Muscle metabolic, SR Ca\(^{2+}\)-cycling responses to prolonged cycling, with and without glucose supplementation

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Duhamel TA, Green HJ, Stewart RD, Foley KP, Smith IC, Ouyang J. Muscle metabolic, SR Ca\(^{2+}\)-cycling responses to prolonged cycling, with and without glucose supplementation. J Appl Physiol 103: 1986–1998, 2007. First published October 4, 2007; doi:10.1152/japplphysiol.01440.2006.—This study investigated the effects of prolonged exercise, with and without glucose supplementation, on metabolism and sarcoplasmic reticulum (SR) Ca\(^{2+}\)-handling properties in working vastus lateralis muscle. Fifteen untrained volunteers [peak \(\text{O}_2\) consumption (\(\text{VO}_2\)peak) = 3.45 ± 0.17 l/min; mean ± SE] cycled at ~60% \(\text{VO}_2\)peak on two occasions, during which they were provided with either an artificially sweetened placebo beverage (NG) or a 6% glucose (G) beverage (~1.00 g carbohydrate/kg body mass). Beverage supplementation started at 30 min of exercise and continued every 15 min thereafter. SR Ca\(^{2+}\)-handling, metabolic, and substrate responses were assessed in tissue extracted from the vastus lateralis at rest, after 30 min and 90 min of exercise, and at fatigue in both conditions. Plasma glucose during G was 15–23% higher (\(P < 0.05\)) than those observed during NG following 60 min of exercise until fatigue. Exercise time to fatigue was increased (\(P < 0.05\)) by ~19% during G (137 ± 7 min) compared with NG (115 ± 6 min). Prolonged exercise reduced (\(P < 0.05\)) maximal Ca\(^{2+}\)-ATPase activity (~18.4%), SR Ca\(^{2+}\) uptake (~27%), and both Phase 1 (~22.2%) and Phase 2 (~34.2%) Ca\(^{2+}\)-release rates during NG. The exercise-induced reductions in SR Ca\(^{2+}\)-cycling properties were not altered during G. The metabolic responses to exercise were all unaltered by glucose supplementation, since no differences in respiratory exchange ratios, carbohydrate and lipid oxidation rates, and muscle metabolite and glycogen contents were observed between NG and G. These results indicate that the maintenance of blood glucose homoeostasis by glucose supplementation is without effect in modifying the muscle metabolic, endogenous glycogen, or SR Ca\(^{2+}\)-handling responses.

Ca\(^{2+}\) regulation; glucose supplementation; human skeletal muscle; metabolism

THE PRIMARY FUNCTION OF THE sarcoplasmic reticulum (SR) in the skeletal muscle cell is the control of cytosolic-free calcium concentration ([Ca\(^{2+}\)]c). The regulation of [Ca\(^{2+}\)]c by the SR is mediated both by the open state of the Ca\(^{2+}\)-release channel (CRC or ryanodine receptor), which controls the release of stored Ca\(^{2+}\) from the SR, and by the activity of the sarco(endoplasmic) reticulum Ca\(^{2+}\)-ATPase (SERCA), the cation pump, which sequesters Ca\(^{2+}\) back into the lumen of the SR (2). In skeletal muscle, the CRC, which consists of a single isoform (ryanodine receptor 1) with a molecular mass of ~560 kDa, is composed of a large cytoplasmic foot structure facing and attached to the T-tubule and anchored to the junctional SR membrane by ~12 transmembrane sequences (51). Calcium release from the SR occurs from the cytoplasmic domain, which consists of a central channel that branches into four radial channels that extend to the lateral surface of the foot.

The SR in skeletal muscle is uniquely structured to perform its primary role, namely the ability to rapidly respond to the transmission of neural command signal by rapid and precise changes in the [Ca\(^{2+}\)]c transient, necessary to activate the myofilibrillar apparatus for the production of a desired force response (51). To regulate [Ca\(^{2+}\)]c, Ca\(^{2+}\) cycling increases dramatically during contractile activity by increasing both Ca\(^{2+}\) release and Ca\(^{2+}\) uptake in a coordinated manner (51). As might be expected, both the CRC and the Ca\(^{2+}\)-ATPase are under complex intrinsic regulatory control (49).

Disturbances in the [Ca\(^{2+}\)]c transient, as measured by Ca\(^{2+}\)-sensitive dyes, are a well-documented effect of repetitive contractile activity (1). The disturbances in the [Ca\(^{2+}\)]c transient have been shown to be accompanied by reductions in both Ca\(^{2+}\) release (1, 5) and Ca\(^{2+}\) uptake (1, 5) in single fibers during induced contractions of the mouse, ostensibly as a result of changes in the intracellular environment mediated by the contractile activity (40). Reductions in Ca\(^{2+}\) release (20, 37), Ca\(^{2+}\) uptake (5, 37), and maximal Ca\(^{2+}\)-ATPase activity (\(V_{\text{max}}\)) (6, 37, 47) have also been shown to occur in rat muscle with repetitive contractions when measured in homogenates “in vitro” under supposedly optimal conditions. However, not all studies employing rats show disturbances in SR Ca\(^{2+}\)-cycling in muscle with prolonged, submaximal exercise (21, 66). Of the few studies that have examined the SR responses to a similar exercise challenge in humans, all have found disturbances in SR Ca\(^{2+}\) handling, namely \(V_{\text{max}}\), Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release (4, 14, 15). Based on the limited evidence available, it appears that no changes occur in coupling efficiency, defined as the ratio between Ca\(^{2+}\) uptake and \(V_{\text{max}}\), or in the sensitivity of the enzyme to Ca\(^{2+}\) (15, 66). However, one study (13) has reported that, late in prolonged exercise, changes in membrane permeability to Ca\(^{2+}\) occurs, which conceivably should alter coupling efficiency.

One factor that appears to affect the SR Ca\(^{2+}\) cycling function during repetitive contractions is the carbohydrate (CHO) status of the muscle cell. Several studies have linked the depletion of muscle glycogen with reduced [Ca\(^{2+}\)]c transients in rat (43), mouse (7), and toad (70), while others (16) have reported that exercise-induced reductions in Ca\(^{2+}\)-handling properties in human muscle measured in vitro occur earlier during low-glycogen compared with high-glycogen states. In this latter study, no changes were observed in the Ca\(^{2+}\) sensitivity of the enzyme, the coupling ratio, or membrane permeability to Ca\(^{2+}\) (16).
The most inviting hypothesis to account for the relationship between muscle SR Ca\(^{2+}\) cycling and CHO status is based on the substrate and/or energy status of the cell. According to this rationale, contracting muscle with depleted glycogen status experience a decreased ability to regenerate ATP. As a consequence, ATP levels decline and metabolic by-products accumulate, both of which may affect Ca\(^{2+}\) release (18) and Ca\(^{2+}\) uptake (41). Evidence also exists to support the existence of a SR-glycogenolytic complex containing glycogen phosphorylase, glycogen debranching enzyme, many of the enzymes involved in the glycolytic pathway, and Cr phosphokinase (86), the dissociation of which could alter Ca\(^{2+}\)-cycling behavior (44, 85). The depletion of cellular glycogen stores could effectively dissociate this complex and result in a disturbance in energy homeostasis in regions close to the Ca\(^{2+}\)-ATPase (12) and CRC (30), or result in structural alterations in the SR, both of which could impact on SR Ca\(^{2+}\) cycling (43).

