Hindawi Publishing Corporation
ISRN Physiology
Volume 2013, Article ID 371235, 8 pages
http://dx.doi.org/10.1155/2013/371235

Research Article

Reduced Muscle Glycogen Differentially Affects Exercise Performance and Muscle Fatigue

Jay H. Williams,[1] Timothy W. Batts,[1] and Simon Lees[1,2]

[1] *Department of Human Nutrition, Foods and Exercise, Virginia Polytechnic Institute & State University, Blacksburg, VA 24060, USA*
[2] *Medical Sciences Division, Northern Ontario School of Medicine, Thunder Bay, ON, Canada P7B 5E1*

Correspondence should be addressed to Jay H. Williams; jhwms@vt.edu

Received 21 August 2012; Accepted 26 September 2012

Academic Editors: J. Cannon and K. Sakuma

This investigation examined the effects of reduced muscle glycogen on exercise performance and muscle fatigue. Male rats were assigned to a low glycogen group (LG) that participated in a protocol of exercise and fasting, a high glycogen group (HG) that exercised but were allowed free access to food, or control group (CON) that did not exercise but were allowed free access to food. Following the protocol, muscle glycogen content of the LG animals was reduced by 45%. The LG animals also performed 79 and 81% less voluntary treadmill exercise than the HG and CON groups. At exhaustion, the LG group had lower blood glucose than HG and CON but exhibited no reduction in sarcoplasmic reticulum (SR) function. During 30 min of *in situ* stimulation, the rates and magnitudes of muscle fatigue were not significantly different between groups, and fatigue-induced reductions in SR function were similar between groups. The results indicate that reduced muscle glycogen markedly impairs voluntary exercise performance but does not appreciably affect isolated muscle function. It is likely that exercise exhaustion due to reduced muscle glycogen is due, in large part, to hypoglycemia and central fatigue as opposed to peripheral mechanisms.

1. Introduction

As early as the 19th century a relationship between carbohydrate metabolism and fatigue was proposed. In 1807, Berzelius suggested "the amount of free lactic acid in skeletal muscle is proportional to the extent to which it has been exercised" [1]. Exhaustive exercise is known to cause to dramatic reductions in muscle glycogen content within the active muscle [2–4]. Also, the onset of exhaustion is dependent on the initial muscle glycogen content [2] and dietary manipulation of muscle glycogen can increase or decrease exercise duration [5]. Based on this, most researchers readily accept the notion that muscle glycogen depletion is somehow involved in the development of fatigue during prolonged exercise.

Despite this, more than 40 years of investigation have failed to identify a specific "cause-and-effect" relationship between muscle glycogen and fatigue during exercise. Newsholm and Leech [6] proposed that during muscular activity, the loss of glycogen results in an inability to sustain an adequate glycolytic flux rate to support ATP regeneration. Reductions in ATP resynthesis would then cause a decline in intracellular ATP concentration, resulting in an inability to meet the energy demands of the contraction process. As a consequence, the muscle would be unable to sustain maximal force output. Green [7] defined this notion as the "energy crisis" hypothesis. While this remains a widely accepted explanation to account for the link between muscle glycogen and fatigue, it has received little experimental support. In fact, it is often observed that during prolonged or intense exercise, there is little or no reduction in intracellular ATP despite near complete depletion of muscle glycogen (see [8]).

The process of voluntary muscle contraction consists of a long chain of events beginning with input to the motor cortex and ending with force production via actin-myosin interaction. If any one of the steps in this sequence fails, force output will be compromised. In simple terms, fatigue can be thought of as "central" or "peripheral" [8]. Central fatigue involves events responsible for α-motoneuron activation. In this case, force output is limited by failure to adequately activate the α-motoneuron pool. Central fatigue can be further described as "supraspinal", failure at the level of the motor cortex, or "spinal", involving afferent activation or

excitability of the α-motoneuron pool [9]. On the other hand, peripheral fatigue occurs distal to the spinal cord and usually refers to mechanisms at the level of the muscle fiber.

In the present study, we sought to gain insight into the mechanisms whereby reduced muscle glycogen limits exercise performance through central or peripheral fatigue mechanisms. We utilized a rodent model of reduced muscle glycogen [10] and examined the effects on exercise performance (central and peripheral mechanisms) and *in situ* muscle function (peripheral mechanisms). We find that reduced muscle glycogen severely limits exercise performance but has little effect on *in situ* muscle force or fatigue.

2. Methods

2.1. Experimental Model. For all experiments, female Sprague-Dawley rats were used (200–250 g). All procedures used were reviewed and approved by the Virginia Tech Animal Care Committee. The experimental model used in this study has been described in detail [10]. Briefly, to reduce muscle glycogen, a protocol combining exercise and fasting was used. Rats were randomly divided into three groups, high glycogen (HG), low glycogen (LG), and control (CON). During the first 24 hrs of the protocol, food was removed from the HG and LG groups. After this initial fast, these animals were placed on a motor-driven treadmill and exercised for 90 min at a running pace of 21 m/min and 5% grade. The treadmill was equipped with an electrical grid that provided a mild external stimulus during exercise. Immediately after exercise, animals were returned to their cages. The HG group was then given free access to standard rodent chow and water 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 and did not participate in the exercise protocol.

