Skeletal Muscle Glycogen Loss Evoked by Resistance Exercise

Per A. Tesch¹, Lori L. Ploutz-Snyder², Linda Yström³, Michael J. Castro⁴, and Gary A. Dudley⁵,⁶

¹Dept. of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden; ²Dept. of Biological Sciences, Ohio University, Athens, OH 45701; ³NASA, Kennedy Space Center, FL 32899; ⁴Dept. of Exercise Science, University of Georgia, Athens, GA 30602.

Reference Data

ABSTRACT
Biopsies were taken from the left m. vastus lateralis of 9 young men and analyzed for mixed muscle and for single fiber glycogen to infer recruitment, especially among fast-twitch subtypes, during knee extensions with loads of 30, 45, and 60% of 1-repetition maximum. The relative decline in mixed muscle glycogen was related to relative exercise load, as were the percentage of fast-twitch fibers showing glycogen loss and the relative cross-sectional area (CSA) of m. vastus lateralis occupied by type Ila or by fast-twitch fibers that showed glycogen loss (p ≤ 0.0478, r ≥ 0.50). The relative decline in mixed m. vastus lateralis glycogen was related to the percentage of both fast-twitch fibers and type Ila fibers, and the relative CSA of m. vastus lateralis occupied by type Ila fibers that showed glycogen loss (p ≤ 0.0436, r ≥ 0.51). Type I and Ila fibers were used for all 3 bouts. Type Ila + Ilb fibers showed glycogen loss for the heaviest load. The results suggest that mixed muscle glycogen loss is related to load, mainly due to fast fiber usage. It also appears that the general understanding put forth for cycling and running—that fast-twitch fiber type use depends on exercise intensity—holds for resistance exercise. Because type Ila + Ilb fibers showed glycogen loss at loads of 60% of maximum, it is suggested that fast-twitch subtypes are used at lower loads than generally appreciated.

Key Words: weight training, skeletal muscle fiber types, recruitment

Introduction
Fiber type utilization during resistance exercise, as described in most textbooks of exercise physiology, is based on studies of motor unit recruitment during isometric actions, as assessed by EMG, or fiber type glycogen loss during running or cycling (e.g., 11, 20). Representative figures generally indicate that fast fibers, especially type Iib, are not used until high forces are needed. The limited study of fiber type recruitment during resistance exercise using glycogen depletion may stem in part from Keul et al.'s (17) suggestion 20 years ago that the energy demands of resistance exercise, simulating competition, could be met by high-energy phosphates stored in skeletal muscle. However, the results of more recent studies clearly show that an acute bout of resistance exercise decreases mixed muscle glycogen about 30% (10, 29).

With this in mind, Robergs et al. (25) recently examined glycogen depletion in slow and fast fibers for knee extensions performed with a load equal to 70 or 35% of the 1-repetition maximum (1-RM). Total work was comparable between regimens because twice as many repetitions were performed with the lower load. Fast fibers showed greater glycogen loss than slow fibers for both loads. While this may in part reflect the greater glycolytic capacity of fast vs. slow fibers, it seems surprising that fast fibers would be used to act against a load that is 35% of maximum for 6 sets of 12 reps. However, Golnick et al. (13) reported that fast fibers begin to be recruited when force exceeds 20% of maximal voluntary isometric. The results of these two studies suggest that fast fibers are used during resistance exercise at loads much less than maximal.

We sought to expand these earlier studies by examining fast fiber subtype use during resistance exercise. Specifically, this study examined glycogen loss, mixed muscle or single fiber, during moderate intensity resistance exercise to assess which fiber type or types were mainly responsible for degradation of this fuel source and infer recruitment, especially among fast-twitch subtypes.

Methods
Subjects and General Protocol
Nine healthy young men (77 ± 3 kg) who had just completed 9 weeks of resistance training served as subjects (22). On the test day, each performed 5 sets of 10 concentric actions with the left m. quadriceps femoris with each of 3 loads. Biopsies of the left m. vastus lateralis were obtained before exercise, and after each 5 × 10 bout.

Prior to training, the young men were untrained in lower body resistance exercise; some had been seden-
tary for over 15 years while others were engaged in recreational activities. The procedures of the study and the risks and benefits of participation were explained, and informed written consent was obtained from each subject. This study was approved by the Human Research Review Board, Kennedy Space Center, FL.

**Exercise Testing**

All strength and exercise testing was conducted on a modified Nautilus™ knee extension machine. Ball bearings were used to replace the silicone sleeve at the lever’s axis of rotation, and the oblong cam was replaced with one of constant radius to provide consistent external resistance throughout the range of motion. Immediately after training, the left *m. quadriceps femoris* was tested for the maximum load that could be raised once, as done previously (4, 6, 12, 22–24). Briefly, the concentric 1-RM was determined as the heaviest load the subject could lift one time as the lever arm of the ergometer passed from 90° below horizontal to 0° (horizontal). Subsequently, subjects performed 5 sets of 10 concentric actions with each of the 3 loads (approx. 30, 45, and 60% of concentric 1-RM) which had been shown to require differential use of *m. quadriceps femoris* (22, 23).

