

## Skeletal muscle adaptation: training twice every second day vs. training once daily

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**Hansen, Anne K., Christian P. Fischer, Peter Plomgaard, Jesper Løvind Andersen, Bengt Saltin, and Bente Klarlund Pedersen.** Skeletal muscle adaptation: training twice every second day vs. training once daily. *J Appl Physiol* 98: 93–99, 2005. First published September 10, 2004; doi:10.1152/jappphysiol.00163.2004.—Low muscle glycogen content has been demonstrated to enhance transcription of a number of genes involved in training adaptation. These results made us speculate that training at a low muscle glycogen content would enhance training adaptation. We therefore performed a study in which seven healthy untrained men performed knee extensor exercise with one leg trained in a low-glycogen (Low) protocol and the other leg trained at a high-glycogen (High) protocol. Both legs were trained equally regarding workload and training amount. On *day 1*, both legs (Low and High) were trained for 1 h followed by 2 h of rest at a fasting state, after which one leg (Low) was trained for an additional 1 h. On *day 2*, only one leg (High) trained for 1 h. *Days 1* and *2* were repeated for 10 wk. As an effect of training, the increase in maximal workload was identical for the two legs. However, time until exhaustion at 90% was markedly more increased in the Low leg compared with the High leg. Resting muscle glycogen and the activity of the mitochondrial enzyme 3-hydroxyacyl-CoA dehydrogenase increased with training, but only significantly so in Low, whereas citrate synthase activity increased in both Low and High. There was a more pronounced increase in citrate synthase activity when Low was compared with High. In conclusion, the present study suggests that training twice every second day may be superior to daily training.

substrate availability; 3-hydroxyacyl-CoA dehydrogenase; citrate synthase

THE ROLE OF SUBSTRATE AVAILABILITY has been a key research area within exercise physiology for many years. When relating to the effect on performance, e.g., running time in a marathon race, it is clear that carbohydrate loading the days before to rebuild muscle glycogen as well as carbohydrate intake during the race enhance performance (28, 31, 37). In analogy, a common belief is that carbohydrate intake during training at high amounts will allow the athlete to train harder and longer and thus achieve a superior training response. However, this argument does not consider the unsolved longstanding question of whether it is a lack or a surplus of a substrate that triggers the training adaptation (14).

In endurance exercise, adaptation includes systemic changes such as improved maximal oxygen uptake ( $\dot{V}O_{2\max}$ ). Adaptation also includes prolonged time until exhaustion at a given workload, which is linked to maximal aerobic power but may

also be linked to local factors within the muscle (46). The multitude of adaptations that occur with training that allow for greater performance also includes an increased number of capillaries (2, 3), an increased density of mitochondria with the activity of enzymes such as 3-hydroxyacyl-CoA dehydrogenase (HAD) and citrate synthase (CS) being elevated, an increased concentration of transport proteins, greater glycogen concentration (24, 27, 30, 46), and a relative increase in the occurrence of type IIA fibers at the expense of type IIX fibers (2, 3). As a result, the ability to metabolize fat is enhanced (24).

When a more molecular view on training adaptation is taken, it is obvious that adaptation is a consequence of accumulation of specific proteins. The gene expression that allows for these changes in protein concentration is pivotal to the training adaptation. Recent studies have demonstrated that exercise induces transcription of several genes (43). Furthermore, it has been demonstrated that muscle glycogen is a determining factor for the transcription of some genes. Exercising when muscle glycogen concentration was low resulted in a greater transcriptional activation of interleukin-6 (32), pyruvate dehydrogenase kinase 4 (23, 42), hexokinase (42), and heat shock protein 72 (22) compared with when muscle glycogen concentration was high or normal at the start of exercise. The role of glycogen could be explained by the fact that several transcription factors include glycogen-binding domains. When muscle glycogen is low, these factors are released and become free to associate with different targeting proteins (4, 18, 44, 48, 49).

