Comparative effects of a low-carbohydrate diet and exercise plus a low-carbohydrate diet on muscle sarcoplasmic reticulum responses in males

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Duhamel, T. A., H. J. Green, J. G. Perco, and J. Ouyang. Comparative effects of a low-carbohydrate diet and exercise plus a low-carbohydrate diet on muscle sarcoplasmic reticulum responses in males. Am J Physiol Cell Physiol 291: C607–C617, 2006. First published May 17, 2006; doi:10.1152/ajpcell.00643.2005.—We employed a glycogen-depleting session of exercise followed by a low-carbohydrate (CHO) diet to investigate modifications that occur in muscle sarcoplasmic reticulum (SR) Ca\(^{2+}\)-cycling properties compared with low-CHO diet alone. SR properties were assessed in nine untrained males [peak aerobic power (\(V_O_2\)\_peak)] = 43.6 ± 2.6 (SE) ml·kg\(^{-1}\)·min\(^{-1}\)] during prolonged cycle exercise to fatigue performed at ~58\% \(V_O_2\)\_peak\ after 4 days of low-CHO diet (Lo CHO) and after glycogen-depleting exercise plus 4 days of low-CHO (Ex+Lo CHO). Compared with Lo CHO, Ex+Lo CHO resulted in 12% lower (\(P < 0.05\)) resting maximal Ca\(^{2+}\)-ATPase activity (\(V_{\text{max}}\) = 174 ± 12 vs. 153 ± 10 μmol·g protein\(^{-1}\)·min\(^{-1}\)) and smaller reduction in \(V_{\text{max}}\) induced during exercise. A similar effect was observed for Ca\(^{2+}\) uptake. The Hill coefficient, defined as slope of the relationship between cytosolic free Ca\(^{2+}\) concentration and Ca\(^{2+}\)-ATPase activity, was higher (\(P < 0.05\)) at rest (2.07 ± 0.15 vs. 1.90 ± 0.10) with Ex+Lo CHO, an effect that persisted throughout the exercise. The coupling ratio, defined as the ratio of Ca\(^{2+}\) uptake to \(V_{\text{max}}\), was 23–30% elevated (\(P < 0.05\)) at rest and during the first 60 min of exercise with Ex+Lo CHO. The ~27 and 34% reductions (\(P < 0.05\)) in phase 1 and phase 2 Ca\(^{2+}\) release, respectively, observed during exercise with Lo CHO were not altered by Ex+Lo CHO. These results indicate that when prolonging exercise precedes a short-term Lo CHO diet, Ca\(^{2+}\) sequestration properties and efficiency are improved compared with those during Lo CHO alone.

Adequate reserves of the endogenous muscle carbohydrate glycogen are recognized as essential for sustaining contractile activity (2, 7, 20). Intuitively, the most obvious mechanism for this effect would appear to relate to its role as a fuel for oxidative phosphorylation and glycolysis, the metabolic pathways involved in regenerating the ATP necessary to supply the energy needs of the active muscle. According to this theory, the depletion of muscle glycogen would be expected to compromise the flux rate through these metabolic pathways resulting in an energy crisis and an inability to supply the ATP requirements of one or more of the ATPases involved in excitation and contraction (20).

Some evidence exists to support this theory. To date, experimental efforts aimed at elucidating the role of muscle glycogen in cellular function have concentrated primarily on the sarcoplasmic reticulum (SR). The existence of a glycogenolytic complex that is physically associated with the SR in skeletal muscle is well established (17, 41, 54). The SR-glycogenolytic complex, which appears to be bound to the SR via a protein phosphatase (41), contains a complex of enzymes involved in both the catabolism and synthesis of glycogen as well as creatine phosphokinase (CPK) (10, 11, 39). Numerous studies have demonstrated a functional coupling between CPK (11, 29, 39) and the glycolytic enzymes (9, 10, 36, 53) associated with the SR and Ca\(^{2+}\) uptake, supposedly via a regional capability to maintain a high phosphorylation potential (29, 39). The depletion of glycogen from the SR may disrupt ATP homeostasis and, consequently, Ca\(^{2+}\) uptake by depressing the activity of key enzymes, as has been shown for phosphorylase (31). Curiously, prolonged exercise to fatigue resulting in near complete depletion of muscle glycogen has minimal effect on globular cellular ATP homeostasis (4). However, it is possible that these global measurements do not reflect regional changes, for example, in the vicinity of the SR.

Another proposed mechanism to explain the apparent essential role of cellular glycogen in muscle contractility is via structural changes in specific organelles such as the SR. According to this theory, the loss of glycogen from the SR modifies selective proteins such as the Ca\(^{2+}\)-ATPase as a result of conformational changes in the region of the adenine nucleotide binding site (8). Such a mechanism could result in a depression in SR Ca\(^{2+}\) uptake in the absence of a disturbance in ATP homeostasis.

Regardless of the mechanism involved, it might be expected that repetitive contractions resulting in depletion of muscle glycogen would result in an impairment in SR Ca\(^{2+}\) uptake. Numerous studies have found this to be the case (3, 5, 13, 31, 50). Moreover, the reduction in Ca\(^{2+}\) uptake appears to be mediated by reductions in Ca\(^{2+}\)-ATPase activity (3, 5, 13, 50), which appears to occur secondary to modifications in the region of the adenine nucleotide of the enzyme (34, 35). These measurements, which are performed in vitro under supposedly optimal conditions by using exogenously generated ATP (47), support the hypothesis that the loss of catalytic activity occurs not as a result of deficiencies in ATP availability but, rather, as a result of structural modifications to the enzyme.