By holding the number of sets and repetitions constant over bouts and varying the load, it was possible to examine the influence of exercise intensity per se on glycogen loss. Two minutes of rest were given between sets and 45 min between bouts. Exercise began with the 30% load, then subjects rested for 45 min, used the 45% load, rested another 45 min, and completed the exercise regimen with the 60% load. For each concentric action, subjects raised the load with the lever arm moving from 90° below horizontal to 0°. A hydraulic device lowered the load so that the next concentric action could begin.

**Skeletal Muscle Biopsies**

Samples were taken from the left *m. vastus lateralis* using the percutaneous biopsy technique, as described by Bergstrom (2). One sample was taken at rest before any exercise, and another immediately after the 5th set of each exercise bout. Each sample was divided into two parts, one for histochemical analysis and the other for mixed muscle glycogen determination. Samples for histochemical analysis were mounted on a tongue blade using a medium of OCT compound and tragacanth gum and were frozen in 2-methyl butane cooled in liquid nitrogen. The portion of the sample for mixed muscle glycogen determination was frozen directly in liquid nitrogen. All samples were stored at −70 °C until analyzed.

Each mounted sample was processed via histochemical and microdensitometric techniques to determine fiber type, cross-sectional area (CSA), and glycogen. Briefly, samples were warmed to −20 °C and serial-sectioned at 10 μm. Fiber type and CSA were determined using histochemical analysis for myofibrillar actomyosin ATPase (mATPase) as done previously (3, 16, 22). Fibers were classified as type I, IIA, IIa, or IIb with recognition of the recent observation that type IIb myosin heavy chain (MHC) of human skeletal muscle is equivalent to rat IIX MHC, not IIb MHC (8). Single fiber glycogen was determined using a PAS stain and the microdensitometric analysis previously described (32, 33). Fiber type specific measures for glycogen were obtained by matching fibers in serial sections assayed for mATPase or glycogen.

For all analyses, assays were run and images of the sections were digitized in 1 day. Images were subsequently analyzed using the public domain NIH Image program (written by Wayne Rasband of U.S. National Institutes of Health, and available from the Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disk from NTIS 5285 Port Royal Rd., Springfield, VA 22161, part no. PB93-504868), as done previously (3, 16, 22, 31). Densitometric measurements for glycogen determination were made after calibration of the relation between gray level and optical density (OD) using neutral density filters. The OD of pixels in the blank field was subtracted from pixel values of a given image to correct for camera field variation. All microscope illumination was provided through a narrow pass interference filter with peak emission at 510 μm.

Fiber type percentage was determined from a mean of 447 ± 98 (mean ± SE) fibers per subject. Type IIb fibers were rare and thus combined with type IIa. Used for CSA analysis were 167 ± 35, 157 ± 50, and 69 ± 33, type I, IIA, and IIa + IIb fibers, respectively. Fiber type percentage and CSA data were used to calculate relative fiber type CSA for each subject. Type I, IIA, and IIa + IIb glycogen content was determined from 67 ± 10, 63 ± 12, and 18 ± 4 fibers for each subject, respectively, and expressed in OD units. The mean OD and its standard deviation for each fiber type for each subject preexercise was used to establish if a fiber showed glycogen loss.

Within each fiber type and subject, fibers with an OD 2 standard deviations below the preexercise mean were classified as showing glycogen loss, and thus having performed recent contractile activity (32). The percentage of fibers showing glycogen loss after a given bout of exercise was calculated for each fiber type by subject. The percentage of *m. vastus lateralis* occupied by the fibers of a given type that showed glycogen loss was calculated by subject using relative fiber type CSA and percent of fibers of a type showing glycogen loss. We were able to match sufficient fibers in the mATPase and PAS sections to obtain data on 23 of the 36 samples.

Whole muscle glycogen was determined for both pre- and postexercise samples. After freeze-drying, blood clots and connective tissue were dissected out under a light microscope at constant room temperature of 20 °C and humidity of 30%. Duplicate portions of samples approximately 1 mg (1.19 ± 0.29 mg) were weighed on a Cahn® electrobalance and hydrolyzed at 100 °C for 2 hours in 1 M hydrochloric acid. Glycogen
concentration was analyzed in duplicate as glucose residues using an enzymatic fluorometric method (9, 19).

