Effect of muscle glycogen content on exercise-induced changes in muscle T2 times

THOMAS B. PRICE AND JOHN C. GORE
Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut 06510

Price, Thomas B., and John C. Gore. Effect of muscle glycogen content on exercise-induced changes in muscle T2 times. J. Appl. Physiol. 84(4): 1178–1184, 1998.—Effects of gastrocnemius glycogen (Gly) concentration on changes in transverse relaxation time (T2; ms) were studied after 5-min plantar flexion at 25% of maximum voluntary contraction (MVC). Gastrocnemius Gly, phosphorus metabolites, and T2 were measured in seven subjects by using interleaved 13C/31P magnetic resonance spectroscopy (MRS) at 4.7 T and magnetic resonance imaging (MRI; 1.5 T). After baseline MRS/MRI, subjects exercised for 5 min at 25% of MVC and were reexamined (MRS/MRI). Subjects then performed ~15 min of single leg toe raises (50 ± 2% of MVC), depleting gastrocnemius Gly by 43%. After a 1-h rest (for T2 return to baseline), subjects repeated the 5-min protocol, followed by a final MRI/MRS. After the initial 5-min protocol, T2 values increased by 5.9 ± 0.8 ms (29.9 ± 0.4 to 35.8 ± 0.6 ms), whereas Gly did not change significantly (70.5 ± 6.8 to 67.6 ± 7.4 mM). After 15 min of toe raises, gastrocnemius Gly was reduced to 40.4 ± 5.3 mM (P ≤ 0.01), recovering to 45.8 ± 5.3 mM (P ≤ 0.05) during a 1-h rest. After the second 5-min bout of plantar flexion (reduced Gly at 25% of MVC), T2 values increased by 5.0 ± 0.8 ms (30.4 to 35.4 ms), whereas muscle Gly rose to 57.6 ± 5.3 mM. We conclude that muscle Gly concentration per se does not affect exercise-induced T2 increases in the human gastrocnemius.

### WHEN SKELETAL MUSCLE IS EXERCISED

...the transverse relaxation time (T2) of tissue water increases, producing increased signal intensity on T2-weighted magnetic resonance (MR) images (6, 9, 10, 14, 28). In healthy individuals this increase is affected by parameters such as the intensity (8, 9, 14, 18, 28), duration (13, 18, 28), and type of exercise (31, 35). Exercise-induced T2 change can also be affected by the presence of disease (7, 11). During dynamic exercise, a muscle's T2 is influenced by exercise intensity and duration; however, the total amount of work performed by the muscle does not appear to affect its T2 (18, 28). This has been shown in a small muscle working at a specific exercise intensity and duration, which exhibited a T2 increase that was the same as that in a large muscle working under identical conditions (28). This observation is important because the different muscles in the human body vary greatly in size and strength, and during systemic exercise each might be expected to contribute differently to the total work performed. Magnetic resonance imaging (MRI) has great potential for evaluating muscle recruitment patterns (9) as well as assessing regions of pathology (6, 24) and injury (35); however, the physiological factors that can affect exercise-induced T2 changes need to be identified and quantified. In this study, the effect of muscle glycogen concentration on exercise-induced T2 changes was evaluated. Although glycogen change has been suggested as a possible cause of exercise-induced T2 increases (11, 14, 36), glycogen content has not been previously correlated with T2 because of the difficulty of obtaining muscle glycogen concentrations with traditional biopsy methods. With recent developments in natural abundance 13C magnetic resonance spectroscopy (MRS), it is now possible to noninvasively measure muscle glycogen (30, 37).

