Effects of prior exercise and a low-carbohydrate diet on muscle sarcoplasmic reticulum function during cycling in women

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Duhamel, T. A., H. J. Green, J. G. Perco, and J. Ouyang. Effects of prior exercise and a low-carbohydrate diet on muscle sarcoplasmic reticulum function during cycling in women. J Appl Physiol 101: 695–706, 2006. First published May 18, 2006; doi:10.1152/japplphysiol.00052.2006.—The effects of exercise and diet on sarcoplasmic reticulum Ca$^{2+}$-cycling properties in female vastus lateralis muscle were investigated in two groups of women following four different conditions. The conditions were 4 days of a low-carbohydrate (Lo CHO) and glycogen-depleting exercise plus a Lo CHO diet (Ex + Lo CHO) (experiment 2) and 4 days of normal CHO (Norm CHO) and glycogen-depleting exercise plus Norm CHO (Ex + Norm CHO) (experiment 1). Peak aerobic power ($V_{O2peak}$) was $38.1 \pm 1.4$ (SE); $n = 9$ and $35.6 \pm 1.4$ ml·kg$^{-1}·min^{-1}$; $n = 9$, respectively. Sarcoplasmic reticulum properties measured in vitro in homogenates (µmol·g protein$^{-1}·min^{-1}$) indicated exercise-induced reductions ($P < 0.05$) in maximal Ca$^{2+}$-ATPase activity ($0 > 30, 60$ min $> $ fatigue), Ca$^{2+}$ uptake ($0 > 30 > 60$, min, fatigue), and Ca$^{2+}$ release; both phase 1 ($0 > 30 > 60$, min, fatigue) and phase 2 ($0 > 30, 60$, min, fatigue; $30 > $ fatigue) in Norm CHO. Exercise was without effect in altering the Hill slope ($n_H$), defined as the slope of relationship between Ca$^{2+}$-ATPase activity and Ca$^{2+}$ concentration. No differences were observed between Norm CHO and Ex + Norm CHO. Compared with Norm CHO, Lo CHO resulted in a lower ($P < 0.05$) Ca$^{2+}$ uptake; phase 1 Ca$^{2+}$ release ($30$, min), and $n_H$. Ex + Lo CHO resulted in a greater ($P < 0.05$) Ca$^{2+}$ uptake and $n_H$ compared with Lo CHO. The results demonstrate that Lo CHO alone can disrupt SR Ca$^{2+}$-cycling and that, with the exception of Ca$^{2+}$ release, a glycogen-depleting session of exercise before Lo CHO can reverse the effects.

Ca$^{2+}$ release; Ca$^{2+}$ uptake; Ca$^{2+}$-ATPase activity; vastus lateralis; submaximal; female

The level of free Ca$^{2+}$ ([Ca$^{2+}$]$_i$) in the skeletal muscle cell that is regulated primarily by the sarcoplasmic reticulum (SR) is vital for a range of cellular functions and, most particularly, for the mechanical response. The SR is an intracellular organelle that contains a collection of special proteins embedded in a phospholipid membrane. Proteins such as the ryanodine receptor (RyR) or Ca$^{2+}$-release channel (CRC) and the Ca$^{2+}$-ATPase are intimately involved in the control of [Ca$^{2+}$]$_i$, given their ability to regulate both the release and uptake of Ca$^{2+}$ by the SR.

With the onset of muscle stimulation, the requirements for Ca$^{2+}$ cycling increase dramatically, necessitating rapid increases in Ca$^{2+}$ release and Ca$^{2+}$ uptake. Precise regulation of the [Ca$^{2+}$]$_i$ transient is intimately dependent on a close coordination between the open state of the CRC and the activation of the Ca$^{2+}$-ATPase. Certain tasks appear to promote a disturbance in Ca$^{2+}$-cycling properties. In prolonged contractile activity, as an example, it has been observed that when assessed in vitro, the maximal catalytic activity of the enzyme ($V_{max}$) is depressed (3, 4, 32) as a result of structural alterations to the enzyme (29) and the RyR channel (14). These events result in a depression in Ca$^{2+}$ uptake (3, 4, 32, 50) and Ca$^{2+}$ release (15, 50). The reductions in $V_{max}$ observed with prolonged activity appear to occur in the absence of changes in Ca$^{2+}$-dependent binding to the enzyme as assessed by the half-maximal activation and the Hill slope ($n_H$)(10, 11). In addition, prolonged activity also appears to be without effect in modifying the integrity of the SR membrane, as assessed by the ratio of $V_{max}$ measured without the Ca$^{2+}$ ionophore (A23187), and the efficiency of Ca$^{2+}$ pumping, as measured by the ratio of Ca$^{2+}$ uptake to Ca$^{2+}$-ATPase activity (10, 11).

The disturbing effects of prolonged exercise on SR Ca$^{2+}$-cycling properties have been most consistently displayed in humans, and, in particular, men (3, 10, 11, 17, 49). It is possible that women may exhibit a different response in SR Ca$^{2+}$-cycling properties to prolonged activity than men, given the gender differences that exist in substrate dependence. Evidence exists to suggest that the muscle carbohydrate (CHO) reserves, and, in particular, muscle glycogen concentration, may affect SR behavior (5, 26). According to this theory, the progressive depletion of muscle glycogen that occurs with prolonged exercise results in the loss of the glycogen-glycolytic complex, which contains a collection of enzymes involved in glycogen metabolism as well as creatine phosphokinase, which is bound to the SR (26, 39, 52). The dissociation of this complex from SR could either alter the structural integrity of the SR (6) or compromise local regeneration of ATP via high-energy phosphate transfer (25, 37) and/or glycolysis (26, 51), which is used to supply the Ca$^{2+}$-ATPase with its energy needs. Conceivably, these effects could alter both Ca$^{2+}$ uptake and Ca$^{2+}$ release by the SR. Because it is well known that women depend less on CHO as a substrate during prolonged exercise than men (46), it is possible that muscle glycogen levels may be better protected and SR function better preserved.

Alternatively, it is possible that the disturbances in Ca$^{2+}$ cycling observed during exercise may not associate with muscle glycogen reserves. Evidence has been published indicating that the structural alterations that occur to the Ca$^{2+}$-ATPase and the CRC are due to oxidation and nitrosylation of these proteins as a result of the accumulation of free radicals (14, 24, 33).