Statistics
Load, relative to the 1-RM, and percentage decrease in mixed m. vastus lateralis glycogen content data were analyzed using a one-way ANOVA with repeated measures over bout. Relations between variables were assessed with simple linear regression.

Results
Average Responses by Exercise Bout
Load, relative to the 1-RM, averaged 30 ± 1, 45 ± 1, and 60 ± 2% for Exercise Bouts 1, 2, and 3, respectively. The decline in mixed m. vastus lateralis glycogen relative to rest increased (p = 0.0109) over the 3 exercise bouts (Figure 1). Type Ila fibers of the m. vastus lateralis showed glycogen loss of about 40% for Bouts 1 and 2 while approximately 70% of these fibers showed glycogen loss after Bout 3 (Figure 1). Type Ilb + Iib fibers showed almost no glycogen loss for Bouts 1 (0%) and 2 (2%), while approximately 30% of these fibers showed glycogen loss after Bout 3. The percentage of type I fibers showing glycogen loss was comparable over bouts, averaging 35 ± 8%. Similar trends were found for the relative CSA of m. vastus lateralis occupied by a given fiber type that showed glycogen loss, with type I fibers averaging 16 ± 5% over exercise bouts (Figure 1).

Biopsy Data and Relative Exercise Load
The relative decline in mixed m. vastus lateralis glycogen was positively related to relative exercise load (% decrease in glycogen = 0.601 [% 1-RM] –3.097%, r = 0.496, p = 0.0099) (Figure 2). The percentage of fast-twitch fibers of m. vastus lateralis showing glycogen loss was also related to relative exercise load (% fast-twitch = 1.513 [% 1-RM] –28%, r = 0.587, p = 0.0168) (Figure 3). The same was the case for the relative CSA of m. vastus lateralis occupied by type Ila and by fast-twitch fibers showing glycogen loss (% CSA type Ila = 0.676 [% 1-RM] –6.83%, r = 0.502, p = 0.0478, and % CSA fast-twitch = 1.043 [% 1-RM] –22%, r = 0.578, p = 0.0303) (Figure 3).

Relationships Between Biopsy Data
The relative decline in mixed m. vastus lateralis glycogen was related to the percentage of fast-twitch fibers that showed glycogen loss (% decrease in glycogen = 0.201 [% fast-twitch] +17%, r = 0.510, p = 0.0436), the percentage of type Ila fibers that showed glycogen loss (% decrease glycogen = 0.205 [% type Ila] +15%, r = 0.567, p = 0.0220), and the relative CSA of m. vastus lateralis occupied by type Ila fibers that showed glycogen loss (% glycogen depletion = 0.349 [% CSA type Ila] +17%, r = 0.566, p = 0.0222) (Figure 4).

Discussion
Resistance exercise is gaining support as an important component of physical fitness and health, especially
Figure 2. % Decline in mixed m. VL glycogen plotted vs. % of 1-RM for each bout of 5 sets of 10 concentric actions with left m. quad. femoris; % decrease in VL mixed Gly = 0.601 [% 1-RM] -3.097%, r = 0.496, p = 0.0099.

among middle-age and older individuals (30). It has also received increased attention as a research topic (18). Yet there are still areas of gross lack of knowledge about resistance exercise. For example, there are contrasting views as to whether neural factors are central in adapting to resistance training (15, 22). It is also generally held that type IIb fibers are recruited so that high forces can be developed, yet several studies have shown that their percentage is reduced by resistance training (18). As it turns out, data on muscle fiber recruitment for resistance exercise are limited.

In light of the above, we took biopsies of m. vastus lateralis before and after several sets of knee extensions to assess muscle fiber glycogen loss, as reflected by reduced OD, to gain insight into fiber use, especially among the fast-twitch subtypes. Our results show that loss of glycogen in type IIb + IIb fibers is dependent on exercise load (Figure 1). With light loads, type IIab + IIb fibers showed no glycogen loss. This was not the case for type I or type IIa fibers, with about half of type IIa fibers showing glycogen loss when the load approached 50% of maximal. When load approached that which might be used in training by novices, or considered moderate by experienced lifters, type Ila + IIb fibers showed glycogen loss.