A limitation in our laboratory’s previous work designed to investigate the relationship between muscle glycogen and SR Ca\(^{2+}\)-handling properties during prolonged whole body exercise (16) was the inability to control blood glucose levels and, consequently, the regulatory hormones, insulin (Ins) and the catecholamines, norepinephrine (NE) and epinephrine (Epi). In our laboratory’s previous work, alterations in the dietary intake of CHO resulted in differences during exercise in blood glucose concentrations that were also accompanied by differences between conditions in plasma NE and Epi (16) and most probably Ins as well (23). Differences in blood glucose availability could alter SR Ca\(^{2+}\)-cycling function via improved energy homeostasis and/or protection of muscle glycogen reserves (44, 85), while differences in the hormonal responses could affect intrinsic behavior through second-messenger regulation (49, 84). As an example, Epi could alter the phosphorylation status of the regulatory protein phospholamban (PLN) via cAMP signaling, resulting in alterations in the catalytic activity of the enzyme (49). Interestingly, as with experiments designed to manipulate muscle glycogen levels by exercise and diet (3), oral glucose supplementation during exercise also has an ergogenic effect (11, 31).

Maintenance of blood glucose homeostasis during prolonged exercise could affect SR Ca\(^{2+}\)-cycling responses, by altering one or more of the factors involved in its regulation. In this regard, the literature is contradictory with regard to the effects of glucose supplementation on muscle metabolism (8, 69, 72), glycogen depletion patterns (11, 55, 72), and blood hormonal responses (10, 24, 31).

The main purpose of this study was to investigate the effects of prolonged exercise and prolonged exercise plus oral glucose supplementation on muscle SR Ca\(^{2+}\)-cycling function. We have hypothesized that prolonged exercise would result in a progressive reduction in the maximal activity of the Ca\(^{2+}\)-ATPase, Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release in active muscle and that the reduction in these properties with exercise would occur in the absence of changes in Ca\(^{2+}\) sensitivity, Ca\(^{2+}\)-transport efficiency, and SR membrane permeability to Ca\(^{2+}\). Oral glucose supplementation is proposed to attenuate the exercise-induced changes in SR Ca\(^{2+}\)-cycling properties by protecting muscle energy homeostasis and glycogen content during exercise.

METHODS

Participants. Fifteen volunteers (14 men and 1 woman) were recruited from the general student population at the University of Waterloo to participate in the study. Volunteers were healthy and recreationally active (assessed by a questionnaire), but not involved in exercise utilizing large-muscle groups on a regular basis (i.e., not more than once per week). The physical characteristics of the participants included the following: age, 19.3 ± 0.4 yr; height, 179 ± 4 cm; and body mass, 78.5 ± 3.7 kg. Peak aerobic power (\(V_{\text{O}_2\text{peak}}\)) was 3.45 ± 0.17 l/min. All volunteers were healthy, as determined by questionnaire. Testing was completed during the midfollicular phase of the menstrual cycle for the female participant in this study. This study received approval from the Office of Research Ethics at the University of Waterloo.

Experimental design. To investigate the effects of oral glucose supplementation on muscle SR Ca\(^{2+}\)-transport properties, two sessions of prolonged, moderate-intensity exercise were employed. One session served as the control, or no glucose (NG), condition, and was used to investigate the effects of exercise in isolation without oral glucose supplementation. The other session served as the glucose supplementation (G) condition and was identical to NG, with the only difference being the ingestion of oral glucose supplements, designed to maintain blood glucose levels during the late stages (i.e., >30 min) of exercise. The order of the two experimental conditions was randomized for 9 of 15 participants. The remaining six participants completed the NG condition before the G condition. The latter sequence allowed for muscle tissue to be sampled at a matched time point in both conditions within this subgroup. The matched time point was selected to correspond to fatigue in the NG condition and was unique to each individual. The time to fatigue in NG was 115 ± 6 min, whereas average time to fatigue in G was 137 ± 7 min. The increase in time to fatigue in G was 19.1% (\(P < 0.05\)).

Before the commencement of each test session, participants were asked to complete a 7-day diet journal to allow for the assessment of average daily nutritional intake. Based on the analysis of these 7-day diet journals, no differences were observed in total caloric intake or average daily nutritional intake. Based on the analysis of these 7-day diet journals, no differences were observed in total caloric intake or macronutrient intake during the lead-in period between experimental conditions.

The prolonged cycle task was performed in a neutral environment (−20°C; ~50% relative humidity) at an intensity that was ~60% \(V_{\text{O}_2\text{peak}}\). Exercise was continued until volitional fatigue or when the participant could not maintain a cadence of at least 50 revolutions/min, with verbal encouragement.

Glucose supplements. The glucose supplement was a 6% solution of glucose, without the addition of any electrolytes. Participants were provided with a drink (volume dependent on body mass; >1.00 g CHO/kg body mass in a 6% solution), starting after 30 min of exercise and continuing every 15 min thereafter. The average volume ingested at each time point ranged between 100 and 300 ml. A placebo (Sugar Twin; Alberto-Culver Canada, Toronto, ON, Canada) consisting of a 7.5% Sugar Twin [water, sodium cyclamate (10%), benzoic acid, methyl paraben] solution was provided according to the same schedule using similar individual volumes, as received during G. Beverages were served at room temperature (~20°C). For participants who completed the NG condition before G, beverage volumes were matched to the volume that was to be consumed during the G condition. Test sessions were separated by at least 4 wk and were conducted in the morning, following an overnight fast.

The total amount of CHO ingested during G was 1.23 ± 0.11 g CHO/kg body mass. The average volume ingested at each time point was 243 ± 17 ml, which amounted to a total volume of 1,564 ± 142 ml for G over the course of the exercise. The volume of fluid ingested at each time point for NG was not different from G.

\(V_{\text{O}_2\text{peak}}\) determination and respiratory gas collection. A progressive exercise test was performed on an electrically braked cycle (Quinton R870), as previously described (35), to measure \(V_{\text{O}_2\text{peak}}\) using
an open-circuit gas collection system with continuous measurements (36). The gas collection system was calibrated daily, 30 min before all test sessions, using standardized gas samples of known concentrations. All exercise sessions were performed on the same cycle ergometer and using the same respiratory gas collection system. Respiratory gas properties measured during the exercise tests include oxygen uptake ($\text{VO}_2$), carbon dioxide production ($\text{VCO}_2$), and respiratory exchange ratio ($\text{RER} = \frac{\text{VCO}_2}{\text{VO}_2}$). From these data, respiratory exchange ratios were calculated. Stoichiometric equations and appropriate caloric equivalents were used to calculate CHO and lipid oxidation rates during the exercise.

**Blood sampling.** Blood samples were collected at rest, during exercise (15, 30, 45, 60, and 90 min), and at fatigue ($\sim 115 \pm 6$ min) from a catheter inserted in the prewarmed dorsal region of the hand during the NG condition. During G, blood samples were collected at rest, during exercise (15, 30, 45, 60, and 90 min), at a time corresponding to fatigue during the NG condition ($\sim 115 \pm 6$ min) and at fatigue ($\sim 137 \pm 7$ min).

The remaining aliquots of whole blood, serum, and plasma samples were processed and frozen at $-20^\circ$C until analyses. Blood glucose and lactate were determined fluorometrically, in triplicate, from the plasma aliquots that were deproteinized using perchloric acid and centrifuged. On a given day, all samples for a given individual for a given condition were analyzed in triplicate.

