Combined effects of exercise and fasting on skeletal muscle glycogen and sarcoplasmic reticulum function

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Abstract

Prolonged exercise is associated with depressed rates of skeletal muscle sarcoplasmic reticulum (SR) Ca$^{2+}$ transport as well as a decline in muscle glycogen. This investigation sought to determine if these two changes are causally related. A protocol involving fasting and prolonged exercise was used to reduce glycogen in rested muscles. Rats in the low glycogen group were fasted for 24 hrs, performed 90 min of treadmill exercise and were fasted for another 24 hrs. The high glycogen group was fasted for 24 hrs, exercised then allowed free access to food for 24 hrs. A control group was fed ad lib and did not participate in the exercise session. The low glycogen animals had 42% less muscle glycogen and 90% less SR glycogen than the other two groups. They also experienced mild hypoglycemia. However, the loss of glycogen resulted in enhanced SR Ca$^{2+}$ pump function as indicated by significantly increased Ca$^{2+}$ uptake rates and Ca$^{2+}$ ATPase activities. This finding held when rates were adjusted for Ca$^{2+}$ pump protein content. These results suggest that the loss of glycogen during prolonged exercise does not contribute to the reductions in SR function that are associated with muscle fatigue.

Key Words: Ca$^{2+}$ ATPase, SERCA, fasting, fatigue, exhaustion

Several groups have shown that contractile activity leading to the development of muscle fatigue is associated with alterations in sarcoplasmic reticulum (SR) function. Specifically, the release and uptake of Ca$^{2+}$ by the SR is depressed in muscle that has been subjected to repetitive stimulation or prolonged exercise (for reviews see [1, 21, 22, 24]). Such changes likely lead to diminished contractile apparatus activation and reduced force output. Unfortunately, the specific “triggers” that cause the reductions in SR Ca$^{2+}$ exchange are not completely understood.

It is well known that exhaustion from prolonged exercise leads to dramatic reductions in muscle glycogen content [13]. It is also well known that the time to exhaustion is linked to initial muscle glycogen levels [6]. Recent work also suggests that glycogen depletion during exercise may lead to alterations in SR function. Several studies provide indirect evidence of a “cause and effect” relationship between muscle glycogen content and SR function. Lees et al. [15] showed that in situ stimulation causes declines in both whole muscle and SR-associated glycogen contents. Their protocol also resulted in depressed SR Ca$^{2+}$ pump function. Batts et al. [5] found that muscle glycogen reduction following acute epinephrine administration leads to changes in SR function that are qualitatively similar to those changes caused by exhaustive exercise. A 24% reduction in muscle glycogen was associated with a 20% reduction in Ca$^{2+}$ uptake and ATPase activity. Using single fibers, Chin and Allen [7] report that allowing a fatigued muscle fiber to recover in a medium that contains glucose and insulin results in the restoration of glycogen content as well as SR Ca$^{2+}$ uptake and release. However, in fibers that were allowed to recover in a glucose-free medium, glycogen content and SR function remained depressed. Taken together, these studies provide circumstantial evidence to suggest that the loss of muscle glycogen during prolonged exercise may directly alter SR function.

In this investigation, we sought to determine if reducing muscle glycogen in rested muscle directly affects SR function. To accomplish this, we utilized an in vivo model of reduced muscle glycogen involving prolonged endurance exercise and fasting. We found that despite lowered muscle glycogen levels, SR function was enhanced rather than adversely affected.
TABLE 1. Muscle Mass, Glycogen Contents and Plasma Glucose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HG</th>
<th>LG</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Muscle Mass (g)</td>
<td>1.921 ± 0.094</td>
<td>1.856 ± 0.125</td>
<td>1.849 ± 0.103</td>
</tr>
<tr>
<td>Dry Muscle Mass (g)</td>
<td>0.587 ± 0.026</td>
<td>0.543 ± 0.046</td>
<td>0.552 ± 0.051</td>
</tr>
<tr>
<td>Liver Glycogen (µmol•g DM⁻¹)</td>
<td>227.44 ±18.48</td>
<td>39.72 ± 2.99a</td>
<td>209.60 ± 14.48</td>
</tr>
<tr>
<td>Muscle Glycogen (µmol•g DM⁻¹)</td>
<td>189.13 ± 9.35b</td>
<td>108.98 ± 6.21a</td>
<td>160.45 ± 7.23</td>
</tr>
<tr>
<td>Plasma Glucose (µmol•ml⁻¹)</td>
<td>10.32 ± 0.56</td>
<td>6.31 ± 0.20a</td>
<td>9.87 ± 0.55</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n).  a, p<.05 vs. HG and CON, b, p<.05 vs CON.  DM, dry mass