The purpose of this study was to examine the effects of prolonged cycle exercise and endogenous glycogen concentra-

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tion on SR Ca\(^{2+}\)-cycling properties in women with respect to both the kinetic behavior of the enzyme and the Ca\(^{2+}\)-uptake and Ca\(^{2+}\)-release properties. We have hypothesized that women would show a time-dependent disturbance with exercise in these properties with progressive depletion of muscle glycogen. Moreover, we have also hypothesized that reduction in muscle glycogen reserves mediated by a low-CHO diet would associate with an increased disturbance in the SR Ca\(^{2+}\)-cycling response in the working muscle of women.

METHODS

Experimental design. To examine the effects of diet on SR Ca\(^{2+}\)-cycling responses, four different conditions were used employing two different experiments and two groups of participants (Fig. 1). In experiment 1, the control condition, the SR responses to prolonged standardized cycle exercise were examined in a group of volunteers both while on a normal habitual diet that we have designated as Norm CHO and after a prolonged session of cycle exercise designed to deplete muscle glycogen followed by 4 days on a normal CHO diet (Ex + Norm CHO). These experiments allowed us to determine the effects of the glycogen-depleting session of exercise by itself while on a normal CHO diet. In experiment 2, essentially the same design was employed, except that a dietary manipulation was incorporated. In this group of volunteers, the standardized exercise was performed both after 4 days of a low-CHO diet (Lo CHO) and after a preliminary session of prolonged exercise, again designed to deplete muscle glycogen reserves, plus 4 days of Lo CHO (Ex + Lo CHO). These conditions allowed investigation of the effects of a low-CHO diet and also the effects of prior exercise while on a low-CHO diet. Comparisons across conditions allowed isolation of the effects both of diet (Norm CHO vs. Lo CHO) and glycogen-depleting exercise plus diet (Ex + Norm CHO vs. Ex + Lo CHO). Participants were randomly assigned to each experiment and each condition with the constraint that the number first assigned a given condition could not exceed five. At least 28 days were provided between conditions. No significant order effect was observed for any of the SR properties examined.

Each volunteer was required to visit the laboratory on four separate occasions. The first visit was used to familiarize the participant with all experimental procedures and to determine peak O\(_2\) consumption (V\(_{\text{O}_2\text{peak}}\)). In the case of the Ex + Norm CHO and Ex + Lo CHO, the second visit involved the performance of prolonged cycling for 2 h designed to substantially deplete muscle glycogen reserves. On average, this exercise session was performed at 55–60% V\(_{\text{O}_2\text{peak}}\). Brief rest periods were provided as required to complete the actual 2 h of exercise. During two additional visits (Norm CHO and Ex + Norm CHO or Lo CHO and Ex + Lo CHO), the volunteers performed a standardized cycle test until fatigue. These tests were performed at ~58 and 61% V\(_{\text{O}_2\text{peak}}\) for the Norm CHO and Ex Lo CHO conditions, respectively. The same absolute power output was used for each condition within each experiment.

For the Norm CHO condition, the participants were instructed to follow a 4-day standardized diet (~2,000 kcal/day; ~55% CHO; ~30% fat; ~15% protein) both before the performance of the cycling test and during the 4-day period after the glycogen-depleting exercise. Average daily caloric intake during this period was tailored to match each participant’s habitual diet. This was achieved by having each participant record their habitual diet for a 4-day period before the start of the experiment. In the Lo CHO experiment, volunteers were asked to follow a low-CHO diet (~2,000 kcal; 20% CHO; ~60% fats; 20% protein) both in the 4-day lead-in period before the cycling test and during the 4-day period after the glycogen-depleting session of exercise.

Subject characteristics. Two groups of female volunteers were recruited for the study. One group (n = 9) was assigned to the Norm CHO conditions. The characteristics of this group included age 21 ± 0.8 yr (mean ± SE), body mass 62.7 ± 2.3 kg, and V\(_{\text{O}_2\text{peak}}\) 38.1 ± 1.4 ml·kg\(^{-1}\)·min\(^{-1}\). The second group (n = 9) was labeled Lo CHO. For this group, the characteristics were age 19 ± 0.3 yr, body mass 71.0 ± 4.4 kg, and V\(_{\text{O}_2\text{peak}}\) 35.6 ± 1.4 ml·kg\(^{-1}\)·min\(^{-1}\). Differences in V\(_{\text{O}_2\text{peak}}\) between groups were not significant. Both groups dis-

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**A** Experiment One

![Experiment One diagram](image)

**B** Experiment Two

![Experiment Two diagram](image)

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played normal hemoglobin (Hb) and hematocrit (Hct) levels before the experimental period. For the Norm CHO group, Hb (%) and Hct (%) values were 14.8 ± 0.9 and 42.5 ± 0.8, respectively. For the Lo CHO group, the respective values for Hb and Hct were 13.9 ± 0.6 g% and 42.6 ± 0.8%. No differences (P > 0.05) were found between groups for any of these physical, physiological, or hematological properties.

All participants were healthy and were not involved in vigorous exercise on a regular basis (i.e., less than once per week) and were not actively participating in an exercise-training program before or throughout the duration of this study. All participants were eumenorrheic with a normal reproductive cycle length. Testing was completed during the midfollicular phase of the menstrual cycle for all women. Twelve of the women were taking triphasic-type oral contraceptives. An equal number of these women participated in the Norm CHO and Lo CHO groups. Plasma 17β-estradiol and progesterone concentrations were determined by using a radioimmunoassay kit (Coat-A-Count). No differences were observed between conditions or between dietary groups for 17β-estradiol or progesterone (Table 1). No differences were determined by using a radioimmunoassay kit (Coat-A-Count). No differences were observed between conditions or between dietary groups for any of these physical, physiological, or hematological properties.

Twelve of the women were taking triphasic-type oral contraceptives. All participants consumed a meal-replacement beverage (Ensure; 250 kcal; 61% CHO; 24% fats; 15% protein) 4 h before reporting to the laboratory. This procedure was utilized to standardize the nutritional intake of all participants before the cycle tests and to minimize the disturbances in blood glucose levels during exercise. Fluid intake before reporting to the laboratory was ad libitum. Upon reporting to the laboratory (~60 min before testing), participants were prepared for tissue and blood sampling. Tissue samples were collected from vastus lateralis by the needle biopsy technique (1) under local anesthesia (2%) xylocaine with epinephrine and using suction to increase yield. During each condition, four sites, randomized between legs, were prepared for tissue extraction. Tissue was extracted before exercise (0 min), during exercise (30 and 60 min), and at fatigue. During an experimental session, the two sites on a given vastus lateralis used for tissue sampling were made within a field free from nerves and blood vessels but widely separated. At fatigue, the time of exercise was 115 ± 14, 108 ± 9.4, 104 ± 4.2, and 94 ± 6.0 for the Norm CHO, Ex+Norm CHO, Lo CHO, and Ex+Lo CHO conditions, respectively. Differences in exercise times between conditions were not significant. Because no differences were found between conditions, we have applied the term “fatigue” to all conditions.