Helge and Kiens (29) studied the role of substrate availability on muscle enzyme activity and found that HAD increased with training after adaptation to a fat-rich diet but not a carbohydrate-rich diet. Transcriptional activities of HAD and CS are only markedly influenced by acute muscle contractions (43). However, it is possible that the accumulation of mRNA for these genes peaks late in the recovery from the exercise and that a low muscle glycogen level may enhance the transcription of these genes.

On this molecular background, we have formulated the overall hypothesis that training on a low muscle glycogen level will improve training adaptation (21). In the present study, we specifically tested the hypothesis that training at a low muscle glycogen content would enhance levels of HAD and CS. Moreover, performance defined as time until exhaustion at a given power output would be more pronounced by training twice every second day compared with training once daily.

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We therefore designed a protocol of two different training regimens in which the cycling of muscle glycogen differed. Critical would be to have direct comparison between legs on the same individual, where one leg has trained markedly more with low glycogen than the other. This can be accomplished by having one leg train twice every second day, whereas the other only trains once daily.

When a subject exercises, muscle glycogen declines and is slowly restored over the following 24 h if carbohydrate intake is normal (14, 26, 34). Therefore, when two exercise sessions of 1 h is separated by 2 h, the second bout of exercise is undertaken with low muscle glycogen at its start, whereas muscle glycogen is restored before each exercise bout when the exercise is separated by 24 h.

## MATERIALS AND METHODS

Seven healthy untrained young men were recruited with a mean age of 26 yr (range 24–29 yr), a mean weight of 86.7 kg (range 53–129 kg), mean height of 180.1 cm (range 171–189 cm). The subjects were exposed to a highly demanding and intensive training program lasting 10 wk with one- and/or two-legged knee extensor exercise (Fig. 1). The study was opposed by the local Ethical Committee of Copenhagen and Frederiksburg Communities and was performed in accordance with the Declaration of Helsinki. The two legs were trained after different schedules. By randomization, all subjects trained one leg twice every second day [low-glycogen training (Low)], whereas the other leg was trained once daily [high-glycogen training (High)]. Each bout of exercise lasted 1 h. On *day 1*, both legs trained simultaneously for 1 h at 75% of maximal power output ( $P_{\max}$ ), followed by 2 h of recovery. Thereafter, the Low leg trained for 1 h at 75% of  $P_{\max}$ . On *day 2*, the High leg trained alone for 1 h at 75% of  $P_{\max}$ . This 2-day training cycle was repeated for 10 wk. Every week, subjects trained 5 days and then rested 2 days. The training sessions were performed in the morning after an overnight fast, the first exercise bout being undertaken between 6:00 and 9:00 AM. The subjects were fasting until the training session was finished. Water intake was ad libitum. The workload of the 10-wk training was initially 75% of  $P_{\max}$  before the training started. Workload was increased individually by 5–10% depending on the progress of each individual. The workload was identical for the two legs. Before and after the 10 wk of training, a  $P_{\max}$  test was obtained for each leg separately using the same cycle ergometer-knee extensor exercise apparatus (Monark, Varberg, Sweden). Thereafter, a 90%  $P_{\max}$  endurance test was performed for each leg.

In the first trial, the subjects performed a  $\dot{V}O_{2\max}$  test on each leg to determine the individual knee  $P_{\max}$ . Both legs were tested on the

same day with at least 30 min of rest in between. The test began with a 10-min warm-up at 20 W followed by a stepwise increase of 10 W in workload every 2 min. Subjects worked until exhaustion.

**Diet.** The subjects consumed a mixed Western diet throughout the study (~16 MJ per day, 70% carbohydrate, 15% protein, 15% fat). Subjects were asked to adhere to the diet and to refrain from strenuous exercise other than that included in the training protocol.

