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

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Running title: Muscle glycogen and SR function in athletes

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ABSTRACT

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 ($VO_{2\text{max}}$ 66.5±1.3 ml O$_2$ · kg$^{-1}$ · min$^{-1}$), performed 4h of glycogen depleting cycling exercise ($HR_{\text{mean}}$ 73±1% of maximum). During the first 4h 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 20h recovery. Results: Immediately following 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 4h recovery with CHO (61±2% of pre) and SR Ca$^{2+}$ release rate returned to pre-exercise level. However, in the absence of CHO during the first 4h recovery, glycogen and SR Ca$^{2+}$ release rate remained depressed, with normalization of both parameters at the end of the 24h 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 4h cycling exercise reduced $W_{peak}$ by 5.5-8.9% at different cadences ($P<0.05$) and $W_{peak}$ was normalized after 4h recovery with CHO whereas $W_{peak}$ remained depressed ($P < 0.05$) following water provision. $W_{peak}$ was fully recovered after 24h 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 hours post exercise. Key words: Calcium regulation, Muscle function, Elite athletes, Recovery, Fatigue
INTRODUCTION

Paragraph 1. 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 pre-exercise 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.

Paragraph 2. 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.
Paragraph 3. Recent findings from human studies support the findings in animal models where Ca\(^{2+}\) kinetics is influenced by glycogen levels (9, 10, 33). Duhamel and colleagues (10) examined the relation between muscle glycogen content and SR vesicle Ca\(^{2+}\) release rate in untrained males during a prolonged fatiguing cycling session at \(\approx 70\%\) of \(\dot{V}O_2\)peak. The cycling session was preceded by a glycogen depleting exercise session and four days 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 to 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 following a fatiguing cross-country skiing exercise (33). Muscle glycogen levels were manipulated in the recovery period by diet intervention where skiers either were provided with either a CHO enriched diet or water during the initial 4h period following exercise. After 4h of recovery with CHO, SR vesicle Ca\(^{2+}\) release rate was normalized and muscle glycogen content were markedly increased compared to post exercise, whereas both SR release and glycogen remained depressed in the group which 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 following e.g. prolonged exercise.

Paragraph 4. Based on existing human data, there is a critical level of muscle glycogen at around 250-300 mmol \(\cdot\) 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 following exercise and the interaction between SR Ca\(^{2+}\) handling and muscle glycogen concentration have employed exercise protocols of 2h or less. Training sessions and competitive events often involve exercising for a period of 3h 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\(_{\text{max}}\)), inducing enormous energy expenditure that primarily relies on endogenous glycogen (1, 20). It remains unclear whether impairments in SR function are present following such long term cycling sessions performed by elite athletes and whether muscle glycogen modulates recovery of SR function following ultra-endurance based training sessions. Also, the association between reduced muscle glycogen, SR Ca\(^{2+}\) release rate and functional muscle performance, as peak power output, is at present unknown. Furthermore, little is known about the glycogen re-synthesis rate in these highly trained endurance athletes.

Paragraph 5. In the present study, we measured the muscle glycogen content, SR function and peak power output in highly trained triathletes before and during a 24h recovery period that followed a 4h glycogen depleting cycling exercise. By allowing or omitting CHO intake during the initial 4h 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 4h 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 re-synthesis in these athletes at elite level.

**METHODS**

*Subjects*

Paragraph 6. Fourteen male elite triathletes were recruited for the study (Table 1). Six triathletes were current or former Danish National Team members, and four 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 Ethics Committee of Southern Denmark (Project-ID S-20090140).

*Experimental overview*

Paragraph 7. At least two days before the experimental part of the study, subjects visited the laboratory for preliminary tests and familiarization to the employed peak power ($W_{\text{peak}}$) test. The experimental part of the study comprised 4hrs of glycogen diminishing cycling exercise performed at 73% of the maximum heart rate ($HR_{\text{max}}$). During the first 4h recovery period that followed the exercise, the subjects were randomized to receive either water (H$_2$O 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 4h of recovery, all subjects received a CHO enriched energy intake for the remaining 20h of the 24h recovery period, thus both groups having received the same total food and beverages after 24h.
Muscle biopsies were obtained from *m. vastus lateralis* before (Pre), immediately after (Post), as well as four (4h) and twenty-four (24h) hours after exercise. Following obtainment of each muscle biopsy, $W_{\text{peak}}$ 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**

Paragraph 8. Quantifications of $W_{\text{peak}}$, mean power output ($W_{\text{max}}$), aerobic capacity ($VO_{2\text{AT}}$), and aerobic power ($VO_{2\text{max}}$) 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.

