Progressive effect of endurance training on metabolic adaptations in working skeletal muscle

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Phillips, S. M., H. J. Green, M. A. Tarnopolsky, G. J. F. Heigenhauser, and S. M. Grant. Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. *Am. J. Physiol.* 270 (*Endocrinol. Metab.* 33): E265–E272, 1996.—We investigated the hypothesis that a program of prolonged endurance training, previously shown to decrease metabolic perturbations to acute exercise within 5 days of training, would result in greater metabolic adaptations after a longer training duration. Seven healthy male volunteers [O_2 consumption = 3.52 ± 0.20 (SE) l/min] engaged in a training program consisting of 2 h of cycle exercise at 59% of pretraining peak O_2 consumption ($\dot{V}O_{2peak}$) 5–6 times/wk. Responses to a 90-min submaximal exercise challenge were assessed pretraining (PRE) and after 5 and 31 days of training. On the basis of biopsies obtained from the vastus lateralis muscle, it was found that, after 5 days of training, muscle lactate concentration, phosphocreatine (PCr) hydrolysis, and glycogen depletion were reduced vs. PRE (all $P < 0.01$). Further training (26 days) showed that, at 31 days, the reduction in PCr and the accumulation of muscle lactate was even less than at 5 days ($P < 0.01$). Muscle oxidative potential, estimated from the maximal activity of succinate dehydrogenase, was increased only after 31 days of training (+41%; $P < 0.01$). In addition, $\dot{V}O_{2peak}$ was only increased (10%) by 31 days ($P < 0.05$). The results show that a period of short-term training results in many characteristic training adaptations but that these adaptations occurred before increases in mitochondrial potential. However, a further period of training resulted in further adaptations in muscle metabolism and muscle phosphorylation potential, which were linked to the increase in muscle mitochondrial capacity.

phosphorylation potential; training duration; energy metabolism

ENDURANCE EXERCISE performed on a regular basis induces extensive alterations in the metabolic characteristics of skeletal muscle (5, 15). In the trained human, one of the most consistent adaptations is an increase in muscle mitochondria, which is reflected in elevations in the maximal activities of representative enzymes of the tricarboxylic acid cycle and β-oxidative pathway (5, 10, 15, 17). Training is also accompanied by pronounced adaptations in the metabolic response in the working muscle. After training, moderate exercise at a given absolute intensity results in less of a decrease in phosphorylation potential, as evidenced by the higher concentration of phosphocreatine (PCr) and lower concentrations of inorganic phosphate (P_i) and calculated free ADP (ADP_f) and free AMP (AMP_f; see Refs. 8–10). Because these effects occur in the absence of changes in O_2 consumption ($\dot{V}O_2$), a tighter metabolic control is indicated whereby recruitment of a given level of mitochondrial respiration is accomplished with less of a perturbation in the adenine nucleotides (15). Training also results in an enhanced utilization of fats, due mainly to an increased oxidation of muscle triglyceride, since extramuscular fat oxidation and turnover are reduced with training (16, 17, 19). Muscle glycogen depletion is also attenuated after training (8, 10, 17, 19). All of these changes have been postulated to be mechanistically linked to a training-induced increase in muscle mitochondrial potential (5, 15).

According to current thinking, the increase in mitochondrial protein, and consequently in the enzymes of β-terminal and end-terminal oxidation, increase respiratory control sensitivity, allowing a given respiratory rate to be achieved at a lower concentration of cytosolic ADP_f or related modulators (5, 15). This regulation has been postulated to occur on the basis of classic Michaelis-Menton kinetics where, as a result of an increase in enzyme concentration, a given reaction velocity can be realized at a lower substrate concentration (15). However, our work using regular daily exercise conducted only for a short period of time has challenged this view. We have found that many if not all of the adaptations in muscle metabolism and substrate oxidation occur soon after the onset of training and before increases in mitochondrial content occur, as measured by the maximal activities of representative enzymes of the tricarboxylic acid cycle (1, 8–10).

This finding brings into question the role that the mitochondrial protein content and oxidative capacity of the muscle serve in respiratory control. It is possible that more than one mechanism may be involved, each dependent on the duration of training. In accordance with this hypothesis, training-induced increases in mitochondrial protein may be important and serve to promote an even tighter metabolic control. Unfortunately, previous training studies have only compared the metabolic and mitochondrial adaptations after a prolonged period of training without regard to time course measurements (5, 15–17).