The general weakness of studies published to date designed to investigate the role of muscle glycogen on SR function during exercise has been an inability to manipulate the glycogen concentration (31). As a result, comparisons of SR Ca\(^{2+}\)-cycling responses at different durations of exercise with different amounts of muscle glycogen have not been possible. In recent work, we have addressed this problem by using a
preliminary session of prolonged cycling designed to substantially deplete glycogen reserves followed by a 4-day period of either a high- or low-carbohydrate diet (14–16). We have found that with this experimental protocol, muscle glycogen levels in vastus lateralis were elevated by 57% in high- compared with low-carbohydrate diets and remained elevated throughout exercise. The high carbohydrate condition also resulted in less of a reduction in maximal Ca\textsuperscript{2+}-ATPase activity, Ca\textsuperscript{2+} uptake, and Ca\textsuperscript{2+} release at different exercise time points. These differences occurred in the absence of changes in affinity of Ca\textsuperscript{2+}-ATPase for Ca\textsuperscript{2+}, the efficiency of Ca\textsuperscript{2+} transport, and membrane permeability for Ca\textsuperscript{2+}. No differences in these SR properties were observed between conditions before exercise.

Although these results strongly suggest a role for muscle glycogen on SR Ca\textsuperscript{2+} cycling during prolonged exercise, limitations in experimental design must be acknowledged. Collectively, exercise and dietary manipulations are effective methods to alter muscle glycogen content in humans. However, these protocols may confound the interpretation of results, because it is difficult to tease out the combined effects of the dietary manipulation plus preceding exercise from the independent effects of the dietary manipulation or the independent effects of the preceding bout of exercise, respectively. It is conceivable that a single bout of exercise, designed to deplete muscle glycogen, may induce a training effect within muscle and influence the metabolic control of specific ATP-generating pathways. Previous work from our laboratory has demonstrated that a single exercise session may alter SR Ca\textsuperscript{2+}-handling properties, assessed during a standardized exercise test, following a 2-day (50) and 4-day (12) recovery period.

We have recently conducted a series of experiments to isolate and characterize the independent and combined effects of preceding exercise and diet on SR function. Our initial study (12) described the effects of a preliminary bout of exercise on metabolic and SR Ca\textsuperscript{2+}-handling properties in males after a 4-day normal carbohydrate diet. In that study, preceding exercise followed by a 4-day normal carbohydrate diet, compared with a 4-day normal carbohydrate diet alone, did not alter SR Ca\textsuperscript{2+} uptake, maximal Ca\textsuperscript{2+}-ATPase activity, or Ca\textsuperscript{2+} release rates. However, preceding exercise plus a normal diet did alter resting Ca\textsuperscript{2+} uptake and prevented alterations in SR membrane permeability to Ca\textsuperscript{2+} at fatigue. These changes occurred in the absence of differences in muscle glycogen concentration either at rest or during exercise. On the basis of these observations, it appears that SR Ca\textsuperscript{2+}-handling properties can be influenced by a preceding bout of exercise when a normal carbohydrate diet is ingested during a 4-day recovery period. Nevertheless, the experimental design utilized in our initial study (12) leaves an important question unanswered, namely, whether the glycogen-depleting conditioning exercise conducted 4 days before the standardized cycling task could induce the changes in SR Ca\textsuperscript{2+}-cycling properties regardless of composition of the diet.

Muscle glycogen content is most effectively influenced through the combination of glycogen depletion exercise followed by a period of dietary manipulation through the ingestion of low- vs. high-carbohydrate diets (2). Although it may be assumed that the dietary manipulation serves only to alter carbohydrate availability and muscle glycogen resynthesis, there is evidence in the literature that indicates a low-carbohydrate diet also can influence protein expression and lead to adaptations in metabolic regulation and substrate utilization (38). For example, the literature has demonstrated that diet-induced changes in pyruvate dehydrogenase kinase protein content and activity can occur within 3 days after starting a high-fat diet (38). To our knowledge, no published study has yet characterized the effects that a low-carbohydrate diet exerts on SR Ca\textsuperscript{2+}-handling properties in muscle.

The primary objective of this study was to compare the independent effects of a 4-day low-carbohydrate diet with the combined effects of a preceding session of prolonged exercise, designed to deplete muscle glycogen, plus a 4-day low-carbohydrate diet. We hypothesized that the exercise-induced reductions in SR Ca\textsuperscript{2+}-ATPase activity, Ca\textsuperscript{2+} uptake, and Ca\textsuperscript{2+} release observed in vitro following a low-carbohydrate diet would not be significantly different from the changes observed when the low-carbohydrate diet was preceded by a prolonged session of exercise. As with the SR Ca\textsuperscript{2+}-cycling responses, we also hypothesized that no differences would occur between conditions in muscle glycogen concentration either at rest or during exercise.

A secondary objective of this study is to characterize the independent effects of a diet low in carbohydrates compared with a normal carbohydrate diet. We are able to determine the independent effects of a low-carbohydrate diet on SR function by comparing the results of this study with the results of our earlier study using a normal carbohydrate diet (12). We postulated that a low-carbohydrate diet would alter SR Ca\textsuperscript{2+}-handling properties as a result of diet-induced differences in muscle glycogen concentration that are known to occur in response to 4-day normal and low-carbohydrate diets.