Paragraph 9. *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 x 5 s of maximal sprints at the following incremental pedaling frequencies: 100, 110, 120, and 130 revolutions per minute (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, $W_{\text{peak}}$ 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 sub-maximal endurance protocol. Initial work load 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 30W every 5 min until blood lactate concentration exceeded 4 mmol·L⁻¹·blood⁻¹. Blood lactate was determined from capillary blood fingertip samples collected over the last 30 s of each 5 min step using YSI Model 1500 Sport (YSI, Inc., Yellow Springs, Ohio, USA). The YSI was calibrated before each test by two standardized solutions containing 5 and 15 mmol lactate. VO₂ was calculated continuously throughout the aerobic capacity test on basis of ventilation and expired gas concentrations using a mixing chamber system (AMIS 2001, Innovision, Odense, Denmark). Prior to each test, the gas analyzer was calibrated by a known gas solution and the ventilation sensors by manual calibration with a 3L syringe. Aerobic capacity was defined as oxygen consumption (ml O₂·kg⁻¹·min⁻¹) at 4 mmol lactate ·L⁻¹·blood.

**Maximal aerobic capacity (VO₂max)** 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. VO₂max was defined as the highest mean VO₂ obtained during a 15 sec period. Heart rate and power output were sampled throughout the test to determine HR_max and W_max, respectively.

**Prolonged cycling exercise**

Paragraph 12. Work load during the bike segment of Ironman triathlon is approximately 83% of HR_max and lasts more than 4h (1, 20). However, requiring subjects in the current study to
maintain a mean work load of 83% of $HR_{\text{max}}$ was not feasible considering that no CHO was supplied during exercise. Thus, mean heart rate ($HR_{\text{mean}}$) during exercise was intended to be 75% of $HR_{\text{max}}$ with a self-selected pedaling frequency. However, if a subject considered that maintaining 75% of $HR_{\text{max}}$ for 4 h was not feasible after 2 h of exercise, the load was down regulated to the highest achievable $HR_{\text{mean}}$. By use of turbo trainers (Elite Crono Mag ElastoGel Trainer, Fontaniva, Italy) subjects were able to employ personal bikes, shoes and pedals. Heart rate was sampled continuously throughout the trial and $HR_{\text{mean}}$ was monitored following exercise. A minimum intake of water corresponding to 1 ml·kg$^{-1}$·h$^{-1}$ was imposed during exercise. Room temperature (~22°C) and humidity (~35%) were standardized throughout the 4h cycling exercise. Subjects were instructed to eat normally and refrain from severe exercise on the day preceding testing.

**Dietary manipulation and test procedure**

Paragraph 13. Dietary intake was controlled and calculated on basis of body mass throughout experimentation. Breakfast in both groups was consumed 90 min and 4h before reporting to the laboratory for the extraction of the first (Pre) and fourth (24h) biopsy, respectively, and consisted of CHO rich foods (i.e. porridge oats, raisins, skimmed milk, orange juice and a sports bar; 82 kJ·kg$^{-1}$ bw). Following the prolonged exercise, the CHO group received a meal (1.07g CHO·kg$^{-1}$ bw·h$^{-1}$) consisting of pasta, chicken, vegetables and a CHO beverage during the initial 2h of the recovery period and a CHO beverage and a sports bar (1.05g CHO·kg$^{-1}$ bw·h$^{-1}$) during the subsequent 2h 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 H₂O group received a supplemental meal in order 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 (≈ 10 g CHO·kg⁻¹ bw·day⁻¹).

Paragraph 14. *Peak power output.* \(W_{\text{peak}}\) 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. \(W_{\text{peak}}\) was expressed relative to body mass (W·kg⁻¹) and relative to Pre (% of pre) in order to compare the effect of exercise and recovery between groups.

**Analytical Techniques**

Paragraph 15. *Muscle biopsies.* Four muscle biopsies (i.e. Pre, Post, 4h and 24h) of 100-150 mg were obtained from the *m. vastus lateralis* portion of *m. quadriceps femoris* using 5 mm Bergström needles. This muscle was chosen since 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 contra-lateral leg. The procedure for 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 *m. vastus lateralis* with a scalpel under local anesthesia (3 to 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, USA) in an ice-cold buffer containing 300 mM sucrose, 1 mM EDTA, 10 mM NaN₃, 40 mM Tris-base, 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.

