of turbo trainers (Elite Crono Mag ElastoGel Trainer, Fontaniva, Italy), subjects were able to use personal bikes, shoes, and pedals. Heart rate was sampled continuously throughout the trial, and HR_{mean} was monitored after exercise. A minimum intake of water corresponding to 1 $mL \cdot kg^{-1} \cdot h^{-1}$ was imposed during exercise. Room temperature (~22°C) and humidity (~35%) were standardized throughout the 4-h cycling exercise. Subjects were instructed to eat normally and to refrain from severe exercise on the day preceding testing.

Dietary Manipulation and Test Procedure

Dietary intake was controlled and calculated on the basis of body mass throughout experimentation. Breakfast in both groups was consumed 90 min and 4 h before reporting to the laboratory for the extraction of the first (Pre) and fourth (24 h) biopsy, respectively, and consisted of CHO-rich foods (i.e., porridge oats, raisins, skimmed milk, orange juice, and a sports bar; 82 $kJ \cdot kg^{-1}$ bw). After the prolonged exercise, the CHO group received a meal (1.07 g $CHO \cdot kg^{-1}$ $bw \cdot h^{-1}$) consisting of pasta, chicken, vegetables, and a CHO beverage during the initial 2 h of the recovery period and a CHO beverage and a sports bar (1.05 g $CHO \cdot kg^{-1}$ $bw \cdot h^{-1}$) during the subsequent 2 h of the recovery. This CHO intake corresponds to the recommendations from the American College of Sports Medicine (37). The alternate group was provided with water only during the initial 4 h of the recovery period. During the remainder 20 h of recovery, both groups received a standardized CHO-enriched meal. In addition, the H_2O group received a supplemental meal to

equalize the total energy intake between groups during the recovery period. In total, subjects received 264 kJ·kg^{-1} bw on the first experimental day corresponding to 17.2 to 22.6 MJ ($\approx$10 g CHO·kg^{-1} bw·d^{-1}).

Peak power output. W_{peak} at 100, 110, 120, and 130 rpm was determined after obtainment of each muscle biopsy as previously described. Hence, there was a delay of 20 min from termination of exercise until the power measurements were conducted. W_{peak} was expressed relative to body mass (W·kg^{-1}) and relative to Pre (% Pre) to compare the effect of exercise and recovery between groups.

Analytical Techniques

Muscle biopsies. Four muscle biopsies (i.e., Pre, Post, 4 h, and 24 h) of 100–150 mg were obtained from the vastus lateralis muscle portion of the quadriceps femoris muscle using 5-mm Bergström needles. This muscle was chosen because it is highly active during a cycling exercise (14). Two biopsies were obtained randomly from the right and left legs with the first and third biopsies from one leg and the second and fourth biopsies from the contralateral leg. The procedure for the extraction of muscle tissue was identical at all time points. After lying horizontally for 5 min, a 1-cm incision was made in the middle region of the vastus lateralis muscle with a scalpel under local anesthesia (3–4 mL of 2% lidocaine) before the biopsy was obtained by the percutaneous needle biopsy technique. Finally, the incision was covered with gauze. Muscle tissue was removed from the needle, placed on filter paper upon an ice-cooled Petri dish, blotted, dissected free from fat and connective tissue, and divided into five specimens with a scalpel. One part was immediately frozen in liquid nitrogen and stored at −80°C for subsequent analysis of citrate synthase (CS) activity, 3-hydroxyacyl-CoA dehydrogenase (HAD) activity, and metabolite content. Another part was manually homogenized with a Potter–Elvehjem glass–glass homogenizer (Kontes Glass Industry, Vineland, NJ) in an ice-cold buffer containing 300 mM sucrose, 1 mM EDTA, 10 mM NaN$_3$, 40 mM Tris base, and 40 mM L-histidine at pH 7.8. The homogenization was performed in a weight to volume ratio of 1:10. Homogenate was divided into parts of 25–100 μL, frozen in liquid nitrogen, and stored at −80°C until further analyses were performed.

SR vesicle Ca^{2+} uptake and release rates. The fluorescent dye technique was used to determine Ca^{2+} uptake and release rates in SR vesicles as previously described in detail elsewhere (31,33). The assay buffer consisted of 165 mM KCL, 22 mM HEPES, 7.5 mM oxalate, 11 mM NaN$_3$, 5.5 μM TPEN, 20 μM CaCl$_2$, and 2 mM MgCl$_2$ (pH 7.0 at 37°C). Briefly, muscle homogenate (70 μl) was mixed with 2 mL of assay buffer. Ca^{2+} uptake was then initiated by adding 2 mM ATP to a final concentration of 5 mM. Free [Ca^{2+}] was determined by the fluorescent Ca^{2+} indicator Indo-1 (1 μM), and fluorescence was sampled every 0.5 s (20 Hz, Ratiomaster RCM; Photon Technology Interna-