To investigate this hypothesis, we have employed a longitudinal design with measurements made soon after the onset of training (5 days) and again after 31 days of regular exercise. These time points were selected based on previous work that has shown that increases in mitochondrial content do not occur until later in training (9). Our results enabled us to conclude that metabolic adaptation during work is enhanced as the training duration is prolonged, raising the possibil-

ity of a role for mitochondrial protein content in enhancing respiratory control sensitivity.

METHODS

Subjects. Seven healthy but untrained male volunteers [age = 23 ± 1 (SE) yr] were recruited from the student population at the University of Waterloo (Ontario, Canada). After being informed of the procedures involved and the possible risks associated with each procedure, each participant signed a consent form, as approved by the Office of Human Research. During the study, the participants were asked to maintain a normal diet. This was checked by periodically requiring subjects to maintain 3-day food records. Analysis of these records, pretraining and during training, indicated that subjects consumed more total calories while training, but the percentage of calories from carbohydrate, fat, and protein did not change significantly (results not shown). Participants were also asked to refrain from consuming alcohol throughout the study. In addition, consumption of foods and liquids containing caffeine was not permitted on the days when exercise tests were administered.

Experimental design. The testing and training protocol was similar in nature to that employed in previous studies (1, 8–10). Two different exercise tests were used. A progressive cycle test was administered 1–2 wk before the beginning of training and after 8 and 31 days of training. This test was used to establish pretraining workloads and to assess changes in peak oxygen consumption ($\dot{V}O_{2peak}$), peak power output, ventilation, and heart rate. $\dot{V}O_{2peak}$ obtained during a maximal progressive exercise test to fatigue, before training (PRE), was 3.52 ± 0.2 l/min [44.3 ± 2.4 (SE) ml·kg^{-1}·min^{-1}]. After 8 days of training, $\dot{V}O_{2peak}$ was unchanged (3.55 ± 0.2 l/min) but increased 10% (3.89 ± 0.18 l/min; $P < 0.05$) after 31 days of training. Changes in $\dot{V}O_{2peak}$ and other measures are reported elsewhere (20). The second test, which involved 90 min of cycling at ~59% of pretraining $\dot{V}O_{2peak}$, was administered PRE and after 5 and 31 days of training. Each challenge ride was performed at a similar temperature and relative humidity.

At least 6 h before each challenge ride, subjects selected a snack (from a list of recommended foods provided for them: bread, cereal, juice), which was recorded, and the identical snack was consumed at the same time before each subsequent challenge ride. The snack provided 1,760 ± 200 kJ and was on average 62% carbohydrate, 12% protein, and 26% fat. It is unlikely that consumption of this snack affected any measures of metabolism, since the concentration of blood glucose, glycerol, lactate, and free fatty acids before exercise were not different during the study (results not shown).

Immediately before and during (15 and 90 min) the prolonged exercise tests, tissue was obtained by needle biopsy from the vastus lateralis muscle. At each time point, two samples were obtained from the same incision. These samples were harvested during brief interruptions in the exercise. In the case of the initial biopsy, the sample was extracted as rapidly as possible, and the needle was quickly plunged into liquid N_2. Samples were stored at −80°C until analysis. Specific details regarding the sites selected and the technique used have been published earlier (1, 8–10).

Training consisted of cycling for 5 consecutive days, for 2 h/day at 59% of pretraining $\dot{V}O_{2peak}$. The subjects then rested for 1 day after which the training cycle began again. Training was at the same absolute power output throughout the study and was initiated 2 days after the first prolonged exercise test and terminated 1 day before each subsequent test at 5 and 31 days. The training rides were only interrupted if the subjects could not complete the prescribed 2 h, and, in such cases, the subjects were allowed rest pauses until they completed a total of 2 h of exercise. All subjects could complete the 2-h training ride, without rest pauses, by *day 4* of the training protocol. Subjects were allowed access to water ad libitum during the training rides.

Analytic procedures: muscle. Muscle biopsy samples were analyzed for PCr and breakdown products, creatine (Cr), and P_i. Glucose, glucose 6-phosphate (G-6-*P*), citrate, lactate, and pyruvate were also measured. These parameters were determined using fluorometric techniques according to procedures employed previously by our laboratory (8, 10, 23). Extraction of muscle constituents was performed according to the general procedures outlined by Harris et al. (12). In addition, measurements of the muscle adenine nucleotides (ATP, ADP, and AMP) and the adenine nucleotide degradation product IMP were made using high-performance liquid chromatography (HPLC) techniques as described previously (11). All measurements, except for glycogen (see third paragraph below), were corrected to total average creatine (TCr) concentration determined for each individual. Our justification for correcting all metabolites, even though not all are restricted to the intracellular space, is that TCr provides a more stable reference base independent of the contamination of blood, connective tissue, and fat (9). Neither exercise nor training altered the TCr content ($P > 0.7$).