We would emphasize that we have defined fatigue as the end point of the exercise. Because our primary focus was to understand the SR Ca2+-cycling responses to prolonged exercise per se, no motivational strategies were provided to encourage additional effort.

To allow for the assessment of muscle metabolic and SR Ca2+-handling properties, two separate tissue samples, using two different needles, were extracted from one site at each time point (~100 mg). At the time of tissue sampling, the participants briefly stopped cycling (~30 s) so that tissue samples could quickly be extracted. The first tissue sample was rapidly frozen in liquid N2 and kept frozen (~80°C) until analysis of the tissue for muscle metabolites. The second tissue sample was used to prepare a crude homogenate, which was stored at ~80°C and subsequently used to assess SR Ca2+-handling properties.

Blood samples were drawn over a 30-s period before exercise (0 min), at 15 min, 30 min, and 60 min of exercise, and immediately before fatigue from a 20-gauge catheter inserted in the dorsal region of the hand, which had been preheated (~20 min before rest sample). Samples were deproteinized and stored at ~80°C before measurement of glucose and lactate. Hct and Hb were measured at the time of sampling by standardized techniques. Respiratory gas samples were collected for a 3- to 4-min period before blood sampling and used to calculate O2 uptake (V˙O2), CO2 output (V˙CO2), and respiratory exchange ratio (RER). From these data, the rates of CHO and fat oxidation were assessed. The exercise tests (progressive and prolonged) were performed on a cycle ergometer (Quinton 870) that was calibrated on a regular basis. Volitional fatigue was defined as the inability to maintain a pedal cadence of at least 50 rpm regardless of the protocol employed. The environment in the laboratory for all test sessions was ~21°C and ~55% relative humidity. No fluid intake was permitted during any of the test sessions.

The progressive exercise test used for measurement of V˙O2peak employed a protocol previously described by our group (19). Gas exchange (V˙O2, V˙CO2, and expired ventilation) for both exercise tests was measured with an open-circuit system regularly employed by our group (22). The gas analyzers (Beckman OM-11 and LB-2) were calibrated on each test day by use of precisely determined reference gases.

### Table 1. Resting blood concentrations of 17β-estradiol and progesterone measured during the midfollicular phase for the different experimental conditions

<table>
<thead>
<tr>
<th>Group</th>
<th>17β-Estradiol, pg/ml</th>
<th>Progesterone, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
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</tr>
<tr>
<td>Norm CHO</td>
<td>30.4±5.0</td>
<td>1.02±0.19</td>
</tr>
<tr>
<td>Ex + Norm CHO</td>
<td>33.4±5.6</td>
<td>1.02±0.05</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lo CHO</td>
<td>35.7±7.0</td>
<td>1.01±0.14</td>
</tr>
<tr>
<td>Ex + Lo CHO</td>
<td>31.1±3.4</td>
<td>1.04±0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 9 per group). Norm CHO, normal carbohydrate; Ex + Norm CHO, exercise plus normal carbohydrate; Lo CHO, low carbohydrate; Ex + Lo CHO, exercise plus low carbohydrate.

### ANALYTICAL TECHNIQUES

**Metabolites.** The initial rapidly extracted tissue samples obtained before exercise and at selected time points during the exercise were used for the measurement of ATP, phosphocreatine (PCr), inorganic phosphate (P), creatine (Cr), inosine monophosphate (IMP), and glycogen. Immediately after withdrawal of tissue sample, the biopsy needle containing the sample was plunged into liquid N2 and stored at ~80°C until analysis. With the exception of the IMP, all metabolites were measured fluorometrically (28) as described previously (18, 20). The content of IMP was measured by HPLC techniques (23). All muscle metabolite content, determined on freeze-dried tissue, were adjusted to total creatine (TCr) content, calculated for each individual. The adjustment to TCr provides a more stable reference base, free from the contamination of blood, connective tissue, and fat. No significant changes in TCr were observed with the experimental protocols employed. Glycogen content was measured as glucosyl units in a separate piece of tissue from the metabolites after hydrolysis with hydrochloric acid. Blood glucose and lactate were analyzed fluorometrically (28) in supernatants obtained after centrifugation of blood that was placed in a prechilled tube containing 0.6 M perchloric acid and neutralized in 1.25 mM KHCO3.

**Sarcoplasmic reticulum.** After removal of the second sample from the vastus lateralis muscle, ~25–30 mg of tissue were separated from the main sample and placed into ice-cold homogenizing buffer (11:1 vol/wt dilution) consisting of (in...
mM) 250 sucrose, 5 HEPES, 10 NaN₃, and 0.2 PMSF (pH 7.5). Homogenization was performed by using a hand-held glass homogenizer (Kontes, Duall 20) (35). The homogenates were then separated into a number of aliquots, rapidly frozen in liquid N₂, and stored at −80°C for later analyses of Ca²⁺-ATPase activity and Ca²⁺-uptake and Ca²⁺-release rates. All properties were expressed per unit protein where protein was measured by the method of Lowry as modified by Schacterle and Pollock (40).

SR Ca²⁺-uptake and Ca²⁺-release rates were measured at pH 7.0 and 37°C in duplicate by a common assay using the Ca²⁺-fluorescent dye indo-1 according to the basic procedures of O’Brien et al. (36) and Ruell et al. (38) as modified in our laboratory (41, 48). Briefly, oxalate-supported Ca²⁺ uptake was measured in a reaction buffer containing (in mM) 200 KCl, 20 HEPES, 15 MgCl₂, 1 mM EGTA, 10 NaN₃, 10 phosphoenolpyruvate, 5 oxalate, and 0.005 TPEN. Before each assay, 1.5 µM indo-1, 18 U/ml lactate dehydrogenase, and 18 U/ml pyruvate kinase were added to 2 ml of reaction buffer followed by the addition of 2.5 µl of CaCl₂ (10 mM), used to produce a consistent starting [Ca²⁺]i of ~3.5 µM, and 40 µl of homogenate. Ca²⁺ uptake was initiated by the addition of 40 µl of homogenate. After active loading of Ca²⁺ into the SR and plateauing of [Ca²⁺]i, Ca²⁺ release was initiated by adding 20 mM of 4-chloro-m-cresol, the Ca²⁺-releasing agent.