2.2. Exercise and Muscle Performance. Exercise performance was determined using treadmill running to exhaustion. Animals were exercised at a pace of 21 m/min, 5% grade. Exhaustion was determined when the animals could no longer keep pace with the treadmill and failed to right themselves when placed on their back.

The *in situ* muscle stimulation was carried out as described by Williams and Ward [11] and Lees et al. [12]. Briefly, animals were anesthetized using ketamine (40 mg/kg) and xylazine (8 mg/kg) delivered *ip*. The left gastrocnemius muscle was isolated; the Achilles tendon was cut at the calcaneus and tied via surgical thread, to an isometric transducer (Harvard Apparatus). Muscle temperature was maintained at 37°C using a warming lamp, and hydration was preserved by bathing muscle with mineral oil and covering the area with polyvinylidene chloride film (Saran). The muscle was stimulated through electrodes placed around the sciatic nerve. Contractions were evoked via 0.5 msec pulses using a voltage that elicited maximal twitch force.

After resting length was determined (i.e., the length that resulted in peak twitch force), three tetanic contractions (333 msec, 100 Hz) were elicited (separated by 1 min) [13]. Next, a fatigue protocol was applied, which consisted of 333 ms trains of pulses (20 Hz) delivered at a rate of one per second for 30 min. Immediately after the fatigue protocol, a single titanic contraction was elicited. All contractions were evoked and sampled via microcomputer then analyzed off-line for peak force and peak rate or relaxation. For the rested condition, the three contractions were averaged. For the fatigued condition, the single contraction was used. This was because there was some, albeit small recovery of force between three postfatigue contractions separated by 1 min.

2.3. Biochemical and Sarcoplasmic Reticulum (SR) Function Measurements. Immediately before (rested) and after (exhausted) the exercise performance treadmill bout, animals were quickly anesthetized with CO_2 inhalation, and a blood sample was obtained for plasma glucose measurement. They were then decapitated, and both gastrocnemius muscles were removed. One muscle was prepared for glycogen analysis and the other prepared for SR function measurements. For comparison with exhausted LG animals, separate groups of HG and CON animals were sacrificed after 35 minutes of exercise (35 min). This resulted in three conditions for these groups: rested, exhausted, and 35 min.

For the *in situ* stimulation experiments, both gastrocnemius muscles were removed (rested and fatigued muscle from each animal) and prepared for glycogen or SR function analysis (separate animals were used for each assay). A blood sample was also obtained for plasma glucose measurement. Animals were then euthanized by anesthesia overdose. In a separate group of animals, muscles were removed for glycogen measurements. They were immediately frozen in liquid N_2 then freeze dried. Freeze-dried samples were weighed and then 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]. Plasma glucose was determined using the glucose oxidase method using a commercially available kit (Stanbio).

ATP, ADP, and PCr measurements were made using high-performance liquid chromatography (HPLC). A portion of each freeze-dried muscle was weighed, minced, and then homogenized in 5 volumes of ice-cold perchloric acid. After incubating the samples on ice for 20 minutes, the pH was neutralized with KOH. Samples were then centrifuged at 1600 xg to sediment proteins and precipitated KCl. HPLC analysis was carried out as described [15, 16] using a Waters HPLC system equipped with a diode array detector and a Supelchem C18 3 μm reversed phased column (0.46 × 15 cm).

For the SR function measurements, dissected muscles were immediately placed in ice-cold buffer containing 20 mM HEPES, 0.2% sodium azide, 0.2 mM PMSF, and 1 mM EDTA (VirtiShear, 3 × 15 s). Differential centrifugation was then used to isolate SR vesicles (8,000–60,000 xg pellet) as described previously [17]. Following centrifugation, the final SR pellets were frozen and stored at −80°C until used.

Ca^{2+} uptake was determined as described by Lees and Williams [17]. For the uptake measurements, SR protein (25 μg) was added to 1.5 mL of buffer (100 mM KCl, 20 mM HEPES, 5 mM $MgCl_2$, 5 mM KH_2PO_4, 2 mM ATP and

250 μM antipyrylazo III, pH. 7.0, 37°C). Uptake was initiated by adding 1.2 μmol/mg $CaCl_2$ and was allowed to continue until free [Ca^{2+}] in the cuvette declined to a plateau. APIII absorbance was monitored at 790 nm and 710 nm using a diode array spectrophotometer (Aligent) and converted to free [Ca^{2+}]. Peak rate of uptake was determined as the steepest slope of the free [Ca^{2+}] time curves.

Ca^{2+} ATPase activities were determined using a coupled-enzyme assay [18]. Samples (20 μg protein) were added to incubation buffer (25 mM HEPES, 100 mM KCl, 10 mM $MgCl_2$, 1 mM EGTA, 0.2% NaN_2, 2 μM A23187, 5 U/mL lactate dehydrogenase, 7.5 U/mL pyruvate kinase, 3.0 mM phosphoenolpyruvate, and 0.6 mM NADH, pH 7.0, 37°C). NADH absorbance was monitored continuously at 340 nm using a diode array spectrophotometer (Aligent). The reaction was started with addition of ATP (5 mM) and basal activity was recorded for 3 minutes. Ca^{2+} was then added (2.0 μM free [Ca^{2+}]), and total activity was recorded for an additional 3 minutes. Ca^{2+}-stimulated activity was computed by subtracting basal activity from total activity.