Because measurements of intramuscular concentrations of ADP and AMP represent total concentration, the majority of which is bound, we also estimated the free concentrations of both metabolites. Calculating ADP_f and AMP_f involves making a number of assumptions regarding the intracellular ionic environment and the status of the creatine phosphokinase (CPK) and adenylate kinase (AK) equilibrium (9). We have discussed these assumptions and outlined how these calculations were performed previously (9).

Muscle citrate was measured fluorometrically using 75 µl neutralized perchloric acid muscle extract (12). The PCA extract was added to a buffer containing tris(hydroxymethyl)aminomethane·HCl (pH 7.6), 10 µM NADH, 50 µM $ZnCl_2$, and malate dehydrogenase (MDH; 0.3 U/ml). Fluorometric readings were taken before and after addition of citrate lyase (0.016 U/ml), which initiated the assay. Citrate lyase was suspended in 50 mM triethanolamine buffer (pH 7.4) made up to 0.03 mM in $ZnCl_2$ and 0.5 M $(NH_4)_2SO_4$. Citrate standards ranging from 3 to 30 µM were used, and the assay was linear over this range. The intra-assay coefficient of variation (CV) for citrate (determined on 8 identical samples) was 7.7 ± 3.1% and the interassay CV (determined on 4 duplicate pairs) was 9.9 ± 2.9%.

Muscle was also assayed for glycogen content using previously established procedures (8–10) on a separate piece of tissue. Muscle intramuscular triglyceride (IMTG) content was also analyzed (in duplicate) on each biopsy sample according to the methods published by Spriet et al. (24). Briefly, triglycerides from muscle (5–8 mg dry wt) were extracted overnight (12–14 h) in $CHCl_3$-methanol (1:2). The top aqueous layer was aspirated and discarded, whereas the organic layer was transferred to another test tube and dried under dry N_2. After drying, the residue was resuspended in 2 ml $CHCl_3$, and 250 µg silic acid (400 mesh; Sigma Chemical, St. Louis, MO) was then added to remove any phospholipid contamination. Samples were spun [10 min, 10,000 revolutions/min (rpm)], and the supernatant was removed and dried under N_2. To the remaining residue, 250 µl ethanolic KOH, was added for saponification of triglycerides. The samples were then heated at 60°C for 1 h and were subsequently neutralized with $MgSO_4$, the precipitate was centrifuged (20 min, 10,000 rpm), and the supernatant was removed and

assayed for glycerol content according to procedures described by Spriet et al. (24). All assays for IMTG concentration for a particular subject were performed at one time to minimize interassay variability. The intra-assay CV, determined on six individual samples from one biopsy, was 5 ± 2.4%, and the interassay CV was 7 ± 3.4% (determined on 10 duplicate samples). Neither muscle glycogen nor IMTG content could be corrected to TCr, since the conditions necessary for analysis of both substrates prevent measurement of muscle Cr content.

We also analyzed each resting biopsy sample for the maximal activities of a number of enzymes according to the procedures of Henriksson et al. (13). Muscle samples were hand homogenized in a phosphate buffer (pH 7.4) containing 0.02% bovine serum albumin (BSA), 5 mM mercaptoethanol, and 0.5 mM EDTA. Homogenates were diluted in 20 mM imidazole buffer with 0.02% BSA. Enzyme assays were performed at room temperature, except for succinate dehydrogenase (SDH) activity, which was measured at 37°C, as outlined previously (10). Measurements of SDH and 1-phosphofructokinase (PFK) activity were made using fresh muscle homogenates. All other enzymes were assayed using frozen aliquots of muscle homogenate that had been stored at −80°C until analysis. Protein was measured using the Lowry technique as modified by Schacterle and Pollock (23). All assays for a particular enzyme, for a particular subject, were performed at one time to minimize the interassay variability. The intra-assay CV for each enzyme assay did not exceed 7 ± 3%, and the interassay CV for any one particular enzyme did not exceed 15 ± 4%. Markers of muscle metabolism were chosen to represent glucose phosphorylation [hexokinase (HK)], glycogen degradation [phosphorylase (PHOS)], glycolysis [PFK and lactate dehydrogenase (LDH)], gluconeogenesis and glyconeogenesis (fructose-1,6-bisphosphatase), the tricarboxylic acid cycle (SDH and MDH), and fat oxidation [β-hydroxyacyl-CoA-dehydrogenase (β-HAD)].