The concentration of extravesicular [Ca²⁺]i was determined fluorometrically (Ratiomaster RCM, Photon Technology International, Brunswick, NJ) by using the Ca²⁺-fluorescent dye indo-1 (excitation wavelength 355 nm and emission wavelengths 405 and 585 nm). Additional details including the calculation of ionized Ca²⁺ concentration and the dissociation constant appear in a recent article from our laboratory (41). Ca²⁺-uptake rates were assessed over a range of [Ca²⁺]i, namely 500, 1,000, 1,500, and 2,000 nM from the single assay. The maximal rate of Ca²⁺ uptake for each [Ca²⁺]i was determined by differentiating the linear fit curve (41). Because the Ca²⁺-uptake rates at each [Ca²⁺]i generally demonstrated the same experimental effects, we have only reported on the results obtained at 2,000 nM in this paper. We would emphasize that because of the limited sensitivity of indo-1, the Ca²⁺-uptake rates are not necessarily maximal. Maximal Ca²⁺-release rates were measured as phase 1 and phase 2, calculated by using the same methods described for Ca²⁺ uptake. We have previously demonstrated that two phases of Ca²⁺ release can be differentiated with our assay procedures, namely a fast, rapid release (phase 1) and a slower, more delayed release (phase 2) (48). In general, phase 1 and phase 2 Ca²⁺-release rates ranged over time periods of 0–2 s and 3–10 s, respectively.

Ca²⁺-induced Ca²⁺-ATPase activity in homogenates was analyzed according to the methods of Simonides and van Hardeveld (43) as modified in our laboratory (41). The reaction buffer contained (in mM) 200 KCl, 20 HEPES, 15 MgCl₂, 1 mM EGTA, 10 NaN₃, 5 ATP, 10 phosphoenolpyruvate with pH adjusted to 7.0 at 37°C. Before starting the reaction, 18 U/ml lactate dehydrogenase and 18 U/ml pyruvate kinase, 0.3 mM NADH, 1 µM Ca²⁺ ionophore A23187 (Sigma C-7522) and 25 µl of homogenate were added to a cuvette containing 1 ml of reaction buffer. The reaction, which was performed at 37°C and 340 nm (Shimadzu UV 160), was initiated by adding 1 µl of 100 mM CaCl₂. Maximal Ca²⁺-ATPase activity (Vmax) and Ca²⁺ dependency of Ca²⁺-ATPase were assessed by successive additions (0.5 µl of 100 mM CaCl₂) until a plateau and subsequent decline in Ca²⁺-ATPase was observed. Basal (i.e., Mg²⁺-ATPase) activity was determined by adding 40 µM cyclopiazonic acid, a specific inhibitor of the Ca²⁺-ATPase (42). The [Ca²⁺]i corresponding to each CaCl₂ was measured fluorometrically as described.

In addition to Vmax we have also assessed one other kinetic property of the enzyme, namely nH, defined as the slope of the relationship between Ca²⁺-ATPase activity and [Ca²⁺]i. For nH, only a portion of the curve that corresponded to between 20 and 80% of maximal activity was used. For determination of nH, Ca²⁺-ATPase was plotted against the negative logarithm of [Ca²⁺]i (pCa) calculated using nonlinear regression with computer software (Graph Pad Software) and a sigmoidal dose response equation (41).

By measuring Vmax with and without the Ca²⁺ ionophore A23187, we were able to assess the potential effects of our experimental manipulations on Ca²⁺ permeability of the SR membrane. We have also calculated the coupling ratio, defined as the ratio between Ca²⁺ uptake and maximal Ca²⁺-ATPase activity, which is an index of the efficiency of Ca²⁺ sequestration. Because we were unsure whether maximal Ca²⁺ uptake was achieved in our assay given the limitations in indo-1, we have called this the apparent coupling ratio.

On a given analytical day and for a given assay, the complete set of samples for a given individual was performed, alternating between conditions for each sample set. In our hands, the coefficients of variability for Ca²⁺-uptake were less than 10%. No differences existed between groups, it was found that, for Norm CHO, percent CHO was higher whereas percent fat and percent protein were lower than in Lo CHO. No differences existed between groups in average daily caloric intake.

**RESULTS**

**Dietary analyses.** Both the average daily caloric intake and the percent calories derived from each foodstuff were measured for the Norm CHO and Lo CHO groups (Table 2). These assessments were performed on each group on two occasions, namely in the 4-day lead-up period before the standardized test and during the 4-day periods (Norm CHO, Lo CHO) between the exercise sessions (Ex + Norm CHO; Ex + Lo CHO). For Norm CHO, neither the average daily caloric intake nor the percent calories derived from CHO, fat, and protein were different between the Norm CHO and Ex + Norm CHO conditions. Similarly, for Lo CHO, no differences in these properties were noted between Lo CHO and at Ex + Lo CHO. When comparisons were made between the experiment 1 and experiment 2 groups, it was found that, for Norm CHO, percent CHO was higher whereas percent fat and percent protein were lower than in Lo CHO. No differences existed between groups in average daily caloric intake.
variables examined. Similarly, in experiment 2, no differences were found between conditions. Similarly, no differences existed between conditions. As with PCr, no differences were found between experiments for any of the measurements throughout the exercise protocol. As with PCr, no differences were also noted between experiments. For Ex + Lo CHO, lower contents were observed at rest and at all exercise time points compared with both Norm CHO and Ex + Norm CHO. Muscle glycogen was also different between experiment 1 and experiment 2 (Fig. 4B). At rest and at 30 min of exercise, glycogen content in Norm CHO and Ex + Norm CHO were higher than in either Lo CHO and Ex + Lo CHO. As expected, glycogen content was substantially depleted by exercise. For experiment 1, the depletion was progressive with time, regardless of condition. In contrast, in experiment 2, initial reductions were noted at 30 min of exercise followed by further reductions at fatigue. The time-dependent effects of exercise on glycogen

Respiratory gas exchange. To assess the effects of diet and preceding exercise on respiratory gas exchange during the standardized test, we have measured VO₂ and VCO₂ (data not represented) and calculated the RER from the VO₂ and VCO₂ assessments (Fig. 2). In experiment 1, no differences between Norm CHO and Ex + Norm CHO were observed for any of the variables examined. Similarly, in experiment 2, no differences were found between Lo CHO and Ex + Lo CHO for these variables. Increases in both VO₂ and VCO₂ and RER were observed with exercise regardless of condition. In the case of VO₂ and VCO₂, time-dependent effects were not differentiated by condition. For VO₂, the increase observed at 15 min of exercise persisted throughout 60 min of exercise before increasing again just before fatigue. In the case of VCO₂, generally a similar pattern was observed except that no further changes were observed beyond 15 min of exercise. To some degree, the drift in RER with exercise was dependent on the experimental condition. For Norm CHO, an initial increase in RER was observed at 15 min of exercise followed by progressive reductions at 60 min of exercise and at fatigue. For Lo CHO, a similar time-dependent change was observed, except that no further alteration was detected between 60 min of exercise and fatigue. Only in the case of RER was a difference observed between experiment 1 and experiment 2. For RER, experiment 1 was greater than experiment 2.