It is generally accepted that exercise-induced T2 increases reflect alterations in the amount and compartmentation of muscle water (1, 6, 11, 12, 19, 23, 27); however, the precise nature of shifts in tissue water and the relationship among exercise, T2, and muscle water content are not well understood. Glycogen, a major energy substrate in liver and muscle, is also a highly hydrated macromolecule. When present in substantial amounts, glycogen binds water molecules and could, in principle, provide pathways for enhanced proton relaxation. The relationship of water content and glycogen concentration to T2 was first reported by Sostman et al. (36) in a 1986 study in mouse liver. The study found that there was a modest but significant (P ≤ 0.01) correlation between liver T2 and its water and glycogen contents (36). Although it is possible that changes in muscle glycogen content could also influence tissue water, the relationship has not been studied in muscle tissue. In human subjects, exercise at 50% of maximum voluntary contraction (MVC) can deplete muscle glycogen by 50–60 mM in as little as 10 min of exercise (29, 31). However, most existing studies of T2 and exercise have reported significant T2 increases on the basis of lighter workloads (9, 18, 27, 28) that do not rapidly deplete muscle glycogen (30). Therefore, knowledge of the relationship between muscle glycogen content and T2 increases after low-intensity exercise could provide insight into the nature of exercise-induced water shifts.

With the use of combined MRI and MRS, the objectives of this study were 1) to examine the effect of two different preexercise glycogen concentrations (normal resting glycogen and low glycogen) on the T2 increase induced by identical 5-min bouts of plantar flexion exercise; 2) to correlate the steady-state concentrations of gastrocnemius glycogen, glucose 6-phosphate (G-6-P), other phosphorus metabolites, and H+ with muscle T2 relaxation times after exercise; and 3) to identify or rule out the resting glycogen concentration as a factor to be considered in conducting MRI studies of exercise.

### METHODS

Subjects. Seven untrained healthy subjects, four men and three women, participated in this study. Subjects were 24 ± 2
yr of age (17–33 yr), weighed 69 ± 4 kg (59–89 kg), and were 173 ± 3 cm (170–180 cm) in height. All subjects were screened according to exercise and dietary habits (30). Those who trained aerobically >5 days/wk and/or those on specialized diets were excluded from the study. Subjects were also screened according to the Yale-New Haven Hospital standard criteria for MRI. On acceptance into the study, each subject gave informed written consent in accordance with a protocol approved by the Yale University Human Investigations Committee.

Exercise protocols. Each subject performed two different exercise protocols. One, a standardized low-intensity plantar flexion protocol, was intended to elicit a known increase in the T2 time of the gastrocnemius (20, 28). The other, a high-intensity plantar flexion protocol, was performed after the initial low-intensity protocol to significantly deplete glycogen stores in the gastrocnemius (20, 29, 31). After a subsequent rest period, the low-intensity protocol was repeated.

During the low-intensity exercise protocol, the medial and lateral heads of the gastrocnemius were rotated through a full range of motion against a constant resistance equal to 25% of each subject’s MVC at an average rate of 35 contractions/min by using a pedal apparatus (28). Dynamic MVC values, obtained for each subject before the study by established methods (28, 30), were 134 ± 10 kg (102–177 kg), yielding 2.87 ± 0.21 kJ total work for the 5-min protocol. To permit exercise within an MR imager, the pedal ergometer was constructed from nonmagnetic materials (28). Resistance was generated by use of a sealed pneumatic cylinder/piston assembly that allowed air pressurization to the desired workload (28). Resting MR images were obtained while subjects lay quietly in the imager. Subjects then performed 5-min plantar flexion exercise, followed by a postexercise MR image.

During the high-intensity exercise protocol (glycogen-depletion protocol), the gastrocnemius was rotated through a full range of motion by having each subject perform single-leg toe raises while standing (29, 31). During exercise, subjects were required to maintain full extension of their knee to isolate the work. Each subject performed the toe raises for 1 min and then rested for 1 min, repeating this minute-on/minute-off rotation until their gastrocnemius glycogen levels had been reduced by ~40%. The on/off exercise protocol allowed for the depletion of glycogen while avoiding anaerobic fatigue. The duration of exercise required to deplete glycogen was 12 ± 1 min. By manipulating their own body weights with an individual muscle (gastrocnemius), subjects were working at 52 ± 2% of measured MVC (44–58%) to perform 17.2 ± 1.3 kJ total work. Resting MR spectra were obtained while subjects lay quietly in the MR spectrometer. Subjects were then removed from the spectrometer so they could perform the toe raises while standing. After the high-intensity protocol, subjects were repositioned in the MR spectrometer and postexercise spectra were obtained.