Paragraph 16. SR vesicle Ca²⁺ uptake and release rates. The fluorescent dye technique was used to determine Ca²⁺ 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 Heps, 7.5 mM oxalate, 11mM NaN₃, 5.5 µM TPEN, 20 µM CaCl₂ and 2 mM MgCl₂ (pH 7.0 at 37°C). Briefly, muscle homogenate (70µl) was mixed with 2 ml of assay buffer. Ca²⁺ uptake was then initiated by adding 2 mM ATP to a final concentration of 5 mM. Free [Ca²⁺] was determined by the fluorescent Ca²⁺ indicator Indo-1 (1µM) and fluorescence was sampled every 0.5 sec. (20 Hz, Ratiomaster RCM; Photon Technology International, Brunswick, NJ). Ca²⁺ uptake was recorded for 3 min., before [Ca²⁺] reached a plateau. Upon measurements of Ca²⁺ uptake, the SR Ca²⁺ ATPase was blocked with cyclopiazonic acid (40µM) before SR vesicle Ca²⁺ release was initiated by addition of 4-chloro-M-Cresol (4-CmC) [5mM]. Raw-data for [Ca²⁺] were imported into Matlab version 7.0.1 (The MathWorks, Natick, MA) and mathematically analyzed (Curve Fitting Toolbox version 1.1.1; The MathWorks). Curve fitting of Ca²⁺ uptake was performed with data points between free [Ca²⁺] of 1000 nM and free [Ca²⁺] 20 s prior to initiation of Ca²⁺
release. Time for free $[^{2+}\text{Ca}]$ to decrease by 63% of the initial free $[^{2+}\text{Ca}]$ ($\tau$) was calculated as $1/b$ from the equation; $y = ae^{-bt} + c$, where $y$ is the free $[^{2+}\text{Ca}]$, $t$ is time and $a$, $b$ and $c$ are constants assigned from Matlab. There were no differences in constant $c$ (Nadir $[^{2+}\text{Ca}]$) between trials, time or within same subject at various time points. SR $[^{2+}\text{Ca}]$ release rate was obtained by mathematically fitting the data points during the first 30 s of release to the equation; $y = a \left[1 - e^{-b(t-c)}\right]$. This was back-extrapolated to Nadir $[^{2+}\text{Ca}]$ and the rate of $[^{2+}\text{Ca}]$ release was determined as the derivate of the initial release. Values obtained for SR $[^{2+}\text{Ca}]$-release rates are relative and therefore expressed as arbitrary units; $[^{2+}\text{Ca}] \cdot \text{g protein}^{-1} \cdot \text{min}^{-1}$. Due to the inter-individual variation in SR $[^{2+}\text{Ca}]$ release rates, results are normalized to pre-values (% of Pre). Assays of uptake and release of $[^{2+}\text{Ca}]$ were performed in triplicates (a few in duplicates due to limited tissue homogenate). Protein content in the muscle homogenate was measured in triplicates using a standard kit (Pierce BCA protein reagent no. 23225). n=7 for both groups, except Post, 4h and 24h were n=6 for both groups due to limited tissue.

Paragraph 17. Muscle glycogen. Muscle glycogen content was determined spectrophotometrically (Beckman DU 650) (35). Freeze dried muscle tissue (1.5mg) was boiled in 0.5 ml 1 M HCL for 150 min before it was quickly cooled, whirl-mixed and centrifuged at 3500g for 10 min. at 4°C. 40 µL of boiled muscle sample and 1 ml of reagent solution containing Tris-buffer (1M), distilled water, ATP (100mM), MgCl$_2$ (1M), NADP$^+$ (100mM) and G-6-PDH were mixed before the process was initiated by adding 10µL of diluted hexokinase. Absorbance was recorded for 60 min. before the glycogen content was calculated. Muscle glycogen was expressed as mmol·kg$^{-1}$ dw. In addition, maximal glycogen resynthesis in the CHO group was calculated on basis of the increase in muscle glycogen from post to 4h and expressed as
mmol·kg\(^{-1}\) dw·h\(^{-1}\). n=7 for both groups at each time point, except for Post CHO and H\(_2\)O and 4h CHO were n=6 due to limited tissue.