Blood metabolites. Neither condition nor exercise was found to affect blood glucose concentration regardless of the experiment (Fig. 3). In both experiment 1 and experiment 2, blood lactate was elevated at 15 min of exercise, the first measurement point, and remained elevated until fatigue.

Muscle metabolites. To examine for differences in phosphorylation potential in vastus lateralis, ATP and PCr and the related metabolites P_i, Cr, and IMP were measured (Table 3). Exercise regardless of time and condition failed to alter ATP content. The content of IMP, regarded as a more sensitive indicator of changes in ATP, was increased at each exercise time point. In contrast, PCr was reduced at 30 min of exercise and remained reduced at fatigue. The change in both P_i and Cr with exercise were similar and near stoichiometric to PCr, namely an early increase (30 min of exercise) that persisted throughout the exercise protocol. As with PCr, no differences existed between conditions. Similarly, no differences were found between experiments for any of the measurements assessed.

Table 2. Dietary analyses for normal and low carbohydrate conditions for women

<table>
<thead>
<tr>
<th>Daily Calories</th>
<th>Dietary Composition</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>kcal</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
</tr>
<tr>
<td>Norm CHO</td>
<td>2,088±155</td>
</tr>
<tr>
<td>Ex + Norm CHO</td>
<td>2,155±140</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
</tr>
<tr>
<td>Lo CHO</td>
<td>2,053±184</td>
</tr>
<tr>
<td>Ex + Lo CHO</td>
<td>1,997±176</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 per group. Daily Calories, average daily calories; CHO, carbohydrate; %, percent of total daily calories. Main effects (∗ P < 0.05) of diet were found for % dietary composition. For dietary composition, CHO, Norm CHO > Lo CHO; for fat and protein, Norm CHO < Lo CHO.

Compared with rest, muscle lactate increased at 30 min of exercise and then remained elevated until fatigue (Fig. 4A). At 30 min of exercise, muscle lactate was higher than at 60 min of exercise and fatigue. Differences were found between conditions within an experiment but only between Lo CHO and Ex + Lo CHO where lactate was higher for Lo CHO. Differences were also noted between experiments. For Ex + Lo CHO, lower contents were observed at rest and at all exercise time points compared with both Norm CHO and Ex + Norm CHO. Muscle glycogen was also different between experiment 1 and experiment 2 (Fig. 4B). At rest and at 30 min of exercise, glycogen content in Norm CHO and Ex + Norm CHO were higher than in either Lo CHO and Ex + Lo CHO. As expected, glycogen content was substantially depleted by exercise. For experiment 1, the depletion was progressive with time, regardless of condition. In contrast, in experiment 2, initial reductions were noted at 30 min of exercise followed by further reductions at fatigue. The time-dependent effects of exercise on glycogen

Fig. 2. O₂ consumption (VO₂; A) and calculated respiratory exchange ratio (RER; B) in response to exercise and dietary manipulation. Values are means ± SE; n = 9 in each experimental group. Main effects (∗ P < 0.05) of exercise were found for VO₂ and RER. For VO₂, 0 < 15, 30 < 60 min < fatigue. For RER in experiment 1, 0 < 15, 30 < 60 min < fatigue. For RER in experiment 2, 0 < 15, 30 < 60 min < fatigue. Main effects (∗ P < 0.05) were also shown for diet. Norm CHO > Lo CHO; Ex + Norm CHO > Ex + Lo CHO.
depletion were not altered by prior exercise in either experiment 1 or experiment 2.

Sarcoplasmic reticulum. Two properties were selected to examine the effects of diet and exercise on Ca$^{2+}$-ATPase kinetics (Fig. 5). These properties included the $V_{\text{max}}$ and $n_{\text{H}}$. Prolonged exercise to fatigue regardless of experimental condition resulted in an ~28% reduction in $V_{\text{max}}$. The pattern of change was such that an initial reduction was observed at 30 min of exercise followed by further reductions at fatigue. No differences in $V_{\text{max}}$ were noted between experiments or between conditions within an experiment. Although exercise was without effect in altering $n_{\text{H}}$, differences were noted between experiments. In experiment 2, both Lo CHO and Ex + Lo CHO conditions were higher than both the Norm CHO and Ex + Norm CHO conditions. As well, $n_{\text{H}}$ for Lo CHO was depressed compared with Ex + Lo CHO. The differences between conditions in $n_{\text{H}}$ were generalized effects, not specific to any time point. Basal ATPase was not altered by exercise or experimental condition (data not presented).

Ca$^{2+}$ uptake was depressed during exercise in all experimental conditions (Fig. 6). Compared with rest, progressive reductions in Ca$^{2+}$ uptake were observed at 30 and 60 min of exercise and then remained unchanged until fatigue. In general, Ca$^{2+}$ uptake was lower in Lo CHO compared with Ex + Lo CHO, Norm CHO and Ex + Norm CHO. No differences were found between Norm CHO and Ex + Norm CHO.

We could find no effect of exercise or experimental condition on the ionophore ratio (Table 4). The ionophore ratio, calculated as the ratio of $V_{\text{max}}$ measured with and without the Ca$^{2+}$ ionophore A23187, is a measure of the membrane permeability to Ca$^{2+}$.