tional, Brunswick, NJ). Ca^{2+} uptake was recorded for 3 min, before [Ca^{2+}] reached a plateau. Upon measurements of Ca^{2+} uptake, the SR Ca^{2+} ATPase was blocked with cyclopiazonic acid (40 μM) before SR vesicle Ca^{2+} release was initiated by the addition of 4-chloro-*m*-cresol (4-CmC) (5 mM). The raw data for [Ca^{2+}] were imported into Matlab version 7.0.1 (The MathWorks, Natick, MA) and mathematically analyzed (Curve Fitting Toolbox version 1.1.1; The MathWorks). The curve fitting of Ca^{2+} uptake was performed with data points between free [Ca^{2+}] of 1000 nM and free [Ca^{2+}] 20 s before initiation of Ca^{2+} release. The time for free [Ca^{2+}] to decrease by 63% of the initial free [Ca^{2+}] (τ) was calculated as $1/b$ from the equation $y = ae^{-bt} + c$, where y is the free [Ca^{2+}], t is time, and a, b, and c are constants assigned from Matlab. There were no differences in constant c (Nadir Ca^{2+}) between trials, time, or within same subjects at various time points. SR Ca^{2+} release rate was obtained by mathematically fitting the data points during the first 30 s of release to the equation $y = a [1 - e^{-b (t - c)}]$. This was back-extrapolated to Nadir [Ca^{2+}], and the rate of Ca^{2+} release was determined as the derivate of the initial release. Values obtained for SR Ca^{2+} release rates are relative and therefore expressed as arbitrary units (Ca^{2+}·g^{-1} protein·min^{-1}). Because of the interindividual variation in SR Ca^{2+} release rates, results are normalized to prevalues (% Pre). Assays of uptake and release of Ca^{2+} were performed in triplicates (a few in duplicates due to limited tissue homogenate). The protein content in the muscle homogenate was measured in triplicates using a standard kit (Pierce BCA protein reagent no. 23225). For both groups, except Post, $n = 7$; 4 h and 24 h were $n = 6$ for both groups because of limited tissue.

Muscle glycogen. Muscle glycogen content was determined spectrophotometrically (Beckman DU 650) (35). Freeze-dried muscle tissue (1.5 mg) was boiled in 0.5 mL of 1 M HCL for 150 min before it was quickly cooled, whirl mixed, and centrifuged at 3500*g* for 10 min at 4°C. Forty microliters of boiled muscle sample and 1 mL of reagent solution containing Tris buffer (1 M), distilled water, ATP (100 mM), MgCl$_2$ (1 M), NADP$^+$ (100 mM), and G-6-PDH were mixed before the process was initiated by adding 10 μL of diluted hexokinase. Absorbance was recorded for 60 min before the glycogen content was calculated. Muscle glycogen was expressed as millimoles per kilogram of dry weight. In addition, maximal glycogen resynthesis in the CHO group was calculated on the basis of the increase in muscle glycogen from post to 4 h and expressed as millimoles per kilogram of dry weight per hour. For both groups at each time point, $n = 7$, except for Post CHO, and for H$_2$O and 4 h CHO, $n = 6$ because of limited tissue.

CS and HAD activity. Enzyme activities were measured in freeze-dried muscle dissected free from nonmuscle constituents (30°C) (35). CS activity was determined by the addition of oxaloacetate to a buffer solution containing muscle homogenate, DTNB buffer, acetyl-CoA, and H$_2$O. β-Hydroxy-acyl coenzyme A dehydrogenase (HAD) activity

was measured after the addition of acetoacetyl-CoA to a buffer solution containing imidazole, NADH, and EDTA. The absorbance of CS and HAD was recorded for 600 s, converted into enzyme activity rates, and expressed as micromoles per gram·of dry weight per minute.

Fiber type distribution. Myosin heavy chain (MHC) composition was determined from homogenate using gel electrophoresis (32). Muscle homogenate (80 μL) was mixed with 200 μL of sample buffer (10% glycerol, 5% 2-mercaptoethanol, and 2.3% sodium dodecyl sulfate (SDS), 62.5 mM Tris, and 0.2% bromophenol blue at pH 6.8.), boiled in water at 100°C for 3 min, and loaded (10–40 μL) on an SDS–PAGE gel (6% polyacrylamide [100:1 acrylamide–*bis*-acrylamide], 30% glycerol, 67.5 mM Tris base, 0.4% SDS, and 0.1 M glycine). Gels were run at 80 V for at least 42 h at 4°C, and MHC bands were made visible by staining with Coomassie. The gels were scanned (Lino-scan 1400 scanner, Heidelberg, Germany) and MHC bands quantified densitometrically (Phoretix 1D, nonlinear, Newcastle, UK) as an average of the three loaded protein amounts (Fig. 1). MHCII was identified with Western blot using monoclonal antibody (Sigma M4276) with the protocol Xcell IITM (Invitrogen, Carlsbad, CA). The MHC composition of each subject was determined as an average of three biopsies from time points Pre, Post, 4 h, and 24 h.