2.4. Statistics. Analyses of variance with Tukey's post-hoc exams were used to identify differences between the HG, LG, and CON groups for all variables recorded. For the stimulation experiments, the analyses of variance were adjusted for repeated measures performed on the same animal (rested and fatigued muscles). Significance was established at the 0.05 level of confidence.

3. Results

3.1. Treadmill Exercise. Animals in the LG group (35.21 ± 6.72 min) exercised for significantly less time than did those in the HG (166.84 ± 23.21 min) and CON (182.40 ± 17.90) groups ($P < .05$, $n = 8$ for each group, mean ± SEM). Means for the later two groups were not significantly different.

Muscle glycogen and plasma glucose levels in rested and exercise animals are shown in Figure 1. At rest, The LG animals displayed reduced levels of both glycogen and glucose. Compared to the HG animals, the LG animals' glycogen was reduced by 45.0%. At exhaustion (~35 minutes of exercise), these animals showed marked glycogen depletion and hypoglycemia. Glycogen was reduced by 85.0% compared to rest and plasma glucose dropped to nearly 2 μmol/mL. On the other hand, the HG and CON animals showed no significant changes in either parameter after 35 minutes of exercise. At exhaustion, these animals did experience significant declines in both glycogen and glucose (compared to rest), but final glycogen and glucose levels were higher than the LG group. Also, plasma glucose in the HG and LG groups remained above 4.0 umol/mL at exhaustion.

Intramuscular ATP, ADP, and PCr levels in rested and exercised muscles are shown in Table 1. Under all three conditions, there were no significant exercise-induced changes in ATP levels but significant increases in ADP and significant decrements in PCr. However, values were not significantly different between conditions in either rested or exhausted animals.

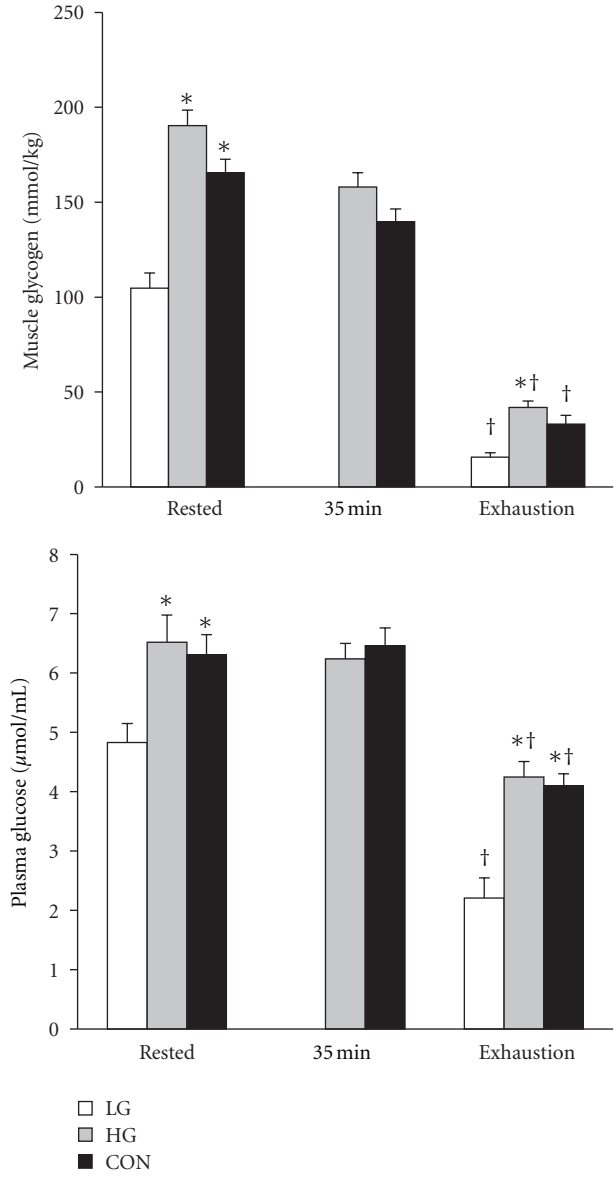

FIGURE 1: Muscle glycogen (top) and plasma glucose (bottom) measured at rest and during exercise. Muscle glycogen values are expressed as glucosyl units per g dry mass. $^{*}P < .05$ versus LG, $^{\dagger}P < .05$ versus rested. $n = 8$ for each group and time point.

As shown previously [10], Both SR Ca^{2+} uptake and Ca^{2+}-stimulated ATPase activity were increased in the LG animals by about 25% compared to the other groups (Table 2). At exhaustion, these animals did not display any exercise-induced reductions in SR function. After 35 minutes of exercise, there were also no changes in SR Ca^{2+} uptake or ATPase activity in the HG or CON animals. At exhaustion, however, these latter groups showed 30–38% reductions in both parameters.

3.2. In Situ Stimulation. Force responses to electrical stimulation are shown in Figure 2. All three groups showed a staircase response during the initial minute. This staircase response was significantly smaller in the LG group than

Table 1: Muscle metabolites in rested and exercised animals. Values are expressed as μmol/g dry mass.