To determine whether the efficiency of Ca$^{2+}$ transport was altered with our experimental conditions, we have calculated the apparent coupling ratios. Although no effect of exercise was observed in the apparent coupling ratio, differences were found between conditions (Table 4). The results indicate that the Lo CHO condition resulted in lower apparent coupling

Table 3. Effects of exercise and dietary manipulation with and without preceding exercise on high-energy phosphates and related metabolites

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time, min</th>
<th>ATP</th>
<th>PCr</th>
<th>Pi</th>
<th>Cr</th>
<th>IMP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norm CHO</td>
<td>0</td>
<td>23.7±0.5</td>
<td>72.4±5.0</td>
<td>34.0±1.3</td>
<td>51.5±1.8</td>
<td>0.035±0.01</td>
</tr>
<tr>
<td>Ex + Norm CHO</td>
<td>30</td>
<td>22.9±0.4</td>
<td>40.7±3.7</td>
<td>66.4±3.7</td>
<td>83.1±4.4</td>
<td>0.146±0.02</td>
</tr>
<tr>
<td>60</td>
<td>22.7±0.7</td>
<td>44.0±4.7</td>
<td>64.7±4.2</td>
<td>78.2±5.9</td>
<td>0.216±0.07</td>
<td>0.345±0.01</td>
</tr>
<tr>
<td>Fatigue</td>
<td>22.3±0.6</td>
<td>41.4±4.5</td>
<td>69.5±4.0</td>
<td>82.5±5.3</td>
<td>0.283±0.07</td>
<td>0.216±0.07</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lo CHO</td>
<td>0</td>
<td>23.2±0.9</td>
<td>75.5±3.7</td>
<td>35.9±1.8</td>
<td>50.8±1.2</td>
<td>0.063±0.02</td>
</tr>
<tr>
<td>Ex + Lo CHO</td>
<td>30</td>
<td>22.7±0.4</td>
<td>45.3±4.5</td>
<td>67.9±5.2</td>
<td>87.7±3.8</td>
<td>0.282±0.09</td>
</tr>
<tr>
<td>60</td>
<td>22.5±0.6</td>
<td>48.7±3.8</td>
<td>64.1±6.0</td>
<td>82.4±5.4</td>
<td>0.227±0.06</td>
<td>0.376±0.02</td>
</tr>
<tr>
<td>Fatigue</td>
<td>22.6±0.5</td>
<td>44.3±3.1</td>
<td>68.7±4.3</td>
<td>83.9±3.7</td>
<td>0.279±0.12</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry wt (n = 9 per group). PCr, phosphocreatine; Pi, inorganic phosphate; Cr, creatine; IMP, inosine monophosphate. Main effects ($P < 0.05$) of time were observed for PCr, P, Cr, and IMP. For PCr, 0 > 30, 60, min, and fatigue; for P, and Cr, 0 < 30, 60 min, and fatigue. For IMP, 0 < 30, 60 min, and fatigue.
We have examined the relationship between SR function and muscle glycogen content by calculating the correlation coefficients for each experiment. For experiment 1 (Norm CHO and Ex-Norm CHO), significant relationships \((P < 0.05)\) were found between glycogen levels and \(V_{\text{max}}\) \((r = 0.319)\), Ca\(^{2+}\) uptake \((r = 0.318)\), and both phase 1 \((r = 0.469)\) and phase 2 \((r = 0.417)\) Ca\(^{2+}\) release. For experiment 2, the correlation between coefficients were only significant \((P < 0.05)\) between glycogen and phase 1 \((r = 0.701)\) and phase 2 \((r = 0.316)\) of Ca\(^{2+}\) release. These comparisons generally indicate low-order relationships between glycogen level and SR function.

**DISCUSSION**

Our results reveal several novel features regarding SR Ca\(^{2+}\)-cycling responses in skeletal muscle of women when challenged by a standardized cycling test after dietary and exercise plus dietary manipulation. Because our hypothesis was critically dependent on producing conditions that displayed differences in preexercise glycogen concentration, we have used a variation of a previously published procedure to lower muscle glycogen, namely a preliminary session of “glycogen-depleting” exercise followed by 4 days of a low-CHO diet \((2)\). These results were compared with a normal CHO diet that was also preceded by a similar session of glycogen-depleting exercise. Because it is conceivable that the 4-day period after the glycogen-depletion session of exercise was insufficient to allow full recovery of SR Ca\(^{2+}\)-cycling responses \((24)\) given the changes occur in Ca\(^{2+}\) uptake and Ca\(^{2+}\) release with the glycogen-depleting protocol \((10)\) and/or the introduction of a conditioning effect, it was necessary to include both a Lo CHO and Norm CHO condition. The Lo and Norm CHO dietary conditions resulted in an \(\sim 37\%\) difference in resting muscle glycogen with no differences observed between conditions within each dietary regime.

To isolate the effects of preceding exercise, conducted 4 days before the standardized test, while on a normal diet, we have compared the Norm CHO and Ex + Norm CHO conditions. The effects of prolonged exercise on SR Ca\(^{2+}\)-cycling behavior in women during Norm CHO are similar, both qualitatively and quantitatively, to what we have recently reported in men \((10)\). In general, exercise resulted in a reduction in \(V_{\text{max}}\) that was found to occur independently of changes in another kinetic property of the enzyme, namely \(n_H\). The reduction in \(V_{\text{max}}\) was also accompanied by reductions in Ca\(^{2+}\)-cycling properties, as indicated by the depression in Ca\(^{2+}\) uptake and Ca\(^{2+}\) release, both phase 1 and phase 2, during exercise. No changes were observed with exercise in the permeability of the SR membrane to Ca\(^{2+}\), as measured by the ionophore ratio or in the efficiency of Ca\(^{2+}\) transport, as measured by the apparent coupling ratio. No differences were found between Norm CHO and Ex + Norm CHO, either at rest or during exercise for any of the SR properties examined. These findings demonstrate that 4 days while on a Norm CHO diet is sufficient to reverse the expected disturbances in Ca\(^{2+}\)-cycling behavior induced by the preliminary session of fatiguing exercise and to neutralize any conditioning effects that may have occurred. On the basis of our previous study conducted under the same conditions, we also generally failed to find any significant effects in men \((10)\).

However, some differences were observed in the male study, namely for resting Ca\(^{2+}\) uptake and the ionophore ratio at...
fatigue, which was higher in Ex + Norm CHO compared with Norm CHO.

Lo CHO compared with Ex + Lo CHO resulted in a lower
\( n_\text{H} \) that was also accompanied by a lower \( \text{Ca}^{2+} \) uptake and a lower apparent coupling ratio. Because these results were main effects, not specific to exercise per se, it would appear that the effect of the preceding exercise was to increase these properties at rest, an effect that persisted during the exercise state. Interestingly, these differences in these SR properties between Lo CHO and Ex + Lo CHO occurred in the absence of differences in muscle glycogen concentration.