**METHODS**

**Participants.** The participants (n = 9), all recruited from the male student population at the University of Waterloo (Waterloo, ON, Canada), were healthy and not involved in vigorous activity on a regular basis for at least 6 mo before the beginning of the study. Age, height, and body mass were 20 ± 0.4 (SE) yr, 178 ± 2.2 cm, and 79.4 ± 3.9 kg, respectively. Peak aerobic power (VO\textsubscript{2 peak}), as determined by a progressive cycling task to fatigue, was 43.6 ± 2.6 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}. Blood hemoglobin (Hb; 16.1 ± 0.7 g%) and blood hematocrit (Hct; 48.7 ± 1.3%), determined before the study, were within the normal range. Volunteers were recruited for the study after the approval of all experimental procedures by the Office of Research Ethics at the University of Waterloo. All volunteers were fully informed of experimental details and the risks involved before written consent was obtained.

**Experimental design.** To investigate our hypothesis, we employed two experimental conditions, each randomly assigned and separated by at least 4 wk. In one condition, the participants were required to follow a low-carbohydrate (Lo CHO) diet, consisting of 20, 60, and 20% of kilocalories derived from carbohydrate, fat, and protein, respectively, for a 4-day period before performing a prolonged exercise task. In the second condition, a glycogen-depleting bout of prolonged exercise preceded the 4-day Lo CHO diet (Ex+Lo CHO) before the prolonged exercise test was performed. Each participant was required to visit the laboratory on five occasions. The first visit was used to familiarize the participant with the experimental protocols used during the study and to determine VO\textsubscript{2 peak}. On a second visit, participants were provided with a 4-day meal plan designed to meet the specific dietary requirements and were instructed on how to record the type and quantity of food and beverage consumed in a diet journal. The remaining three visits involved performing prolonged cycle exercise, either to investigate the effects of Lo CHO and Ex+Lo CHO...
or to deplete muscle glycogen. All volunteers were requested to refrain both from any vigorous exercise and from alcohol and caffeine consumption during the experimental periods.

**Exercise protocols.** A progressive test to fatigue was performed on an electrically braked cycle (Quinton 870), calibrated on a daily basis, for measurement of \( VO_2 \text{peak} \). The protocol employed, previously described by Hughson et al. (27), involved cycling at 60 revolutions per minute (rpm) for a baseline period of 4 min at 25 W, followed by 15-W increases per minute as a continuous ramp. Exercise continued until fatigue, defined as an inability to maintain at least 50 rpm with verbal encouragement. \( VO_2 \text{peak} \) was defined as the highest \( VO_2 \) value obtained, averaged over a 30-s period.

For the prolonged cycling task, designed to measure muscle metabolism and the SR Ca\(^{2+}\)-cycling properties, a workload was selected to elicit 55–60% \( VO_2 \text{peak} \). For these tests, respiratory gas exchange was measured during the experimental periods.

**Muscle glycogen and metabolites.** Muscle glycogen and selected metabolites were measured on freeze-dried tissue free of visible connective tissue, fat, and blood. Total muscle glycogen concentration, assessed as glucosyl units, was determined fluorometrically after hydrolysis with hydrochloric acid (25). Measurement of ATP, creatine phosphate (CrP), creatine (Cr), inorganic phosphate (P\(_i\)), and lactate (Lac) were assessed fluorometrically according to published procedures (23, 25). All metabolites for each sample were corrected to the average total creatine (TCr) content for each individual. Neither exercise nor the dietary manipulation altered the TCr content. During a given analytical session, all samples for a given metabolite and for a given individual were analyzed together with each individual sample measured in duplicate. Because of technical difficulties, rest values are not provided.

**Sarcoplasmic reticulum.** For the measurement of SR Ca\(^{2+}\)-cycling properties, muscle samples (40–60 μg) were diluted 1:11 (w/vt) in ice-cold homogenizing buffer (pH 7.5) containing (in mM) 250 sucrose, 5 HEPES, 0.2 phenylmethylsulfonyl fluoride, and 0.2% sodium azide (Na\(_3\)N). The samples were mechanically homogenized with a Duall glass on a glass hand homogenizer (Kontes Class, Duall 20). The homogenate, which was prepared on ice, was divided into aliquots, rapidly frozen in liquid nitrogen, and stored at −80°C pending analyses of SR function. Protein determination of homogenates was made by the method of Lowry as modified by Schacterle and Pollack (43).

The measurements of Ca\(^{2+}\) uptake and Ca\(^{2+}\) release were performed on the same homogenate sample in a coupled assay. With this procedure, oxalate-supported Ca\(^{2+}\) uptake rates are initially determined according to the ratiometric method developed by Ruell et al. (40) as modified by our laboratory (51). Extravesicular Ca\(^{2+}\) was measured fluorometrically (Ratiomaster system; Photon Technology International) using the Ca\(^{2+}\) indicator indo-1 [excitation wavelength, 355 nm; emission wavelengths, 485 and 405 nm for free Ca\(^{2+}\) (G) and bound Ca\(^{2+}\) (F), respectively]. The concentration of cytosolic free Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_c\)) was calculated by measuring the ratio of F to G using the equation of Grynkiewicz et al. (24). The \( K_d \) value employed for this interaction of Ca\(^{2+}\) and indo-1 for muscle homogenates was 250 nM. The reaction buffer (pH 7.0) contained (in mM) 200 KC1, 20 HEPES, 10 Na\(_2\)HPO\(_4\), 10 MgCl\(_2\), 10 Na\(_3\)HPO\(_4\), 10 phosphoenolpyruvate (PEP), and 5 oxalate plus 5 μM N,N,N,N-tetrakis(2-pyridylmethyl)ethyleneamine. Before each assay, 1.5 μM indo-1, 18 U/ml lactate dehydrogenase (LDH), and 18 U/ml pyruvate kinase (PK) were added to 2 ml of reaction buffer. Immediately before collection of emission spectra, 60 μl of muscle homogenate were added to the cuvette containing the reaction buffer. After the initiation of data collection, 2.5 μl of 10 mM CaCl\(_2\) were added to the cuvette (producing a consistent starting [Ca\(^{2+}\)] of ~3.5 μM), followed by 5 mM ATP to initiate Ca\(^{2+}\) uptake. Fluorescence ratio values were sampled at 2 Hz. Ca\(^{2+}\) uptake rates, generated from the [Ca\(^{2+}\)]\(_c\) vs. time curve, were smoothed over 21 points using the Savitsky-Golay algorithm. Linear regression was performed to determine Ca\(^{2+}\) uptake rates at [Ca\(^{2+}\)] values of 500, 1,000, 1,500, and 2,000 nM by using data representing each [Ca\(^{2+}\)] ± 100 nM. Because the effects of exercise and diet were similar...
regardless of the $[\text{Ca}^{2+}]_i$, we have only reported the $\text{Ca}^{2+}$ uptake at a $[\text{Ca}^{2+}]_i$ of 2,000 nM.