TABLE 2. SR Glycogen, SERCA and GP Content

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HG</th>
<th>LG</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR Glycogen (µg•mg SR protein⁻¹)</td>
<td>636.23 ± 142.13</td>
<td>69.56 ± 15.03a</td>
<td>602.56 ± 151.89</td>
</tr>
<tr>
<td>SERCA (µg•mg SR protein⁻¹)</td>
<td>245.31 ± 18.22</td>
<td>315.72 ± 20.75a</td>
<td>240.33± 19.54</td>
</tr>
<tr>
<td>GP (µg•mg SR protein⁻¹)</td>
<td>159.81 ± 12.62</td>
<td>84.62 ± 10.19a</td>
<td>146.64 ± 18.31</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n).  a, p<.05 vs. HG and CON, b, p<.05 vs CON.

Materials and Methods

Animals. For all experiments, 12 female Sprague-Dawley rats were used (200-250g). The experimental procedures used in this study were reviewed and approved by the Virginia Tech Animal Care Committee.

Experimental Model. Rats were randomly divided into three groups, a high glycogen (HG) group, a low glycogen group (LG) and a control group (CON). At 08:00 on day one, food was removed from the HG and LG groups. At 08:00 on day two, after the 24hr fast, HG and LG animals were placed on a motor driven treadmill and exercised for 90 min at a running pace of 21m•min⁻¹ and 5% grade. Immediately after exercise, animals were returned to their cages. The HG group was then given free access to standard rodent chow and water. For these animals, water was supplemented with 5% sucrose. The LG group was given free access to water (no sucrose) and fasted for another 24 hrs. The CON group was given free access to food and water throughout the 48hr treatment period and they did not participate in the exercise protocol.

At 08:00 on day three, animals were anesthetized with sodium pentobarbital (60 mg•kg⁻¹) delivered ip. One muscle as well as the liver was immediately removed, weighed and frozen in liquid nitrogen for measurement of glycogen. The other gastrocnemius was removed and placed in ice-cold homogenizing buffer (see below) for preparation of SR vesicles. In addition, a blood sample was obtained from the aortic vein and the plasma was frozen for analysis of plasma glucose.

Muscle Glycogen and Plasma Glucose Measurements. For the muscle and liver glycogen measurements, muscle samples were first freeze dried. They were then weighed and homogenized in 5 vol of ice-cold perchloric acid. Glycogen content was determined using the glucoamylase (E.C. 3.2.1.3) method described by Keppler and Decker [14]. Glycogen associated with the SR was also measured using the glucoamylase method as described by Lees et al. [15, 16] based on the method of Keppler and Decker [14]. In each, whole muscle or SR samples (see below) were incubated with glucoamylase for 60 min (pH 4.5, 40°C). Glucose released from glycogen was then determined spectrophotometrically. Plasma glucose was determined using the glucose oxidase method using a commercially available kit (Stanbio).

Sarcoplasmic Reticulum Function Measurements. Sarcoplasmic reticulum vesicles were isolated as described by Williams et al. [25] and Lees et al [16]. Briefly, muscles were homogenized in an ice-cold buffer containing 20mM HEPES, 0.2% sodium azide, 0.2mM PMSF, and 1mM EDTA (VirtiShear, 3 x 15s). Differential centrifugation was then used to isolate SR vesicles (12,000-60,000 x g pellet). Following centrifugation, the final SR pellets were frozen and stored at −80°C until used. The samples used to determine SR glycogen were stored in the homogenizing buffer whereas the samples used for the SR function measurements and SDS-PAGE were stored in homogenizing buffer supplemented with 300mM sucrose.
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Ca$^{2+}$ uptake rates were determined as described by Williams et al. [23, 25, 26]. For the uptake measurements, SR vesicles (25µg) were placed in a buffer containing 100mM KCl, 20mM HEPES, 1mM MgCl$_2$, 5mM KH$_2$PO$_4$, 2mM ATP and 2µM fura-2 (pH 7.0, 37°C). Uptake was initiated by adding 1.2 µmol•mg$^{-1}$•min$^{-1}$ CaCl$_2$ and was allowed to continue until free [Ca$^{2+}$] in the cuvette declined to a plateau. A Jasco CAF-110 fluorometer was used with excitation wavelengths of 340 and 380 and an emission wavelength of 500. Fura-2 signals were converted into free [Ca$^{2+}$] as described by Grynkiewicz et al. [12]. The steepest negative slope of the [Ca$^{2+}$]-time curve was used as the measure of peak uptake rate.