**Tissue sampling.** Four tissue samples for each condition were collected from the vastus lateralis under local anesthesia (2% xylcaine). For NG, tissue samples were collected at rest, after 30 and 90 min of exercise, and at fatigue. During the G condition, tissue samples were collected at rest, after 30 and 90 min of exercise, and at fatigue in 9 of 15 participants. In the remaining six participants, the resting tissue sample in G was omitted and replaced with a tissue sample taken at a time corresponding to fatigue during the NG condition (i.e., matched NG fatigue). The decision to replace the resting tissue sample with a matched placebo fatigue tissue sample was based on observations made in this study (data not shown), in addition to numerous experiments from our laboratory (14-16) that have shown no differences in resting SR $\text{Ca}^{2+}$-handling or metabolic properties between conditions when the experimental treatment was preceded by a standardized diet. This approach allowed for direct comparison of tissue samples collected at a time corresponding to the placebo fatigue time point in NG and G. Statistical analyses of all data were adjusted to account for this subgroup of participants, as described in Statistical analyses below.

Two tissue samples were taken from each site at each sampling time. The first sample was immediately placed into liquid $N_2$ and stored at $-80^\circ$C until metabolite analyses were performed. The second tissue sample was used for determination of SR $\text{Ca}^{2+}$-handling properties.

**Assessment of muscle metabolites.** Muscle metabolite analyses were performed as previously reported (27), using fluorometric procedures and HPLC techniques (38). The specific metabolites were performed as previously reported (27), using fluorometric procedures. The concentrations of free ADP (ADP$_f$) and free AMP (AMP$_f$) were calculated, as previously described (61).

Quantification of total glycogen, proglycogen, and macroglycogen was accomplished using fluorometric techniques (50). All measurements, with the exception of muscle glycogen content, were performed using an extract from the same piece of freeze-dried tissue and were corrected to the average total Cr content (TCr = PCR + Cr$_t$) of all tissue samples collected for each individual. Neither exercise nor glucose supplementation altered TCr. During a given analytic session, all tissue samples from three individuals were analyzed in duplicate for a given metabolite.

**Assessment of SR $\text{Ca}^{2+}$-handling properties.** To assess changes in $\text{Ca}^{2+}$ transport across the SR membrane, a variety of functional properties of SERCA, the CRC, and the SR membrane were performed using crude muscle homogenates. Muscle samples (40–60 $\mu$g) were diluted 1:11 (wt/vol) in ice-cold homogenizing buffer (pH 7.5) containing (in mM) 250 sucrose, 5 HEPES, 0.2 PMSF, and 0.2% sodium azide (NaSz). Dithiothreitol was not used in the preparation of crude muscle homogenates, since dithiothreitol could potentially reverse exercise-induced sulfhydryl oxidation during sample preparation (64). The muscles were hand homogenized with a Duall glass on glass hand homogenizer (Kontes Glass). Tissue samples were stored on ice from the time of extraction until homogenized. The total time between tissue extraction and homogenization was typically $< 10$ min.

Muscle homogenate aliquots ($\sim 115$ $\mu$l per aliquot; approximately four aliquots) were rapidly frozen in liquid $N_2$ and stored at $-80^\circ$C for future analysis of SR function. Assessment of the kinetics parameters of SERCA included $V_{\text{max}}$, Hill coefficient ($n_H$) (defined as the relationship between SERCA activity and $[\text{Ca}^{2+}]/(V_{\text{max}}^{[\text{Ca}^{2+}]})$, for $10$–90% $V_{\text{max}}$), which is a measure of the cooperative binding behavior of SERCA for $\text{Ca}^{2+}$, and the $C_{50}$ (defined as the $[\text{Ca}^{2+}]_r$ at 50% $V_{\text{max}}$), which is also a measure of cooperative binding behavior. Additionally, we determined the relative amounts of passive $\text{Ca}^{2+}$ leak across the SR membrane by comparing the ratio between $V_{\text{max}}$ in the presence and absence [$V_{\text{max}}^{[\text{Ca}^{2+}]}/V_{\text{max}}$] of 1 $\mu$M $\text{Ca}^{2+}$ ionophore A23187 (ionophore ratio).

**SRCA activity.** Measurement of SERCA kinetic properties was performed using a spectrophotometric assay (68), as modified in our laboratory (75). Assay protocols were further adapted for use on a plate reader (SPECTRAMax Plus; Molecular Devices). With this protocol, we analyzed three different muscle samples simultaneously on a single plate. Each sample was prepared by adding 40 $\mu$L of crude muscle homogenate to 5 ml cocktail buffer, and then each sample was aliquoted (300 $\mu$L) into 16 Eppendorf tubes and mixed with $\text{Ca}^{2+}$ to generate 14 different $\text{Ca}^{2+}$ concentrations, ranging between 7.6 and 4.7 pCa units. The difference in assay conditions was limited to one Eppendorf tube, in which $\text{Ca}^{2+}$ ionophore A23187 was not included in the reaction cocktail. In all other Eppendorf tubes and for all 14 $\text{Ca}^{2+}$ concentrations used to generate the substrate-SERCA activity curve, the $\text{Ca}^{2+}$ ionophore A23187 (1 $\mu$M Sigma C-7522) was included. The $\text{Ca}^{2+}$ ionophore A23187 was used to prevent the formation of a large $\text{Ca}^{2+}$ gradient across the SR membrane. By measuring maximal SERCA activity in the presence ($V_{\text{max}}$) and in the absence [$V_{\text{max}}^{[\text{Ca}^{2+}]}/V_{\text{max}}$] of $\text{Ca}^{2+}$ ionophore A23187, passive $\text{Ca}^{2+}$ leak through the SR membrane was assessed. Assessment of $V_{\text{max}}$, $V_{\text{max}}^{[\text{Ca}^{2+}]}/V_{\text{max}}$, and calculation of ionophore ratios [$V_{\text{max}}^{[\text{Ca}^{2+}]}/V_{\text{max}}$] were completed at a pCa of 4.9 for all samples. On a given analytic day, complete sets of samples from six individuals were analyzed for SERCA kinetics in duplicate.

In the measurement of $V_{\text{max}}$, phosphatase inhibitors were not employed in the extraction buffer. The possibility exists that exercise and/or glucose effects on phosphorylation on the CRC or SERCA directly or via PLN could be obviated via activation of phosphatases during homogenization. To examine this possibility, we have measured $V_{\text{max}}$ in four to six individuals at rest and during exercise, with and without phosphatase inhibitors (Phosphatase Inhibitor Cocktail I, Sigma P2850) in the extraction buffer. For the control or NG condition, the difference between means for the two conditions ranged between 2.0 and 6.3%. For the experimental or G condition, the differences at similar exercise times ranged between 0.6 and 6.1%. These data suggest that $V_{\text{max}}$ is not affected by the addition of phosphatase inhibitors.

**$\text{Ca}^{2+}$ uptake.** Oxalate-supported $\text{Ca}^{2+}$-uptake rates were measured using the $\text{Ca}^{2+}$ fluorescent dye indo 1, according to published methods (56, 57), as modified by Ruel et al. (60) and our laboratory (76). Fluorescence measurements were made on a spectrophotometer (Ratiomaster system, Photon Technology International) equipped with dual-emission monochromators. The measurement of $[\text{Ca}^{2+}]_r$ using this procedure is based on the difference in maximal emission wavelengths between the $\text{Ca}^{2+}$-bound indo 1 complex and the $\text{Ca}^{2+}$.
free indo 1 complex, as described previously in this paper and earlier publications (56, 57).