Paragraph 18. CS and HAD activity. Enzyme activities were measured in freeze-dried muscle dissected free from non-muscle constituents (30 °C), (35). Citrate synthase (CS) activity was determined by 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 addition of acetoacetyl CoA to a buffer solution containing Imidazol, NADH and EDTA. Absorbance of CS and HAD were recorded for 600 sec, converted into enzyme activity rates, and expressed as µmol·g\(^{-1}\) dw·min\(^{-1}\).

Paragraph 19. 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% SDS, 62.5 mM Tris and 0.2% bromophenolblue at pH 6.8.), boiled in water at 100°C for 3 min. and loaded (10-40µL) on a SDS-PAGE gel (6% polyacrylmide (100:1 acrylmid : bis-acrylmid), 30% glycerol, 67.5 mM tris-base, 0.4% SDS, and 0.1M glycine). Gels were run at 80V for at least 42 h at 4°C and MHC bands 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 (Figure 1). MHCII was identified with Western blot using monoclonal antibody (SigmaM4276) with the protocol Xcell II (Invitrogen, Carlsbad, CA, USA). The MHC composition of each subject was determined as an average of 3 biopsies from time points Pre, Post, 4h, 24h.
Paragraph 20. 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, 4h, 24h) and group (CHO, H2O) as fixed effects. Variables with skewed distributions were appropriately transformed (boxcox) 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

Paragraph 21. Baseline. Anthropometrics, physiological characteristics and training information of the subjects are presented in table 1. Except from VO2max, no parameters were different between groups (CHO: 68.3 ± 1.4 ml·kg⁻¹·min⁻¹ vs. H2O: 63.5 ± 1.8 ml·kg⁻¹·min⁻¹, $P<0.05$).

Paragraph 22. 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 to the H2O group at all time points and a corresponding lower MHCIIa content ($P<0.05$). The relative fibre type distribution remained unchanged within the two groups between Pre, Post, 4h and 24h (Avg. from at least three time points: MHCI; CHO: 73.5 ± 1.9 vs. H2O: 55.4 ± 2.1 and MHCIIa; CHO: 26.4 ± 1.9 vs. H2O: 42.3 ± 2.4). CS and HAD activities were on
average 111 ± 6 µmol · g⁻¹ dw · min⁻¹ and 138 ± 7 µmol · g⁻¹ dw⁻¹ · min⁻¹. CS activity was similar between the CHO and H₂O group, whereas HAD activity was 23% higher in the CHO group compared to the H₂O group (P<0.05).

### Table 1 near here

Paragraph 23. *Exercise intensity.* All subjects accomplished the prolonged cycling exercise, although some individuals were unable to maintain the pre-determined exercise intensity of 75% \(HR_{\text{max}}\). Consequently, \(HR_{\text{mean}}\) was 73 ± 1% of \(HR_{\text{max}}\), (CHO 74 ± 1% and H₂O 71 ± 0 %) which, estimated from the sub-maximal test, equaled approximately 56 % of \(VO_{2\text{max}}\).

Paragraph 24. *Muscle glycogen.* Resting muscle glycogen concentration in *m. vastus lateralis* 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 x time interaction was evident for muscle glycogen concentration, i.e. the change in muscle glycogen concentration was different between groups from post to 4h (P<0.05). The 4h exercise reduced muscle glycogen to a similar level in both groups, corresponding to 32 ± 4% of pre values or 225 ± 28 mmol · kg⁻¹ dw for combined data (CHO: 28% of pre, 205 ± 31 mmol · kg⁻¹ dw ; H₂O: 36 % of pre, 245 ± 49 mmol · kg⁻¹ dw) (Figure 2a, \(P<0.01\)). Due to the CHO manipulation, muscle glycogen changed differently between groups from post to 4h (\(P<0.05\)). Thus, muscle glycogen remained depressed in the H₂O group (264.0 ± 31.0 mmol·kg⁻¹ dw, ns) whereas muscle glycogen was significantly elevated in the CHO group in comparison to the Post
measurement (449 ± 27 mmol·kg\(^{-1}\) dw, \(P<0.05\)) (Figure 2a). 24 h following the cycling exercise, muscle glycogen content attained pre-levels in both groups (Figure 2a).