Statistics. Data were analyzed using a two-way analysis of variance with repeated measures. Training status (PRE, 5 days, or 31 days) and time were the independent variables. When significant differences were found, a Student-Newman-Keuls post hoc test was used to locate differences between specific conditions. A one-way analysis of variance was used to analyze data when only single measurements were made (i.e., enzyme activity) at different times throughout the training protocol. Significance was set at the 0.05 level. Data are presented as means ± SE.

RESULTS

High-energy phosphate metabolism. The concentrations of adenine nucleotides (ATP, ADP, and AMP) determined by HPLC were unaltered by either training or exercise (Table 1). However, both training and exercise duration had a significant effect on the levels of muscle IMP. Before training (PRE), the concentration of IMP increased ~2.5 times resting ($P < 0.01$) by 15 min of exercise. The concentration of IMP over the ensuing 75 min of exercise increased a further ~70% ($P < 0.01$) to a level that was about four times greater than the resting value (Table 1). After 5 days of training, the rise in IMP concentrations ([IMP]) was attenuated at both 15 and 90 min vs. PRE (Table 1). Training for 31 days reduced the accumulation of IMP even further, and concentrations at 15 and 90 min were lower than both PRE and 5-day values (Table 1).

Table 1. *Changes in adenine nucleotides and IMP, determined by HPLC, with exercise and training*

	Exercise Time, min		
	0	15	90
ATP			
PRE	24.3 ± 0.6	24.2 ± 0.8	24.1 ± 0.9
5D	25.2 ± 0.6	24.3 ± 0.8	24.1 ± 0.5
31D	24.6 ± 0.3	24.8 ± 0.8	24.2 ± 0.4
ADP			
PRE	4.32 ± 0.45	4.11 ± 0.61	3.94 ± 0.27
5D	4.26 ± 0.42	4.02 ± 0.51	4.11 ± 0.38
31D	4.51 ± 0.33	4.31 ± 0.22	3.87 ± 0.76
AMP			
PRE	0.183 ± 0.11	0.212 ± 0.09	0.192 ± 0.08
5D	0.165 ± 0.08	0.187 ± 0.06	0.167 ± 0.05
31D	0.172 ± 0.07	0.212 ± 0.12	0.196 ± 0.13
IMP			
PRE	0.121 ± 0.02	0.300 ± 0.07*	0.504 ± 0.14*†
5D	0.127 ± 0.03	0.169 ± 0.04‡	0.213 ± 0.11*†‡
31D	0.088 ± 0.09	0.091 ± 0.05‡§	0.102 ± 0.06*‡§

Values are means ± SE in mmol/kg dry wt; $n = 7$ subjects. PRE, pretraining; 5D, 5 days of training; 31D, 31 days of training. $P < 0.01$: *significantly different from 0 min; †significantly different from 15 min; ‡significantly different from PRE; §significantly different from 5D.

Muscle PCr concentration at 15 min of exercise decreased from rest by 42% below the resting level at PRE, by 28.5% at 5 days, and by 22.9% at 31 days (Table 2). From 15 to 90 min, there was no further reduction in PCr (Table 2). The rest-to-exercise reduction in PCr was significantly smaller with each training duration ($P < 0.01$). The progressively smaller hydrolysis of muscle PCr was supported by parallel changes in levels of muscle Cr and P_i, the concentrations of which were dependent on training duration and independent of exercise duration (Table 2).