Ca$^{2+}$ ATPase activity was determined using the enzyme-linked assay described by Luckin et al. [17]. Samples (20mg) were incubated in buffer containing 25 mM HEPES, 100 mM KCl, 10 mM MgCl$_2$, 1 mM EGTA, 0.2% NaN$_2$, 2 µM A23187, 5 U•ml$^{-1}$ lactate dehydrogenase, 7.5 U•ml$^{-1}$ pyruvate kinase, 3.0 U•ml$^{-1}$ phosphoenolpyruvate and 0.6 mM NADH (pH 7.0, 37°C). The disappearance of NADH was monitored (340nm) and converted to ATPase activity. Basal or Mg$^{2+}$-stimulated activity was recorded for 3 min. Total activity was then recoded after adding 1.2 µM•mg$^{-1}$ CaCl$_2$. Ca$^{2+}$ stimulated activity was determined by subtracting basal from total.

Content of the SR Ca$^{2+}$ pump (SERCA) was determined using SDS-PAGE as described by Lees & Williams [16]. Protein standards were run on the same gel and optical densities of the SERCA bands were quantified based on the standard curve. Ca$^{2+}$ uptake and ATPase activities were then normalized using SERCA content measurements.

Statistics. Analyses of variance with Student-Newman-Kewals post-hoc exams were used to identify differences between the HG, LG and CON groups for all variables recorded. Significance was established at the 0.05 level of confidence.

Results
Animal and Muscle Data. There was no indication of any adverse effect of the prolonged fast in the LG animals. All animals appeared alert and active in their cages and readily initiated the treadmill exercise. During the removal of tissues, there were no signs of tissue damage or dehydration.

Wet and dry gastrocnemius muscle masses were not significantly different between the LG, HG and CON groups (Table 1). Table 1 also shows the liver and muscle glycogen contents in the three groups as well as plasma glucose. As can be seen, the LG group had 42% less muscle glycogen and 83% less liver glycogen than did the HG group. In addition, muscle glycogen in the HG was significantly greater that that of the CON group (15%). The LG group also showed slight hypoglycemia in that blood glucose was lower in this group compared to the other two groups.

The amount of glycogen associated with the SR was also significantly lower in the LG animals compared to the HG and CON groups (Table 2). The reduction in SR glycogen in the LG group was proportionately
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SR Function. Measurements of SR function are shown in Figures 1 and 2. In Figure 1, the Ca\(^{2+}\) uptake and ATPase activity rates are expressed per mg of total protein in the sample. The peak rate of Ca\(^{2+}\) uptake was 47% greater in the LG group compared to the HG and CON groups. Likewise, both basal (Mg\(^{2+}\)-stimulated) and Ca\(^{2+}\)-stimulated ATPase activities were increased by 42 and 43%, respectively.

A portion of the increase in SR function in the LG groups can be accounted for by increases in the amount of SR Ca\(^{2+}\) pump protein in the samples. When normalized by the SERCA content, both Ca\(^{2+}\) uptake and Ca\(^{2+}\)-stimulated ATPase activity remained increased over the HG and CON samples by 13 and 10%, respectively (Figure 2). Basal activity, normalized by SERCA content, was not different between groups.

Discussion
The goal of this investigation was to examine the effects of a protocol that combined exercise and fasting on muscle glycogen and SR performance. Using the approach described here, we were able to substantially reduce whole muscle glycogen in resting muscle as well as the amount of glycogen associated with the SR. Resting blood glucose and liver glycogen contents were also lowered. In contrast to prolonged exercise, the treatment was associated with increases in SR Ca\(^{2+}\) uptake rate and Ca\(^{2+}\) ATPase activity. These changes were evident when rates were adjusted for SERCA content. Thus, it appears that the loss of glycogen during prolonged exercise does not account for the reductions in SR Ca\(^{2+}\) pump function.

Others have used dietary manipulations to lower muscle glycogen and examine SR function. Mishima et al. [18] used a program of starvation (1-3 days) to reduce glycogen content of the rat gastrocnemius by approximately 25%. Duhamel et al. [10] placed human subjects on a low CHO diet for four days and reduced glycogen of the vastus lateralis by around 35%. In neither study was the SR Ca\(^{2+}\) ATPase significantly affected. However, both of these investigations used muscle homogenate fractions rather than purified SR vesicles. It is possible that a more purified preparation, one in which the Ca\(^{2+}\) ATPase is a greater fraction of total protein content, is more sensitive to changes in muscle glycogen or glycogen associated with the SR.