$\text{Ca}^{2+}$ release rates were measured after the active loading of $\text{Ca}^{2+}$ into the SR when $[\text{Ca}^{2+}]_i$ plateaued by adding 20 mM 4-chloro-m-cresol (4-Cmc), an agent that induces $\text{Ca}^{2+}$ release via the $\text{Ca}^{2+}$ release channel (CRC) (26). The 4-Cmc induces a biphasic $\text{Ca}^{2+}$ release that we have labeled phase 1 and phase 2 (49). Maximal $\text{Ca}^{2+}$ release rates for each phase were calculated using the same method as for $\text{Ca}^{2+}$ uptake, where the ionized $\text{Ca}^{2+}$ concentration is calculated using the equation of Grynkiewicz et al. (24). Differentiating the linear-fit curves allows determination of $\text{Ca}^{2+}$ release rates. Phase 1 $\text{Ca}^{2+}$ release is characterized by a rapid early release (~0–2 s), whereas phase 2 $\text{Ca}^{2+}$ release is characterized as the slower, more delayed release (~5–15 s) (13, 49).

In this study, we measured $\text{Ca}^{2+}$ release rates following oxalate-dependent $\text{Ca}^{2+}$ loading into the SR. It is possible that the $\text{Ca}^{2+}$ release rates could be biased by the dissociation of $\text{Ca}^{2+}$ from oxalate. $\text{Ca}^{2+}$ loading of the SR without oxalate takes 40–60 min. We find the homogenate preparation unstable during this time period. It should be noted that in previous papers from our group (13, 44, 50), estimates of $\text{Ca}^{2+}$ uptake and $\text{Ca}^{2+}$ release were 100 times too high. This occurred because of errors in the software used to calculate $[\text{Ca}^{2+}]_i$ in conjunction with a dilution error.

The procedures for measuring $\text{Ca}^{2+}$-ATPase activity in our laboratory have been described in detail (45, 50). Essentially the same procedures were employed as for measuring $\text{Ca}^{2+}$ uptake. The protocol, originally developed by Simonides and Van Hardeveldt (47) on rat muscle tissue and adapted for human muscle (40), involves measuring the change in $[\text{Ca}^{2+}]_i$ with successive additions of 0.5 μl of $\text{Ca}^{2+}$ until a plateau and subsequent decline in $\text{Ca}^{2+}$-ATPase activity is observed. The reaction buffer contained (in mM) 200 KC1, 20 HEPES, 15 MgCl2, 10 Na2N3, 10 PEP, 5 ATP, and 1 EDTA. The pH of the buffer was adjusted to 7.0 at 37°C. Immediately before the reaction was started, 18 U/ml LDH, 18 U/ml PK, 0.3 mM NADH, and 25 μM of homogentisate were added to 1 ml of reaction buffer. Samples were run both with and without 1 μM $\text{Ca}^{2+}$ ionophore A23187 (C-7522, Sigma Chemical, St. Louis, MO). Assays were performed at 37°C and 340 nm (Shimadzu UV 160). Once the recording of baseline absorbance was completed, the reaction was initiated by adding 1 μl of 100 mM CaCl2 and monitored for ~2 min. After the successive additions of 0.5 μl of $\text{Ca}^{2+}$, basal or $\text{Mg}^{2+}$-ATPase activity was determined by adding 1 μl of the $\text{Ca}^{2+}$-ATPase specific inhibitor cyclopiazonic acid (46). $\text{Ca}^{2+}$-ATPase activity was then plotted against the negative logarithm of $[\text{Ca}^{2+}]_i$ (pCa). The $[\text{Ca}^{2+}]_i$ needed to elicit 50% of $V_{\text{max}}$ (Ca0) and the Hill coefficient (nH) were calculated as described previously (45). In a given analytical session, and for a given protocol, all tissues for a given individual were measured in duplicate. The order of analyses for a given property was randomized.

We calculated the ratio between $\text{Ca}^{2+}$ uptake and $\text{Ca}^{2+}$-ATPase activity to gain insight into whether the energy costs associated with $\text{Ca}^{2+}$ transport were altered. It should be emphasized that the ratio was determined at submaximal levels of $\text{Ca}^{2+}$ uptake, whereas the $\text{Ca}^{2+}$-ATPase activity was maximal. Because the ratio was determined under different conditions for the two properties, we have used the term “apparent coupling ratio.”