The reaction buffer (pH 7.0) for muscle homogenates contained 200 mM KCl, 20 mM HEPES, 10 mM MgCl₂, 10 mM NaN₃, 10 mM phosphoenolpyruvate, 5 mM oxalate, and 5 µM N,N,N',N'-tetraakis(2-pyridylmethyl)ethylenediamine. Before each assay, 1.5 µM indo 1, 18 U/ml Lac dehydrogenase, and 18 U/ml pyruvate kinase were added to the 2 ml of reaction buffer. Immediately before collection of emission spectra, a volume of muscle homogenate was added to the cuvette containing the reaction buffer. Following initiation of data collection, 2.5 µl of 10 mM CaCl₂ were added to the cuvette, which produces a consistent starting [Ca²⁺]ᵢ of ~3.5 µM. Shortly after the attainment of a constant [Ca²⁺]ᵢ, 5 mM ATP were added to the cuvette to initiate Ca²⁺ uptake. The curve generated from [Ca²⁺]ᵢ vs. time was then smoothed over 21 points using the Savitsky-Golay algorithm. Linear regression was performed on values ranging ±100 nM, at [Ca²⁺]ᵢ of 500, 1,000, 1,500, and 2,000 nM. Differentiating the linear fit curve allowed determination of Ca²⁺-uptake rates. On a given analytic day, complete sets of samples from three individuals were analyzed for Ca²⁺-uptake kinetics in duplicate.

We have also assessed the apparent coupling ratio, which provides an indication of the efficiency of SERCA to transport Ca²⁺ from the cytosol into the lumen of the SR per ATP hydrolyzed, by calculating the ratio between Ca²⁺-uptake rates and the rates were determined in the absence of Ca²⁺ ionophore A23187 and at [Ca²⁺]ᵢ ~12.5 µM, whereas, Ca²⁺-uptake rates were determined in the absence of Ca²⁺ ionophore A23187. Additionally, our measurements of Ca²⁺-uptake are submaximal, since the Ca²⁺ sensitivity of indo 1 is limited at high [Ca²⁺]ᵢ. We have used the term “apparent coupling ratio” to indicate that the parameters used to calculate this ratio were measured under different assay conditions.

SR Ca²⁺ release. SR Ca²⁺-release rates were measured according to the methods of Ruell et al. (60), as modified by our group (76). SR Ca²⁺-release assays were conducted similar to the Ca²⁺-uptake assay procedures, where a dual-emission spectrophotometer (Ratiosystem master, Photon Technology International) records simultaneous photon counts per second for indo 1 emission wavelengths previously defined. To assess Ca²⁺-release kinetics, homogenate samples were actively loaded with Ca²⁺ until a characteristic plateau in [Ca²⁺]ᵢ was achieved. At this point, 20 mM 4-chloro-m-cresol were added to the assay mixture to chemically stimulate Ca²⁺ release in vitro. Two different properties of Ca²⁺ release have been reported in this paper. Phase 1 Ca²⁺ release has been characterized as the more prolonged, slower rate of Ca²⁺ release lasting from ~4–10 s. Both phases of Ca²⁺ release have been calculated using the same methods as described for Ca²⁺ uptake, where the ionized Ca²⁺ concentration is calculated as described previously (29). Subsequently, differentiating the linear fit curves allows determination of Ca²⁺-release rates. On a given analytic day, Ca²⁺-release kinetics were assessed from complete sets of samples from three individuals using the same assay sample as that used to assess Ca²⁺ uptake.

Protein determination of homogenates was made by the method of Lowry and Passonneau (46), as modified by Schacterle and Pollock (63). Samples were analyzed in triplicate to determine protein concentration.

Western blot analysis. To assess PLN phosphorylation site-specific polyclonal antibodies for anti-serine 16 (Ser16) PLN (sc-12963) and anti-threonine 17 (Thr17) PLN (sc-17024), phosphorylated forms of PLN were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Samples (5–50 µg protein with quantity dependent on specific antibody) were loaded on 12.5% polyacrylamide gels. Proteins were separated using standard SDS-PAGE protocols and transferred to nitrocellulose membranes. After blocking with a 5% skim milk suspension, the membranes were treated with site-specific polyclonal antibodies raised against the Ser16 and Thr17 phosphorylated forms of PLN, washed in Tris-buffered saline/0.1% Tween, and treated with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology). Membranes were washed in Tris-buffered saline, 0.1% Tween, and the signals were detected with an enhanced chemiluminescence kit (Amersham Biosciences) using a bio-imaging system and the GeneSnap software (Syngene) obtained from Fisher. Relative phosphorylation levels were determined by quantifying the optical density of bands at ~10 and ~25 kDa, as specified on the material data sheet for the PLN antibodies. For each antibody, the linearity of progressive increases in protein content was established before experiments were conducted (data not shown). Relative protein levels were determined by scanning densitometry, and values were expressed as a percentage of standard. When direct comparisons were made between sampling times, values were normalized to tissue samples collected at rest (0 min) and expressed as percentage of rest. All samples were analyzed in duplicate and on different gels.

Statistical analyses. Data are presented as means ± SE. A one-way ANOVA for repeated measures was utilized to compare differences between the exercise times within each condition. A two-way ANOVA (2 repeated measures) was utilized to discriminate between differences resulting from experimental condition and exercise time for matched samples. The data collected in the subgroup of participants (i.e., 6 of 15 volunteers) who had a tissue sample collected at a matched time corresponding to fatigue in NG (i.e., 115 ± 6 min) during both conditions were analyzed using a different two-way

Table 1. Gas exchange measurements collected at rest and during prolonged exercise in the no glucose and glucose conditions

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>115 ± 6 min</th>
<th>137 ± 7 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂, ml/min NG</td>
<td>376 ± 39</td>
<td>1,970 ± 105</td>
<td>2,047 ± 103</td>
<td>2,083 ± 114</td>
<td>2,130 ± 77</td>
<td>2,234 ± 123</td>
<td>2,255 ± 90</td>
</tr>
<tr>
<td>G</td>
<td>369 ± 26</td>
<td>1,987 ± 86</td>
<td>2,060 ± 92</td>
<td>2,140 ± 85</td>
<td>2,194 ± 97</td>
<td>2,094 ± 98</td>
<td>2,255 ± 90</td>
</tr>
<tr>
<td>VCO₂, ml/min NG</td>
<td>290 ± 31</td>
<td>1,872 ± 106</td>
<td>1,959 ± 99</td>
<td>1,923 ± 101</td>
<td>1,932 ± 71</td>
<td>2,059 ± 113</td>
<td>2,069 ± 95</td>
</tr>
<tr>
<td>G</td>
<td>284 ± 22</td>
<td>1,881 ± 88</td>
<td>1,935 ± 92</td>
<td>1,971 ± 86</td>
<td>2,010 ± 93</td>
<td>1,910 ± 86</td>
<td>2,069 ± 95</td>
</tr>
<tr>
<td>RER</td>
<td>0.77 ± 0.01</td>
<td>0.95 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.93 ± 0.01</td>
<td>0.91 ± 0.01</td>
<td>0.92 ± 0.01</td>
<td>0.92 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15. VO₂, oxygen uptake; VCO₂, carbon dioxide production; NG, no glucose; G, glucose; RER, respiratory exchange ratio. Rest represents 0 min. RER is calculated as the ratio between VCO₂ and VO₂. Main effects of exercise (P < 0.05) were found for VO₂, VCO₂, and RER. For VO₂, rest < 15 min, 30 min < 60 min, 90 min, 115 ± 6 min, and 137 ± 7 min. For VCO₂, rest < 15 min, 30 min, 60 min, 90 min, 115 ± 6 min, and 137 ± 7 min. For RER, rest < 15 min, 30 min > 60 min, 90 min, 115 ± 6 min, and 137 ± 7 min.
**RESULTS**

**Respiratory gas and substrate oxidation.** The relative exercise intensities based on measurements performed at 15 min were 57.2 ± 1.9 and 57.8 ± 1.2% \( V_{O2peak} \) for the NG and G conditions, respectively. No differences in \( V_{O2}, V_{CO2}, \) or RER were observed between conditions at rest or during exercise (Table 1). However, main effects of exercise were found for \( V_{O2}, V_{CO2}, \) and RER. For \( V_{O2} \), rest < 15 min, and 30 min < 60 min, 90 min, 115 ± 6 min, and 137 ± 7 min. For \( V_{CO2} \), rest < 15 min, 30 min, 60 min, 90 min, 115 ± 6 min, and 137 ± 7 min. For RER, rest < 15 min, and 30 min > 60 min, 90 min, 115 ± 6 min, and 137 ± 7 min.