		LG group	HG group	CON group
ATP	Rested	22.12 ± 1.80	25.34 ± 2.03	25.46 ± 1.98
	35 min	—	24.92 ± 2.20	25.65 ± 1.39
	Exhausted	19.36 ± 2.45	20.56 ± 3.11	22.38 ± 2.06
ADP	Rested	3.12 ± 0.41	2.98 ± 0.56	3.20 ± 0.69
	35 min	—	4.94 ± 1.02†	5.22 ± 1.34†
	Exhausted	5.36 ± 1.34†	5.06 ± 0.93†	5.27 ± 2.03†
PCr	Rested	78.33 ± 4.22	81.24 ± 3.22	80.44 ± 5.10
	35 min	—	19.34 ± 4.00†	20.67 ± 4.68†
	Exhausted	17.56 ± 4.10†	18.21 ± 3.29†	22.64 ± 4.06†

$^{\dagger}P < .05$ versus rested. $n = 8$ for each group and time point.

Table 2: Effects of treadmill exercise on SR function. Values are expressed per mg of protein.

		LG group	HG group	CON group
Ca^{2+} Uptake rate (μmol/mg/min)	Rested	1.25 ± 0.19	1.03 ± 0.12*	0.97 ± 0.19*
	35 min	—	1.13 ± 0.10	1.08 ± 0.11
	Exhausted	1.18 ± 0.13	0.64 ± 0.09*†	0.68 ± 0.08*†
Ca^{2+}-Stim ATPase (μmol/mg/min)	Rested	3.16 ± 0.25	2.55 ± 0.39*	2.67 ± 0.29*
	35 min	—	2.85 ± 0.28	2.56 ± 0.33
	Exhausted	3.14 ± 0.21	1.76 ± 0.12*†	1.77 ± 0.19*†

$^{*}P < .05$ versus LG, $^{\dagger}P < .05$ versus rested. $n = 8$ for each group and time point.

the other groups (Table 2). This was followed by a steady decline in force during the following 90 sec. From minutes 3 to 30, force showed a much slower steady reduction in force. To characterize the rate and magnitude of fatigue for each animal, these forces were fit by nonlinear regression to an equation of the form: $P/P_o = a \cdot e^{-k \cdot t} + b$, where $-k$ is the rate constant for force loss or fatigue and b is the final force. Neither of these variables was significantly different between the three groups. That is neither the rate nor magnitude of fatigue during stimulation differed between groups.

Tetanic forces, recorded before stimulation, were not significantly different between groups (Table 3). After stimulation, titanic forces were reduced by 46–50% but were not significantly different between groups. Relaxation rate in rested muscles was significantly higher in the LG group (Table 2). In the fatigued muscles, these rates were decreased compared to the rested condition in all three groups but were not significantly different between groups.

Muscle glycogen recorded in the rested and stimulated limbs is shown in Figure 3. As can be seen, the rested limb of the LG animals had significantly lower glycogen content than those of the other two groups. In the stimulated limb, glycogen was lower than the rested limb in all three groups. However, the stimulated limb of the LG animals contained less glycogen than that of the HG and CON groups. Also, plasma glucose at the end of stimulation was significantly lower in the LG group than in the HG and CON animals (5.03 ± 0.52, 5.92 ± 0.61 and 6.01 ± 0.58 μmol/mL, resp.).

Table 4 shows intramuscular ATP, ADP, and PCr levels in rested and stimulated muscles. There were no significant

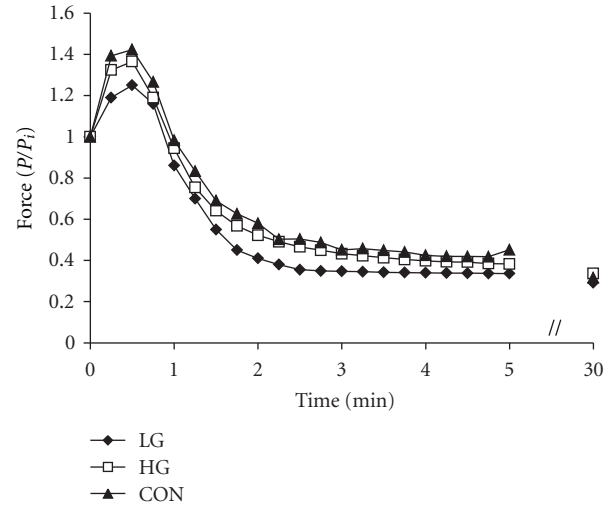

Figure 2: Forces generated during constant 30 Hz stimulation. Values are means for each group. Error bars are omitted for clarity. $n = 16$ for each group.

stimulation-induced changes in ATP levels in any of the three groups of animals. However, significant increases in ADP and significant decrements in PCr occurred in all three. Values were not significantly different between conditions in either rested or fatigued muscles.

Both Ca^{2+} uptake and ATPase activity recorded in the rested limb of the LG animals were greater than values of the

Table 3: Effects of fatiguing stimulation of tetanic force and fatigue.

		LG group	HG group	CON group
Staircase (P/P_i)		1.25 ± 0.07	1.40 ± 0.08*	1.42 ± 0.07*
Rate constant ($/s$)		1.54 ± 0.09	1.39 ± 0.09	1.47 ± 0.07
Final force (P/P_i)		0.29 ± 0.03	0.34 ± 0.03	0.32 ± 0.04
Tetanic force (mN/gm)	Rested	36.98 ± 3.42	33.15 ± 3.62	35.22 ± 4.14
	Fatigued	18.33 ± 2.11†	17.53 ± 1.77†	19.02 ± 2.06†
Relaxation rate (mN/ms)	Rested	61.9 ± 2.0	55.0 ± 2.5*	51.3 ± 2.1*
	Fatigued	21.7 ± 2.7†	20.3 ± 3.1†	19.8 ± 2.6†

P_i: initial force. Tetanic forces are expressed per gram of dry muscle mass. *$P < .05$ versus LG, †$P < .05$ versus rest. $n = 16$ for each group and condition (rest or fatigue).