**Figure 1 and 2 near here**

Paragraph 25. *Muscle glycogen re-synthesis rate*. Muscle glycogen re-synthesis rate during the initial 4h of recovery was on average 61 mmol·kg\(^{-1}\) dw·h\(^{-1}\) (from 205 to 449 mmol·kg\(^{-1}\) dw) and calculated on basis of all subjects from the CHO group.

Paragraph 26. *SR vesicle Ca\(^{2+}\) release rate*. A group x time interaction was evident for SR Ca\(^{2+}\) release rate, i.e. the change in SR Ca\(^{2+}\) release rate from Pre to 4h was different between the CHO and H\(_2\)O group (\(P<0.05\)) (Figure 2b and Table 2). SR Ca\(^{2+}\) release rate was equally reduced in both groups immediately following the prolonged cycling exercise, corresponding to -14.3 ± 2.2 % for combined data (CHO: -12.5 ± 3.9% ; H\(_2\)O: -16.2 ± 2.1% (\(P<0.05\)) (Figure 2b). Following 4 h of recovery, SR vesicle Ca\(^{2+}\) release rate attained pre-levels in the CHO group (-2 ± 5% of pre, ns), whereas it remained depressed in the H\(_2\)O group compared to pre-exercise (-17.5 ± 3.1%, \(P<0.05\)). In addition, SR vesicle Ca\(^{2+}\) release was significantly different between groups following the 4h recovery with or without optimal CHO intake (\(P<0.05\)) (Figure 2b). During the subsequent 20h recovery period, SR vesicle Ca\(^{2+}\) release rate returned to pre-levels in the H\(_2\)O group, whereas no further changes were observed in the CHO group.
Paragraph 27. **SR vesicle Ca\textsuperscript{2+} uptake rate.** SR vesicle Ca\textsuperscript{2+} uptake rate ($\tau$) was not affected by exercise or recovery in neither the CHO nor the H\textsubscript{2}O group and no differences were observed between groups at any time point (Table 2).

**Table 2 near here**

Paragraph 28. **Correlation between muscle glycogen and SR vesicle Ca\textsuperscript{2+} release rate.** Alterations in muscle glycogen and SR vesicle Ca\textsuperscript{2+} release rate followed a similar pattern (Fig. 2). Plotting the individual data on SR Ca\textsuperscript{2+} release rate and muscle glycogen for all time points, demonstrated a significant linear correlation ($P < 0.01$, $r^2 = 0.32$). In order to exclude the possible influence of acute effects of exercise on SR Ca\textsuperscript{2+} release rate and take advantage of the glycogen manipulating design, we also plotted data without the Post data (i.e. Pre, 4h and 24h), which revealed a significant correlation between SR vesicle Ca\textsuperscript{2+} release rates and muscle glycogen contents ($P=0.01$, $r^2 = 0.30$, Fig. 3). Thus, SR Ca\textsuperscript{2+} release rate and muscle glycogen content is temporarily associated (Fig. 2) and individual data are linear correlated (Fig. 3). Importantly, taking advantage of the study design, the SR Ca\textsuperscript{2+} release rate and glycogen content was closely associated after 4h recovery with and without CHO. Hence, both muscle glycogen and SR Ca\textsuperscript{2+} release rate remained depressed in the H\textsubscript{2}O group whereas muscle glycogen and SR Ca\textsuperscript{2+} release were significantly elevated in the CHO group in comparison to the Post measurement.

**Figure 3 near here**
Paragraph 29. Peak power output. Before exercise, $W_{\text{peak}}$ corresponded to 13.2 ± 0.6 W·kg\(^{-1}\), 13.6 ± 0.6 W·kg\(^{-1}\), 14.0 ± 0.6 W·kg\(^{-1}\) and 13.2 ± 0.6 W·kg\(^{-1}\), at 100, 110, 120 and 130 RPM, respectively, and there were no significant differences in $W_{\text{peak}}$ between pedaling frequencies nor between CHO and H\(_2\)O groups. Since no differences were observed between the pedaling frequencies, representative data for the cadence with the highest $W_{\text{peak}}$ values (120 RPM) are described. An overall group x time interaction was evident for $W_{\text{peak}}$ at 120 RPM ($P<0.05$), i.e. $W_{\text{peak}}$ changed in a different manner from post to 4h ($P<0.05$). The 4h prolonged cycling exercise reduced $W_{\text{peak}}$ to a similar extent in both groups, corresponding to 8.8 ± 1.7% (CHO: -11.1 ± 2.9%; H\(_2\)O: -6.0 ± 1.3 %) (Figure 4, $P<0.05$). $W_{\text{peak}}$ was normalized after 4h recovery with CHO provision (-1.7 ± 1.7 %, ns), however, remained depressed by 5.3 ± 2.3% ($P<0.01$) in the H\(_2\)O group (Figure 4). $W_{\text{peak}}$ was fully restored in both groups following additional 20 h of recovery.