The SR $\text{Ca}^{2+}$-ATPase isoforms (SERCA1 and SERCA2a) were measured with the use of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and immunoblotting with the primary monoclonal antibodies specific for human SERCA1 (MA3-912; Affinity Bioreagents) and SERCA2a (MA3-919; Affinity Bioreagents) as detailed previously (45). A 7.5% polyacrylamide SDS gel and a 3.75% stacking gel were employed. Muscle homogenates (10 μg), made as a suspension of 1.0 mg/ml protein, were diluted to 0.5 mg/ml protein with a solution (1:1 ratio) of distilled water and sample buffer (146 mM sucrose, 0.5 M Tris-HCI, 20% SDS, 0.1% bromophenol, 2 mM DTT, and 0.2 EDTA). After transfer of the protein to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad) and incubation of the PVDF membrane with the primary antibodies, a secondary antibody (anti-mouse IgG1 conjugated to horseradish peroxidase) protein quantification was performed with the use of densitometry and an enhanced chemiluminescence immunodetection procedure (Amersham ECL-RPN2106P1).

The blots were developed in Kodak GBX developing solution after exposure to photographic film (Kodak Hyperfilm-ECL). All samples, for both isoforms, were compared with a standard (tissue sample of human vastus lateralis collected independently), and the relative protein level was calculated. To determine the effect of the Ex+Lo CHO condition, the protein level was determined relative to that in the Lo CHO condition and expressed as a percentage. The linearity of protein content versus antibody over the range of protein levels used was established before the measurements of SERCA1 and SERCA2a.

All SR properties except $\text{Ca}^{2+}$-ATPase were measured in duplicate. $\text{Ca}^{2+}$ release was measured in a single trial. For a given individual and a given property, the measurements were performed in the same analytical session.

Statistical analyses. To determine the effects of the different treatments on the variables studied, we employed one-, two- and three-way analyses of variance (ANOVA) procedures. Where significant differences were found, Newman-Keuls post hoc procedures were employed to compare specific means. Statistical significance was set at $P < 0.05$.

RESULTS

Dietary analysis. On average, the participants consumed 1,793 ± 111 and 1,613 ± 101 kcal/day during the Lo CHO and Ex+Lo CHO conditions, respectively. The daily caloric intake was not different between conditions. For the Lo CHO condition, the daily caloric intake consisted of 22 ± 2% carbohydrate, 56 ± 2% fat, and 22 ± 1% protein. For Ex+Lo CHO, the daily caloric intake consisted of 20 ± 2% carbohydrate, 57 ± 2% fat, and 22 ± 1% protein and was not different from Lo CHO.

Respiratory gas exchange. Exercise increased $V_{\text{O}2}$ to ~58% of $V_{\text{O}2_{\text{peak}}}$ by 15 min of exercise in Lo CHO (Table 1). Thereafter, $V_{\text{O}2}$ remained at steady state until fatigue, where a small but significant increase of 8% was observed. Essentially, the same response was observed for $V_{\text{CO}2}$, with the exception that no increase in this property was observed at fatigue. No difference in the response of either $V_{\text{O}2}$ or $V_{\text{CO}2}$ was observed between the Lo CHO and Ex+Lo CHO conditions. Similarly, we could find no difference in RER between conditions, either at rest or during exercise. In general, RER increased by 15 min of exercise and then remained stable throughout the remainder of the prolonged cycling task.

Cycling times to fatigue were 102.8 ± 10.8 and 96.9 ± 6.4 min for the Lo CHO and Ex+Lo CHO conditions, respectively. The difference observed between Lo CHO and Ex+Lo CHO was not significant.

Substrate oxidation rates. Exercise increased both carbohydrate and lipid oxidation rates (Table 1). In the case of carbohydrates, increases in oxidation rate were fully manifested by 15 min of exercise regardless of condition. In contrast, lipid oxidation rates not only increased by 15 min of exercise but showed further increases at both 60 min of exercise and at fatigue. No differences were found in lipid or carbohydrate oxidation rates between Lo CHO and Ex+Lo CHO conditions.

Blood metabolites. Blood glucose concentrations were observed to be affected by both exercise sessions in a manner that
was specific to the condition (Fig. 1). During exercise in Lo CHO, blood glucose remained stable throughout the initial 30 min of exercise before progressively declining at 60 min and at fatigue. At fatigue, blood glucose was reduced by ~15%. Compared with Lo CHO, the initial decline in blood glucose concentration in Ex+Lo CHO was observed earlier in exercise, namely, at 15 min. Thereafter, blood glucose progressively declined until 60 min of exercise. Compared with Lo CHO, blood glucose concentrations were lower during Ex+Lo CHO at 30 and 60 min and at fatigue. For blood lactate, an elevation was observed at 15 min of exercise that was sustained until 30 min of exercise before declining at 60 min of exercise. Blood lactate was not different between 60 min of exercise and fatigue. No differences in blood lactate were observed between conditions.

Muscle glycogen and metabolites. As expected, muscle glycogen concentration declined progressively with the duration of exercise (Fig. 2). There were no differences in glycogen reserves between the Lo CHO and Ex+Lo CHO conditions. At fatigue, muscle glycogen concentration was depleted on average by 63%. During exercise, no differences were observed between conditions for any of the muscle high-energy phosphates (ATP, PCR) and related metabolites (Cr, Pi) or muscle lactate concentration (Table 2).