Experimental protocol. To set up the two gastrocnemius glycogen conditions, each study was performed in four stages (Fig. 1): 5-min standardized exercise at 25% of MVC (normal glycogen condition); glycogen-depleting exercise at 50% of MVC (glycogen-depletion protocol); 65-min rest period (recovery period); and repeat of 5-min standardized exercise at 25% of MVC (low-glycogen condition). Magnetic resonance spectroscopic information was obtained on two different instruments: a 1.5-T GE Signa system (General Electric, Milwaukee, WI) for MRI and a 4.7-T Bruker Biospec spectrometer (30-cm-diameter magnet bore) for MRS. Studies were performed according to the following time line. First, resting MR spectra were obtained to document basal metabolite levels (Fig. 1). Second, an MR image was obtained at rest, followed by 5-min plantar flexion at 25% of MVC and a post-5-min-exercise MR image (Fig. 1). Third, post-5-min-exercise MR spectra were obtained, and subjects were asked to perform the glycogen-depletion exercise protocol, which was followed by post-glycogen-depletion MR spectra (Fig. 1). Fourth, MR images were acquired every 15–18 min over the next 65- to 75-min rest period. Fifth, before a second 5-min exercise protocol, MR spectra were obtained. Sixth, an MR image was obtained, followed by a second 5-min plantar flexion at 25% of MVC (Fig. 1) and a postexercise MR image. Finally, MR spectra were collected to complete the protocol (Fig. 1). The MR images were used to assess exercise-induced T2 changes (ms). Natural abundance 13C-MR spectra were obtained to assess gastrocnemius glycogen concentrations (mM), and 31P-MRS was used to assess gastrocnemius pH as well as concentrations of creatine phosphate (PCr), P, and G-6-P (mM).

MRI. Single-slice MRI was performed on a 1.5-T GE Signa system (General Electric) at 63 MHz. Subjects were positioned supine within the magnet, with an extremity coil positioned midcalf and with one foot positioned in the pedal assembly of the exercise apparatus. Transverse midcalf images (1.5 cm slice thickness; field of view = 20 cm; 128 × 256 matrix; NEX = 1) were obtained by using a multiple spin-echo sequence (repetition time = 1,000; echo time = 30, 60, 90, 120; total scan time = 2 min 20 s). T2 values before and after exercise were calculated in a region of interest (ROI = 0.8 × 0.8 × 1.5 cm) within the gastrocnemius. Each ROI was selected so that visible blood vessels and fat were avoided. In exercised muscles, several ROIs were selected to compare the uniformity of the T2 change throughout the muscle. T2 values were calculated by fitting four data points (4 echoes) to a monoexponential decay by using a least-squares algorithm.

MRS. Interleaved natural abundance 13C/31P-NMR spectroscopy was performed at 4.7 T. During the measurements, subjects remained supine, with one leg positioned within the