Figure 4 near here
DISCUSSION

Paragraph 30. 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 following 4h endurance exercise, 4h recovery with either CHO or water supplementation and additional 20 h recovery with a CHO enriched diet. Importantly, our experimental design specifically identified that muscle SR Ca\textsuperscript{2+} release rate and muscle power capacity remained depressed at post-exercise levels after the 4h 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 Ca\textsuperscript{2+} release rate. Thus, the present findings provide further support to the hypothesis that glycogen is a prerequisite for normal SR Ca\textsuperscript{2+} release, and thereby a key regulator of muscle cytosolic levels of Ca\textsuperscript{2+} in contracting skeletal muscle. This may eventually, at least in part, explain the reduced $W_{peak}$ observed after recovery with water provision.

Paragraph 31. SR vesicle Ca\textsuperscript{2+} regulation following exercise. A role of reduced SR Ca\textsuperscript{2+} release in fatigue was first proposed by Eberstein and Sandow in 1963 (11) and today it is generally accepted that deteriorations in SR function, and SR Ca\textsuperscript{2+} release rate in particular, contributes to muscle fatigue in a variety of exercise types (2, 10, 34). Studies in untrained humans have demonstrated reductions in both SR vesicle Ca\textsuperscript{2+} release and uptake rates during and following prolonged cycling exercise (8, 10, 24). By the present results we have extended these findings by demonstrating that SR vesicle Ca\textsuperscript{2+} release rate was also impaired in highly trained elite endurance athletes following 4h prolonged cycling exercise. SR vesicle Ca\textsuperscript{2+} release rate was reduced by 14 % following exercise, which is lower than previously reported in humans
following short term high intensity exercise (16, 24) but in agreement with reports examining the influence of prolonged cycling exercise on SR Ca\(^{2+}\) release rate in untrained subjects (8, 10, 24). Of note is that even relatively small decreases in SR Ca\(^{2+}\) release and thereby \([\text{Ca}^{2+}]_i\), will significantly affect the force production due to the sigmoidal shape of the force-\([\text{Ca}^{2+}]_\text{free}\) relationship. Thus, alterations in force, due to reduced Ca\(^{2+}\) transients, are more apparent at sub-maximal contractions like those from cycling exercise versus maximal contractions.

Paragraph 32. *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 and colleagues (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).

Paragraph 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) following 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, i.e. 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.

Paragraph 33. *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 following 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 (Figure 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, 4h and 24h 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 following 4 h recovery with either CHO or water consumption. In addition, two studies from Duhamel and colleagues observed that pre exercise muscle glycogen content was associated with SR vesicle Ca$^{2+}$ release rate during exercise in untrained males and females (9, 10). Following 4 days 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 to the high CHO diet, indicating that total glycogen has a direct impact on SR Ca$^{2+}$ release. Another
study from the same group of researchers supported these observations by reporting a similar association following 4 days 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 and colleagues and Ørtenblad and colleagues (10, 33). As SR Ca\(^{2+}\) regulation is quantitatively, but not qualitatively, different between fiber types with an approximately 4 times higher SR uptake and -release rates in MHC II fibers (33). Impaired SR function following 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 present study speculatively partly could explain differences between groups independent of glycogen contents.

Paragraph 34. 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 and colleagues demonstrate that these associations are dynamic and dependent on the glycogen state in the muscle cell (21, 22). 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 SR \(Ca^{2+}\) release is ATP dependent and that release properties are modulated by local \([ATP]\) or endogenous ATP production.