Statistical analysis. Statistical analysis was performed using a linear mixed model (STATA 10.1; StataCorp, College Station, TX). All variables were analyzed with subject ID as a random effect and with time (Pre, Post, 4 h, and 24 h) and group (CHO and H_2O) as fixed effects. Variables with skewed distributions were appropriately transformed (Box-Cox) before analysis. Statistical analyses of the degree of association between glycogen and SR function were performed using linear regression (regress). Values are presented as mean ± SE. The level of statistical significance was set at $P < 0.05$.

RESULTS

Baseline. Anthropometrics, physiological characteristics, and training information of the subjects are presented in Table 1. Except from $\dot{V}O_{2max}$, no parameters were different between groups (CHO: 68.3 ± 1.4 mL·kg^{-1}·min^{-1} vs H_2O: 63.5 ± 1.8 mL·kg^{-1}·min^{-1}, $P < 0.05$).

MHC distribution and enzyme activity. By combining data from both groups, the relative distribution of

MHCI, MHCIIa, and MHCIIx was 64.7% ± 2.1%, 34.0% ± 1.9%, and 1.3% ± 0.3%, respectively. There was a consistent higher relative content of MHCI in the CHO group compared with the H_2O group at all time points and a corresponding lower MHCIIa content ($P < 0.05$). The relative fiber type distribution remained unchanged within the two groups between Pre, Post, 4 h, and 24 h (average from at least three time points: MHCI: CHO = 73.5 ± 1.9 vs H_2O = 55.4 ± 2.1; MHCIIa: CHO = 26.4 ± 1.9 vs H_2O = 42.3 ± 2.4). CS and HAD activities were on average 111 ± 6 and 138 ± 7 μmol·g^{-1} dw·min^{-1}. CS activity was similar between the CHO and the H_2O groups, whereas HAD activity was 23% higher in the CHO group compared with the H_2O group ($P < 0.05$).

Exercise intensity. All subjects accomplished the prolonged cycling exercise, although some individuals were unable to maintain the predetermined exercise intensity of 75% HR_{max}. Consequently, HR_{mean} was 73% ± 1% of HR_{max}, (CHO 74% ± 1% and H_2O 71% ± 0%), which, estimated from the submaximal test, equaled approximately 56% of $\dot{V}O_{2max}$.

Muscle glycogen. Resting muscle glycogen concentration in vastus lateralis muscle was on average 699 ± 26 mmol·kg^{-1} dw (range = 519–883 mmol·kg^{-1} dw), with two athletes demonstrating resting glycogen contents of more than 875 mmol·kg^{-1} dw. An overall group–time interaction was evident for muscle glycogen concentration, that is, the change in muscle glycogen concentration was different between groups from Post to 4 h ($P < 0.05$). The 4-h exercise reduced muscle glycogen to a similar level in both groups, corresponding to 32% ± 4% of prevalues or 225 ± 28 mmol·kg^{-1} dw for combined data (CHO: 28% Pre, 205 ± 31 mmol·kg^{-1} dw; H_2O: 36% Pre, 245 ± 49 mmol·kg^{-1} dw) (Fig. 2a, $P < 0.01$). Because of the CHO manipulation, muscle glycogen changed differently between groups from Post to 4 h ($P < 0.05$). Thus, muscle glycogen remained depressed in the H_2O group (264.0 ± 31.0 mmol·kg^{-1} dw, NS), whereas muscle glycogen was significantly elevated in the CHO group in comparison with the Post measurement (449 ± 27 mmol·kg^{-1} dw, $P < 0.05$) (Fig. 2a). Twenty-four hours after the cycling exercise, muscle glycogen content attained prelevels in both groups (Fig. 2a).

Muscle glycogen resynthesis rate. Muscle glycogen resynthesis rate during the initial 4 h of recovery was on average 61 mmol·kg^{-1} dw·h^{-1} (from 205 to 449 mmol·kg^{-1} dw) and calculated on the basis of all subjects from the CHO group.

