Eccentric exercise-induced muscle damage impairs muscle glycogen repletion

KEVIN P. O'REILLY, MICHAEL J. WARHOL, ROGER A. FIELDING, WALTER R. FRONTERA, CAROL N. MEREDITH, AND WILLIAM J. EVANS
Human Physiology Laboratory, US Department of Agriculture/Human Nutrition Research Center on Aging, Tufts University, Boston 02111; and Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02135

O'REILLY, KEVIN P., MICHAEL J. WARHOL, ROGER A. FIELDING, WALTER R. FRONTERA, CAROL N. MEREDITH, AND WILLIAM J. EVANS. *Eccentric exercise-induced muscle damage impairs muscle glycogen repletion.* J. Appl. Physiol. 63(1): 252–256, 1987.—Five healthy untrained young male subjects were studied before, immediately after, and 10 days after a 45-min bout of eccentric exercise on a cycle ergometer (201 W). The subjects were sedentary at all other times and consumed a eucaloric meat-free diet. Needle biopsies of the vastus lateralis muscle were examined for intracellular damage and glycogen content. Immediately after exercise, muscle samples showed myofibrillar tearing and edema. At 10 days, there was myofibrillar necrosis, inflammatory cell infiltration, and no evidence of myofibrillar regeneration. Glycogen utilization during the exercise bout was 33 mmol glycosyl units/kg muscle, consistent with the metabolic intensity of 44% of maximal O_2 uptake; however, the significant glycogen use by type II fibers contrasted with concentric exercise performed at this intensity. At 10 days after exercise, muscle glycogen was still depleted, in both type I and II fibers. It is possible that the alterations in muscle ultrastructures were related to the lack of repletion of muscle glycogen. Damage produced by eccentric exercise was more persistent than previously reported, indicating that more than 10 days may be necessary for recovery of muscle ultrastructure and carbohydrate reserves.

eccentric exercise; muscle damage; muscle ultrastructure

ECCENTRIC EXERCISE INVOLVES the forced lengthening of active muscles and the transfer of external power from the environment to the subject (19). Research studies have shown that eccentric exercise is associated with muscle soreness (5, 14, 25, 30), increased plasma levels of creatine kinase (CK) and other intracellular enzymes (9, 23, 30), and ultrastructural changes indicating marked intracellular damage (12, 22, 24). These changes have been described between 1 and 7 days after the eccentric contractions took place. A recent study in our laboratory showed that an increase in muscle protein breakdown, estimated from urinary excretion of 3-methylhistidine, reached the highest levels ~10 days after 45 min of high-intensity eccentric exercise (9), suggesting that the effects on muscle were more prolonged or delayed than previously considered. In addition, postexercise muscle glycogen repletion, which is rapid following concentric contractions (3), is delayed after exercise that causes muscle damage, such as after a marathon (31). This suggests that the prolonged damage caused by eccentric exercise would have a significant effect on the rate of glycogen repletion. The hypothesis for the delay is that ultrastructural damage to the sarcolemma may impair glucose transport into the cell, affecting the energy state of the fiber and the ability of the fiber to repair itself. To this end, the present study was designed to examine the ultrastructural evidence of long-term muscle damage suggested by the delayed increase observed in actomyosin breakdown. The second objective was to measure glycogen repletion after a 10-day recovery period and to determine the fiber type-specific pattern of glycogen depletion and repletion.

METHODS

Subjects. Five untrained healthy men (aged 24 ± 2 yr) participated in the study, after giving their informed consent. The research protocol was approved by the New England Medical Center-Tufts University Human Investigation Review Committee. Aerobic capacity ($\dot{V}O_{2\,max}$) was determined by use of a continuous incremental protocol on an electronically braked concentric cycle ergometer at least 2 wk before the study. Body fat was determined by hydrostatic weighing (4, 28, 32), and muscle mass was calculated from 24-h urinary creatinine excretion after the subjects had adapted to a meat-free diet (16).

Experimental design. The subjects lived in a metabolic ward for 21 days, maintaining sedentary habits and consuming a eucaloric diet (Ensure HN, Ross Laboratories, OH). The diet provided 54% of calories as carbohydrate and 1.5 g protein·kg^{-1}·day^{-1}.