**Muscle biopsies.** Muscle biopsy samples were obtained before and after 10 wk of training from the vastus lateralis muscle of both legs. The volunteers were told to abstain from any strenuous exercise 48 h before these biopsies. In addition, muscle biopsies were obtained in relation to training sessions (before, immediately after the first bout of exercise, after 2 h of rest, and immediately after the second bout of exercise). Muscle biopsies were analyzed for glycogen by using enzymatic analyses with fluorometric detection (19). In addition, biopsies were analyzed for CS and HAD activity (20).

**Hormones.** Glucose and lactate were measured by use of an automated analyzer (Cobas Fara, Roche, Basel, Switzerland). Plasma insulin (Insulin RIA 100, Amersham Pharmacia Biotech, Uppsala, Sweden), glucagon (Linco Research, St. Charles, MO), and cortisol (Diagnostic Products, Los Angeles, CA) were determined by RIA, and plasma epinephrine and norepinephrine were determined by HPLC (9, 47).

**Fiber types and capillaries.** Serial sections (10  $\mu\text{m}$ ) of the muscle biopsy samples were cut in a cryostat at  $-20^{\circ}\text{C}$ , and routine ATPase histochemistry analysis performed after preincubation at pH 4.37, 4.60, and 10.30 (10). Five different fiber types were defined: types I, I/IIA, IIA, IIAX, and IIX. The terms "IIAX" and "IIX" have been used instead of "IIAB" and "IIB," to match the predominant nomenclature used for the human myosin heavy chain (MHC) isoforms (1). Fibers determined to be type II fibers, but showing an intermediate staining with pH 4.60 preincubation, were categorized as type IIAX fibers. These fibers covered a wide range from fibers with only a light staining (i.e., fibers with predominately MHC IIA expression) to fibers with a much darker staining (i.e., fibers with predominantly MHC IIX expression). In some individuals, the number of the minor fiber types (I/IIA, IIAX, and IIX) were so small that a reliable statistical comparison of changes in fiber-type size was impossible. Therefore, calculations of fiber-type size were performed for three major categories of fiber types (type I, type IIA, and type IIAX). Staining of capillaries was performed by using the double-staining method (45).

The serial sections of the various ATPase and capillary stainings were visualized and analyzed for fiber-type percent, fiber-type area percent, fiber size, and capillary density expressed as capillaries per fiber and as capillaries per millimeter squared by using a TEMA image analyzing system (Scanbeam, Hadsund, Denmark) as used

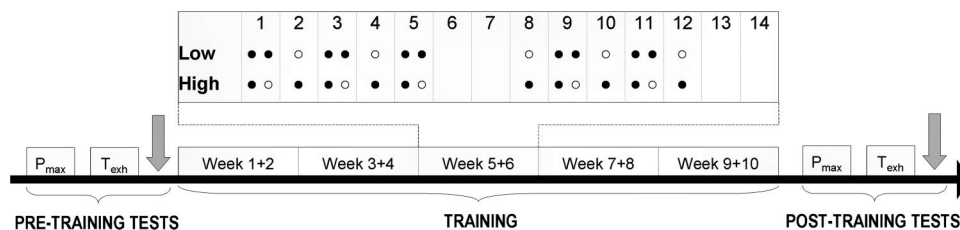


Fig. 1. Schematic overview showing the design of the study. Before onset of the training period, the maximal power output ( $P_{\max}$ ) and the time to exhaustion ( $T_{\text{exh}}$ ) at 90% of  $P_{\max}$  were determined for each leg on separate occasions. At least 48 h after the last performance test, a blood sample as well as a muscle biopsy from the vastus lateralis (gray arrows) were obtained during rest after an overnight fast. After the first set of performance tests, the participants trained both legs for 10 wk, followed by a second posttraining set of performance tests similar to those performed before training. The training consisted of a 14-day cycle repeated 5 times. During one 14-day period (box at top), one leg (Low) was trained for 5 days by the subjects performing 2 bouts of dynamic knee extensor exercise, each bout lasting 1 h and separated by a 2-h break. Meanwhile, the other leg (High) was trained every day for 5 days/wk by the subjects performing 1 bout of dynamic knee extensor exercise for 1 h. Numbers in the box at top denote the number of the day during the 14-day training cycle. The workload was initially set to 75% of the pretraining  $P_{\max}$ , and increased by 5–10% every 14-days, depending on the progress of each participant. Of note, the workload for each leg was equal during the training sessions; thus the total work for each leg during the training period was equal. ●, One 1-hour training bout; ○, contralateral resting leg.