RERs were used to calculate substrate oxidation rates through the use of indirect calorimetry (Table 2). Main effects of exercise were found for both CHO and lipid oxidation rates. For CHO oxidation, rest > 15 min, 30 min, 60 min, 90 min, 120 min, and fatigue. For lipid oxidation, rest, 15 min, and 30 min < 60 min, 90 min, 120 min, and fatigue. No differences between NG and G conditions were observed for CHO or lipid oxidation rates.

**Blood metabolites.** During NG, blood glucose concentrations were maintained for the initial 30 min of exercise, followed by a reduction at 90 min and 115 ± 6 min of exercise (Fig. 1A). During G, the normal exercise-induced reductions in blood glucose were prevented by the glucose supplementation protocol. Comparisons across conditions indicated that plasma glucose concentrations during G were 15, 19, 23, and 24% higher than those observed during NG at 60, 90, and 115 ± 6 min of exercise, and at fatigue in G (137 ± 7 min) compared with fatigue during NG (115 ± 6 min), respectively.

Blood Lac concentrations measured during NG increased above resting levels with the onset of exercise and peaked between 15 and 30 min of exercise (Fig. 1B). Similar changes in plasma Lac concentrations were observed during G at 15 and 30 min of exercise. No differences in plasma Lac concentrations were observed during the initial 60 min of exercise between NG and G. However, blood Lac concentrations were lower during G, compared with NG, at 90 and 115 ± 6 min of exercise and at fatigue in G (137 ± 7 min) compared with fatigue during NG (115 ± 6 min).
AMP concentration. Main effects of exercise (IMP, mmol/kg dry wt Cr, mmol/kg dry wt Pi, mmol/kg dry wt Table 4. were found for ADPf and AMPf (Table 3). For both ADPf and this exercise effect. min) was found for IMP. Glucose supplementation did not alter glucose, mmol/kg dry wt

<table>
<thead>
<tr>
<th>Rest</th>
<th>30 min</th>
<th>90 min</th>
<th>115±6 min</th>
<th>137±7 min</th>
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<tbody>
<tr>
<td>ATPf, mmol/kg dry wt</td>
<td></td>
<td></td>
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<tr>
<td>NG</td>
<td>24.0±0.6</td>
<td>23.6±0.7</td>
<td>23.8±0.5</td>
<td>22.5±0.7</td>
</tr>
<tr>
<td>G</td>
<td>23.8±1.0</td>
<td>23.2±0.7</td>
<td>23.8±0.5</td>
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<tr>
<td>PCr, mmol/kg dry wt</td>
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<tr>
<td>NG</td>
<td>73.7±3.3</td>
<td>34.7±4.5</td>
<td>38.0±3.6</td>
<td>35.2±4.4</td>
</tr>
<tr>
<td>G</td>
<td>75.2±3.1</td>
<td>40.5±3.6</td>
<td>37.3±4.0</td>
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</tr>
<tr>
<td>Pi, mmol/kg dry wt</td>
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<td></td>
<td></td>
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<tr>
<td>NG</td>
<td>43.8±4.4</td>
<td>86.6±9.4</td>
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<td>G</td>
<td>38.5±4.4</td>
<td>80.0±9.0</td>
<td>87.9±10.0</td>
<td>96.4±12.5</td>
</tr>
<tr>
<td>IMP, mmol/kg dry wt</td>
<td></td>
<td></td>
<td></td>
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<td>NG</td>
<td>51.3±3.4</td>
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<td>86.9±5.1</td>
<td>89.8±5.6</td>
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<tr>
<td>G</td>
<td>46.3±5.0</td>
<td>84.5±4.8</td>
<td>87.7±6.3</td>
<td>95.5±6.5</td>
</tr>
<tr>
<td>ADPf, μmol/kg dry wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NG</td>
<td>0.133±0.02</td>
<td>0.354±0.08</td>
<td>0.492±0.12</td>
<td>0.617±0.13</td>
</tr>
<tr>
<td>G</td>
<td>0.132±0.16</td>
<td>0.321±0.09</td>
<td>0.400±0.10</td>
<td>0.475±0.12</td>
</tr>
<tr>
<td>AMPf, μmol/kg dry wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NG</td>
<td>0.7±0.2</td>
<td>14.4±6.6</td>
<td>7.1±1.6</td>
<td>11.6±3.4</td>
</tr>
<tr>
<td>G</td>
<td>0.5±0.1</td>
<td>10.1±6.5</td>
<td>9.5±2.6</td>
<td>9.0±2.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15. PCr, phosphocreatine; Pᵮ, inorganic phosphate; Cr, creatine; ADPᵮ, calculated free ADP concentration; AMPᵮ, calculated free AMP concentration. Main effects of exercise (P < 0.05) were found for PCr, Pᵮ, Cr, IMP, ADPᵮ, and AMPᵮ. For PCr, rest > 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min. For Pᵮ and Cr, rest < 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min. For IMP, rest < 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min. For ADPᵮ and AMPᵮ, rest < 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min.

min) was found for IMP. Glucose supplementation did not alter this exercise effect.

Main effects of exercise but not glucose supplementation were found for ADPᵮ and AMPᵮ (Table 3). For both ADPᵮ and AMPᵮ, rest < 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min.

Muscle glycogen content was reduced by 40, 62, 68, and 72% after 30, 90, 115 ± 6, and 137 ± 7 min of exercise, respectively. Similar percent changes were also observed during exercise for propylate and macroglycerogen. SR properties. Resting and exercise Vmax activities were not different between NG and G conditions (Table 5; Fig. 3A). Exercise reduced Vmax during both NG and G conditions. In general, the reduction in Vmax by the end of exercise amounted to between 17 and 18% for both conditions. Other Ca²⁺-dependent catalytic properties, including RH and CASO, were not altered by exercise, regardless of condition. Passive leak of Ca²⁺ through the SR membrane assessed by Vmax of Ca²⁺ ionophore A23187 was not altered during exercise or by experimental condition. Ionophore ratios, defined as the ratio of Vmax/Vmax(-), were also unaltered with exercise, both with and without glucose.