On *day 6*, the subjects participated in the eccentric exercise protocol (Table 1). The exercise was performed on an eccentric cycle ergometer (19). Each subject exercised for three 15-min periods at watt settings equal to 90, 80, and 70%, respectively, of power output at $\dot{V}O_{2\,max}$. Subjects rested for 5 min between each 15-min exercise period. Expired air was collected into Douglas bags during the final 5 min of each exercise period. Expired air volume was measured with a Tissot spirometer and analyzed for CO_2 and O_2 composition (Beckman LB-2, Anaheim, CA; Applied Electrochemistry S-3A, Sunnyvale, CA). Needle biopsies of the vastus lateralis muscle were obtained before exercise, immediately after the exercise (I-Post), and 10 days after the exercise (10D-

TABLE 1. *Eccentric exercise protocol*

	Load, W	Relative Load Setting, % max	Actual Metabolic Cost		O_2 Uptake/Watt, ml $O_2 \cdot W^{-1} \cdot min^{-1}$
			l O_2/min	% max	
0–15 min	218±18	90	1.63±0.14	48±6	7.66±0.93
20–35 min	201±15	80	1.52±0.16	42±5	7.72±1.20
40–55 min	180±12	70	1.46±0.17	41±5	8.38±1.59

Values are means ± SE; $n = 5$ subjects. Relative load set according to maximal O_2 uptake determined before the study by concentric cycle ergometer.

Post) (2, 11). Both biopsies on the day of exercise were taken at approximately the same site to minimize trauma to the subject. Ten days later, the biopsy was taken near the scar left by the previous biopsy. Biopsy material was divided into pieces for histochemical analysis, the determination of glycogen concentration, and ultrastructural analysis.

For histochemical analysis, a 5- to 10-mg piece was embedded in mounting medium (Tissue Tek, Miles, WI), frozen in isopentane cooled to the temperature of liquid nitrogen (−180°C), and stored at that temperature before being sectioned and stained. Serial cross sections (8 μm) were obtained at −20°C in a microtome cryostat (Damon/IEC, MA). The sections were incubated for myofibrillar adenosine triphosphatase (ATPase) activity to determine fiber type distribution (26). Sections were stained for glycogen content by the periodic acid-Schiff (PAS) reaction (7). Type I and II fibers were classified according to the intensity of the PAS stain in an average of 570 fibers/section as glycogen filled, moderately filled, or empty (15).

For glycogen determination, frozen biopsy material was divided into three weighed parts, hydrolyzed in 2 N HCl at 100°C for 2 h, and neutralized with 2 N NaOH. The resulting glucose was determined by an automated system (Cobas, Switzerland) utilizing the method of Passoneau and Lauderdale (27). Glycogen concentration averaged from the three pieces was expressed in glycosyl units per kilogram wet weight after tissue weights were corrected for evaporation.

For ultrastructural analysis, a fragment was finely minced (1 mm), placed in half-strength Karnovsky's fixative, postfixed in osmium tetroxide, dehydrated in alcohol, and embedded in Polygon. Thin sections were stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (JEOL EM 100). A minimum of 20 fibers was examined from each sample (33), and the preexercise sample was used as a control.

Data were expressed as means ± SE. Results were analyzed by one-way analysis of variance with repeated measures, and differences over time were identified by the Newman-Keuls test ($P < 0.05$).

RESULTS

Subject characteristics. The subjects in this study were similarly lean and untrained, as shown by their weight (70.7 ± 3.2 kg), height (180.4 ± 2.4 cm), body composition (16.2 ± 1.3% fat, 48.9 ± 1.7% muscle), and $\dot{V}O_{2\,max}$ (3.50 ± 0.23 l/min). Metabolic energy expenditure ranged from 48% of $\dot{V}O_{2\,max}$ during the first 15 min to 41% of $\dot{V}O_{2\,max}$ during the final 15 min. The metabolic cost of the eccentric exercise was 53–59% of the estimated cost of a concentric bout at a similar load, as shown by the relationship between the actual metabolic cost and the relative load setting (Table 1).

Glycogen utilization and repletion with eccentric exercise. The exercise resulted in significant glycogen depletion that was not restored 10 days after the exercise. Muscle glycogen levels before exercise were 85 ± 6 mmol glycosyl units/kg (Fig. 1). Glycogen utilization during the exercise bout was 33 ± 12 mmol/kg wet wt, equal to an average 39% of initial values (Fig. 1). In the I-Post sample, glycogen depletion was observed in both type I and II fibers; however, fewer type I than type II fibers were classified as glycogen filled ($P < 0.05$), suggesting a preferential type I fiber type-specific pattern of glycogen use during exercise (Fig. 2). The biopsy taken at rest 10 days after the eccentric exercise bout showed no selective repletion in type I or II fibers. Glycogen content in the 10D-Post exercise sample was 37 ± 11 mmol/kg wet wt, significantly lower than the resting preexercise level ($P < 0.05$) and not different from the immediately postexercise level (Fig. 1).