FIGURE 1—Representative gel analysis of the MHC isoform composition. Bands with MHC I, IIa, and IIx are identified by arrows. The whole muscle homogenate MHC bands made visible by staining with Coomassie and the relative distribution of the MHC isoform bands was estimated by densitometrically quantification and given as an average of three separate lanes for each biopsy (A1–A3, B1–B3, etc.). The MHC composition of each subject was determined as an average of three biopsies. Three MHC isoforms (MHC I, IIA, and IIX) are detectable in a mixed sample of human vastus lateralis muscle (SD, lane 1).
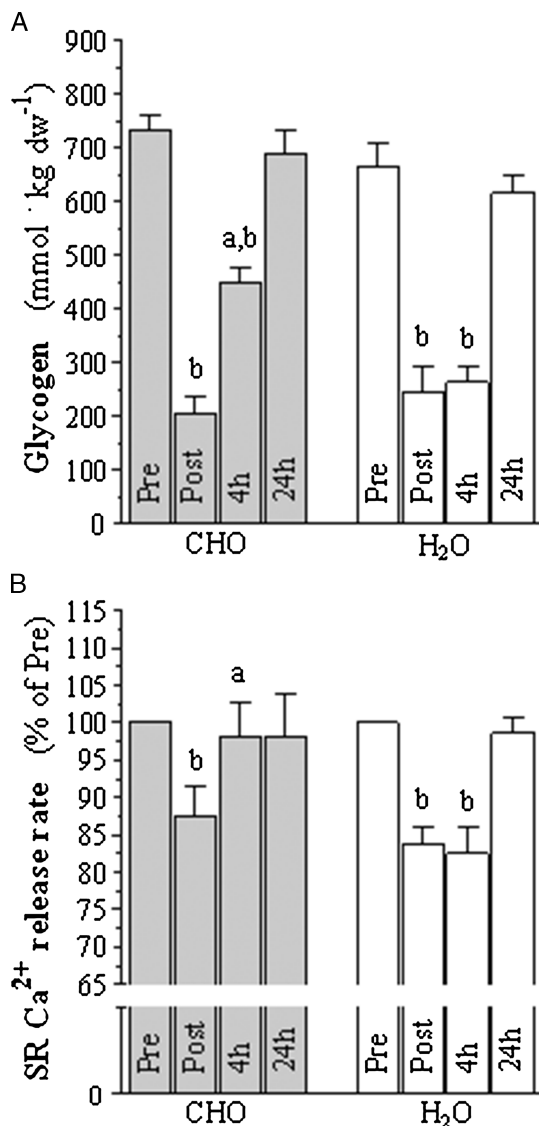
FIGURE 2—A. Absolute changes in muscle glycogen content in the CHO (gray bars) and H_2O group (open bars) throughout the experimental period. B. Relative changes in SR vesicle Ca^{2+} release rate in the CHO (gray bars) and H_2O group (open bars) throughout the experimental period. [a]Significantly different from H_2O ($P < 0.05$). [b]Significantly different from Pre ($P < 0.05$).

SR vesicle Ca^{2+} release rate. A group–time interaction was evident for SR Ca^{2+} release rate, that is, the change in SR Ca^{2+} release rate from Pre to 4 h was different between the CHO and H_2O group ($P < 0.05$) (Fig. 2b and Table 2). SR Ca^{2+} release rate was equally reduced in both groups immediately after the prolonged cycling exercise, corresponding to $-14.3\% \pm 2.2\%$ for combined data (CHO: $-12.5\% \pm 3.9\%$; H_2O: $-16.2\% \pm 2.1\%$ ($P < 0.05$) (Fig. 2b). After 4 h of recovery, SR vesicle Ca^{2+} release rate attained prelevels in the CHO group ($-2\% \pm 5\%$ Pre, NS), whereas it remained depressed in the H_2O group compared with preexercise ($-17.5\% \pm 3.1\%$, $P < 0.05$). In addition, SR vesicle Ca^{2+} release was significantly different between groups after the 4-h recovery with or without optimal CHO intake ($P < 0.05$) (Fig. 2b). During the subsequent 20-h recovery period, SR vesicle Ca^{2+} release rate returned to prelevels in the H_2O group, whereas no further changes were observed in the CHO group.

SR vesicle Ca^{2+} uptake rate. SR vesicle Ca^{2+} uptake rate (τ) was not affected by exercise or recovery in neither the CHO nor the H_2O group, and no differences were observed between groups at any time point (Table 2).

Correlation between muscle glycogen and SR vesicle Ca^{2+} release rate. Alterations in muscle glycogen and SR vesicle Ca^{2+} release rate followed a similar pattern (Fig. 2). Plotting the individual data on SR Ca^{2+} release rate and muscle glycogen for all time points demonstrated a significant linear correlation ($P < 0.01$, $r^2 = 0.32$). To exclude the possible influence of acute effects of exercise on SR Ca^{2+} release rate and to take advantage of the glycogen manipulating design, we also plotted data without the Post data (i.e., Pre, 4 h and 24 h), which revealed a significant correlation between SR vesicle Ca^{2+} release rates and muscle glycogen contents ($P = 0.01$, $r^2 = 0.30$; Fig. 3). Thus, SR Ca^{2+} release rate and muscle glycogen content are temporarily associated (Fig. 2), and individual data are linear correlated (Fig. 3). Importantly, taking advantage of the study design, the SR Ca^{2+} release rate and glycogen content were closely associated after a 4-h recovery period with and without CHO. Hence, both muscle glycogen and SR Ca^{2+} release rate remained depressed in the H_2O group, whereas muscle glycogen and SR Ca^{2+} release were significantly elevated in the CHO group in comparison with the Post measurement.