Paragraph 35. **Resting muscle glycogen and glycogen re-synthesis rate.** In accordance with previous studies, the present study demonstrates enlarged glycogen storage capacities in endurance trained athletes compared to previous reports from untrained subjects \((12)\). Resting glycogen content of the \(m. vastus lateralis\) was \(699 \pm 26 \text{ mmol·kg}^{-1} \text{ dw}\) \((\text{range: 519 to 883})\), which agrees with a recent study from Branth and colleagues that reported an average resting glycogen content of \(707 \pm 32 \text{ mmol·kg}^{-1} \text{ dw}\) in endurance trained males from the Swedish national mountain bike team \((4)\). These resting glycogen levels exceed by far previous values reported in moderately trained endurance athletes \((\approx 380–460 \text{ mmol·kg}^{-1} \text{ dw})\) and approach or even attain muscle glycogen contents observed following loading regimes in the same subjects \((17)\). Generally, the rate of glycogen re-synthesis has been reported to be in the range of \(20-50 \text{ mmol·kg}^{-1} \text{ dw·h}^{-1}\) following glycogen diminishing exercise \((\text{for review see;} (17))\). Half of the highly trained endurance athletes from the present study were provided with \(1.03\text{g CHO·kg}^{-1}·\text{h}^{-1}\) during the initial 4h recovery period. Although not fully depleted following exercise, subjects demonstrated an average muscle glycogen synthesis rate of \(61.0 \text{ mmol·kg}^{-1} \text{ dw·h}^{-1}\) \((205 \text{ to } 449 \text{ mmol·kg}^{-1} \text{ dw})\) during recovery, measured as an average over 4h and therefore likely higher during the first hour of recovery. Such a glycogen re-synthesis rate is to our knowledge the highest reported, and may reflect adaptations to the very prolonged muscle glycogen depletion exercise these athletes perform on weekly basis.
Paragraph 36. *Power generating capacity after prolonged exercise.* Traditionally, alterations in force generating capacity following prolonged cycling exercise have been quantified by measurements of maximal voluntary contraction (MVC) of the knee extensors. Following cycling protocols, comparable to 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). Following exercise, maximal power generating capacity was significantly reduced by 5.5-8.9% at different cadences (i.e. 100, 110, 120 and 130 RPM). Due to the muscle biopsy extraction, there was a delay of 20 min from termination of prolonged cycling exercise until determination of $W_{\text{peak}}$ and it is thus very likely that the observed reduction in PPO was, in reality, higher immediately following 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 20 min recovery. However, both muscle glycogen content and SR vesicle Ca$^{2+}$ release rate were reduced following exercise and followed a pattern similar to that of $W_{\text{peak}}$ that were irrespective of supplementation following 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.

Paragraph 37. *Limitations of the study.* Consistently, there was a higher relative content of MHCI in the CHO group compared to the H$_2$O 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 since 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 pre-values (% of Pre), thus providing a reference for comparison. Accordingly, the changes observed in SR Ca\textsuperscript{2+} release rate are relative and independent of fiber type differences. Although glycogen utilization may be fiber type specific (i.e. active MHCI fibers use relatively more glycogen, which could affect SR function more than in MHCII fibers), SR Ca\textsuperscript{2+} release rate was equally reduced in both groups immediately following the prolonged cycling exercise (-16 ± 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 due to 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 4h of recovery. However, it seems unlikely that alterations in these factors explain the observed difference in SR Ca\textsuperscript{2+} release since they have not been shown to affect SR Ca\textsuperscript{2+} release.

Conclusions

Paragraph 38. In summary, the present results demonstrates that 4 h of glycogen diminishing cycling exercise in highly trained endurance athletes is associated with reductions in SR vesicle Ca\textsuperscript{2+} release rate and reductions in peak power output. The impaired SR vesicle Ca\textsuperscript{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 re-synthesis rate of 61 mmol \( \cdot \) kg\(^{-1}\) \( \cdot \) dw \( \cdot \) h\(^{-1}\) within the first 4 h of recovery.

Paragraph 39. Acknowledgements and conflict of interest. The experiments were performed at the Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark, DK-5230 M, Denmark. The study was supported by The Danish Ministry of Culture, the Committee on Sports Research. There are no further disclosures to report and no conflict of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine.
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Figure captions

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 Comassie 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 (A1-3, B1-3 etc.). The MHC composition of each subject was determined as an average of 3 biopsies. Three MHC isoforms (MHC I, IIA, and IIX) are detectable in a mixed sample of human vastus lateralis muscle (std, lane 1).

Figure 2 Absolute changes in muscle glycogen content in the CHO (grey bars) and H2O group (open bars) throughout the experimental period (A). Relative changes in SR vesicle Ca2+ release rate in the CHO (grey bars) and H2O group (open bars) throughout the experimental period (B). a: significantly different from H2O (P<0.05), b: significantly different from pre (P < 0.05).