No differences were observed for basal ATPase activities during exercise or between conditions at matched time points in NG and G. SR Ca²⁺-uptake rates (nmol·mg protein⁻¹·min⁻¹) between NG and G conditions were assessed at 2,000 nM (Fig. 3B). No

Table 3. Selected muscle metabolite concentrations at rest and during prolonged exercise in the no glucose and glucose conditions

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>30 min</th>
<th>90 min</th>
<th>115±6 min</th>
<th>137±7 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/kg dry wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NG</td>
<td>1.75±0.17</td>
<td>4.23±0.82</td>
<td>2.50±0.35</td>
<td>1.79±0.30</td>
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</tr>
<tr>
<td>G</td>
<td>2.11±0.26</td>
<td>4.57±0.98</td>
<td>2.32±0.21</td>
<td>2.04±0.40</td>
<td></td>
</tr>
<tr>
<td>Lactate, mmol/kg dry wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NG</td>
<td>5.29±0.62</td>
<td>25.7±4.4</td>
<td>14.9±3.2</td>
<td>9.87±2.2</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>5.80±1.2</td>
<td>21.1±4.1</td>
<td>13.1±2.6</td>
<td>11.0±3.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; (n = 15). A main effect of exercise (P < 0.05) was found for glucose. For glucose, rest < 30 min, 90 min; 30 min > 90 min, 115 ± 6 min, and 137 ± 7 min. For lactate, rest < 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min; 30 min > 90 min, 115 ± 6 min, and 137 ± 7 min.
The magnitude of change induced by exercise. In addition to Ca\(^{2+}\)-uptake measurements made at 2,000 nM, we also assessed Ca\(^{2+}\)-uptake rates at 1,500, 1,000, and 500 nM (date not presented). At these Ca\(^{2+}\) concentrations, a similar pattern of change occurred during exercise as at 2,000 nM. Glucose supplementation did not further alter the exercise-induced reductions in Ca\(^{2+}\)-uptake rates at the lower Ca\(^{2+}\) concentrations.

To assess the effects of exercise and glucose supplementation on the efficiency of Ca\(^{2+}\) transport across the SR membrane, we have calculated apparent coupling ratios (Ca\(^{2+}\)-uptake rate at 2,000 nM/V\(_{\text{max}}\) (Fig. 3C). No changes in the apparent coupling ratio were found with exercise for both NG and G.

No differences in resting Ca\(^{2+}\)-release rates for either Phase 1 or Phase 2 were observed between NG (Phase 1, 23.4 ± 1.3; Phase 2, 7.3 ± 0.4) and G (Phase 1, 23.0 ± 1.1; Phase 2, 7.3 ± 0.5), respectively (Fig. 4). Exercise reduced both Phase 1 and Phase 2 Ca\(^{2+}\)-release rates in both NG and G conditions. For Phase 1 Ca\(^{2+}\) release, rest > 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min, and 30 min > 115 ± 6 min and 137 ± 7 min. For Phase 2 Ca\(^{2+}\) release, rest > 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min, and 90 min > 115 ± 6 min and 137 ± 7 min. Glucose supplementation did not alter the exercise-induced reductions in Ca\(^{2+}\)-release kinetics.

**PLN phosphorylation.** No effect of exercise or glucose supplementation was found for the 25- and 10-kDa bands, representing Ser16 PLN phosphorylation (Table 6). Similarly, the 25-kDa Thr17 phosphorylation band was unaltered by exercise or by glucose supplementation. In contrast, a main effect of exercise was found for the 10-kDa Thr17 phosphorylation band. For this band, rest < 30 min > 90 min, 115 ± 6 min, and 137 ± 7 min. Glucose supplementation did not alter this response. Representative immunoblots are presented in Fig. 5.

**DISCUSSION**

As hypothesized, we have found that prolonged, submaximal exercise in humans resulted in disturbances in SR Ca\(^{2+}\)-handling properties in the active vastus lateralis muscle, as indicated by the reductions in both Ca\(^{2+}\) uptake and Ca\(^{2+}\) release. The reduction in Ca\(^{2+}\) uptake occurred in conjunction with reductions in the maximal catalytic activity of the enzyme (V\(_{\text{max}}\)), but not in the Ca\(^{2+}\) affinity of the enzyme, as assessed by K\(_{\text{H}}\) and C\(_{\text{so}}\). We also report that prolonged exercise failed to effect changes in the ionophore ratio and the apparent coupling ratios, indirect measures of the permeability of the SR membrane for Ca\(^{2+}\) and the efficiency of Ca\(^{2+}\) transport, respectively. Collectively, these observations contribute to a growing body of evidence, particularly in the human, that sustained, contractile activity of moderate intensity induces SR Ca\(^{2+}\)-cycling alterations in skeletal muscle (4, 14, 15, 77).

Contrary to our hypothesis, we found no effect of oral glucose supplementation in modifying the SR Ca\(^{2+}\)-cycling responses to prolonged exercise. In our experimental design, oral glucose supplementation began at 30 min of exercise, providing a lead-in period to allow for the exercise response to become established. Differences between NG and G in the SR properties examined were expected at 90 min of exercise, the first tissue sampling point after the beginning of glucose

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**Fig. 2.** Total muscle glycogen (A), macroglycogen (C), and proglycogen (B) before and during prolonged exercise, with and without glucose supplementation. Values are means ± SE (n = 15). Exercise end points represent the time to fatigue for NG and G. For NG and G, the times to fatigue were 115 ± 6 and 137 ± 7 min, respectively. A main effect (P < 0.05) of exercise was found for total, pro-, and macroglycogen content. For exercise, 0 > 30 min > 90 min > 115 ± 6 min > 137 ± 7 min.

Differences in resting Ca\(^{2+}\)-uptake rates were observed between NG (6.4 ± 0.3) and G (6.3 ± 0.4). Exercise reduced (main effect; rest > 30 min and 90 min > fatigue) Ca\(^{2+}\)-uptake rates during both NG and G conditions. During NG, Ca\(^{2+}\)-uptake rates were reduced by 10.8, 15.8, and 26.9% after 30, 90, and 115 ± 6 min of exercise, respectively. Similar reductions were observed in the G condition, at comparable time points. Glucose supplementation was without effect in altering
supplementation and at the matched time point beyond 90 min of exercise, representing the point of fatigue in NG. Since our hypothesis was based on several previous studies using repetitive contractions, which demonstrated a protective effect on a SR Ca\(^{2+}\) function during increased CHO availability, at least with regard to endogenous muscle glycogen levels (7, 12, 16, 43), the failure to find an experimental effect invites further examination.

Oral glucose supplementation could promote a variety of responses, many of which could potentially affect SR function during the exercise state. To examine these possibilities, it was first necessary to determine that our glucose supplement schedule could abolish the reduction in blood glucose observed at 90 min of exercise and beyond. As expected, based on previous studies (9, 11, 55), blood glucose remained stable throughout exercise in G.

A second objective of this study was to determine whether maintenance of blood glucose concentration could alter muscle substrate utilization and metabolism. Glucose supplementation did not change the metabolic efficiency of the exercise, as indicated by the lack of differences in VO\(_2\) between conditions or the contribution of either CHO or fats in substrate oxidation. In addition, we found no effect of glucose supplementation on either glycogen content or cellular energy responses during the exercise. In this regard, our data support previous studies that have not found an effect of glucose supplementation on the magnitude of exercise-induced glycogen depletion (11, 55). Given the potential functional differences between the different forms of glycogen (50, 67), we have also measured macro- and proglycogen contents in addition to total glycogen. Our results indicate that no differences existed between NG and G conditions in the pattern of depletion that occurred, regardless of the glycogen moiety examined.

Similar to previous studies (55, 72), there was a failure of G to alter the change in the high-energy phosphates, ATP and PCr, and their metabolites, P\(_1\) and Cr, that normally occurs with exercise. We had anticipated that G would attenuate the increase in IMP, recognized as a more sensitive indication of ATP (32) observed with exercise based on a previous study (69). Although we found a clear trend toward lower IMP after glucose feeding, the difference was not significant. Our failure to find an effect on IMP accumulation between conditions, as shown previously (69), could be due to differences in exercise protocol and/or the training state of the participants, as well as the glucose supplement schedule. It should be noted that a variety of glycolytic intermediates were measured, all of which were not significantly different, either at rest or during exercise between conditions (data not presented). The lack of an effect of glucose supplementation on the metabolic properties could contribute to our negative results, since accumulation of one or more of these compounds has been shown to alter both SR Ca\(^{2+}\) release (18) and Ca\(^{2+}\) uptake (73). Since muscle glycogen levels appear to be directly involved in modifying the contractile-induced effect on SR Ca\(^{2+}\) cycling (7, 16, 43), the failure of our intervention to modify glycogen contents may also be important in our inability to realize an experimental effect on SR responses.