Peak power output. Before exercise, W_{peak} corresponded to 13.2 ± 0.6, 13.6 ± 0.6, 14.0 ± 0.6, and 13.2 ± 0.6 $W \cdot kg^{-1}$ at 100, 110, 120, and 130 rpm, respectively, and there were no significant differences in W_{peak} between pedaling frequencies nor between CHO and H_2O groups. Because no differences were observed between the pedaling frequencies, representative data for the cadence with the

TABLE 2. SR vesicle Ca^{2+} uptake and release

Variable		Pre	Post	4 h	24 h
τ (s)	CHO	45.9 ± 5.4	52.6 ± 3.5	50.9 ± 7.3	38.0 ± 3.3
	H_2O	40.8 ± 7.3	39.7 ± 6.1	37.9 ± 5.2	34.8 ± 3.2
SR vesicle Ca^{2+} uptake at 800 nM Ca^{2+}	CHO	3.4 ± 0.2	2.7 ± 0.1	2.8 ± 0.3	3.5 ± 0.4
	H_2O	3.6 ± 0.3	3.3 ± 0.3	3.2 ± 0.2	3.6 ± 0.3
SR vesicle Ca^{2+} release rate	CHO	2.7 ± 0.2	2.2 ± 0.1^a	2.6 ± 0.1	2.8 ± 0.2
	H_2O	2.8 ± 0.3	2.3 ± 0.2^a	2.3 ± 0.1^a	2.8 ± 0.2

The SR vesicle Ca^{2+} uptake and release were analyzed fluorometrically in crude muscle homogenate. The τ is the inverse rate constant representing the time for 63% of the Ca^{2+} to be taken up by the SR vesicles. Uptake and release rates are expressed in arbitrary μmol $Ca^{2+} \cdot g^{-1}$ protein·min^{-1}.
[a]Sign different from Pre.
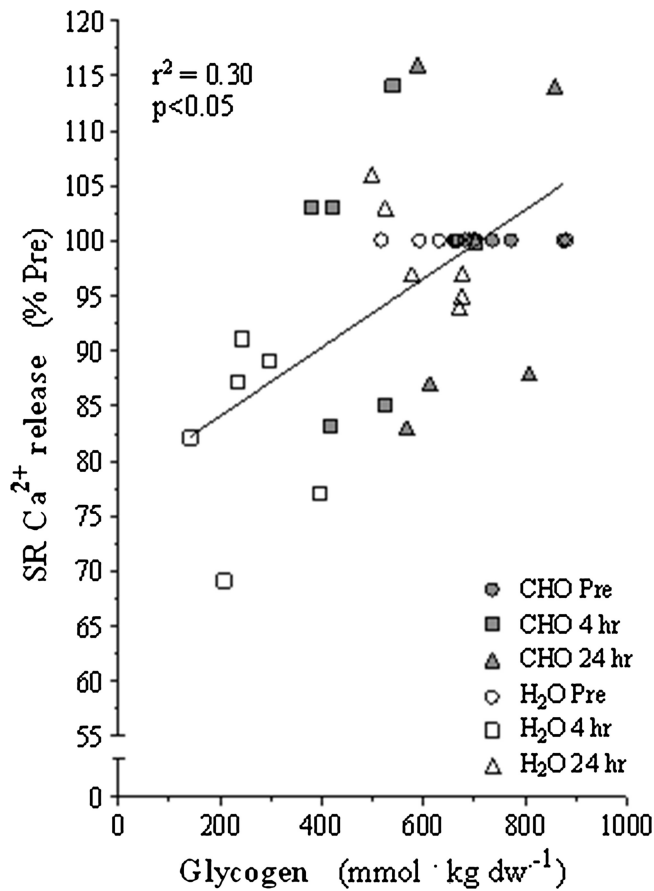
FIGURE 3—Correlation of SR Ca²⁺ release rate and total glycogen concentration in biopsies from elite triathletes. Data points are three time points (Pre, 4 h, and 24 h) for both conditions (CHO and H₂O). The line indicates the best fit of all the data points ($r^2 = 0.30$, $P < 0.01$). For Pre (CHO and H₂O), $n = 7$, and due to the missing values in either glycogen content or SR Ca²⁺ release rate, $n = 5$ (4 h CHO) and $n = 6$ (4 h H₂O and 24 h CHO and H₂O).