![Fig. 1. Experimental time course. Magnetic resonance (MR) images spectra were obtained at different time points on 2 separate MR systems. MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MVC, maximum voluntary contraction; 25% MVC, 5-min plantar flexion at 25% MVC at rest and after 1–1.25-h resting recovery from glycogen-depleting exercise; 50% MVC, 15-min glycogen-depleting plantar flexion at 50% of MVC.](http://jap.physiology.org/)
homogeneous volume of the magnet and with the lower portion of that leg resting on the stage of a surface coil radio frequency (RF) probe. The spectrometer was equipped with an RF relay switch that allowed the hardware to switch the RF power between $^{13}$C (50.4 MHz) and $^{31}$P (81.1-MHz) channels with a 10-ms switching time (4). The pulse sequence allowed switching of the acquisition parameters and preamplifiers between the two channels during the 10-ms switching time. A 5.1-cm-diameter circular $^{13}$C/$^{31}$P double-tuned surface RF coil was used for interleaved acquisitions (4). The double-tuned circuit was optimized for the $^{31}$P channel so that the NMR sensitivity would be enhanced to detect G-6-P. Shimming, imaging, and $^1$H decoupling at 200.4 MHz were performed with a $9 \times 9$-cm series butterfly coil. Proton line widths were shimmed to <50 Hz. A microsphere containing $^{13}$C and $^{31}$P reference standards was fixed at the center of the double-tuned RF coil for calibration of RF pulse widths. Subjects were positioned by an image-guided localization routine that employed a T1-weighted gradient-echo image (repetition time = 82 ms, echo time = 21 ms). The subjects' lower legs were typically positioned so that the isocenter of the magnetic field was ~1 cm into the medial head of the gastrocnemius muscle. By determining the 180$^\circ$ flip angles at the center of the observation coil from the microsphere standard, RF pulse widths were set so that the 90$^\circ$ pulse was sent to the center of the muscle. This maximized suppression of the lipid signal that arises from the subcutaneous fat layer and optimized signal from the muscle.

The interleaved $^1$H-decoupled $^{13}$C/$^{31}$P RF pulse sequence was designed so that 72 $^{31}$P transients were acquired during the same period in which 2,736 $^{13}$C transients were obtained (38 $^{13}$C scans/31P relaxation period), and free induction decays were saved separately in two blocks (4). The repetition time for $^{31}$P acquisition was 4.6 s to allow for the long T1 of 31P resonances. The acquisition times of both channels had to be the same period in which 2,736 $^{13}$C transients were obtained and optimized signal from the muscle.

Power deposition, as determined relative to Pi, rather than to PCr. Pan et al. (26) have shown that, over the pH range of 6.60–7.05, exercise-induced changes in the chemical shift of Pi paralleled those of G-6-P. The basal G-6-P concentration was determined by subtracting resting spectra from spectra obtained after exercise, the increase in G-6-P after exercise was cleanly resolved at 2.29 ppm. Measurement of G-6-P by $^{31}$P-NMR has been validated in an animal model by comparison with chemical assay of G-6-P in rat muscle frozen in situ (2).

Statistical analysis. All data are presented as means ± SE of all calculated T2 values and physiological concentrations. T2 values at different points along the time line are compared with paired two-tailed t-tests and with ANOVA. Physiological concentrations at different points along the time line are compared similarly.

RESULTS

MRS and MRI baseline values were obtained from each subject before the first exercise protocol. Mean gastrocnemius glycogen was 70.5 ± 6.8 mmol/kg wet wt at rest, whereas resting levels of PCr, P$_i$, and G-6-P were 15.2 ± 3.3, 2.0 ± 0.4, and 0.093 ± 0.010 mmol/kg wet wt, respectively (Table 1) and the resting gastrocnemius pH was 7.04 ± 0.01 (Table 1). From resting MR images, the mean T2 was 29.9 ± 0.4 ms. Immediately (1.2 min) after 5-min plantar flexion at 25% of MVC (normal resting glycogen condition), T2 times rose significantly in the gastrocnemius to 35.8 ± 0.6 ms (P = 0.001) [change in ($\Delta$)T2 = 5.9 ± 0.8 ms] (Fig. 2C). MR

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<th>Table 1. Concentrations of intramuscular metabolites and pH values at different time points during exercise and recovery protocol</th>
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Values are means ± SE with correlation to transverse relaxation time (T2) in parentheses. Gly, glycolgen; G-6-P, glucose 6-phosphate; PCr, phosphocreatine. *P ≤ 0.05 relative to resting value.
There was a 3.4-fold rise in G-6-P (mmol/kg wet wt) (Fig. 2A) immediately after 15-min plantar flexion at 50% of MVC (Ex50), 1 h into resting recovery, and 10 min after a 2nd bout of plantar flexion at 25% of MVC. Values are means ± SE; n = 7. B: gastrocnemius glycogen concentrations (mmol/kg wet wt) at rest, 10 min after 5-min plantar flexion at 25% of MVC, immediately after 15-min plantar flexion at 50% of MVC, 1 h into resting recovery, and 10 min after a 2nd 5-min bout of plantar flexion at 25% of MVC. Values are means ± SE; n = 7. C: transverse relaxation time (T2) times (ms) at rest and after exercise (n = 7). T2 recovery was followed over 65 ± 4 min resting recovery from glycol-depleting exercise. Values are means ± SE. *P ≤ 0.05.