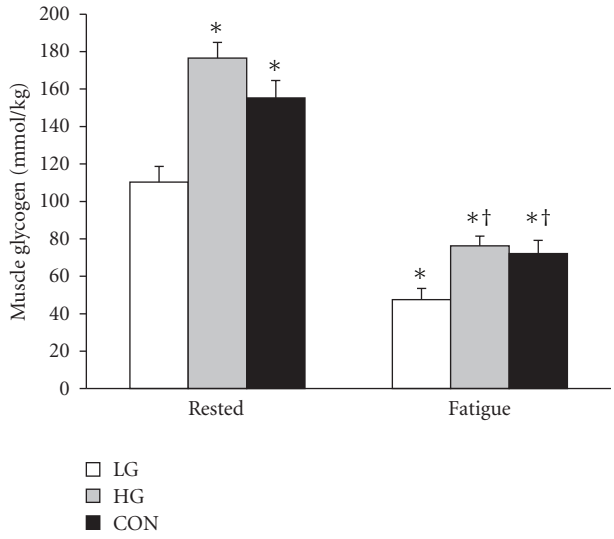

Figure 3: Muscle glycogen rested muscles and muscle subjected to electrical stimulation for 30 min. *$P < .05$ versus LG, †$P < .05$ versus rest. $n = 8$ for each group and condition.

HG and CON animals (Table 5). However, all three groups demonstrated significant reductions in SR function following electrical stimulation. Ca^{2+} uptake was reduced by 30–35%, and ATPase activity was lowered by 30–32% by stimulation.

4. Discussion

Reducing muscle glycogen by approximately 45% through a protocol of exercise and fasting [10] resulted in a dramatic reduction in treadmill exercise performance. However, *in situ* muscle force and fatigue were not affected. Also, reduced muscle glycogen resulted in greater hypoglycemia following both treadmill exercise and *in situ* stimulation. Thus, when the central nervous system (CNS) is required for muscle activity (e.g., treadmill exercise), glycogen depletion and hypoglycemia markedly impair performance. But, when the CNS is bypassed by *in situ* stimulation, the effects are minimal. Taken together, this supports the notion that reduced muscle glycogen affects blood glucose and contributes to the development of central fatigue rather than peripheral fatigue.

It should be pointed out that the *in situ* stimulation protocol used here did not completely mimic the activation pattern of the gastrocnemius muscle during treadmill exercise. For the pace and incline used here, it is estimated that the gastrocnemius muscle was activated at 2–2.5 Hz [19, 20]. Also the duration of activation was estimated at 200–225 ms or a 50% duty cycle [19, 20]. For the repetitive stimulation protocol, contractions were evoked at 1 Hz with a duty cycle of 33%. Also, activation of the gastrocnemius is submaximal during treadmill exercise (at least during the early stages of exercise), whereas with nerve stimulation it is likely that all motor units were activated. Lastly, stimuli during each evoked contraction were delivered at 20 Hz to avoid possible high-frequency fatigue. As for treadmill running, it is difficult to determine an average motor unit activation frequency for a mixed muscle such as the gastrocnemius during activity. However, 20 Hz is well within the range of firing frequencies of other muscles performing submaximal contractions [21]. Thus, there were important differences in the muscle activation pattern between treadmill exercise and *in situ* stimulation. Despite these, it seems reasonable to suggest that the differences between the two muscle activation protocols were small enough such that they did not substantially contribute to the differential effects of reduced muscle glycogen on treadmill running performance and *in situ* fatigue.

The peak staircase response was significantly less in the LG animals. This is likely due to the effects of reduced muscle glycogen on SR function and relaxation rate. As shown earlier by Batts et al. [10] reducing muscle glycogen through diet and exercise leads to increased SR Ca^{2+} uptake rate and ATPase activity. Lees and Williams [17] also found that extraction of glycogen from the SR had the same effect on SR Ca^{2+} pump function. Increasing the rate of SR Ca^{2+} uptake should accelerate relaxation and $-dP/dt$ (as shown here). This, in turn would lead to a diminished staircase response during repetitive stimulation. Thus it is likely that the reduced staircase response was a direct effect of reduced glycogen on the SR Ca^{2+} ATPase.

We did not assess *in situ* muscle function immediately after treadmill exercise. Thus it is not clear to what extent

Table 4: Muscle metabolites in muscles and muscle subjected to stimulation. Values are expressed as μmol/g dry mass.

		LG group	HG group	CON group
ATP	Rested	26.45 ± 2.61	25.84 ± 3.02	27.19 ± 3.12
	Fatigued	21.55 ± 3.56	22.32 ± 4.20	23.10 ± 3.09
ADP	Rested	2.95 ± 0.55	2.79 ± 0.65	3.06 ± 0.96
	Fatigued	5.64 ± 1.44†	5.57 ± 1.00†	5.63 ± 1.56†
PCr	Rested	82.11 ± 4.33	80.26 ± 4.02	80.65 ± 4.65
	Fatigued	29.32 ± 4.32†	27.64 ± 4.03†	27.99 ± 3.96†

$^{\dagger}P < .05$ versus rested. $n = 8$ for each group and time point.