Table 1. Maximal power output and time until exhaustion at 90% of maximal power output before and after 10 wk of training and total work before and after 10 wk of training

Parameter	Pretraining		Posttraining	
	Low	High	Low	High
$P_{\max}$ , W	74±7	77±6	107±7*	106±6†
$T_{\text{exh}}$ , min	5.0±0.7	5.6±1.2	19.7±2.4*‡	11.9±1.3†
Total work, kJ	22±5	25±7	114±14*‡	69±8†

Values are means ± SE. Low, leg trained with low muscle glycogen protocol; High, leg trained with high muscle glycogen protocol;  $P_{\max}$ , maximal power output;  $T_{\text{exh}}$ , time until exhaustion; total work,  $P_{\max} \times T_{\text{exh}}$ . \*Significant difference ( $P < 0.05$ ) from pretraining in Low. †Significant difference ( $P < 0.05$ ) from pretraining, in High. ‡Significant difference ( $P < 0.05$ ) between Low and High.

previously by our laboratory (45). We examined an average of 105 ± 16 fibers in each biopsy.

## RESULTS

**$P_{\max}$  and time until exhaustion.** In response to 10 wk of training,  $P_{\max}$  increased significantly, being the same in the two legs (Table 1). The endurance at 90% of this new  $P_{\max}$  was markedly increased for both legs, but time until exhaustion was twice as long for Low compared with High. In accordance, the actual work performed by Low was also markedly larger compared with High.

**Hormones and lactate in relation to a training session.** Plasma insulin, glucagon, norepinephrine, epinephrine, cortisol, and lactate were measured before and after 1 h of knee extensor exercise with both legs, which corresponded to the first exercise at that particular day; before and after knee extensor exercise for 1 h with the Low leg, which was performed 2 h after the exercise with both legs; as well as before and after 1 h of exercise with the High leg, which corresponded to the first bout of exercise on the following day. During both exercises with two legs as well as one leg, plasma insulin decreased, whereas plasma glucagon, norepinephrine, and epinephrine increased (Table 2). These changes occurred to the same extent when exercise was performed with two legs and when exercise was performed with the Low leg. The hormonal responses to exercise with the Low leg were in general more pronounced compared with exercise performed with the High leg. The difference in responses between the Low and High legs was significant for norepinephrine and epinephrine. Plasma cortisol did not change in response to exercise.

Table 2. Hormone and lactate levels

	Both Legs		Low		High	
	Pretraining	Posttraining	Pretraining	Posttraining	Pretraining	Posttraining
Insulin	47.2±12.76	19.56±3.37*	28.29±8.60	17.86±7.81*	35.54±7.98	21.17±3.36*
Glucagon	90.1±15.61	137.48±24.23*	74.33±8.37	129.52±17.99*	75.68±8.69	109.55±17.09*
Norepinephrine	2.1±0.25	5.79±0.82*	2.21±0.19	4.65±0.35*	2.11±0.26	3.37±0.36†
Epinephrine	0.3±0.07	0.74±0.16*	0.28±0.05	0.89±0.18*	0.30±0.07	0.66±0.14†
Cortisol	16.3±2.46	14.30±4.17	15.48±4.07	11.09±1.60	16.20±3.51	12.53±2.00
Lactate	1.1±0.37	3.28±0.66	1.13±0.13	3.11±0.70	1.38±0.11	2.08±0.34

Values are means ± SE. No significant differences were observed between pretraining values for Low and High legs. \*Difference between pretraining and posttraining;  $P < 0.05$ . †Difference between Low and High legs posttraining value,  $P < 0.05$ .