highest W_{peak} values (120 rpm) are described. An overall group–time interaction was evident for W_{peak} at 120 rpm ($P < 0.05$), that is, W_{peak} changed in a different manner from Post to 4 h ($P < 0.05$). The 4-h prolonged cycling exercise reduced W_{peak} to a similar extent in both groups, corresponding to 8.8% ± 1.7% (CHO: −11.1% ± 2.9%; H₂O: −6.0% ± 1.3%) (Fig. 4, $P < 0.05$). W_{peak} was normalized after 4 h of recovery with CHO provision (−1.7% ± 1.7%, NS); however, it remained depressed by 5.3% ± 2.3% ($P < 0.01$) in the H₂O group (Fig. 4). W_{peak} was fully restored in both groups after additional 20 h of recovery.

DISCUSSION

In the present study, we investigated the role of glycogen availability on muscle SR function and peak power output in highly trained male endurance athletes after a 4-h endurance exercise, a 4-h recovery period with either CHO or water supplementation, and an additional 20-h recovery period with a CHO-enriched diet. Importantly, our experimental design specifically identified that muscle SR Ca²⁺ release

rate and muscle power capacity remained depressed at postexercise levels after the 4-h recovery period if muscle glycogen was maintained low with water and was markedly elevated if muscle glycogen was restored with CHO. Further, multiple linear regression analysis demonstrated an association between muscle glycogen content and SR Ca²⁺ release rate. Thus, the present findings provide further support to the hypothesis that glycogen is a prerequisite for normal SR Ca²⁺ release and thereby a key regulator of muscle cytosolic levels of Ca²⁺ in contracting skeletal muscle. This may eventually, at least in part, explain the reduced W_{peak} observed after recovery with water provision.

SR vesicle Ca²⁺ regulation after exercise. A role of reduced SR Ca²⁺ release in fatigue was first proposed by Eberstein and Sandow (11) in 1963, and today, it is generally accepted that deteriorations in SR function and SR Ca²⁺ release rate, in particular, contribute to muscle fatigue in a variety of exercise types (2,10,34). Studies in untrained humans have demonstrated reductions in both SR vesicle Ca²⁺ release and uptake rates during and after prolonged cycling exercise (8,10,24). By the present results, we have extended these findings by demonstrating that SR vesicle Ca²⁺ release rate was also impaired in highly trained elite endurance athletes after 4 h of prolonged cycling exercise. SR vesicle Ca²⁺ release rate was reduced by 14% after exercise, which is lower than previously reported in humans after short-term high-intensity exercise (16,24) but in agreement with reports examining the influence of prolonged cycling exercise on SR Ca²⁺ release rate in untrained subjects (8,10,24). Of note is that even relatively small decreases in SR Ca²⁺ release and thereby [Ca²⁺]ᵢ will significantly affect the force production because of the sigmoidal shape of the force–[Ca²⁺]_free relationship. Thus, alterations in force because of reduced Ca²⁺ transients are more apparent
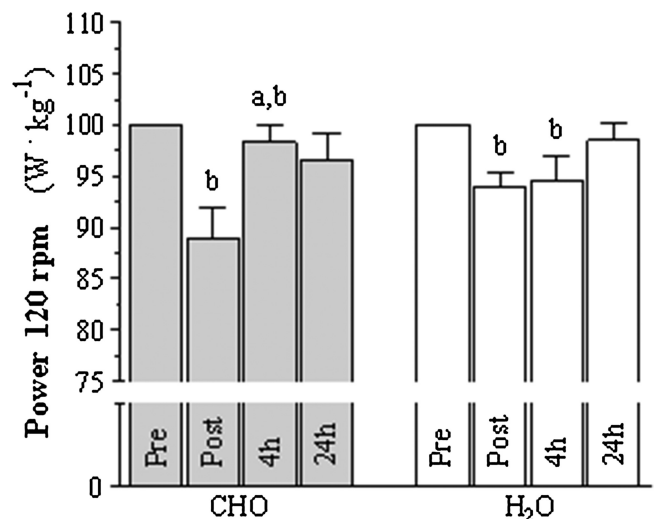
FIGURE 4—Relative changes in W_{peak} in the CHO (gray bars) and H₂O group (open bars) throughout the experimental period. [a]Significantly different from H₂O ($P < 0.05$). [b]Significantly different from Pre ($P < 0.05$).

at submaximal contractions like those from cycling exercise versus maximal contractions.