Unclear from the above comparisons is whether differences did, in fact, occur between the two dietary conditions, namely Norm CHO and Lo CHO, when studied in isolation. A comparison between these conditions indicated a higher \( n_\text{H} \) (no difference in \( V_{\text{max}} \)), a lower \( \text{Ca}^{2+} \) uptake and apparent coupling ratio, and a lower phase 1 \( \text{Ca}^{2+} \) release at 30 min of exercise for Lo CHO. These results illustrate that 4 days on a low-CHO diet alone can independently compromise \( \text{Ca}^{2+} \) uptake and \( \text{Ca}^{2+} \) release (phase 1) and increase the affinity of the \( \text{Ca}^{2+} \)-ATPase for \( \text{Ca}^{2+} \). Because the differences in SR \( \text{Ca}^{2+} \)-sequestration properties were not specific to any exercise time point, it would appear that the primary effect of Lo CHO is to alter the preexercise potential. Because muscle glycogen levels were lower in Lo CHO compared with Norm CHO, it is possible that the differences observed between conditions in SR regulation are related to the reserve of this endogenous substrate as we have hypothesized.

The role of the preliminary session of exercise was investigated by comparing the responses across the normal diet (Norm CHO and Ex + Norm CHO) and the low-CHO diet (Lo CHO vs. Ex + Lo CHO). Although \( V_{\text{max}} \) was not affected, \( n_\text{H} \) values of Lo CHO and Ex + Lo CHO were greater than either Norm CHO or Ex + Norm CHO. In addition, \( \text{Ca}^{2+} \) uptake and the apparent coupling ratio in Lo CHO was lower than both Norm CHO and Ex + Norm CHO. For \( \text{Ca}^{2+} \) release, a lower \( \text{Ca}^{2+} \) release was observed for phase 1 at 30 min of exercise for Lo CHO and Ex + Lo CHO compared with Norm CHO and Ex + Norm CHO. When these effects are considered in conjunction with the differences between Lo CHO and Ex + Lo CHO in isolation, it is apparent that the preliminary session of glycogen-depletion exercise before Lo CHO serves to reverse the disturbances in \( \text{Ca}^{2+} \) uptake and the apparent coupling ratio observed with Lo CHO by normalizing the values to those observed with the normal CHO diet. In the case of \( n_\text{H} \), Ex + Lo CHO exaggerated the effects of Lo CHO alone, resulting in higher \( n_\text{H} \) than observed with either Norm CHO and Ex + Norm CHO. In contrast, Lo CHO resulted in a more rapid decline in \( \text{Ca}^{2+} \) release (phase 1) early in exercise that was not modified by Ex + Lo CHO compared with the normal
CHO conditions. With the potential exception of Ca\(^{2+}\) release (phase 1), these differences between conditions cannot be explained by differences in muscle glycogen content.

While on a normal CHO diet, reductions in these SR Ca\(^{2+}\)-cycling properties is a common observation (47), particularly in humans during fatiguing effort (3, 11, 21, 27, 49). The depression in Ca\(^{2+}\) uptake appears to be mediated by inactivation of the Ca\(^{2+}\)-ATPase secondary to structural changes in vicinity of the adenine nucleotide binding site (24, 29, 33).

Exercise-induced structural changes to the CRC have also been implicated in the reductions in Ca\(^{2+}\) release that have been observed (14). The disturbances that we have observed in SR Ca\(^{2+}\) cycling during prolonged exercise in this study demonstrate, at least qualitatively, that untrained women respond similarly to untrained men, ostensibly by mechanisms related to structural alterations in the Ca\(^{2+}\)-ATPase and CRC. As with men, we have also found that, with prolonged exercise in women, no changes occur in Ca\(^{2+}\)-affinity of the Ca\(^{2+}\)-ATPase as measured by \(n_H\), the apparent coupling ratio, a measure of the efficiency of Ca\(^{2+}\)-transport estimated from the ratios of Ca\(^{2+}\) uptake and Ca\(^{2+}\)-ATPase activity or the permeability of the SR membrane to Ca\(^{2+}\), as measured by the ionophore ratio.

The increased affinity of the enzyme for Ca\(^{2+}\) observed during the low-CHO conditions could occur as a consequence of oligomerization where aggregation from less active to more active enzymes occurs (16, 30, 31). An SR-glycogen-glycolytic complex has been characterized that consists of glycogen particles, glycogen phosphorylase, glycogen debranching enzyme, many of the enzymes involved in glycogenolysis, and creatine phosphokinase (8, 37, 52), which appear coupled to the SR by a protein phosphatase regulatory subunit (39). Low cellular glycogen levels observed with the low-CHO conditions could dislodge this complex from the SR, resulting in structural alterations to the SR and Ca\(^{2+}\)-ATPase in particular (8, 26). Other factors would appear involved, however, because Ex + Lo CHO resulted in a greater \(n_H\) than Lo CHO when no differences existed between conditions in resting glycogen level.

It is also conceivable that the increase in \(n_H\) that we have observed in vitro with low-CHO is related to differences between conditions in the intracellular metabolic environment. Numerous studies have stressed the importance of local ATP regeneration in Ca\(^{2+}\) uptake and Ca\(^{2+}\)-ATPase function (25, 37), which can occur in the region of the SR via the glycogen-glycolytic complex (7, 9, 37, 51). It is possible that the lower glycogen levels observed with Lo CHO compared with Norm CHO could have impaired SR Ca\(^{2+}\)-ATPase function as a result of the dissociation of the enzymes involved in high-energy phosphate transfer and glycolysis from the SR. This possibility appears remote because cellular energetic status was minimally compromised regardless of condition. This observation is consistent with what has been reported in other studies (34, 44). Although the possibility that SR regional disturbances in ATP homeostasis between conditions could explain the differences in SR regulation observed, limitations in assay conditions must be acknowledged. The in vitro assay for measurement of Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) uptake utilizes endogenously synthesized ATP similar to that which occurs in vivo. However, in vitro, these enzymes involved in the generation of ATP exist in solution and are not bound to the SR as might be expected in vivo. Accordingly, differences in assay conditions could have masked any experimental effects.

The higher \(n_H\) should translate into a higher rate of Ca\(^{2+}\) uptake at submaximal [Ca\(^{2+}\)]\(_{\text{c}}\) and a higher apparent coupling ratio. This appears to be the case for Ex + Lo CHO compared with Lo CHO where a higher Ca\(^{2+}\) uptake was accompanied by a higher \(n_H\) and a higher apparent coupling ratio. However, this was not the case across dietary conditions where lower Ca\(^{2+}\) uptake rates and apparent coupling ratios were observed in Lo CHO compared with Norm CHO and Ex + Norm CHO.