We interpret these results as suggesting that type IIa + IIb fibers are recruited for moderate to heavy loads as might be used in resistance exercise, while type IIa are used for even, light lifts. These results support the general approach of using glycogen loss data from cycling or running studies or of motor unit recruitment firing during isometric actions, as assessed by EMG, to infer muscle fiber type use during resistance exercise (11, 20). The findings of type Ila fiber use for light loads and type IIa + IIb fiber recruitment for moderate loads,

Figure 3. % Fast-twitch fibers of m. VL showing glycogen loss (Dep), and % CSA of m. VL occupied by type Ila fibers and by fast fibers showing Gly loss plotted vs. % of 1-RM used for each bout of 5 sets of 10 concentric actions with left m. quad. femoris. Upper panel: % fast fibers [% 1-RM] showing Gly loss = 1.513 – 28%, r = 0.587, p = 0.0168; Middle: % CSA Type Ila showing Gly loss = 0.676 – 6.83%, r = 0.502, p = 0.0478; Lower: % CSA fast fibers showing Gly loss = 1.043 – 22%, r = 0.578, p = 0.0303.
however, suggest fast fiber usage at exercise intensities lower than generally appreciated. These results are supported by the earlier work of Robergs et al. (25) and Gollnick et al. (13), which showed glycogen loss in fast fibers at forces as low as 20% of maximal voluntary isometric contraction.

In this study, it could be argued that defining glycogen loss as meaningful only when fiber OD was 2 standard deviations below that at rest underestimates recruitment. However, we employed the same approach as that used previously to infer fiber type recruitment during cycling (32).

It could be suggested that performance of the first 2 bouts of resistance exercise in this study resulted in sufficient glycogen loss in type I and/or IIa fiber so that type IIab + IIb fibers had to be recruited to perform Bout 3. However, it is highly unlikely that glycogen levels were sufficiently reduced in type I or IIa fibers after Bouts 1 and 2, necessitating the use of type IIb fibers to accomplish Bout 3 (5). For this to occur, levels must decrease to 100 to 150 mmol · kg⁻¹ dry wt, yet values for the present study were over 300 mmol · kg⁻¹ dry wt after Bout 3. In addition, both mixed muscle and fiber type specific glycogen loss were comparable over bouts (Figure 1).

The use of recreationally trained subjects and/or only concentric actions could also be construed as confounding the results of the present study. Subjects who had just completed 9 weeks of resistance training were studied in order to examine recruitment typical of resistance exercise. Individuals who did not yet have neural adaptations to training (22, 27) might have shown a much different response.

The use of concentric actions probably did not appreciably limit glycogen loss because shortening actions are responsible for 85% or more of the energy cost of resistance exercise (7). Our finding of fast fiber subtype use at lower loads than generally expected would not have been affected had eccentric actions been performed. If anything, this may have resulted in type IIa and/or type IIab + IIb fiber usage at even lower loads because fast fibers are preferentially recruited during lengthening actions, especially at high speeds (21).

The results of this study show that fiber type glycogen loss is related to reductions in mixed muscle glycogen content, especially of type IIa fibers (Figure 4). The 40% or so reduction in mixed muscle glycogen may seem surprising since only 50 actions were done per bout, but Robergs et al. (25) reported comparable results after performance of 6 sets of 6 knee extensions with a load of 70% 1-RM. Moreover, we found that the extent of glycogen loss was related to exercise load (Figures 2 and 3). Thus it appears resistance exercise evokes impressive use of glycogen as a fuel source (10, 29) as a function of load, and this occurs mainly in fast fibers.

The intriguing aspect of muscle fiber type use and resistance exercise is that it seems type IIb fibers are
needed to perform such exercise with moderate to heavy loads, yet if resistance training is performed, the majority of them "disappear" (1, 14). We first noted this apparent contradiction over a decade ago (28), and this does indeed appear to be the case. It may be, as previously suggested, that type IIb fibers are recruited last, not because they are ideal for force development during intense efforts but because they are poorly designed for such activity. Repeated high-force contractile activity would dictate an impressive energy demand, eventually calling upon support systems, especially the cardiovascular, to function at an impossible level. Instead, fast fibers, especially the majority of type IIb, are ill-equipped for energy supply. Thus fatigue soon ensues with their use, thereby reducing energy demand.

We took the opportunity in this study to infer the relative cross-sectional area of m. vastus lateralis that was used to perform resistance exercise. Glycogen loss of a given fiber type was considered in light of the relative area of m. vastus lateralis it occupied. The results suggest that 60% or so of the m. vastus lateralis was used to perform knee extension with a load of 60% 1-RM. This would suggest that 100% of the muscle would have to be recruited for a 1-RM. While this seems logical, it may conflict with the notion that it is difficult to recruit the entire motor pool during voluntary effort. However, these subjects had just completed resistance training and thus probably enjoyed the well-noted increase in the ability to call upon high-threshold fast-twitch motor units (26, 27).

Practical Application

The results of this study show that even moderate resistance exercise can evoke meaningful glycogen loss in fast and slow muscle fibers. While we did not address diet and exercise, it seems reasonable to suggest that weight trainees, especially those conducting exercise more vigorous than that used in this study, should consume sufficient carbohydrate to limit overtraining due to chronic glycogen depletion.

References


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Note: Lori Ploutz-Snyder is now with the Dept. of Exercise Science at Syracuse University, Syracuse, NY 13244.