Muscle glycogen content and SR function. The current study findings suggest that the content of glycogen in skeletal muscle cells affect SR vesicle function. Using a similar methodology, Duhamel et al. (9,10) have shown that there is a clear association between muscle glycogen content and SR vesicle function during prolonged exercise when starting exercise with high and low glycogen levels, respectively. In accordance to these results and those from the present study, we have recently shown that in elite trained humans, the detrimental change in SR function is also associated with fatigue, which is glycogen dependent (33).

SR vesicle Ca^{2+} uptake rate. We did not observe an effect from 4 h of prolonged cycling exercise on SR vesicle Ca^{2+} uptake rate. SR vesicle Ca^{2+} uptake have been reported to be both reduced (10,26) and unaltered (19,33) after exercise. These discrepancies in research may be related to the exercise mode and training status of individuals. Further, the intracellular glycogen localization may explain the observed discrepancies. Glycogen is localized in distinct subcellular compartments, and we have previously demonstrated that the particular localization of intermyofibrillar glycogen is associated with the muscle fiber relaxation rate, that is, SR Ca^{2+} uptake rate. There is also evidence that the glycogen distribution in the muscle fiber is dependent on training status and exercise mode (28,30), and hence, it is difficult to quantify the effect of exercise on SR Ca^{2+} uptake rate without considering individual and exercise factors.

SR vesicle Ca^{2+} release rate. Recent studies in humans have reported that muscle glycogen depletion is associated with a reduction in SR vesicle Ca^{2+} release during and after prolonged exercise (9,10,33). The present results provide support for this idea by showing a direct association between muscle glycogen content and SR vesicle Ca^{2+} release rate during recovery from prolonged cycling exercise in highly trained endurance athletes (Fig. 3). Both SR vesicle Ca^{2+} release rate and muscle glycogen content were markedly elevated by CHO provision during the initial 4 h of recovery, whereas both parameters remained depressed when water was provided to the athletes. Pooling of values from Pre, 4 h, and 24 h revealed a significant correlation between muscle glycogen content and SR vesicle Ca^{2+} release. Recently, we have demonstrated a direct association between SR vesicle Ca^{2+} release rate and glycogen content in arms of highly trained cross-country skiers during recovery from 1 h of exhaustive skiing exercise (33). Hence, muscle glycogen and SR vesicle Ca^{2+} release rate were significantly correlated in the arms after 4-h recovery period with either CHO or water consumption. In addition, two studies from Duhamel et al. (9,10) observed that preexercise muscle glycogen content was associated with SR vesicle Ca^{2+} release rate during exercise in untrained males and females. After 4 d of either a low or high CHO diet, resting muscle glycogen contents in the untrained men were markedly different between the two situations. Measurements of SR vesicle function during a prolonged cycling exercise revealed that fatigue and reductions in SR vesicle Ca^{2+} release occurred earlier after ingestion of a low CHO diet compared with the high CHO diet, indicating that total glycogen has a direct effect on SR Ca^{2+} release. Another study from the same group of researchers supported these observations by reporting a similar association after 4 d of low or high CHO diet in untrained women (9). Thus, there are convincing data demonstrating a direct association between SR vesicle Ca^{2+} release rate and muscle glycogen content, and these data gives further support to the existence of a critical threshold of glycogen required to protect SR Ca^{2+} release as previously suggested by Duhamel et al. (10) and Ørtenblad et al. (33). SR Ca^{2+} regulation is quantitatively but not qualitatively different between fiber types with an approximately four times higher SR uptake and release rates in MHC II fibers (33). Impaired SR function after exercise may be speculated to be fiber type dependent, independent of glycogen content and/or fiber activation; however, to our knowledge, such differences between fiber type SR exercise susceptibility or regulation is at present unknown. Hence, differences in average MHC distribution between groups in the present study speculatively partly could explain differences between groups independent of glycogen contents.

Coupling between muscle glycogen and SR Ca^{2+} release rate. The mechanism by which glycogen modulates SR function remains speculative. According to the present and also previous studies, muscle glycogen seems to be a regulator of Ca^{2+} release and, as a direct consequence, a regulator of contractile activity and ATP usage. It is recognized that glycogen, glycogen-regulating proteins (i.e., glycogen phosphorylase, glycogen synthase, and glycogen debranching enzyme), and glycolytic enzymes (e.g., PK, GADPH, aldolase, etc.) are physically associated with the SR membrane in skeletal muscle (6,21,22,39). Studies from Lees et al. (21,22) demonstrate that these associations are dynamic and dependent on the glycogen state in the muscle cell. Hence, a dissociation of this glycogenolytic complex from SR has been observed during glycogen-diminishing exercise and proposed to induce a structural modification of the SR, eventually altering its behavior (21,22). Another hypothesis is that the SR Ca^{2+} release is ATP dependent and that release properties are modulated by local [ATP] or endogenous ATP production.