**Fig. 6. Response of Ca\(^{2+}\) uptake to prolonged cycling after dietary and exercise plus dietary alterations. Values are means ± SE; \(n = 9\) for each experimental condition. Ca\(^{2+}\) uptake was measured at free Ca\(^{2+}\) concentration of 2,000 nM. A: Norm CHO and Ex + Norm CHO. B: Lo CHO and Ex + Lo CHO; Norm CHO, normal carbohydrate diet; Lo CHO, low-carbohydrate diet; Ex + Lo CHO, exercise plus low-carbohydrate diet. A main effect (\(P < 0.05\)) of exercise was observed. For exercise, 0 > 30 > 60 min and fatigue. Interaction effects (\(P < 0.05\)) were noted between dietary conditions. *Lo CHO < Norm CHO, Ex + Norm CHO and Ex + Lo CHO.**
Table 4. Ionophore ratios and apparent coupling ratios determined at rest and during prolonged cycling after exercise and exercise plus dietary manipulations

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ionophore ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norm CHO</td>
<td>3.60±0.33</td>
<td>3.34±0.25</td>
<td>3.71±0.53</td>
<td>3.52±0.30</td>
</tr>
<tr>
<td>Ex+Norm CHO</td>
<td>3.45±0.35</td>
<td>3.59±0.55</td>
<td>2.98±0.43</td>
<td>2.99±0.21</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lo CHO</td>
<td>3.24±0.26</td>
<td>3.59±0.22</td>
<td>3.52±0.45</td>
<td>3.49±0.42</td>
</tr>
<tr>
<td>Ex+Lo CHO</td>
<td>3.80±0.46</td>
<td>3.47±0.41</td>
<td>3.23±0.37</td>
<td>3.91±0.41</td>
</tr>
<tr>
<td><strong>Apparent coupling ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norm CHO</td>
<td>0.024±0.002</td>
<td>0.022±0.001</td>
<td>0.024±0.002</td>
<td>0.023±0.002</td>
</tr>
<tr>
<td>Ex+Norm CHO</td>
<td>0.023±0.002</td>
<td>0.023±0.002</td>
<td>0.020±0.003</td>
<td>0.024±0.002</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lo CHO</td>
<td>0.019±0.001*</td>
<td>0.020±0.002*</td>
<td>0.019±0.002*</td>
<td>0.020±0.001*</td>
</tr>
<tr>
<td>Ex+Lo CHO</td>
<td>0.024±0.001</td>
<td>0.023±0.001</td>
<td>0.023±0.001</td>
<td>0.024±0.002</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 9 per group). The ionophore ratio was calculated as the ratio of maximal Ca^{2+}-ATPase activity (V_{max}) determined in the presence and absence of the Ca^{2+} ionophore A23187. The apparent coupling ratio is defined as the ratio between Ca^{2+} uptake (determined at 2,000 nM) and maximal Ca^{2+}-ATPase activity. Interaction effects (P < 0.05) between dietary conditions were found. *Lo CHO < Norm CHO, Ex + Norm CHO and Ex + Lo CHO.
in spite of a higher $n_H$. In the case of Ex + Lo CHO, $n_H$ was higher than either Norm CHO and Ex + Norm CHO but no differences were observed between these conditions for Ca$^{2+}$ uptake and the apparent coupling ratio. Although not reported, the same results were found with $[Ca^{2+}]_i$ of 500, 1,000, and 1,500 nM as we report for 2,000 nM (unpublished observations). Men also appear to exhibit the same adaptive effects as women to a preliminary session of glycogen-depleting exercise followed by a low-CHO diet (Ex + Lo CHO) compared with a low-CHO (Lo CHO) diet alone, namely a higher $n_H$, elevated submaximal Ca$^{2+}$ uptake and a higher apparent coupling ratio (12, 13). As observed in women, a dissociation between these properties also occurred in men between dietary conditions. The failure to find that increases in submaximal Ca$^{2+}$ uptake accompanied a higher $n_H$ could be explained by membrane leakiness to Ca$^{2+}$. An increase in Ca$^{2+}$ leakiness from the lumen of the SR via the RyR, Ca$^{2+}$-ATPase or the phospholipid membrane into the cytosol could result in a lower net Ca$^{2+}$ uptake even though total Ca$^{2+}$ sequestration may not have changed or was elevated (48).

We also provide evidence that Ca$^{2+}$ release from the SR was also different between conditions, an effect that could be attributed solely to the 4 days of the low-CHO diet. The dietary effect was very specific to phase 1 and only occurred as a result of a more rapid depression in Ca$^{2+}$-release kinetics observed early in exercise. There is evidence of a similar effect for phase 2; however, the differences observed were not significant. In our studies, we routinely report on two phases of Ca$^{2+}$-release kinetics, namely an early, rapid phase (phase 1) and a more delayed, slower phase (phase 2) based on clear differences in the slope of the Ca$^{2+}$-release curves using 4-chloro-m-cresol as the releasing agent (48). Typically, others generally report on phase 2, the more delayed phase of Ca$^{2+}$ release. It is unclear whether each phase is under different regulatory mechanisms and whether the differences are physiologically significant.

We generally find that exercise induces similar depressions in both phases of Ca$^{2+}$ release (10, 11), and it is reasonable to conclude that this is what occurred in the present study. On the basis of previous exercise studies, it would appear that the more rapid depression in Ca$^{2+}$-release kinetics with Lo CHO is mediated by structural alterations in the CRC (14). Because the effects were specific to the Lo CHO conditions, which displayed a lower glycerogen content than Norm CHO conditions, this substrate may well be associated with this alteration in CRC. At present, there is little published evidence linking the SR-glycogen-glycolytic complex with regulation of Ca$^{2+}$-release kinetics.

In summary, our study has generated several novel findings regarding the impact of a low-CHO diet with and without preceding exercise on SR Ca$^{2+}$ regulation in muscle. Conspicuous among the effects was the increase in affinity of the Ca$^{2+}$-ATPase for Ca$^{2+}$ that occurred in the absence of changes in the maximal catalytic activity of the enzyme. Accompanying these changes was a decrease in Ca$^{2+}$ uptake with Lo CHO that could be offset if exercise preceded the Lo CHO diet and a decrease in phase 1 Ca$^{2+}$-release kinetics early in exercise that could not be offset by preceding exercise. These results serve to emphasize that, in addition to exercise, a short-term diet low in CHO by itself and a low-CHO diet plus preceding exercise also serve to modify some of the regulatory properties of the SR. It must be acknowledged that the effects that we have observed in SR regulation could reflect not differences in CHO but differences in fat intake. Regardless, our findings serve to emphasize that muscle SR Ca$^{2+}$-cycling behavior is subject to regulatory factors other than initial glycogen reserves.

**REFERENCES**