Table 5: Effects of fatiguing stimulation on SR function.

		LG group	HG group	CON group
Ca^{2+} uptake rate (μmol/mg/min)	Rested	1.23 ± 0.18	0.91 ± 0.15*	1.02 ± 0.16*
	Fatigued	0.79 ± 0.10†	0.59 ± 0.12†	0.62 ± 0.12†
Ca^{2+}-stimulated ATPase (μmol/mg/min)	Rested	2.95 ± 0.32	2.13 ± 0.31*	2.22 ± 0.25*
	Fatigued	0.91 ± 0.19†	0.64 ± 0.17*†	0.72 ± 0.19†

$^{*}P < .05$ versus LG, $^{\dagger}P < .05$ versus Rested. $n = 8$ for each group and condition.

the LG animals were experiencing muscle fatigue at the point of exhaustion. However, we did measure SR function. A number of groups have shown that muscle fatigue following stimulation is clearly associated with depressions in SR Ca^{2+} uptake and ATPase activity (see [22, 23]). Also, in well-feed animals, exhaustion after treadmill exercise is associated with reductions in SR function. In the exhausted state, the LG animals showed no change in SR function. Likewise, the HG and CON groups showed no change in the SR function at approximately the same exercise duration (35 min). However, both the HG and CON groups showed marked depressions in SR Ca^{2+} pump function at exhaustion. Following stimulation where force was reduced by nearly 70%, all three groups showed reduced SR Ca^{2+} function. While SR Ca^{2+} uptake and ATPase activity not necessarily indicated fatigue, these results are consistent with the idea that the LG animals likely experienced little muscle fatigue at the point of exhaustion, at least not to the extent as the HG and CON groups.

It should be pointed out that Karelis et al. [24] showed that glucose infusion during *in situ* stimulation attenuated the extent of fatigue and partially restored force in fatigued muscle, possibly by maintaining Na/K pump function. This suggests that blood glucose may have a direct effect on muscle function during fatiguing exercise. It also raises the possibility that the hypoglycemia experienced by the LG animals during exercise may have induced some degree of peripheral fatigue during treadmill exercise. However, in the Karelis et al. [24] study, plasma glucose levels were very high during infusion, nearly twice the initial and control levels and, in the control condition, there was no decline in blood glucose. Further, the slight hypoglycemia experience by the LG animals during stimulation (present study) was not associated with alterations in force or fatigue. While it is possible that plasma glucose may directly affect muscle force during exercise, more evidence is needed to support such a notion.

Others have reported a link between muscle glycogen, excitation-contraction coupling, and SR function using dietary manipulation or chemical extraction [10, 17, 25–27]. These studies suggest that the loss of glycogen during exercise may affect force during fatigue via direct actions on SR Ca^{2+} uptake and release. In addition, a recent study by Ørtenblad et al. [28] used glycogen-depleting exercise coupled with carbohydrate supplementation and found a close relationship between low muscle glycogen levels and reduced SR Ca^{2+} release. They found that depressed SR function returned to normal four hours postexercise when carbohydrate was provided. Without supplementation, both glycogen and SR Ca^{2+} release remained depressed. However, Ørtenblad et al. [28] did not measure muscle function or exercise performance. Thus it is not clear to what extent the muscle had recovered and it is not clear if the same relationship between glycogen and SR function would hold in a rested muscle that is deplete of glycogen. In the present study, the muscle function was allowed to full recovery for 24 hours (as indicated by *in situ* force measurements) after exercise. This allowed for the relationship between glycogen and SR function to be examined in the absence of fatigue. In this case, a reduction in glycogen resulted in increased SR Ca^{2+} uptake and ATPase activity. Given this, it is possible that a more complex relationship exists between muscle glycogen and SR function at rest and during recovery from exercise.

When viewed as a whole, the results of this investigation suggest that reduced muscle glycogen during exercise can lead to the development of hypoglycemia and central fatigue. Mosso [29] was one of the first to suggest that "mental fatigue" contributes to muscular performance. The idea that central fatigue contributes to force loss during exercise and that it may be linked to hypoglycemia is not novel (e.g., [7, 30–32]). Glucose oxidation provides a major energy source for the brain [33]. Since the brain maintains very

limited supplies of endogenous glycogen, plasma glucose uptake and oxidation plasma is an essential fuel source [33]. In fact, cerebral glucose uptake declines when arterial concentration drops below 3.6 mM [34]. Coyle et al.[31] showed that carbohydrate feeding during prolonged exercise delayed the development of fatigue, but only in subjects that experienced hypoglycemia. Nybo et al. [33, 35] found that exercise-induced hypoglycemia reduced both voluntary force and the level of muscle activation. In addition, cerebral blood flow, brain glucose, and oxygen uptakes were reduced as well. These changes were prevented when glucose supplementation was used and hypoglycemia was avoided. Thus, there is a strong case that muscle glycogen depletion is linked to voluntary exhaustion via hypoglycemia and central fatigue.