Sarcoplasmic reticulum. During exercise in Lo CHO, V\textsubscript{max} was progressively reduced at 30 and 60 min of exercise and at fatigue (Table 3). During Ex+Lo CHO, initial reductions in V\textsubscript{max} were not observed until 60 min. Thereafter, no further changes were observed. At fatigue, reductions in V\textsubscript{max} of 41 and 26% were observed for Lo CHO and Ex+Lo CHO, respectively. The smaller reduction in V\textsubscript{max} in Ex+Lo CHO was related to the lower V\textsubscript{max} observed for this condition before exercise compared with Lo CHO. Neither exercise nor condition altered basal ATPase activity. Of the two measures used to assess alterations in Ca\textsuperscript{2+}-binding affinity, namely, n\textsubscript{H} and C\textsubscript{50}, only n\textsubscript{H} was affected by condition. The n\textsubscript{H} was higher during Ex+Lo CHO compared with Lo CHO, an effect that was not specific to any time point. Although, on average, the C\textsubscript{50} was generally higher during Ex+Lo CHO, the difference between the two conditions was not significant. Exercise, regardless of condition, was without effect in altering n\textsubscript{H} and C\textsubscript{50}.

The ratio of V\textsubscript{max}, measured with and without the Ca\textsuperscript{2+} ionophore A23187 was also different between conditions but only at fatigue (Ex+Lo CHO > Lo CHO) (Fig. 3). An effect of exercise on the ionophore ratio was observed, but only for Lo CHO. At fatigue in Lo CHO, the ionophore ratio was lower than at 30 min of exercise.

Clear effects of exercise and condition were observed for Ca\textsuperscript{2+} uptake (Fig. 4). Regardless of condition, exercise resulted in progressive reductions in Ca\textsuperscript{2+} uptake at all of the times examined. The reductions in Ca\textsuperscript{2+} uptake with exercise, which ranged between 38 and 41% at fatigue, were not different between conditions. However, Ca\textsuperscript{2+} uptake was higher in Ex+Lo CHO compared with Lo CHO. This was a main effect.

Apparent coupling ratios, defined as the ratio of Ca\textsuperscript{2+} uptake to Ca\textsuperscript{2+}-ATPase activity, were higher during the Ex+Lo CHO compared with the Lo CHO condition before exercise and at 30 and 60 min but not at fatigue (Fig. 5). During Ex+Lo CHO, a reduction in the coupling ratio was observed with exercise, but only at fatigue. No exercise-induced changes in the coupling ratio were observed during Lo CHO.

Two different phases (phases 1 and 2) of Ca\textsuperscript{2+} release were assessed (Fig. 6). Both phases showed the same response to exercise, namely, an initial reduction observed at 30 min of exercise. Thereafter, no further changes were observed throughout the exercise. No differences were observed between Lo CHO and Ex+Lo CHO in either phase 1 or phase 2 Ca\textsuperscript{2+} release.

Measurement of the SERCA isoform changes between the experimental conditions was accomplished using Western immunoblotting techniques. These results indicated that there were no differences in either SERCA1 or SERCA2a isoforms between Lo CHO and Ex+Lo CHO. For the Ex+Lo CHO
condition, the SERCA1 and SERCA2a were 98.6 ± 29 and 99.4 ± 43% of that for the Lo CHO condition, respectively.

DISCUSSION

In this article, we report several novel findings regarding the interaction between diet and exercise on SR function. Our results demonstrate that when a glycogen depletion session of exercise precedes a 4-day low-carbohydrate diet (Lo CHO), several alterations occur in SR Ca\(^{2+}\)-cycling properties that are different from those observed after a 4-day low-carbohydrate diet (Lo CHO) in isolation. With Ex+Lo CHO, \(V_{\text{max}}\) was lower at rest and at 30 min of exercise than with Lo CHO, whereas the \(n_H\) was persistently higher during both rest and exercise. We also have found that the ionophore ratio, a measure of membrane integrity, was higher at fatigue for Ex+Lo CHO. These effects were also accompanied by a generally higher Ca\(^{2+}\) uptake that was not restricted to a time point and a higher coupling ratio that was specific to rest and the first 60 min of exercise. The Ex+Lo CHO condition was without effect in modifying the reduction in Ca\(^{2+}\) release, either phase 1 or phase 2, from that observed during exercise in the Lo CHO condition.

Collectively, these results suggest that the primary effect of the glycogen depletion bout of exercise was to improve Ca\(^{2+}\) transport into the SR at reduced or unchanged \(V_{\text{max}}\). Because most of these effects are expressed at rest, it appears that the differences in exercise between the two conditions reflect, in part, the adaptations that occur before exercise. A critical point is that an exercise session before the Lo CHO diet is needed to induce the adaptations that are observed. Interestingly, the changes in SR Ca\(^{2+}\) cycling noted between conditions at rest and during the standardized exercise occurred in the absence of differences in muscle glycogen concentration.