The concentrations of ADP_f and AMP_f, calculated from the CPK and AK reactions, respectively (9), were altered by both exercise and training (Fig. 1). Not surprisingly, given the changes that we observed in the

Table 2. *Changes in high-energy phosphate metabolites with exercise and training*

	Exercise Time, min		
	0	15	90
PCr			
PRE	80.7 ± 0.7	46.6 ± 2.9	43.7 ± 4.1
5D	78.8 ± 1.0	56.2 ± 3.6*	54.8 ± 3.8*
31D	80.4 ± 0.7	62.7 ± 2.9*†	60.8 ± 2.3*†
Cr			
PRE	44.7 ± 2.0	75.2 + 3.4	76.0 + 4.1
5D	42.6 ± 2.2	66.2 ± 3.8*	67.8 ± 3.2*
31D	44.5 ± 2.1	59.5 ± 2.9*†	62.3 ± 2.3*†
P_i			
PRE	39.1 ± 2.1	82.3 ± 3.3	79.3 ± 3.5
5D	39.6 ± 2.8	65.0 ± 6.0*	64.3 ± 3.4*
31D	37.4 ± 2.3	58.4 ± 5.2*†	54.2 ± 6.7*†

Values are means ± SE in mmol/kg dry wt; $n = 7$ subjects. PCr, phosphocreatine; Cr, creatine. All exercise values were significantly different from rest for PCr, Cr, and P_i ($P < 0.001$). Significant interaction effects were observed for PCr, Cr, and P_i ($P < 0.001$). *Significantly different from PRE ($P < 0.01$). †Significantly different from 5D ($P < 0.01$).

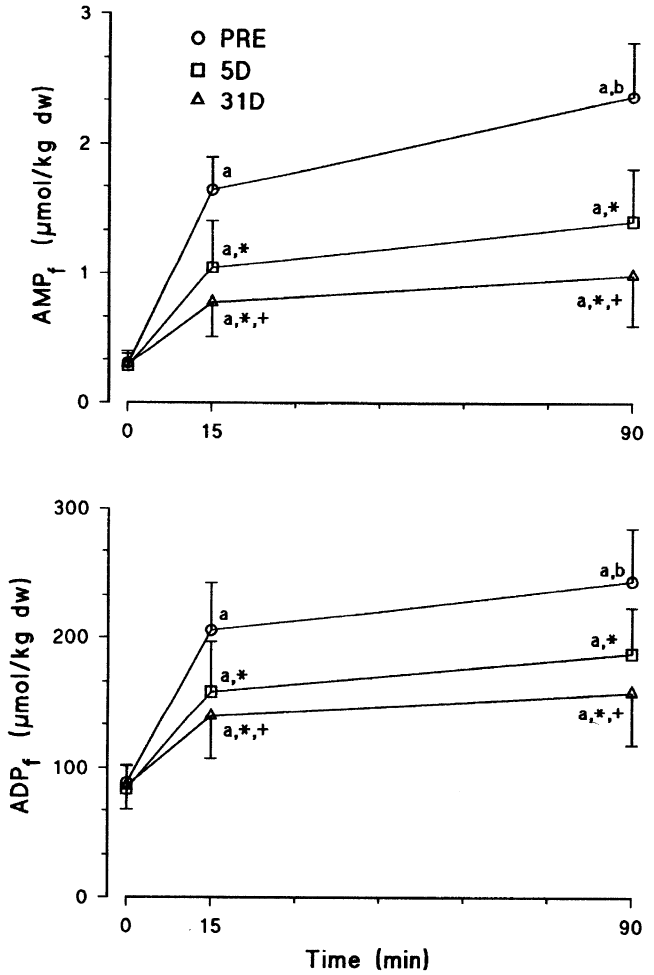

Fig. 1. Effect of exercise and training on calculated values of free AMP (AMP_f) and free ADP (ADP_f). Values are means ± SE (n = 7 subjects), in μmol/kg dry wt (dw). ○, pretraining (PRE); □, 5 days of training (5D); △, 31 days of training (31D). [a]Significantly ($P < 0.01$) different from 0 min. [b]Significantly ($P < 0.05$) different from 15 min. *Significantly ($P < 0.01$) different from PRE. +Significantly ($P < 0.01$) different from 5D.

high-energy phosphate metabolites, exercise resulted in a significant increase in calculated ADP_f and AMP_f that was progressive over time PRE. A similar pattern of change emerged with training, with progressively lower concentrations of both ADP_f and AMP_f with increasing training duration.

Muscle metabolites: lactate, pyruvate, and the lactate-to-pyruvate ratio. On average, the concentration of muscle lactate was increased at 15 min of exercise vs. both rest and 90 min for all training conditions. As the training duration was extended, the increase was progressively smaller (Table 3). The concentration of muscle lactate at 15 min was reduced ~44% by 5 days and was reduced by another 15% at 31 days. Muscle lactate concentration ([lactate]) at 90 min of exercise was not different from rest for all training times (Table 3).