Ultrastructural effects of eccentric exercise. The I-post muscle biopsies revealed numerous ultrastructural abnormalities (Fig. 3). The most obvious change was the loss of granular glycogen in the cytoplasm, identified as the "clear" areas in the micrograph. Five to 10% of the muscle fibers exhibited various degrees of interstitial and intracellular edema, as well as focal myofibrillar lysis and loss of Z-band material (Fig. 3, arrow). A more subtle change in the fibers was the damage to the sarcoplasmic reticulum, making it impossible to identify the fine tubular network. Examination of the 10D-Post fibers showed frankly necrotic muscle fibers, characterized by loss of myofibrillar organization, mitochondrial alterations, incomplete glycogen repletion, and inflammatory cell infiltration. Figure 4 shows histiocytes (macrophages) within the residual basal lamina of the myotube and within the interstitium of a necrotic fiber. There was no evidence of increased rough endoplasmic reticulum, satellite cells, or other signs of myofibrillar regeneration.

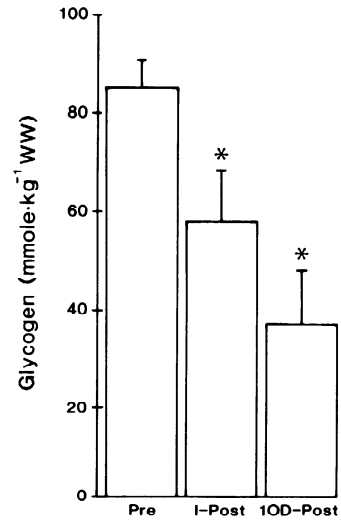

FIG. 1. Muscle glycogen content before (Pre) and after (I-Post) eccentric exercise and 10 days after (10D-Post) exercise. * Difference from Pre ($P < 0.05$).