It is also possible that muscle glycogen depletion and hypoglycemia during exercise result in brain glycogen depletion. Matsui et al. [36] found that prolonged exercise resulted in low levels of glycogen in discrete areas of the brain. They argued that low blood glucose triggered increased astrocytic glycogenolysis leading to reduced brain glycogen and possible central fatigue. Unfortunately, they did not measure the recovery of brain glycogen following exercise. Thus, it is not clear if our LG animals began their exercise bout with normal or reduced brain glycogen. It is possible that the early hypoglycemia experienced by the LG group leads to reductions in brain glycogen, central fatigue, and diminished exercise performance. However, more information on potential links between changes in muscle glycogen and central nervous system metabolism during prolonged exercise and recovery is needed.

Lastly, Racinais et al. [37] and Girard et al. [38] suggest that spinal mechanisms may contribute to central fatigue during voluntary running. This could result in decreased motor neuron activation by Ia afferent inputs and/or decreased excitability of the motor neuron pool. Unfortunately, it is not known at this time if glycogen depletion and/or hypoglycemia affect this aspect of central fatigue.

In summary, this study supports the notion that muscle glycogen depletion is linked to central fatigue. The reduction of muscle glycogen and subsequent development of hypoglycemia markedly impaired treadmill exercise. However, when central activation of the muscle was bypassed via nerve stimulation, muscle fatigue was relatively unaffected. This suggests that a key link between glycogen depletion and exhaustion during exercise is the development of hypoglycemia, possible brain glycogen depletion, and central fatigue. Changes in glycogen or blood glucose appear to have little effect on muscle fatigue.

References

[1] D. Needham, *Machina Carnis*, Cambridge University Press, Cambridge, UK, 1971.

[2] B. Ahlborg, J. Bergstrom, L. G. Ekelund, and E. Hultman, "Muscle glycogen and muscle electrolytes during prolonged exercise," *Acta Physiologica Scandinavica*, vol. 70, pp. 129–142, 1967.

[3] J. Bergström and E. Hultman, "A study of the glycogen metabolism during exercise in man," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 19, no. 3, pp. 218–228, 1967.

[4] L. Hermansen, E. Hultman, and B. Saltin, "Muscle glycogen during prolonged severe exercise," *Acta Physiologica Scandinavica*, vol. 71, no. 2, pp. 129–139, 1967.

[5] J. Bergström, L. Hermansen, E. Hultman, and B. Saltin, "Diet, muscle glycogen and physical performance," *Acta Physiologica Scandinavica*, vol. 71, no. 2, pp. 140–150, 1967.

[6] E. A. Newsholm and A. R. Leech, *Biochemistry for the Medical Sciences*, John Wiley & Sons, New York, NY, USA, 1983.

[7] H. J. Green, "How important is endogenous muscle glycogen to fatigue in prolonged exercise?" *Canadian Journal of Physiology and Pharmacology*, vol. 69, no. 2, pp. 290–297, 1991.

[8] R. H. Fitts, "Substrate supply and energy metabolism during brief high intensity exercise: importance in limiting performance," in *Perspectives in Exercise Science and Sports Medicine*, D. R. Lamb and C. V. Gisolfi, Eds., vol. 5 of *Energy Metabolism in Exercise and Sport*, pp. 53–106, Wm. C. Brown, Dubuque, Iowa, USA, 1992.

[9] S. C. Gandevia, "Spinal and supraspinal factors in human muscle fatigue," *Physiological Reviews*, vol. 81, no. 4, pp. 1725–1789, 2001.

[10] T. W. Batts, S. J. Lees, and J. H. Williams, "Combined effects of exercise and fasting on skeletal muscle glycogen and sarcoplasmic reticulum function," *Basic and Applied Myology*, vol. 19, pp. 247–252, 2009.

[11] J. H. Williams and C. W. Ward, "Changes in skeletal muscle sarcoplasmic reticulum function and force production following myocardial infarction in rats," *Experimental Physiology*, vol. 83, no. 1, pp. 85–94, 1998.

[12] S. J. Lees, P. D. Franks, E. E. Spangenburg, and J. H. Williams, "Glycogen and glycogen phosphorylase associated with sarcoplasmic reticulum: effects of fatiguing activity," *Journal of Applied Physiology*, vol. 91, no. 4, pp. 1638–1644, 2001.

[13] S. J. Lees, Y. T. Chen, and J. H. Williams, "Glycogen debranching enzyme is associated with rat skeletal muscle sarcoplasmic reticulum," *Acta Physiologica Scandinavica*, vol. 181, no. 2, pp. 239–245, 2004.

[14] D. Keppler and K. Decker, "Glycogen," in *Methods of Enzymatic Analysis*, H. U. Bergmyer, Ed., pp. 11–18, Verlag Chimie, Weinheim, Germany, 1984.

[15] P. Bernocchi, C. Ceconi, A. Cargnoni, P. Pedersini, S. Curello, and R. Ferrari, "Extraction and assay of creatine phosphate, purine, and pyridine nucleotides in cardiac tissue by reversed-phase high-performance liquid chromatography," *Analytical Biochemistry*, vol. 222, no. 2, pp. 374–379, 1994.

[16] J. H. Williams, S. E. Vidt, and J. Rinehart, "Measurement of sarcoplasmic reticulum Ca^{2+} ATPase activity using high-performance liquid chromatography," *Analytical Biochemistry*, vol. 372, no. 2, pp. 135–139, 2008.

[17] S. J. Lees and J. H. Williams, "Skeletal muscle sarcoplasmic reticulum glycogen status influences Ca^{2+} uptake supported by endogenously synthesized ATP," *American Journal of Physiology*, vol. 286, no. 1, pp. C97–C104, 2004.