**Muscle glycogen.** Muscle glycogen content was measured at rest before, after 5 wk, and after 10 wk of training (Fig. 2A). Training induced a marked increase in muscle glycogen. This effect was, however, only significant for Low. Muscle glycogen was also measured in relation to two training sessions. Muscle glycogen declined during the first bout of exercise. Because the subjects were not allowed to eat in the recovery period of the first bout of exercise, the second bout of exercise undertaken by Low was initiated at a low muscle glycogen level. Therefore, every second time the Low leg trained, it was with a markedly low muscle glycogen level, whereas the High leg initiated each training session with a high muscle glycogen content (Fig. 2B).

**Mitochondrial enzymes** The activities of the mitochondrial enzymes HAD and CS were measured in muscle biopsies obtained at rest before and after 5 and 10 wk of training (Fig. 3). HAD activity increased with training, but only significantly so in Low, whereas CS activity increased in both Low and High. When the relative change from pretraining to after 10 wk of training was estimated, there was a significantly more pronounced increase in CS activity when Low was compared with High.

**Muscle fibers and capillaries.** Percent number and percent area of type IIX fibers decreased significantly in High, but there was no difference between the two legs postexercise (Table 3). There were no significant effects of training and no difference between Low and High with regard to distribution of fiber types or size or with regard to capillaries.

## DISCUSSION

The main findings of the present study were that 1) that time until exhaustion, 2) resting muscle glycogen concentration, and 3) CS activity were enhanced by training twice every second day when compared with training once daily. The protocol allowed us to compare the work performed in the prolonged time trial with each leg at the same absolute as well as exercise intensity. Using a study design where the two legs were trained at different protocols further allowed us to distinguish possible systemic and local effects. Thus systemic concentrations of, for example, hormones and glucose were equal for the two legs, and the study design therefore only measured possible local differences as a consequence of different training schedules. The present study was based on an overall hypothesis, which can be expressed as follows: "Muscle glycogen: train low, compete high." This hypothesis refers to the fact that, whereas numerous studies have demonstrated that low muscle glycogen