Another argument for glucose supplementation to effect changes in SR function during exercise is via differences in selected blood hormonal responses. Blood glucose levels have a potent effect in regulating the secretion of both Ins from the pancreas and the catecholamines from the adrenal medulla (24, 79). Our results demonstrate marked differences between conditions in the response of these hormones to exercise (25). With G, the normal reduction in serum Ins to prolonged exercise was blunted, while both the Epi and NE time-dependent increases with exercise were substantially reduced. Our findings clearly indicate that, by preventing declines in blood glucose during prolonged exercise, the concentration of these hormones can be substantially altered. Our observations are in accord with other studies, which have reported similar effects on these hormones, with glucose supplementation administered during sustained submaximal exercise (24).

The blunting of the catecholamine response with glucose phosphorylation would be expected to modify the intrinsic regulation of both the CRC and SERCA. It is generally ac-

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Table 5. Sarcoplasmic reticulum Ca\(^{2+}\)-ATPase kinetic properties measured in muscle homogenates at rest and during exercise in the no glucose and glucose conditions

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>30 min</th>
<th>90 min</th>
<th>115±6 min</th>
<th>137±7 min</th>
</tr>
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<tbody>
<tr>
<td>nH(_f)</td>
<td>NG</td>
<td>G</td>
<td>NG</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>1.60±0.05</td>
<td>1.53±0.04</td>
<td>1.57±0.04</td>
<td>1.63±0.07</td>
<td>1.59±0.04</td>
</tr>
<tr>
<td>Ca(_{50}), nM</td>
<td>NG</td>
<td>711±58</td>
<td>694±64</td>
<td>644±50</td>
<td>730±34</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>713±46</td>
<td>711±67</td>
<td>625±50</td>
<td>641±49</td>
</tr>
<tr>
<td>V(_{max}-1), nmol·mg protein(^{-1})·min(^{-1})</td>
<td>NG</td>
<td>35±2</td>
<td>34±2</td>
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<td></td>
<td>G</td>
<td>37±2</td>
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<td>33±2</td>
<td>31±1</td>
</tr>
<tr>
<td>Ionophore ratio</td>
<td>NG</td>
<td>5.06±0.20</td>
<td>4.86±0.22</td>
<td>4.87±0.27</td>
<td>4.67±0.18</td>
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<tr>
<td></td>
<td>G</td>
<td>4.81±0.15</td>
<td>5.17±0.29</td>
<td>4.99±0.24</td>
<td>5.01±0.18</td>
</tr>
<tr>
<td>Basal ATPase, nmol·mg protein(^{-1})·min(^{-1})</td>
<td>NG</td>
<td>11.6±1.8</td>
<td>13.6±2.5</td>
<td>12.0±1.8</td>
<td>9.9±1.8</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>9.8±1.6</td>
<td>11.4±1.4</td>
<td>13.3±1.9</td>
<td>10.2±1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15. nH\(_f\), Hill slope, defined as the relationship between sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) activity and cytosolic free Ca\(^{2+}\) concentration for 90–90% V\(_{max}\). Ca\(_{50}\), the Ca\(^{2+}\) concentration at 1/2 V\(_{max}\). Assessment of V\(_{max}\), V\(_{max}-1\), and calculation of ionophore ratios [V\(_{max}\)/V\(_{max}-1\)] was completed at a pCa of 4.9 for all samples. V\(_{max}\) was assessed in the presence of Ca\(^{2+}\) ionophore A23187, whereas V\(_{max}-1\) was assessed in the absence of Ca\(^{2+}\)-ionophore A23187. Ionophore ratios were calculated as the ratio between V\(_{max}\) and V\(_{max}-1\). pCa is the negative logarithm of the Ca\(^{2+}\) concentration. Basal ATPase rates were measured in the presence of 1 μl cyclopiazonic acid, which is a specific inhibitor of SERCA. V\(_{max}\) appears in Fig. 3.
cepted that Epi can effect increases in PLN phosphorylation through cAMP-dependent mechanisms, resulting in an increase in Ca$^{2+}$/H$^{+}$ sensitivity in the absence of changes in $V_{\text{max}}$ of the enzyme (48). Since we failed to observe changes in SR Ca$^{2+}$-cycling sensitivity with prolonged exercise, an increase in plasma Epi concentration by itself is not effective in modifying the sensitivity of the enzyme to Ca$^{2+}$. The failure of Epi to alter Ca$^{2+}$/H$^{+}$ sensitivity is also supported by our measurements of site-specific phosphorylation of PLN at Ser16 and Thr17, where expected increases (59) with G were not observed.

Epi and Ins signaling can activate various protein kinase and phosphatase pathways to alter CRC phosphorylation (45, 48, 58), thereby regulating the open probability of the CRC. However, since we did not observe any changes in SR Ca$^{2+}$-release kinetics between conditions, it appears unlikely that increases in CRC phosphorylation were induced by glucose supplementation. Since plasma Epi decreased and serum Ins increased with G during prolonged exercise, it is possible that contrasting effects of the two hormones negated a net phosphorylation.

Another possibility to explain the apparent insignificant effect of glucose supplementation on SR behavior during exercise relates to the analytic approach to measuring the SR Ca$^{2+}$-cycling properties. Our measurements are based on in vitro techniques performed on crude homogenates under supposedly optimal conditions. As such, the increases in Ca$^{2+}$ release and SR Ca$^{2+}$ uptake that undoubtedly occurs “in vivo” in the transition from rest to exercise remain undetected. It is possible that changes in vivo mediated by our experimental conditions remain obscure because of analytic limitations. However, in recent work (T. A. Duhamel and H. J. Green, unpublished observations), we have been able to demonstrate that, under supposedly optimal assay conditions, the effect of changes in selected hormones and selected protein kinase and phosphatase signaling pathways on the kinetic behavior of the

![Fig. 3](image-url) Maximal sarcoplasmic reticulum Ca$^{2+}$-ATPase activity (A), Ca$^{2+}$ uptake (B), and coupling ratio (C) during prolonged exercise, with and without glucose supplementation. Values are means ± SE ($n = 15$). Exercise end points represent the time to fatigue for NG and G. For NG and G, the times to fatigue were 115 ± 6 and 137 ± 7 min, respectively. $V_{\text{max}}$, maximal Ca$^{2+}$-ATPase activity; pro, protein. Ca$^{2+}$ uptake was determined at 2,000 nM Ca$^{2+}$. Apparent coupling ratio is defined as the ratio between Ca$^{2+}$ uptake and $V_{\text{max}}$. Main effects ($P < 0.05$) of exercise were found for $V_{\text{max}}$ and Ca$^{2+}$ uptake. For exercise, rest, 30 min > 90 min > 115 ± 6 min, 137 ± 7 min.