Despite differences between the present results and those of Mishima et al. [18] and Duhamel et al. [11], it is important to point out that the three studies clearly indicate that the loss of muscle glycogen does not directly impair the SR Ca\(^{2+}\) pump.

It should be noted that the extent of muscle glycogen reduction in the LG animals is not as great as that...
obtained with prolonged exercise or electrical stimulation. Studies using exercising rats or in situ stimulation report reductions in muscle glycogen of more than 80% [2, 3, 15, 20]. Thus it is possible that greater reductions in glycogen could lead to decreased Ca$^{2+}$ pump function. However, we do not think this is likely in light of the data presented by Lees and Williams [16] and Mishima et al. [18]. Both of these studies used chemical and enzymatic extraction to remove >80% of glycogen from SR vesicles. In neither study was SR Ca$^{2+}$ ATPase activity or Ca$^{2+}$ uptake negatively affected. In fact, in the study by Lees and Williams [16], SR function was slightly enhanced. Thus, it is difficult to argue that a greater loss of muscle glycogen than found here would directly impair SR function.

Dietary and exercise manipulation of muscle glycogen resulted in noticeably less glycogen begin associated with the SR. The reduction in SR glycogen in the LG group (nearly 90%) is similar to that reported to occur immediately following electrical stimulation [15]. However, the reduction in muscle glycogen found here (42%) was not nearly as large as that induced by stimulation (86%) [15]. On the other hand, Batts et al. [5] saw relatively similar changes in muscle and SR glycogen following acute epinephrine administration (~25%). This suggests that there may be a non-linear relationship between the loss of whole muscle and SR glycogen contents. It is also possible that the post-exercise recovery of these glycogen stores is differentially affected by further energy restriction. Derave et al. [9] and Asp et al. [4] found that localized, subcellular fractions of glycogen could be preferentially used during various types of exercise. It is possible that SR associated glycogen is preferentially used under different exercising and metabolic stress conditions. Clearly, more work is needed to clarify the relationship between whole muscle and SR glycogen stores during and following exercise. Batts et al. [5] used acute epinephrine treatment to reduce muscle glycogen in rested animals. In contrast to the present results, they showed that glycogen loss resulted in depressed SR Ca$^{2+}$ uptake rate and ATPase activity. While epinephrine treatment reduces muscle glycogen, it likely causes other effects that may affect SR function. These include elevated muscle temperature, altered intracellular metabolite levels and increased reactive oxygen species. In fact, Batts et al. [5] argue that metabolic stress caused by epinephrine may directly affect SR function rather than the loss of glycogen. In the present study, it is unlikely that metabolic stress affected SR function. The HG and LG animals were allowed to recover from exercise for 24 hours before muscles were removed. Also, the HG animals responded similar to control animals that were not exercised nor subjected to fasting. Thus, our results are consistent with the idea that factors other than glycogen content, such as metabolic stress, negatively affect SR function during exercise.

It should be pointed out that the present investigation does not rule out possible in vivo effects of glycogen depletion on the SR Ca$^{2+}$ pump. It is possible that the loss of glycogen in the vicinity of the Ca$^{2+}$ pump could lead to altered Ca$^{2+}$ handling. We and others have argued that the local environment may play a critical role in SR function [8, 15, 19, 22, 24]. Sarcoplasmic reticulum Ca$^{2+}$ uptake can be supported solely through the enzymatic breakdown of glycogen [8, 19]. Thus, loss of glycogen during exercise may lead to reduced ATP and an increased ADP/ATP ratio, both of which could diminish maximal Ca$^{2+}$ uptake rate. In our in vitro system, exogenous ATP was added to the system and endogenous production of ATP from glycogen was not possible. Thus, we were unable to determine the effects of fasting and prolonged exercise on the local microenvironment in vivo.

In summary, the present investigation shows that a protocol of fasting and prolonged exercise leads to modest reductions in whole muscle glycogen. This effect is associated with a large decline in SR glycogen but no effect on SR Ca$^{2+}$ pump function. Thus, it is unlikely that the loss of glycogen during prolonged exercise causes diminished SR function. It seems reasonable to suggest that some other aspect of the metabolic stress encountered during exercise triggers the decline in SR Ca$^{2+}$ uptake and ATPase activity.

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