Figure 3. Correlation of SR Ca2+ release rate and total glycogen concentration in biopsies from elite triathletes. Data points are three time points (Pre, 4h and 24h) for both conditions (CHO and H2O). The line indicates best fit of all the data points (r² = 0.30, P<0.01). n = 7 for Pre (CHO and H2O), and due to missing values in either glycogen content or SR Ca2+ release rate n = 5 (4h CHO) and n = 6 (4h H2O and 24h CHO and H2O).

Figure 4. Relative changes in WPeak in the CHO (grey 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).
Table 1  Anthropometric, physiological and training characteristics from the CHO and H₂O group as well as pooled data from both groups.

<table>
<thead>
<tr>
<th></th>
<th>CHO + H₂O</th>
<th>CHO</th>
<th>H₂O</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27.2 ± 0.9</td>
<td>27.4 ± 1.0</td>
<td>27.1 ± 1.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>183 ± 2</td>
<td>182 ± 2</td>
<td>184 ± 2</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>75.3 ± 1.4</td>
<td>75.0 ± 1.6</td>
<td>77.7 ± 2.5</td>
</tr>
<tr>
<td>History as elite athlete (years)</td>
<td>4.8 ± 0.7</td>
<td>4.9 ± 1.0</td>
<td>4.7 ± 1.8</td>
</tr>
<tr>
<td>Training volume (h·week⁻¹)</td>
<td>16.4 ± 0.9</td>
<td>17.6 ± 1.3</td>
<td>15.3 ± 1.2</td>
</tr>
<tr>
<td>VO₂max (l·min⁻¹)</td>
<td>5.0 ± 0.1</td>
<td>5.1 ± 0.2</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>VO₂max (ml·kg⁻¹·min⁻¹)</td>
<td>66.5 ± 1.3</td>
<td>68.3 ± 1.4 a</td>
<td>63.5 ± 1.8</td>
</tr>
<tr>
<td>VO₂AT (ml·kg⁻¹·min⁻¹)</td>
<td>52.0 ± 1.6</td>
<td>54.4 ± 1.8</td>
<td>48.7 ± 2.4</td>
</tr>
<tr>
<td>Wattmax (W)</td>
<td>384 ± 13</td>
<td>399 ± 18</td>
<td>371 ± 17</td>
</tr>
</tbody>
</table>

Wattmax defines the mean power output during the 5 min maximal open mode cycling protocol and VO₂AT the relative VO₂ at 4 mmol lac·L⁻¹ blood. a: significantly different from H₂O, P < 0.05.
<table>
<thead>
<tr>
<th>variable</th>
<th>Pre</th>
<th>Post</th>
<th>4h</th>
<th>24h</th>
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<tbody>
<tr>
<td>SR vesicle Ca(^{2+}) uptake and release</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\tau) (tau) (s)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CHO</td>
<td>45.9 ± 5.4</td>
<td>52.6 ± 3.5</td>
<td>50.9 ± 7.3</td>
<td>38.0 ± 3.3</td>
</tr>
<tr>
<td>H(_2)O</td>
<td>40.8 ± 7.3</td>
<td>39.7 ± 6.1</td>
<td>37.9 ± 5.2</td>
<td>34.8 ± 3.2</td>
</tr>
<tr>
<td>SR vesicle Ca(^{2+}) uptake at 800 nM Ca(^{2+})</td>
<td></td>
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<tr>
<td>CHO</td>
<td>3.4 ±0.2</td>
<td>2.7 ± 0.1</td>
<td>2.8 ± 0.3</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>H(_2)O</td>
<td>3.6 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>3.2 ± 0.2</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>SR vesicle Ca(^{2+}) release rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>2.7 ± 0.2</td>
<td>2.2 ± 0.1 *</td>
<td>2.6 ± 0.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>H(_2)O</td>
<td>2.8 ± 0.3</td>
<td>2.3 ± 0.2 *</td>
<td>2.3 ± 0.1 *</td>
<td>2.8 ± 0.2</td>
</tr>
</tbody>
</table>

The SR vesicle Ca\(^{2+}\) uptake and release were analysed fluorometrically in crude muscle homogenate. The \(\tau\) (tau) 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 \(\mu\)mol Ca\(^{2+}\) : g\(^{-1}\) : protein \(^{-1}\) : min \(^{-1}\). * sign different from pre.