In general, pyruvate concentrations increased at both 15 and 90 min of exercise (Table 3). Pyruvate concentration was affected by training duration only at 31 days, and only at the 15-min time point, where pyruvate was lower than PRE values.

The lactate-to-pyruvate ratio (L/P), which was taken to be an indicator of the cellular redox potential (3), was elevated only at 15 min of exercise (Table 3). At 15 min, training resulted in a reduction of the L/P of 40% at 5 days with no further reduction observed at 31 days. The L/P at 90 min of exercise was not significantly different from rest in any of the training conditions.

We also estimated the cytosolic redox potential and pH (Table 3) as outlined previously (9). The results showed that the calculated NAD^+-to-NADH ratio (NAD^+/NADH) was reduced by 15 min of exercise but returned to resting levels by 90 min. In addition, the reduction in the NAD^+/NADH at 15 min was less after 5 days of training than PRE and remained less after 31 days of training. Estimated pH showed similar results to that of the cytosolic redox potential, that is, that exercise resulted in a lower pH at 15 min vs. 0 and 90 min but only in the PRE training condition. Training for both 5 and 31 days resulted in a smaller reduction in pH at 15 min of exercise after 5 and 31 days of training vs. PRE.

Glucose, G-6-P, and citrate. Exercise resulted in an increase in muscle glucose and G-6-*P* at all exercise time points (Table 4). Training duration only affected muscle glucose at 31 days, where the glucose concentration at 90 min was higher than the concentration measured at PRE (Table 4). Muscle G-6-*P* concentrations were also affected by training duration. At 15 min of exercise and after 31 days of training, G-6-*P* concentrations were 36% lower than PRE levels. Exercise, in general, resulted in an increased G-6-*P* concentration at both 15 and 90 min.

Table 3. *Changes in muscle lactate, pyruvate, lactate-to-pyruvate ratio, pH, and calculated cytosolic redox potential with exercise and training*

	Exercise Time, min		
	0	15	90
Lactate			
PRE	6.5 ± 0.4	29.8 ± 3.6*	10.2 ± 0.5
5D	6.2 ± 0.5	16.8 ± 2.3*†	8.8 ± 0.7
31D	6.2 ± 0.5	12.3 ± 2.4*†‡	6.8 ± 0.6
Pyruvate			
PRE	0.210 ± 0.01	0.320 ± 0.03	0.300 ± 0.03
5D	0.170 ± 0.01	0.280 ± 0.03	0.274 ± 0.03
31D	0.166 ± 0.02	0.218 ± 0.04†	0.264 ± 0.04
L/P			
PRE	31.6 ± 2.1	106 ± 28*	35.0 ± 4
5D	34.8 ± 2.7	63.5 ± 12*†	33.5 ± 4
31D	38.7 ± 2.0	56.4 ± 7*†	28.2 ± 4
pH			
PRE	7.03 ± 0.011	6.93 ± 0.030*	7.02 ± 0.021
5D	7.03 ± 0.012	7.00 ± 0.025†	7.02 ± 0.020
31D	7.03 ± 0.008	7.00 ± 0.014†	7.03 ± 0.009
NAD^+/NADH			
PRE	270 ± 41	113 ± 22*	256 ± 33
5D	228 ± 51	154 ± 26*†	268 ± 50
31D	225 ± 20	165 ± 31*†	328 ± 44

Values are means ± SE, in mmol/kg dry wt, except for lactate-to-pyruvate ratio (L/P), pH, and NAD^+-to-NADH ratio (NAD^+/NADH); n = 7 subjects. Significant interaction effect was observed for all variables ($P < 0.01$). *Significantly ($P < 0.05$) different from 0 and 90 min; †significantly ($P < 0.01$) different from PRE; ‡significantly ($P < 0.01$) different from 5D.

Table 4. *Changes in muscle glucose, G-6-P, and citrate with exercise and training*

	Exercise Time, min		
	0	15	90
Glucose			
PRE	1.83 ± 0.25	3.83 ± 0.50*	2.30 ± 0.32*
5D	2.21 ± 0.24	3.62 ± 0.30*	3.37 ± 0.48*
31D	2.61 ± 0.45	3.65 ± 0.30*	3.50 ± 0.41*†
G-6-*P*			
PRE	0.63 ± 0.12	2.04 ± 0.28*	1.32 ± 0.17*
5D	0.80 ± 0.08	1.96 ± 0.15*	1.17 ± 0.26*
31D	0.65 ± 0.10	1.30 ± 0.12*†	1.15 ± 0.15*
Citrate			
PRE	0.56 ± 0.10	0.80 ± 0.18	0.84 ± 0.10*
5D	0.58 ± 0.07	0.79 ± 0.16	0.93 ± 0.21*
31D	0.61 ± 0.07	0.82 ± 0.12	1.09 ± 0.09*†‡§