Resting muscle glycogen and glycogen resynthesis rate. In accordance with previous studies, the present study demonstrates enlarged glycogen storage capacities in endurance-trained athletes compared with previous reports from untrained subjects (12). Resting glycogen content of the vastus lateralis muscle was 699 ± 26 mmol·kg^{-1} dw (range = 519 to 883), which agrees with a recent study from Branth et al. (4) that reported an average resting glycogen content of 707 ± 32 mmol·kg^{-1} dw in endurance-trained males from the Swedish national mountain bike team. These resting glycogen levels exceed by far previous values reported in moderately trained endurance athletes

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(≈380–460 mmol·kg^{-1} dw) and approach or even attain muscle glycogen contents observed after loading regimes in the same subjects (17). Generally, the rate of glycogen resynthesis has been reported to be in the range of 20–50 mmol·kg^{-1} dw·h^{-1} after glycogen-diminishing exercise (for a review, see [17]). Half of the highly trained endurance athletes from the present study were provided with 1.03 g CHO·kg^{-1}·h^{-1} during the initial 4-h recovery period. Although not fully depleted after exercise, subjects demonstrated an average muscle glycogen synthesis rate of 61.0 mmol·kg^{-1} dw·h^{-1} (205 to 449 mmol·kg^{-1} dw) during recovery, measured as an average for 4 h and therefore likely higher during the first hour of recovery. To our knowledge, such a glycogen resynthesis rate is the highest reported and may reflect adaptations to the very prolonged muscle glycogen depletion exercise these athletes perform on weekly basis.

Power-generating capacity after prolonged exercise. Traditionally, alterations in force-generating capacity after prolonged cycling exercise have been quantified by measurements of maximal voluntary contraction of the knee extensors. After cycling protocols, comparable with the present, studies have reported reductions in isometric strength between 9% and 18% (23,27). To our knowledge, this is the first study to evaluate alterations in mechanical muscle outputs from a long-term exercise using a locomotion-specific quantifier (i.e., peak power output from a sprint cycling protocol). After exercise, maximal power-generating capacity was significantly reduced by 5.5%–8.9% at different cadences (i.e., 100, 110, 120, and 130 rpm). Because of the muscle biopsy extraction, there was a delay of 20 min from termination of prolonged cycling exercise until determination of W_{peak}, and it is thus very likely that the observed reduction in PPO was, in reality, higher immediately after exercise (36,38). Further, with the delay between termination of exercise and biopsy, the observed impairment in maximal power-generating capacity can probably not be explained by muscle depolarization and/or metabolic changes except from alterations in glycogen contents (decrease in ATP and PCr and increased P_i, H^+, or Cr), which would be normalized within a 20-min recovery. However, both muscle glycogen content and SR vesicle Ca^{2+} release rate were reduced after exercise and followed a pattern similar to that of W_{peak} that were irrespective of supplementation after exercise. This could indicate that the loss in maximal power generation that follows a prolonged endurance exercise is associated with muscle glycogen content and SR Ca^{2+} release rate.

Limitations of the study. Consistently, there was a higher relative content of MHCI in the CHO group compared with the H_2O group at all time points, as well as a higher HAD activity. This could theoretically influence the present study results and conclusions, as MHCI fibers have a lower SR content and because the fiber activation may differ between fiber isoforms. However, the SR uptake and release rates did not differ significantly between groups, and the SR vesicle function values were normalized to prevalues (% Pre), thus providing a reference for comparison. Accordingly, the changes observed in SR Ca^{2+} release rate are relative and independent of fiber type differences. Although glycogen use may be fiber type specific (i.e., active MHCI fibers use relatively more glycogen, which could affect SR function more than that in MHCII fibers), SR Ca^{2+} release rate was equally reduced in both groups immediately after the prolonged cycling exercise ($-16\% \pm 2\%$). Although the present design circumvents the acute effects of exercise, it should be noted that differences in other physiological parameters than muscle glycogen was likely induced because of the diet manipulation during recovery from cycling exercise. In particular, differences in plasma glucose, insulin levels, and free fatty acids were likely present during the first 4 h of recovery. However, it seems unlikely that alterations in these factors explain the observed difference in SR Ca^{2+} release because they have not been shown to affect SR Ca^{2+} release.

CONCLUSIONS

In summary, the present results demonstrate that 4 h of glycogen-diminishing cycling exercise in highly trained endurance athletes is associated with reductions in SR vesicle Ca^{2+} release rate and reductions in peak power output. The impaired SR vesicle Ca^{2+} release is associated with muscle glycogen content below a critical level. These findings support the idea of a modulating effect of muscle glycogen on SR function. Finally, the high-level endurance athletes here investigated demonstrated extraordinary well-developed glycogen kinetics as demonstrated by a resynthesis rate of 61 mmol·kg^{-1} dw·h^{-1} within the first 4 h of recovery.

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