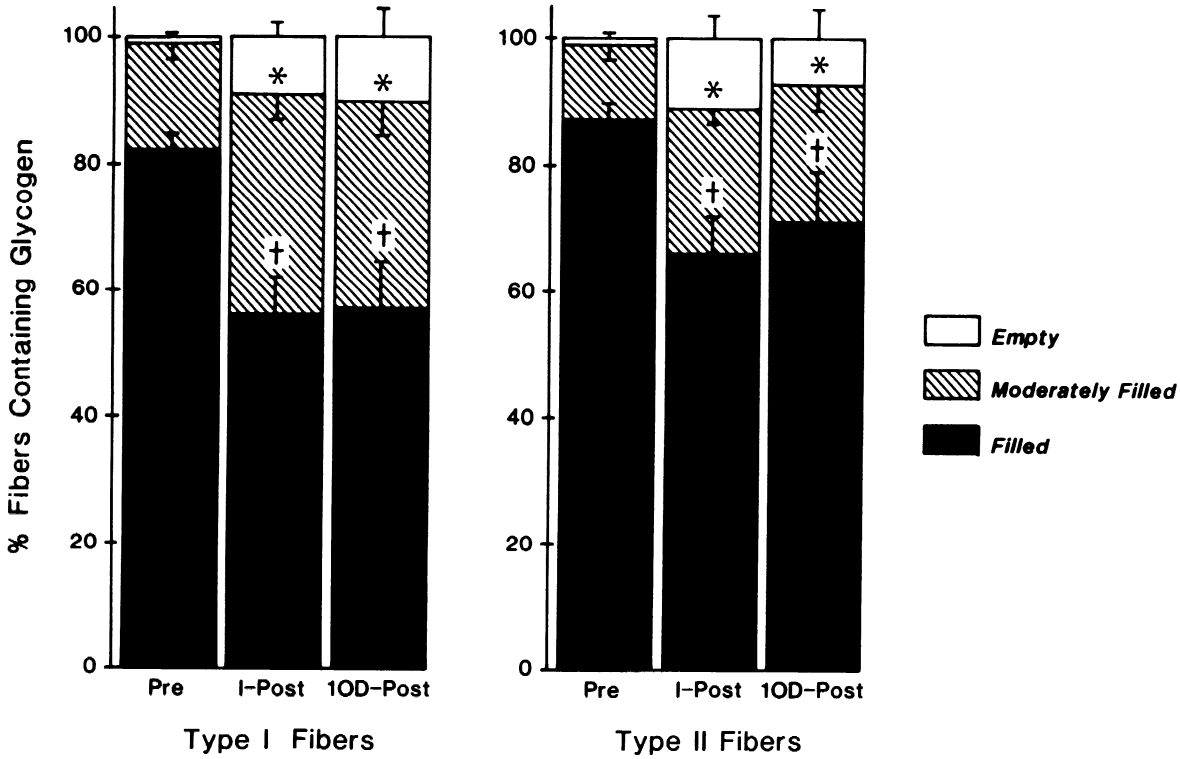

FIG. 2. Glycogen depletion patterns for type I (*left*) and II (*right*) fibers before (Pre), immediately, after (I-Post), and 10 days after (10D-Post) eccentric exercise. Glycogen content was classified histochemically as described in text. Data are expressed in percent (mean ± SE). * Moderately filled different from Pre ($P < 0.05$). † Filled different from Pre ($P < 0.05$).

DISCUSSION

Eccentric exercise characteristics. Studies in animals and humans have shown that, despite the low energy cost of eccentric muscle contractions, they cause more profound and more delayed changes in muscle structure and function than concentric contractions (1, 23, 24). Eccentric exercise has a greater effect on untrained men than in habitually active men (9, 12) or men who become trained on the eccentric cycle (10). In the present study, the subjects were selected to exclude men who exercised regularly so as to obtain more consistent and pronounced results.

The energy cost of performing the eccentric exercise in this study was similar to that reported previously in similarly untrained men (10). Although the load on the cycle was decreased at the end of each 15-min period, three of the five men had a larger O_2 uptake ($\dot{V}O_2$) per watt during the final 15 min compared with the initial, higher intensity 15 min. Thus the linear relationship between mechanical load and $\dot{V}O_2$ that has been described for men accustomed to the cycle ergometer was not observed in the present study (19). It is possible that, with the effort of maintaining coordination, the subjects added isometric contractions of the upper torso and some concentric contractions of the legs, leading to a larger active muscle mass and increased $\dot{V}O_2$.

Glycogen utilization by the vastus lateralis during eccentric exercise (Fig. 1) was similar to utilization reported for fasted subjects during concentric cycling at a similar metabolic rate, but at a lower power output (18), and to values previously reported for eccentric exercise for 45 min at 250 W (10). The substantial type II fiber involvement with eccentric exercise (Fig. 2) contrasts with concentric exercise performed at the same metabolic cost, in which type I fibers are recruited almost exclusively, and was more typical of a concentric effort close to $\dot{V}O_{2\,max}$ (15).

Ultrastructural analysis of the muscle biopsies revealed that the two predominant findings 10 days after exercise were glycogen depletion and myofibrillar lysis. Muscle glycogen was not repleted by 10 days after exercise, despite the fact that the subjects remained inactive and consumed an average of 360 g carbohydrate each day following the exercise bout. The low glycogen levels were in contrast to the observation that, after a bout of submaximal concentric cycling, muscle glycogen returned to preexercise levels within 24 h in subjects consuming a high-carbohydrate diet (3) and became supranormal within a few days (3, 20). Kuipers et al. (21) observed in well-trained cyclists that 24 h after 30 min of eccentric exercise at an intensity similar to the present study glycogen levels were below preexercise values. The present study suggests that this phenomenon continues for an extended time in untrained men.

The relationship between delayed glycogen repletion and myofibrillar lysis is unclear from morphological analysis; however, it is possible that alterations in the membranes of muscles involved in the exercise may interfere with glucose uptake into the cell. The alterations to the sarcolemma in the present study were similar to the findings of Friden and co-workers (13, 14), reflecting a possible loss of membrane integrity. The appearance of large proteins such as CK, lactate dehydrogenase, and myoglobin into the interstitial space and the blood sug-

FIG. 3. An electron micrograph of muscle immediately after eccentric exercise. "Clear" appearance of cytoplasm results from absence of glycogen. There are multiple foci of myofibrillar lysis (*arrows*) with loss of Z-band material. There has also been loss of sarcoplasmic reticulum in areas of myofibrillar lysis. (Original magnification ×20,000; *bar* = 150 nm.)