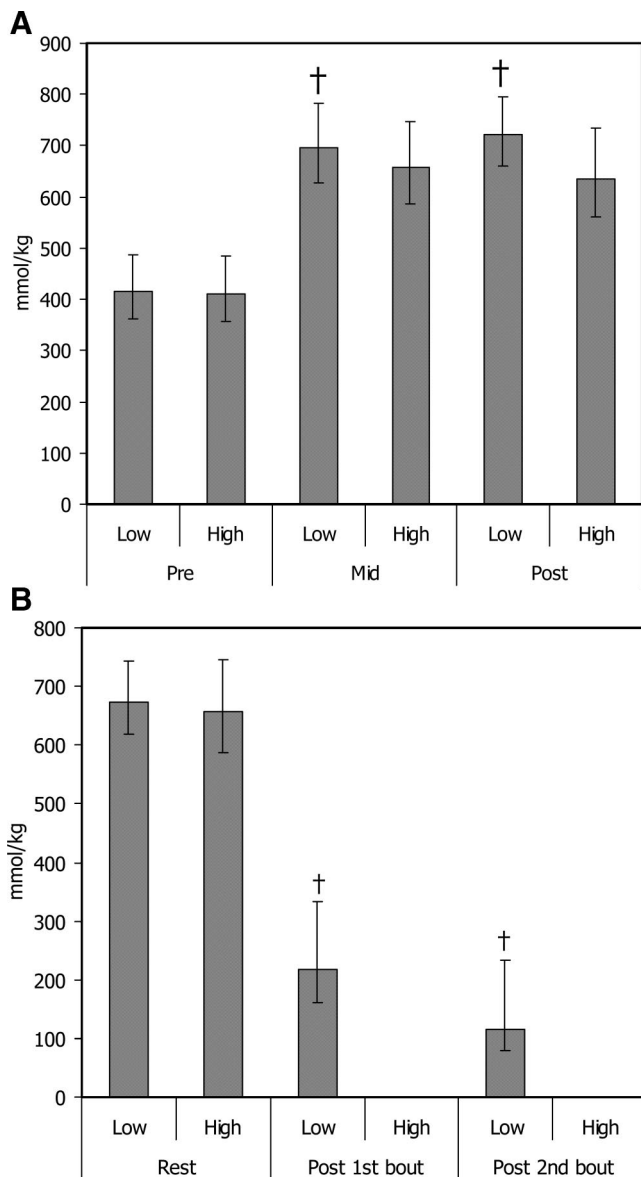


Fig. 2. A: resting muscle glycogen concentration before (pre), halfway through the training period (mid), and at the end (post) of the training period. Values are geometric means  $\pm$  SE. †Difference from pretraining level,  $P < 0.05$ . B: muscle glycogen content at rest, after one bout of two-legged training (Post 1st bout), and after the subsequent bout of one-legged training (Post 2nd bout; with the Low leg). The biopsies obtained after the 1st and 2nd bouts are from the Low leg only. Values are geometric means  $\pm$  SE. †Difference from pretraining level,  $P < 0.05$ .

content is a limiting factor with regard to performance (6, 15, 28, 31, 36), this may not be valid when it comes to training adaptation. In fact, data have accumulated showing that low muscle glycogen content enhances the transcription and the

transcription rate of a number of genes involved in training adaptation (22, 32, 42).

In the present study, the two legs were trained according to different protocols: one leg performed one training session