Spectra, obtained ~10 min after cessation of exercise, revealed that, although there were no significant changes in gastrocnemius glycogen (67.7 ± 7.4 mmol/kg wet wt) (Fig. 2B), pH (7.03 ± 0.01), PCR, or P, (Table 1), there was a 3.4-fold rise in G-6-P (0.317 ± 0.040 mmol/kg wet wt; P ≤ 0.001) (Fig. 2A, Table 1). Subjects next performed 12 ± 1 min plantar flexion (single-leg toe raises) at 52% ± 2% of MVC (glycogen-depletion protocol). After exercise (~7 min), glycogen levels in the gastrocnemius declined to 57% of resting concentration (40.4 ± 5.3 mmol/kg wet wt; P ≤ 0.01) (Fig. 2B). PCR and P, (Table 1) were not significantly increased after the high-intensity protocol; however, pH was decreased (6.84 ± 0.04; P ≤ 0.001), and G-6-P was further increased to 5.2-fold above resting concentration (0.487 ± 0.154 mmol/kg wet wt; P ≤ 0.05) (Fig. 2A). MR images, obtained ~15 min after cessation of exercise, revealed that T2 remained significantly elevated (36.3 ± 1.37 ms; P ≤ 0.001) despite 15 min of resting recovery (Fig. 2C).

To allow exercise-induced T2 increases to return to baseline values, subjects were asked to continue to rest quietly over the next 50 min (65-min total recovery time). Gastrocnemius T2 recovery was monitored with MR images at 35 (33.3 ± 0.8 ms; P ≤ 0.01), 50 (32.2 ± 0.5 ms; P ≤ 0.01), and 65 min (30.4 ± 0.4 ms; not significantly different vs. resting T2) (Fig. 2C). The T2 recovery rate was ~0.115 ms/min during the period from 15 to 65 min of resting recovery. MR spectra were obtained at 58 min of resting recovery. Whereas gastrocnemius glycogen remained significantly depleted (45.8 ± 5.3 mmol/kg wet wt; P ≤ 0.05) (Fig. 2B) after ~1 h of recovery (+5.4 mmol/kg wet wt, recovery), muscle pH (7.03 ± 0.01) and G-6-P (0.115 ± 0.014 mmol/kg wet wt) had returned to resting levels (Table 1, Fig. 2A). Gastrocnemius PCR, P, and P, did not change significantly during the recovery period (Table 1).

After the MR images at 65 min of recovery, when T2 times had returned to baseline values and glycogen remained low (low-glycogen condition), subjects repeated the initial exercise protocol (5-min plantar flexion at 25% of MVC). Immediately (1.2 min) after the second 5-min exercise protocol, T2 times were again significantly elevated (35.4 ± 0.8 ms; P ≤ 0.001; ∆T2 = 5.0 ± 0.8 ms) (Fig. 2C). A final MR spectrum, obtained ~10 min after cessation of the second 5-min protocol, revealed that gastrocnemius glycogen increased significantly compared with immediately before the protocol (57.6 ± 5.3 mmol/kg wet wt; P ≤ 0.05; ∆glycogen = 11.8 mmol/kg wet wt) (Fig. 2B). After exercise, G-6-P again rose significantly (0.224 ± 0.041 mmol/kg wet wt; P ≤ 0.05) (Fig. 2A, Table 1). Gastrocnemius PCR, P, and pH were not altered as a result of the second exercise protocol (Table 1). The T2 increases induced by 5-min dynamic plantar flexion at 25% of MVC were not significantly different at 70.5 vs. 45.8 mM glycogen. When T2 time (ms) was correlated with PCR, P, and glycogen concentrations (mmol/kg wet wt), the correlation was weak (r = 0.098, 0.215, and 0.210, respectively). However, there was a marginal correlation between T2 time (ms) and pH (r = 0.581) and a good correlation with G-6-P concentration (r = 0.861) (Table 1).