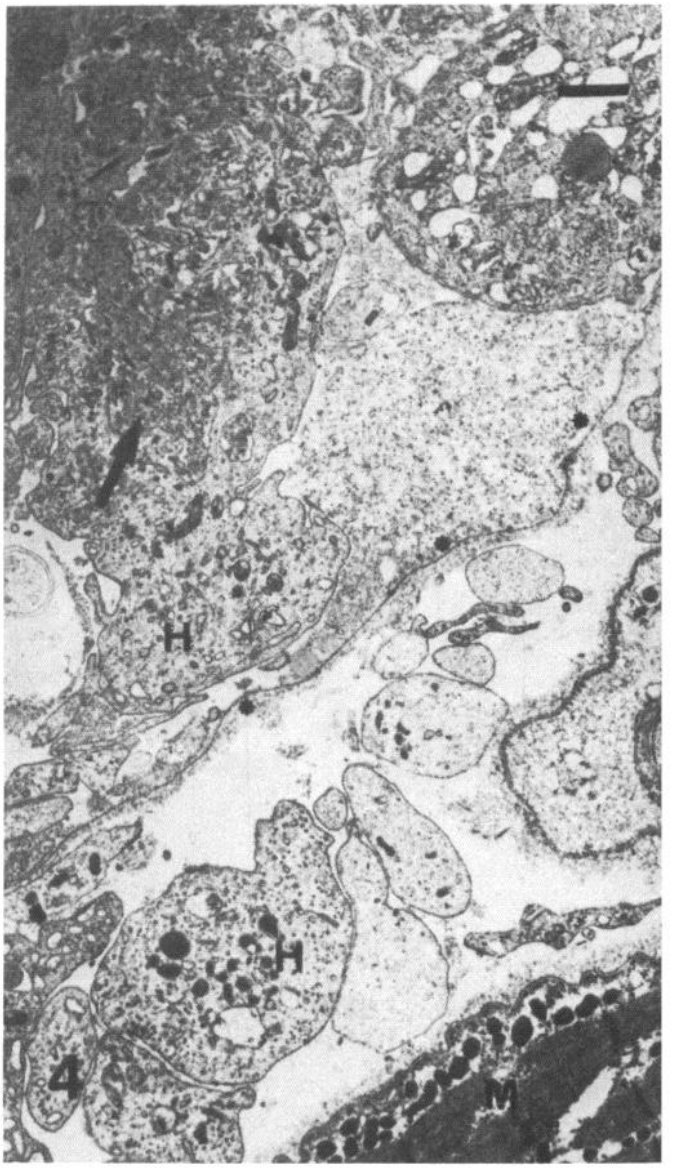

FIG. 4. An electron micrograph of skeletal muscle 10 days after eccentric exercise. A relatively normal muscle fiber (M) is seen at *lower right-hand corner*. Present both in interstitium as well as within a degenerating myofibril are histiocytes (H; macrophages). Lysosomal granules are clearly visible within their cytoplasm. Residual basal lamina of necrotic muscle fiber is marked with *asterisks*. Surrounded by a histiocyte are residual myofibrils (*arrow*). (Original magnification ×8,000; *bar* = 1 μm.)

gests that eccentric exercise may alter the membranes of the muscles involved (6, 30). As glucose transport into the muscle cell has been shown to be the rate-limiting step in glucose utilization in resting muscle following exercise, the effect of these membrane alterations could have resulted in decreased glucose availability in the cell for glycogen resynthesis (8, 29).

An association has been described between delayed ultrastructural damage and impaired glycogen repletion in marathon runners consuming a high-carbohydrate diet before and after the race (31). In biopsies taken before, immediately after, and periodically for 7 days after a 42.2-km race, there was evidence of prolonged ultrastructural damage (17) and of slow postexercise repletion of muscle glycogen. Muscle glycogen was supercompensated before the race, and by 24 h after the race, muscle glycogen stores were repleted to 40% of premarathon levels. Five days after the race, glycogen stores returned to normal levels for trained runners; however, they were still only 67% of prerace levels. The rate of glycogen repletion after the marathon was intermediate between the rates described for concentric exercise (3) and for eccentric exercise in the present study, although in each case subjects consumed a carbohydrate-rich diet. Glycogen repletion in the marathon runners following the race was probably enhanced by their training status and their severely depleted glycogen levels; however, the delay in the repletion of glycogen to prerace levels is probably due to muscle membrane damage resulting from the race, which prevented efficient glucose uptake and utilization, despite normal glycogen synthase activities (31). In the present study with untrained male subjects, the eccentric exercise resulted in prolonged

ultrastructural damage and the longest delay in glycogen repletion.