As expected, our standardized cycling protocol induced disturbances in a number of SR Ca\(^{2+}\)-cycling properties in exercising vastus lateralis muscle when assessed in vitro. These disturbances, measured after the Lo CHO diet, included reductions in \(V_{\text{max}}\), Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release. The reduc-

Table 2. Effects of Lo CHO and Ex+Lo CHO on concentration of high-energy phosphates and metabolites in vastus lateralis muscle

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<td>ATP</td>
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<td>Lo CHO</td>
<td>22.2±1.2</td>
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<td>Ex+Lo CHO</td>
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<td>Lo CHO</td>
<td>37.4±5.5</td>
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<tr>
<td>Ex+Lo CHO</td>
<td>43.1±5.2</td>
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<tr>
<td>Cr</td>
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<tr>
<td>Lo CHO</td>
<td>80.4±4.2</td>
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<tr>
<td>Ex+Lo CHO</td>
<td>78.4±7.2</td>
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<td>Pi</td>
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<tr>
<td>Lo CHO</td>
<td>74.5±3.0</td>
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<tr>
<td>Ex+Lo CHO</td>
<td>73.6±5.5</td>
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<tr>
<td>Lac</td>
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<tr>
<td>Lo CHO</td>
<td>18.4±5.3</td>
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<tr>
<td>Ex+Lo CHO</td>
<td>23.5±5.0</td>
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Values are means ± SE in mmol/kg dry weight; \(n = 9\). PCr, phosphocreatine; Cr, creatine; Pi, inorganic phosphate; Lac, lactate. Only the exercise values are provided.
of condition was found for the Hill coefficient: Ex+/H11001
Lo CHO significantly different from 30 min.‡
significantly different from Lo CHO.

Similarly, the prolonged exercise following Lo CHO failed to alter the coupling ratio,
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and Ex + Lo CHO in this study. This observation is potentially significant given studies that implicate glycogen reserves as an important determinant of SR Ca\(^{2+}\)-cycling behavior (6, 31).

A secondary objective of this study was to determine whether a Lo CHO diet can influence SR Ca\(^{2+}\)-handling properties. We have addressed the issue by comparing the results of our earlier work using a normal CHO diet (12) with the results of the current experiment using Lo CHO. Based on a two-way ANOVA for repeated measures and the same probability level for statistical significance, we found that the Lo CHO compared with the normal CHO resulted in an elevated \(V_{\text{max}}\) (before and at 30 min of exercise) and a lower Ca\(^{2+}\) uptake (at 30 and 60 min of exercise and at fatigue). Also observed were a higher ionophore ratio and a lower apparent coupling ratio, both of which were main effects independent of time. Differences also were observed between Lo CHO and normal CHO in phase 2 Ca\(^{2+}\) release, where higher values were observed at rest and during exercise in normal CHO. As expected, muscle glycogen was lower at all time points in the Lo CHO compared with the normal CHO condition. Collectively, these differences serve to emphasize that the Lo CHO diet for 4 days in isolation causes substantial changes in SR function. It is significant that for all properties except Ca\(^{2+}\) uptake, the differences observed during exercise can be attributed to the differences observed at rest. In the case of Ca\(^{2+}\) uptake, differences existed at rest that could explain the exercise effects, but no significance was found.

It should be emphasized that to isolate the independent and combined effects of exercise, diet, and exercise plus diet, we examined several experimental conditions in separate groups of volunteers. These experimental conditions were conducted concurrently, and the tissue properties from each condition were measured during the same analytical sessions. This strategy was used to avoid any systematic bias that would potentially occur by analyzing the tissue at the different times. In addition, all volunteers were untrained and recruited from the same student population.

The changes that we have observed in SR Ca\(^{2+}\)-cycling behavior during exercise associated with glycogen depletion support the results of previous studies showing that reduced muscle glycogen levels are associated with an increased depression in SR Ca\(^{2+}\) uptake (31, 32). However, our Ca\(^{2+}\) release data are not consistent with the proposed disruptive effect of glycogen on Ca\(^{2+}\) release (6), because we found that Ca\(^{2+}\) release was only depressed during the earlier phase of exercise before substantial depletion of glycogen occurred. No further reductions in Ca\(^{2+}\)-release kinetics were observed after 30 min of exercise, even though muscle glycogen content continued to be reduced during the cycling protocol. Because Chin and Allen (6) measured Ca\(^{2+}\) release in single fibers, it is not clear whether distinct phases of Ca\(^{2+}\) release occur.

![Fig. 5. Apparent coupling ratios determined during prolonged exercise in vastus lateralis muscle following Lo CHO or Ex + Lo CHO. Values are means ± SE; n = 9. Coupling ratio is defined as Ca\(^{2+}\) uptake/Ca\(^{2+}\)-ATPase activity. *P < 0.05, significantly different from 0 min. †P < 0.05, significantly different from 30 min. ‡P < 0.05, significantly different from 60 min. #P < 0.05, significantly different from Lo CHO.](http://ajpcell.physiology.org/)

![Fig. 6. Changes in sarcoplasmic reticulum Ca\(^{2+}\) release in vastus lateralis muscle induced by 4-chloro-m-cresol during prolonged exercise following Lo CHO or Ex + Lo CHO. Values are means ± SE in \(\mu\)mol g protein\(^{-1}\) min\(^{-1}\); n = 9. A: phase 1, the initial, rapid rate of Ca\(^{2+}\) release. B: phase 2, the more prolonged, slower rate of release that follows phase 1. Main effects (P < 0.05) were observed for both phase 1 and phase 2 Ca\(^{2+}\) release: for phase 1, 0 min > 30 min = 60 min = fatigue; for phase 2, 0 min > 30 min = 60 min = fatigue.](http://ajpcell.physiology.org/)
According to current theory, the direct association between muscle glycogen content and Ca\(^{2+}\) uptake occurs as a result of a glycogen-glycolytic complex that is attached to the SR. The loss of glycogen dissociates the complex from the SR and modifies Ca\(^{2+}\)-uptake behavior (31). The modification of Ca\(^{2+}\)-uptake behavior could occur as a direct result of structural alterations to the SR or secondary to energetic considerations. Glycolytically derived ATP resynthesis is believed to improve ATP availability and energetic efficiency by increasing ATP/ADP ratios (30). It must be acknowledged that our measurement of muscle glycogen was assessed globally and not regionally. It is possible that glycogen levels may be different between different cellular compartments with prolonged exercise (18). Potentially important as well is whether differences existed between the two conditions in the intracellular metabolic stress that occurred during the exercise. Because we found no differences between conditions in phosphorylation potential and related metabolites, metabolic stress would not appear to be different. The changes that we have observed in the metabolic response during exercise are as expected for the protocol employed (21).