![Fig. 4](image-url) Sarcoplasmic reticulum Ca$^{2+}$-release rates for Phase 1 (A) and Phase 2 (B) during prolonged exercise, with and without glucose supplementation. Values are means ± SE ($n = 15$). Exercise end points represent the time to fatigue for NG and G. For NG and G, the times to fatigue were 115 ± 6 and 137 ± 7 min, respectively. For both Phase 1 and Phase 2 Ca$^{2+}$-release rates, a main effect ($P < 0.05$) of exercise was found. For Phase 1, rest > 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min. For Phase 2, rest > 30 min, 90 min, 115 ± 6 min, and 137 ± 7 min.
Ca²⁺-ATPase can be detected in vitro. These results suggest that at least some of the potential mechanisms whereby glucose can alter SR Ca²⁺ handling can be assessed in vitro under our assay conditions.

A limitation of the present study was our failure to investigate the potential role of the extraction procedure on the activation of select protein kinases and phosphatases on the phosphorylation of the proteins involved in SR Ca²⁺ cycling. It is important to note, as an example, that the lack of inclusion of protein phosphatase inhibitors within the extraction buffer may have not allowed the proper assessment of changes in PLN phosphorylation (59) and conceivably in vitro SR function. The activation of selected protein kinases and phosphatases could alter the phosphorylation status of the CRC and SERCA directly or, in the case of SERCA via the regulatory proteins, PLN and sarcolipin (SLN). Although we have examined the effect of several phosphatases on the V\textsubscript{max} behavior of SERCA on the limited amount of tissue remaining after the initial measurements performed without any phosphatase inhibitors and found no effect, the possibility remains that the sensitivity of SERCA to Ca²⁺ may have been altered. Measurements of Ca²⁺ sensitivity would appear to be particularly important if the phosphorylation status of PLN and/or SLN were altered.

It is also possible that the isolated effects of glucose supplementation on SR responses are not conspicuous because of the effect of exercise per se on the CRC and the SERCA. Repetitive contractile activity results in a decrease in V\textsubscript{max}, an effect that has been attributed to structural alterations in the region of the nucleotide binding site of the enzyme (47, 54). The structural alterations have been attributed to protein oxidation and nitration induced by accumulation of reactive oxygen species (39, 53). In the face of these changes, which result in reductions in V\textsubscript{max}, it is unclear whether the mechanisms proposed to alter kinetic properties of the Ca²⁺-ATPase with glucose can act independently.

Accompanying the exercise-induced reductions in V\textsubscript{max} and Ca²⁺-uptake rates were reductions in SR Ca²⁺-release kinetics. We have utilized a two-phase kinetic model to characterize distinct phases of Ca²⁺ release that occur following the addition of the Ca²⁺-release agent, 4-chloromer-cresol. Phase 1 Ca²⁺ release has been characterized as the initial fast phase of Ca²⁺ release that lasts for ~1–3 s in duration, while Phase 2 Ca²⁺ release has been characterized

### Table 6. Phospholamban phosphorylation measured at rest and during prolonged exercise in the no glucose and glucose conditions

<table>
<thead>
<tr>
<th>Ser16 ~25-kDa band</th>
<th>Rest</th>
<th>30 min</th>
<th>90 min</th>
<th>115±6 min</th>
<th>137±7 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG</td>
<td>100.0</td>
<td>107.4±7.7</td>
<td>106.2±8.0</td>
<td>110.2±8.9</td>
<td>102.2±8.2</td>
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<tr>
<td>G</td>
<td>100.0</td>
<td>99.3±7.4</td>
<td>100.5±7.8</td>
<td>99.3±7.7</td>
<td>102.2±8.2</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Thr17 ~25-kDa band</th>
<th>Rest</th>
<th>30 min</th>
<th>90 min</th>
<th>115±6 min</th>
<th>137±7 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG</td>
<td>100.0</td>
<td>110.8±7.7</td>
<td>99.6±6.1</td>
<td>102.2±8.9</td>
<td>105.6±10.6</td>
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<tr>
<td>G</td>
<td>100.0</td>
<td>101.0±5.9</td>
<td>96.9±4.4</td>
<td>96.1±7.6</td>
<td>105.6±10.6</td>
</tr>
</tbody>
</table>

Values are means ± SE in arbitrary units expressed relative to the rest value for each condition (%rest); n = 15. Ser16, the phosphorylated form of phospholamban on serine 16; Thr17, the phosphorylated form of phospholamban on threonine 17. A main effect of exercise (P < 0.05) was found for the 10-kDa band of Thr17. For exercise, rest < 30 min > 90 min, 115 ± 6 min, and 137 ± 7 min.

Fig. 5. Representative immunoblots for phospholamban phosphorylation measured at rest and during exercise in the NG and G conditions for Ser16 (A) and Thr17 (B). Rest, before exercise; 30, 90, 115 ± 6, and 137 ± 7, time of exercise; Ser16, phospholamban phosphorylation at serine −10 and −25 kDa; Thr17, phospholamban phosphorylation at threonine −10 and −25 kDa.
as the more prolonged, slower rate of Ca\(^{2+}\) release occurring from ~4- to 10-s duration (76). In this study, exercise-induced reductions occurred for both Phase 1 and Phase 2 Ca\(^{2+}\) release rates, with the reductions being similar in both time and magnitude. Although the physiological significance of each Ca\(^{2+}\)-release phase remains unclear, our previous work demonstrates that two separate phases of Ca\(^{2+}\) release occur (76). Based on previous studies employing prolonged exercise in rats (18), it would appear that the exercise-induced reductions in Ca\(^{2+}\)-release kinetics can be attributed to a reduction in the number of functional CRCs, possibly as a result of protein oxidation associated with the accumulation of reactive oxygen species (19).

The interpretation of the findings of this study must also consider the mixed fiber-type composition of the human vastus lateralis. Since type I and type IIa fibers (28, 78) represent ~90% of the fiber population in the human vastus lateralis (62), the exercise-induced reductions in SR Ca\(^{2+}\)-handling properties observed in this study represent the net change in SR function for all fiber types present in each muscle sample and, consequently, do not represent a fiber-type-specific response. We cannot discount the possibility that our data were influenced by greater reductions in one fiber type compared with another, since differences in fiber-type activation during prolonged exercise are known to exist (28, 78). Moreover, differences in SERCA protein isoform expression (83) or the expression of other SR-associated proteins known to influence the intracellular regulation of SR properties (73) may have also caused SR properties to be affected to a greater extent in one fiber type compared with another. It would have been beneficial to determine the fiber-type-specific changes in SR properties during exercise for type I vs. type II fibers; however, this was not possible, given technical limitations in measuring SR Ca\(^{2+}\)-cycling properties in single fibers.

In summary, our findings demonstrate that prolonged exercise leads to a progressive loss of SR Ca\(^{2+}\)-handling properties in human skeletal muscle when assessed in vitro. Furthermore, it appears that the ingestion of oral glucose supplements initiated after the beginning of exercise does not alter the reductions in SR Ca\(^{2+}\)-handling properties. We have also been able to demonstrate that, with our exercise protocol, glucose supplementation did not alter the metabolic response or the glycogen depletion patterns. However, glucose supplementation did modify both the plasma catecholamine and serum Ins responses. Although glucose supplementation did increase cycle time to fatigue, our SR Ca\(^{2+}\)-cycling responses do not explain this ergogenic effect.

However, in a companion paper, we have been able to determine that glucose supplementation increases Na\(^{+}\)-K\(^{+}\)-ATPase activity (25), which occurs in conjunction with an improved protection of membrane activity (71). It should be noted that our glucose feeding schedule was designed to prevent the reduction in blood glucose concentration that typically occurs with prolonged exercise. This we have been able to accomplish. As such, our results may differ from other studies that have used different glucose dosages and schedules designed to elevate blood glucose above resting levels during sustained exercise.

**REFERENCES**

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