The results of this investigation indicate that recovery from eccentric exercise is a prolonged process. Ultrastructural evidence of muscle damage 10 days after exercise supports the observation of a delayed increase in actomyosin breakdown (9). Delayed glycogen repletion was shown by low glycogen levels 10 days after exercise. Histological evidence suggests that both type I and II fibers were recruited during eccentric exercise and supports the biochemical evidence of delayed glycogen repletion. The results suggest that when exercise involves eccentric contractions, there is delayed repair of ultrastructural damage and impaired repletion of muscle glycogen.

This project was supported by the US Department of Agriculture Human Nutrition Research Center on Aging. The authors wish to thank Dr. H. G. Knuttgen and Dr. J. F. Patton at US Army Research Institute of Environmental Medicine, Natick, MA, for their helpful comments and for the use of the eccentric cycle ergometer. The authors thank the participants in this study for their time and effort. These data were presented at the Annual Meeting of the American College of Sports Medicine, in Indianapolis, IN, in May 1986.

Address for reprint requests: W. J. Evans, USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington St., Boston, MA 02111.

Received 25 August 1986; accepted in final form 12 February 1987.

REFERENCES

1. ARMSTRONG, R., R. OGILVIE, AND J. SCHWANE. Eccentric exercise-induced injury to rat skeletal muscle. *J. Appl. Physiol.* 54: 80–93, 1983.
2. BERGSTRÖM, J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand. J. Clin. Invest.* 35: 609–615, 1975.
3. BERGSTRÖM, J., AND E. HULTMAN. Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. *Nature Lond.* 210: 309–310, 1966.
4. BROZEK, J., F. GRANDE, J. ANDERSON, AND A. KEYS. Densitometric analysis of body composition: revision of some quantitative assumptions. *Ann. NY Acad. Sci.* 110: 113–140, 1963.
5. BYRNES, W., P. CLARKSON, J. WHITE, S. HSIEH, P. FRYKMAN, AND R. MAUGHAN. Delayed onset muscle soreness following repeated bouts of downhill running. *J. Appl. Physiol.* 59: 710–715, 1985.
6. CERNEY, F., AND G. HARALAMBIE. Exercise-induced loss of muscle enzymes. In: *Biochemistry of Exercise*, edited by H. Knuttgen, J. Vogel, and J. Poortmans. Champaign, IL: Human Kinetics, 1983, p. 441–446.
7. DUBOWITZ, V., AND M. BROOKE. *Muscle Biopsy: A Modern Approach.* Philadelphia, PA: Saunders, 1973.
8. ELBRINK, J., AND I. BIHLER. Membrane transport: its relation to cellular metabolic rates. *Science Wash. DC* 188: 1176–1184, 1975.
9. EVANS, W., C. MEREDITH, J. CANNON, C. DINARELLO, W. FRONTERA, V. HUGHES, B. JONES, AND H. KNUTTGEN. Metabolic changes following eccentric exercise in trained and untrained men. *J. Appl. Physiol.* 61: 1864–1868, 1986.
10. EVANS, W., J. PATTON, E. FISHER, AND H. KNUTTGEN. Muscle metabolism during high intensity eccentric exercise. *Biochemistry of Exercise*, edited by H. Knuttgen, J. Vogel, and J. Poortmans. Champaign, IL: Human Kinetics, 1983, p. 225–228.
11. EVANS, W., S. PHINNEY, AND V. YOUNG. Suction applied to a muscle biopsy maximizes sample size. *Med. Sci. Sports* 14: 101–102, 1982.
12. FRIDÉN, J., J. SEGER, M. SJÖSTRÖM, AND B. EKBLOM. Adaptive response in human skeletal muscle subjected to prolonged eccentric training. *Int. J. Sports Med.* 4: 177–183, 1983.
13. FRIDÉN, J., M. SJÖSTRÖM, AND B. EKBLOM. Myofibrillar damage following eccentric exercise in man. *Int. J. Sports Med.* 4: 170–176, 1983.