[18] K. A. Luckin, T. G. Favero, and G. A. Klug, "Prolonged exercise induces structural changes in SR Ca^{2+}-ATPase of rat muscle," *Biochemical Medicine and Metabolic Biology*, vol. 46, no. 3, pp. 391–405, 1991.

[19] E. F. Hodson-Tole and J. M. Wakeling, "Motor unit recruitment patterns 1: responses to changes in locomotor velocity and incline," *Journal of Experimental Biology*, vol. 211, no. 12, pp. 1882–1892, 2008.

[20] R. R. Roy, D. L. Hutchison, D. J. Pierotti, J. A. Hodgson, and V. R. Edgerton, "EMG patterns of rat ankle extensors and flexors during treadmill locomotion and swimming," *Journal of Applied Physiology*, vol. 70, no. 6, pp. 2522–2529, 1991.

[21] M. R. Roos, C. L. Rice, D. M. Connelly, and A. A. Vandervoort, "Quadriceps muscle strength, contractile properties, and motor unit firing rates in young and old men," *Muscle and Nerve*, vol. 22, no. 8, pp. 1094–1103, 1999.

[22] A. R. Tupling, "The sarcoplasmic reticulum in muscle fatigue and disease: role of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase," *Canadian Journal of Applied Physiology*, vol. 29, no. 3, pp. 308–329, 2004.

[23] J. H. Williams and G. A. Klug, "Calcium exchange hypothesis of skeletal muscle fatigue: a brief review," *Muscle and Nerve*, vol. 18, no. 4, pp. 421–434, 1995.

[24] A. D. Karelis, F. Péronnet, and P. F. Gardiner, "Glucose infusion attenuates muscle fatigue in rat plantaris muscle during prolonged indirect stimulation *in situ*," *Experimental Physiology*, vol. 87, no. 5, pp. 585–592, 2002.

[25] M. Barnes, L. M. Gibson, and D. G. Stephenson, "Increased muscle glycogen content is associated with increased capacity to respond to T-system depolarisation in mechanically skinned skeletal muscle fibres from the rat," *Pflugers Archiv*, vol. 442, no. 1, pp. 101–106, 2001.

[26] J. Nielsen, H. D. Schrøder, C. G. Rix, and N. Ørtenblad, "Distinct effects of subcellular glycogen localization on tetanic relaxation time and endurance in mechanically skinned rat skeletal muscle fibres," *Journal of Physiology*, vol. 587, no. 14, pp. 3679–3690, 2009.

[27] D. G. Stephenson, L. T. Nguyen, and G. M. M. Stephenson, "Glycogen content and excitation-contraction coupling in mechanically skinned muscle fibres of the cane toad," *Journal of Physiology*, vol. 519, no. 1, pp. 177–187, 1999.

[28] N. ørtenblad, J. Nielsen, B. Saltin, and H. C. Holmberg, "Role of glycogen availability in sarcoplasmic reticulum Ca^{2+} kinetics in human skeletal muscle," *Journal of Physiology*, vol. 589, no. 3, pp. 711–725, 2011.

[29] A. Mosso, *Fatigue, Drummond M & Drummond WB*, Swan Sonnenschein, London, UK, 1904.

[30] B. Bigland Ritchie, D. A. Jones, G. P. Hosking, and R. H. T. Edwards, "Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle," *Clinical Science and Molecular Medicine*, vol. 54, no. 6, pp. 609–614, 1978.

[31] E. F. Coyle, J. M. Hagberg, and B. F. Hurley, "Carbohydrate feeding during prolonged strenuous exercise can delay fatigue," *Journal of Applied Physiology Respiratory Environmental and Exercise Physiology*, vol. 55, no. 1 I, pp. 230–235, 1983.

[32] J. A. Kent-Braun, "Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort," *European Journal of Applied Physiology and Occupational Physiology*, vol. 80, no. 1, pp. 57–63, 1999.

[33] L. Nybo and N. H. Secher, "Cerebral perturbations provoked by prolonged exercise," *Progress in Neurobiology*, vol. 72, no. 4, pp. 223–261, 2004.

[34] P. J. Boyle, R. J. Nagy, A. M. O'Connor, S. F. Kempers, R. A. Yeo, and C. Qualls, "Adaptation in brain glucose uptake following recurrent hypoglycemia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 20, pp. 9352–9356, 1994.

[35] L. Nybo, K. Møller, B. K. Pedersen, B. Nielsen, and N. H. Secher, "Association between fatigue and failure to preserve cerebral energy turnover during prolonged exercise," *Acta Physiologica Scandinavica*, vol. 179, no. 1, pp. 67–74, 2003.

[36] T. Matsui, S. Soya, M. Okamoto, Y. Ichitani, K. Kawanaka, and H. Soya, "Brain glycogen decreases during prolonged exercise," *Journal of Physiology*, vol. 589, no. 13, pp. 3383–3393, 2011.

[37] S. Racinais, O. Girard, J. P. Micallef, and S. Perrey, "Failed excitability of spinal motoneurons induced by prolonged running exercise," *Journal of Neurophysiology*, vol. 97, no. 1, pp. 596–603, 2007.

[38] O. Girard, G. P. Millet, J.-P. Micallef, and S. Racinais, "Alteration in neuromuscular function after a 5 km running time trial," *European Journal of Applied Physiology*, vol. 112, no. 6, pp. 2323–2330, 2012.