**DISCUSSION**

The results of this study were essentially negative, demonstrating no correlation between the observed changes in T2 and either the primary metabolite assessed (glycogen) or two of the secondary metabolites (PCR, P,). and only a marginal correlation between T2 and pH. However, T2 and G-6-P were correlated, suggesting that exercise-induced transport of glucose into the exercised muscle (29, 33, 34) may play a role in exercise-induced T2 increases. To obtain the primary result, we exploited the longer recovery time of muscle glycogen after intensive exercise and compared T2 changes after two identical exercise regimes: one performed from a normal resting glycogen concentration (70.5 ± 6.8 mmol/kg wt) and the other performed from a significantly lower glycogen concentration (45.8 ±
glycogen content varied between 1.1 and 4.5% (61–250 mmol/kg wet wt; P ≤ 0.05). Exercise-induced T2 responses were similar (ΔT2 = 5.0 ± 0.8 and 5.9 ± 0.8 ms, respectively) after standardized 5-min exercise protocols at both glycogen concentrations. They were also similar to those in an earlier study (ΔT2 = 6.0 ± 1.4 ms; 5-min plantar flexion) (28), demonstrating that a standardized plantar flexion protocol is capable of producing a predictable T2 increase in the gastrocnemius. In addition, the postexercise T2 increase was seen under three different conditions, in which glycogen remained unchanged (<4% decrease; ΔT2 = 5.9 ± 0.8 ms), decreased by 40% (ΔT2 = 6.4 ± 1.2 ms 12 min after cessation of exercise), and increased by 25% (ΔT2 = 5.0 ± 0.8 ms). Although it is widely held that the products of glycogenolysis, particularly lactate, are important effectors of postexercise water shifts (5, 11, 22, 31, 39), the role of glycogen in exercise-induced T2 increases has not been examined other than as a source of lactate (11, 12). Understanding the contribution of the preexercise glycogen concentration to exercise-induced T2 changes is important in the development of MRI techniques to assess muscle recruitment patterns. Although this study does not examine the effect of extremely high glycogen concentrations (carbohydrate loaded) or low glycogen concentrations (exhaustion), it does demonstrate that, in a specific muscle within a normal range of glycogen concentrations, similar T2 increases might be expected from a standardized exercise protocol independent of that muscle’s preexercise glycogen concentration.

When compared with the 1986 paper by Sostman et al. (36), the results of this study suggest that, if glycogen concentration exerts an effect on postexercise T2 changes, it is minimal compared with other effectors. In the study by Sostman et al., T2 times ranged from 27.7 to 31.5 ms (ΔT2 = 3.8 ms) because the liver glycogen content varied between 1.1 and 4.5% (61–250 mmol/kg wet wt; Δglycogen = 189 mM) (36). A subsequent study by Gore et al. (14) reported the efficacy of glycogen as a relaxation agent in solution and showed that there was a relationship between the percent glycogen and the transverse relaxation rate [1/T2 (s)]. In that study, the glycogen content ranged from 0 to 11% (>600 mM), and at 11% glycogen the solution T2 remained >300 ms, suggesting that in physiological systems glycogen is only a minor relaxation agent and that protein-water interactions may be more important. A possible explanation for the different findings in this study is that the glycogen content (%glycogen) in skeletal muscle at rest is lower (%glycogen = 0.7–3%; 27–400 mmol/kg wet wt) than in liver, where it can approach 8% (~450 mmol/kg wet wt) after a meal. The structural differences between liver glycogen particles (α-particle rosettes composed of β-particles) and muscle glycogen particles (single β-particles) may also contribute to the different results between this study and the earlier study (22, 36). Another result of the present study that does not support glycogen as an effector of T2 increases is the glycogen response after the second low-intensity exercise protocol, which was reversed such that gastrocnemius glycogen increased (Δglycogen = +11.8 mM) concurrently with T2. The effect of active recovery on glycogen synthesis rates has been reported with glucose infusion (17, 38), and the active resynthesis rates seen in this study were similar (14.9 ± 6.7 mmol·kg wet wt⁻¹·h⁻¹) to previously reported rates during the same type of exercise protocol (11.8 ± 2.2 mmol·kg wet wt⁻¹·h⁻¹) without glucose infusion (30). The results of the present study therefore demonstrate that, within the range of glycogen concentrations studied, changes in muscle glycogen, composed solely of β-particles, do not exert an effect on changes in muscle T2.