All values are means ± SE in mmol/kg dry wt; n = 7 subjects. G-6-*P*, glucose 6-phosphate. Significant interaction effect was observed for glucose and G-6-*P* ($P < 0.01$). *Significantly different ($P < 0.01$) from 0 min; †significantly ($P < 0.01$) different from PRE; ‡significantly ($P < 0.05$) different from 15 min; §significantly ($P < 0.01$) different from 5D.

Mean muscle citrate concentrations were remarkably consistent at rest in all training conditions, ranging only between 0.56 and 0.61 mmol/kg dry wt. Citrate concentration was not increased after 15 min of exercise, although by 90 min of exercise the concentration was greater than rest in all training conditions. In addition, the concentration of citrate at 90 min was greater after 31 days of training than at 5 days and PRE (Table 4).

Muscle substrate metabolism: glycogen and IMTG. The effects of exercise and training on muscle glycogen concentration and muscle triglyceride are shown in Fig. 2. A main effect for both training and exercise was observed for muscle glycogen concentration. In general, glycogen concentration was progressively higher with training duration. Because the differences in resting muscle glycogen with training obscured the effect of training on glycogen depletion during exercise, the values were normalized to preexercise levels for each condition, and an analysis of variance was performed on the percent change. The statistical analysis showed that there was an interaction between training and time ($P < 0.05$). The percent of resting muscle glycogen concentration at 15 min was as follows: PRE = 60 ± 3%, 5 days = 75 ± 4%, and 31 days = 85 ± 3%. The values at 5 and 31 days were higher than PRE ($P < 0.01$). At 90 min, the percent resting muscle glycogen was as follows: PRE = 17 + 3%, 5 days = 43 ± 4%, and 31 days = 64 ± 4%. All values are different from each other ($P < 0.01$).

At 31 days of training, an increase in the concentration of IMTG stores was observed at rest and at 15 min of exercise compared with both 5 days and PRE (Fig. 2). With exercise, IMTG concentration was reduced at 90 min vs. all other time points regardless of training status. The overall depletion (rest to 90 min) of IMTG was 5.1 ± 1.0 mmol/kg dry wt and was not significantly different PRE vs. 5 days. However, the overall depletion of IMTG was increased by 56% after 31 days vs. PRE and 5 days ($P < 0.01$).

Muscle enzymes. A variety of muscle enzymes were assayed in an attempt to assess the training-induced changes in the capacity of the major metabolic pathways and other relevant biochemical reactions (Table 5). Training increased the maximal activity of muscle HK by 24% at 5 days, an increase that persisted over the training duration. Total PHOS, PFK, and fructose-1,6-bisphosphatase activity remained unchanged after both 5 and 31 days of training. Training did have an effect on the maximal activity of LDH, which decreased at 31 days vs. PRE and 5 days between 11 and 13% (Table 5). Training had no effect on the maximal activities of MDH and SDH at 5 days but did increase the maximal activities of both enzymes by 31 days of training. The maximal activity of β-HAD also increased in a fashion similar to that of MDH and SDH, namely only after 31 days of training (Table 5).