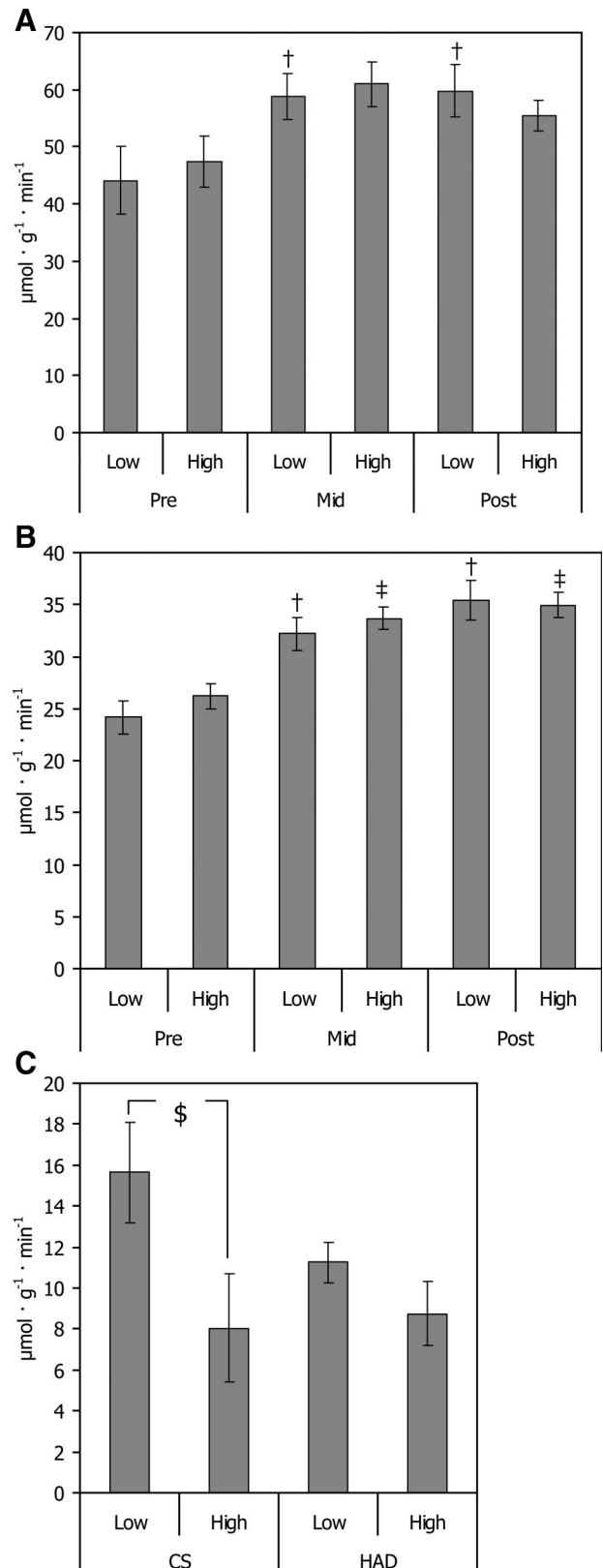


Fig. 3. A: resting muscle citrate synthase (CS) activity pretraining, midtraining, and posttraining. Values are means  $\pm$  SE. †Difference from pretraining level in Low,  $P < 0.05$ . ‡Difference from pretraining level in High  $P < 0.05$ . B: resting muscle 3-hydroxyacyl-CoA dehydrogenase (HAD) activity pretraining, midtraining, and posttraining. Values are means  $\pm$  SE. †Difference from pretraining level in Low,  $P < 0.05$ . ‡Difference from pretraining level in High,  $P < 0.05$ . C: change in resting muscle CS and HAD activity from pretraining to posttraining. Values are means  $\pm$  SE. \$Difference between Low and High,  $P < 0.05$ .



Table 3. Muscle fiber and capillary characteristics pretraining and posttraining

	Pretraining		Posttraining	
	Low	High	Low	High
Percent number				
Type I	67.0±5.1	62.7±6.7	64.7±5.7	61.8±5.1
Type I/IIA	0.2±0.1	0.1±0.1	0.8±0.4	0.2±0.1
Type IIA	25.0±3.4	27.2±4.2	26.3±4.9	35.2±5.9
Type IIAX	5.0±2.1	6.5±2.0	7.5±3.8	2.3±1.0
Type IIX	2.4±1.3	3.2±1.1	0.4±0.3	0.3±0.3*
Percent area				
Type I	65.2±5.0	58.8±6.3	63.7±6.3	61.8±6.6
Type I/IIA	0.2±0.1	0.0±0.0	0.8±0.4	0.2±0.1
Type IIA	28.8±4.3	31.8±4.9	27.9±5.3	35.2±7.2
Type IIAX	3.9±1.5	6.3±1.8	6.8±3.3	2.3±1.1
Type IIX	1.7±0.9	2.8±0.9	0.5±0.3	0.2±0.2*
Fiber size				
Type I	4,963±843	5,061±893	5,216±659	5,564±781
Type II	5,394±926	5,867±676	5,319±550	5,464±558
Capillaries				
Capillaries/type I fiber	5.6±0.6	5.5±0.6	6.0±0.3	6.5±0.4
Capillaries/type II fiber	5.3±0.7	5.7±0.5	5.7±0.4	6.1±0.5
Capillaries/mm <sup>2</sup>	555±16	552±37	598±27	610±33

Values are means ± SE. \*Difference from pretraining,  $P < 0.05$ .

daily, whereas the other leg performed two training sessions separated by only 2 h every second day. The latter training schedule resulted in a marked decrease in muscle glycogen content after the first bout of exercise. Therefore, when the second bout of exercise was performed within the same day, it was undertaken with very low muscle glycogen content. Thus we succeeded in developing a protocol that allowed us to compare training at a low muscle glycogen with training at a high muscle glycogen content. The finding that the catecholamine response to exercise performed at low muscle glycogen was higher than at exercise performed at high muscle glycogen concentration demonstrates that a higher stress response was elicited when the muscle glycogen was low.