This study does not contradict the idea that exercise-induced T2 increases are driven by water shifts that result from transient changes in tissue osmolality (osmosis) and perfusion (22, 32). Previous studies have shown that, although exercise stimulates increased blood flow and tissue perfusion, these increases are not required for a T2 increase to be observed (1, 10). This points to tissue osmolality, an important factor in driving the water shifts that result from exercise. The earlier studies also found that, when blood flow was restored, there was an increase in T2 (1, 10), suggesting an additive effect. The correlation between G-6-P concentration and T2 increases (0.861) is consistent with the idea that the exercise-induced change in intracellular osmolality may affect the water shifts that accompany exercise, and the time course of return of G-6-P to baseline values is similar to the time course for lactate recovery that was reported by Pan et al. (25). However, the G-6-P increase is small in relation to normal tissue osmolality and would account for a <0.3% change in total osmolality. It should be noted also that, although we saw only a marginal correlation between T2 and pH (0.581), transient changes in pH could have initiated intracellular mechanisms that may have persisted, causing or contributing to the observed T2 increase. A transient pH change of this nature would not have been detected in our study because of the time resolution that was required to obtain the G-6-P data.

Exercise-generated muscle lactate may be the best candidate for what drives the water shifts that occur after exercise. When the lactate recovery time course (25) is compared with T2 recovery patterns seen in previous MRI studies (28), there is a high degree of correlation. This correlation suggests that exercise may initiate changes in tissue metabolites that, in turn, alter tissue osmolality, and this may cause shifts in tissue water. It should be noted that the Pan et al. (26) study had subjects exercising to exhaustion, producing lactate concentrations that approached 30 mM and corresponded to as much as a 10% change in osmolality. It is unlikely that the present exercise protocol produced such high lactate concentrations; hence, the overall change in tissue osmolality would be significantly less. Glycolytic intermediates may also make a transient contribution to the change in osmolality. On the whole, the response to exercise by the various metabolites involved in muscle contraction may act in concert to initiate water shifts. Ultimately, all of these...
metabolites constitute <5% of the muscle, whereas protein constitutes up to 20%. Whether the exercise-induced changes in intracellular composition or osmolarity affect the manner in which water protons are relaxed at protein or other macromolecular interfaces remains an open question. However, lactate accumulation as an osmotic force leading to increased intramural free water content remains a plausible mechanism that is supported by the observed correlation between G-6-P and T2.

In summary, the glycogen concentration present in the gastrocnemius at the start of a standardized 5-min protocol did not significantly affect the change in T2 resulting from the 5-min protocol. Although the intensity, duration, and type of exercise affect the exercise-induced T2 increases, within the range examined in this study, changes in glycogen concentration per se did not exert an effect on the skeletal muscle T2 response to exercise, which was similar regardless of whether there was an increase or a decrease in glycogen concentration.

Address for reprint requests: T. B. Price, Yale Univ. School of Medicine, Dept. of Diagnostic Radiology, 333 Cedar St., New Haven, CT 06510 (E-mail: price@boreas.med.yale.edu).

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