14. FRIDÉN, J., M. SJÖSTRÖM, AND B. EKBLOM. A morphological study of delayed muscle soreness. *Experientia* 37: 506–507, 1981.
15. GOLLNICK, P., K. PIEHL, AND B. SALTIN. Selective glycogen depletion pattern in human skeletal muscle fibers after exercise of varying intensity and pedalling rates. *J. Physiol. Lond.* 241: 45–57, 1974.
16. HEYMSFIELD, S., C. ARTEAGA, C. MCMANUS, J. SMITH, AND S. MOFFITT. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am. J. Clin. Nutr.* 37: 478–494, 1983.
17. HIKIDA, R., R. STARON, F. HAGERMAN, W. SHERMAN, AND D. COSTILL. Muscle fiber necrosis associated with human marathon runners. *J. Neurol. Sci.* 59: 185–203, 1983.
18. JANSSON, E., P. HJEMDAHL, AND L. KAIJSER. Epinephrine-induced changes in muscle carbohydrate metabolism during exercise in male subjects. *J. Appl. Physiol.* 60: 1466–1470, 1986.
19. KNUTTGEN, H., J. PATTON, AND J. VOGEL. An ergometer for concentric and eccentric muscular exercise. *J. Appl. Physiol.* 53: 784–788, 1982.
20. KOCHAN, R., D. LAMB, S. LUTZ, C. PERRELL, E. REIMANN, AND K. SCHLENDER. Glycogen synthase activation in human skeletal muscle: effects of diet and exercise. *Am. J. Physiol.* 236 (*Endocrinol. Metab. Gastrointest. Physiol.* 5): E660–E666, 1979.
21. KUIPERS, H., H. KEIZER, F. VERSTAPPEN, AND D. COSTILL. Influence of a prostaglandin-inhibiting drug on muscle soreness after eccentric work. *Int. J. Sports Med.* 6: 336–339, 1985.
22. MCCULLEY, K., AND J. FAULKNER. Injury to skeletal muscle fibers in mice following lengthening contractions. *J. Appl. Physiol.* 59: 119–126, 1985.
23. NEWHAM, D., D. JONES, AND R. EDWARDS. Plasma creatine kinase changes after eccentric and concentric contractions. *Muscle Nerve* 9: 59–63, 1986.
24. NEWHAM, D., G. MCPHAIL, K. MILLS, AND R. EDWARDS. Ultrastructural changes after concentric and eccentric contractions of human muscle. *J. Neurol. Sci.* 61: 109–122, 1983.
25. NEWHAM, D., K. MILLS, B. QUIGLEY, AND R. EDWARDS. Pain and fatigue after concentric and eccentric muscle contractions. *Clin. Sci. Lond.* 64: 55–62, 1983.
26. PADYKULA, H., AND E. HERMAN. Factors affecting the activity of adenosine triphosphatase and other phosphatases as measured by histochemical techniques. *J. Histochem. Cytochem.* 3: 161–195, 1955.
27. PASSONNEAU, J., AND V. LAUDERDALE. A comparison of three methods of glycogen measurement in tissues. *Anal. Biochem.* 60: 405–412, 1974.
28. RAHN, H., W. FENN, AND A. OTIS. Daily variation of vital capacity, residual air and expiratory reserve including a study of the residual air method. *J. Appl. Physiol.* 1: 725–736, 1949.
29. RICHTER, E., L. GARETTO, M. GOODMAN, AND N. RUDERMAN. Muscle glucose metabolism following exercise in the rat. *J. Clin. Invest.* 69: 785–793, 1982.
30. SCHWANE, J., S. JOHNSON, C. VANDENAKKER, AND R. ARMSTRONG. Delayed-onset muscular soreness and plasma CPK and LDH activities after downhill running. *Med. Sci. Sports Exercise* 15: 51–56, 1983.
31. SHERMAN, W., D. COSTILL, W. FINK, F. HAGERMAN, L. ARMSTRONG, AND T. MURRAY. Effect of a 42.2-km footrace and subsequent rest or exercise on muscle glycogen and enzymes. *J. Appl. Physiol.* 55: 1219–1224, 1983.
32. VON DOBELN, W. Human standard and maximal metabolic rate in relation to fat free body mass. *Acta Physiol. Scand. Suppl.* 126: 5–37, 1956.
33. WARHOL, M., A. SIEGEL, W. EVANS, AND L. SILVERMAN. Skeletal muscle injury and repair in marathon runners after competition. *Am. J. Pathol.* 118: 331–339, 1985.