In the present study, resting muscle glycogen content increased with training in accordance to numerous previous studies (25). However, the increase in muscle glycogen was only significant for Low. This indicates that training at the Low protocol may be a more efficient training mode with respect to enhancing muscle glycogen stores. It has long been known that glycogen synthase (GS) activity is closely coupled to the muscle glycogen content in both rodent (16, 17) and human skeletal muscle (7), both in the resting state and after muscle contraction (16, 38, 51). The rate-limiting conversion of UDP-glucose to glycogen is catalyzed by GS, which in skeletal muscle is known to be bound to glycogen particles (5, 35) and myofibrils (33, 50). Rat studies have demonstrated that contraction-induced increase in GS activity is strongly dependent on muscle glycogen (38). Exercise regulation of GS is characterized by great complexity (39). GS is a substrate of kinases and phosphatases acting on several phosphorylation sites of GS, and exercise seems to activate both stimulatory and inhibitory regulators of GS, including activation of 5'-AMP-activated protein kinase (11, 13, 40). The mechanisms responsible for inhibition and especially activation are poorly understood. It may be proposed that the GS activity during exercise may

depend on the relative strength of opposing signals. Glycogen breakdown may be considered the major stimulatory signal. The finding that training on the Low and High protocols influence glycogen metabolism differently talks in favor of the idea that glycogen breakdown and low muscle glycogen are important stimulatory GS signals, which result in a total increase in muscle glycogen concentration.

The effect on resting muscle glycogen does, however, not explain the difference in "time until exhaustion" because this test was carried out on a relatively high intensity, which did not allow the volunteers to exercise for more than a maximum of 25 min. Therefore, muscle glycogen content was not a limiting factor.

The study did not aim to measure peak muscle oxygen consumption, and therefore we did not consider measuring the rate-limiting enzyme  $\alpha$ -keto acid glutamate dehydrogenase (8). Rather, we focused on citrate synthase as a more general marker of the tricarboxylic acid cycle flux and HAD as the most-used marker enzyme for the  $\beta$ -oxidation.

The activity of the mitochondrial enzymes HAD and CS increased with training in both Low and High. However, regarding CS activity, this increase was more pronounced in the leg that was trained in the low muscle glycogen protocol. Transcriptional activities of HAD and CS are only markedly influenced by acute muscle contractions (43).

However, the possibility exists that the mRNA for these genes peak late in the recovery phase. The effect of low muscle glycogen on HAD and CS gene activation has not been studied. The finding that the low muscle glycogen protocol induced a more pronounced enhancement of CS activity may represent one mechanism explaining the enhanced endurance time in the low muscle glycogen-trained leg.

We were unable to identify any major effects of training on muscle fiber types, fiber size, or capillaries, although there was the expected decrease in the amount of type 2X fibers in the High leg, with a corresponding tendency in the Low leg. Similarly, there was a clear, but not significant, tendency of an increased capillary density in both legs. This lack of significant adaptations in fiber types and capillary density is most likely due to the major limitation of the present study: the training protocol was demanding to such an extent that the cost limited us to carry through only seven subjects. An  $n$  value of seven is sufficient to study parameters with little variation, but can be a major limitation in relation to adaptations in fiber types and capillarization.

The present study should be viewed as one among hopefully many studies to be conducted in the coming decade, investigating the effect of muscle glycogen content on training adaptation using molecular biological methods as well as exercise physiological parameters. Coaches and athletes should be careful not to draw practical consequences of the present study with regard to training regimens. In the real world, training on a high muscle glycogen content may allow the athlete to train for longer periods and thereby obtain better results. In addition, training schedules that allow muscle glycogen to decrease to low values may increase the risk for the so-called overtraining syndrome (41).

In summary, in a human experimental laboratory setting, training twice every second day was superior to training once daily. The present study therefore suggests that perhaps some adaptations to physical activity may require a cycling of

muscle glycogen stores as recently suggested by Chakravarthy and Booth (12).

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