The major findings of the current study indicate that the superimposition of a prolonged session of exercise before a 4-day period of Lo CHO designed to maintain a reduced muscle glycogen reserve lower than what would be expected with normal CHO has extensive effects in eliciting additional modifications to SR Ca\(^{2+}\)-cycling properties over those realized by the Lo CHO diet in isolation. The potent effect of an exercise session preceding the Lo CHO diet in modifying the effects of the Lo CHO diet alone suggests adaptations at both the membrane and pump level. These modifications, which result in improved Ca\(^{2+}\) uptake, mediated over a range of [Ca\(^{2+}\)]\(_i\) during both rest and exercise, appear to be accomplished via increases in Ca\(^{2+}\)-binding affinity of the enzyme and decreased energy costs of Ca\(^{2+}\) transport per ATP hydrolyzed as assessed in vitro. These alterations appear to be desirable adaptations to a diet low in carbohydrate and high in fat, where the cost of ATP production via oxidative phosphorylation may be compromised, given the greater fat utilization that occurs. Interestingly, these effects are only realized when exercise is used to precondition the muscle to the Lo CHO diet. As emphasized, many of these effects are expressed at rest and persisted during exercise.

The results that we have reported regarding the alterations in SR behavior with exercise and dietary manipulation must be qualified in light of the limitations of our assay procedures. We assessed the kinetic properties of the Ca\(^{2+}\)-ATPase using the Ca\(^{2+}\) ionophore A23187 to prevent back inhibition of the enzyme by accumulation of [Ca\(^{2+}\)]\(_i\) in the lumen of the SR. Moreover, given the limited sensitivity of indo-1, we were unable to measure Ca\(^{2+}\) uptake at maximal activation levels of [Ca\(^{2+}\)]\(_i\). Consequently, the coupling ratios presented must be put in context. With our protocol, the coupling ratios allow a comparison between the experimental conditions under very defined conditions. We also did not examine passive leak across the SR by selectively inhibiting both the CRC and the Ca\(^{2+}\)-ATPase. In samples in which sufficient homogenates remained, we examined for passive leak from the CRC by using ruthenium red. Our limited results indicate no differences between Lo CHO and Ex+Lo CHO conditions (unpublished).

Ideally, Ca\(^{2+}\) release should be measured following Ca\(^{2+}\) loading without oxalate. However, with homogenates, we have found that Ca\(^{2+}\) loading without oxalate takes \(\sim 60\) min. The homogenate is not stable during this time and, as a result, decreases in Ca\(^{2+}\) release occur (unpublished). The use of DTT to prevent the apparent oxidative damage that occurs is problematic given that exercise in itself appears to cause oxidative damage. The possibility exists of reversing the experimental effect with DTT. For this reason, we did not use DTT in our homogenizing buffer. We have shown that Ca\(^{2+}\) release is graded to the concentration of 4-CMC, the releasing agent that we employed (49). Although it might be expected that Ca\(^{2+}\) release would be enhanced with oxalate, our 4-CMC results demonstrate an important role for the open state of the CRC. Equally important is the fact that our results demonstrating exercise-induced reductions in Ca\(^{2+}\) release are entirely consistent with the literature, which shows that Ca\(^{2+}\) loading has been accomplished with and without oxalate. Our results provide a comprehensive analysis of SR Ca\(^{2+}\)-cycling properties in working muscle, many of which were modified by our experimental manipulations. These measurements were performed on a limited amount of tissues, typically extracted from humans by the biopsy technique.

Human vastus lateralis muscle is composed of a mixture of fiber types, with type I and type IIA fibers (22, 52) representing in excess of 90% of the fiber population (42). Therefore, the changes in SR Ca\(^{2+}\)-handling properties observed in our study represent global changes in SR properties from all fiber types represented within each tissue sample rather than fiber type-specific responses. The literature has shown that exercise protocols of comparable intensity and duration does induce a progressive reduction in muscle glycogen content within both type I and type IIA fibers (22, 52). It is possible that the changes in SR function that were observed during exercise could be restricted to a specific fiber-type population, because we did not assess fiber type-specific glycogen utilization in this study. In addition, differences in SR Ca\(^{2+}\)-handling properties during this study. Our results can now serve as a catalyst for more mechanistic studies using other species where the amount of tissue available is not a limitation. An intriguing question is whether there are species differences.

In summary, prolonged exercise following a Lo CHO diet results in reductions in maximal Ca\(^{2+}\)-ATPase activity, Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release when assessed in vitro. Changes in maximal Ca\(^{2+}\)-ATPase activity occur in the absence of changes in the Ca\(^{2+}\) sensitivity, as measured using the Hill coefficient and Ca\(_{50}\), and in the absence of changes in the coupling ratio. When prolonged exercise precedes the Lo CHO diet, resting maximal Ca\(^{2+}\)-ATPase activity is depressed and Ca\(^{2+}\)-uptake is elevated, resulting in a higher coupling ratio than observed for Lo CHO alone. These
effects persist during the exercise, the duration depending on the property in question. Interestingly, the differences observed between conditions, both at rest and during exercise, occur in the absence of differences in muscle glycogen concentration.

**GRANTS**

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**REFERENCES**