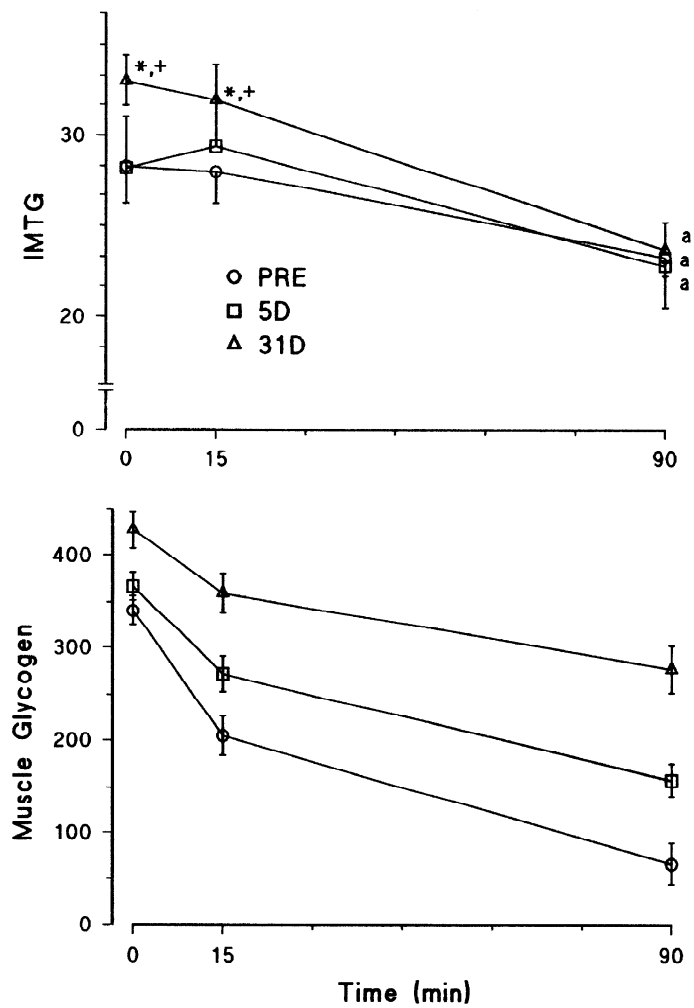

Fig. 2. Effect of exercise and training on intramuscular triglycerides (IMTG) and muscle glycogen concentration. Values are means ± SE (n = 7 subjects), in mmol/kg dry wt for IMTG and mmol glucosyl U/kg dry wt for glycogen. Significant interaction effect ($P < 0.001$) was found for IMTG. Significant main effect for both training and time was found for muscle glycogen ($P < 0.001$). [a]Significantly ($P < 0.01$) different from 0 min. *Significantly ($P < 0.01$) different from PRE. +Significantly ($P < 0.01$) different from 5D.

Table 5. *Effects of training on maximal muscle enzyme activities*

	Training Duration		
	PRE	5D	31D
PHOS	14.9 ± 0.5	15.2 ± 0.7	15.1 ± 0.6
HK	0.365 ± 0.02	0.453 ± 0.02*	0.466 ± 0.02*
PFK	11.2 ± 0.7	11.8 ± 0.6	11.7 ± 0.6
LDH	53.7 ± 1.6	52.2 ± 0.8	46.6 ± 1.2*†
Fru-1,6-Pase$_2$	0.512 ± 0.02	0.505 ± 0.03	0.491 ± 0.02
SDH	3.8 ± 0.7	3.9 ± 0.6	5.4 ± 0.8*†
MDH	13.5 ± 0.8	14.0 ± 1.6	18.6 ± 1.1*†
β-HAD	6.3 ± 0.3	6.3 ± 0.5	7.8 ± 0.4*†

Values are means ± SE, in mol·kg protein^{-1}·h^{-1} at room temperature, except for succinate dehydrogenase (SDH), which was measured at 37°C; n = 7 subjects. PHOS, total phosphorylase; HK, hexokinase; PFK, 1-phosphofructokinase; LDH, lactate dehydrogenase; Fru-1,6-Pase$_2$, fructose-1,6-bisphosphatase; MDH, malate dehydrogenase; β-HAD, β-hydroxyacyl-CoA-dehydrogenase. $P < 0.01$: *significantly different from PRE; †significantly different from 5D.

DISCUSSION

These results showed that 5 days of daily training can induce a shift in the metabolic response of the working muscle toward a tighter metabolic control where the balance between high-energy phosphate utilization and production is more closely matched (3, 14). The tighter metabolic control was also reflected in some other metabolic systems, including glycolysis and glycogenolysis. All of the changes in muscle metabolism that occurred at 5 days were observed before changes in muscle mitochondrial content, as indicated by the maximal activities of two representative tricarboxylic acid cycle enzymes SDH and MDH. However, after 31 days of training when a significant increase in muscle oxidative capacity was observed, many of the changes in metabolism were further potentiated. It is reasonable, therefore, to hypothesize that the increase in muscle mitochondrial capacity resulted in at least part of the greater training-induced changes in muscle metabolism. It should also be emphasized that the early (5 days) training-induced changes in muscle metabolism occurred in the absence of an increase in $\dot{V}O_{2peak}$ and submaximal steady-state $\dot{V}O_2$ (20). Because oxidative phosphorylation was not changed, and because ADP_f and related modulators decreased, it would appear that training increased respiratory control sensitivity.

The present conclusion is contrary to previous speculations from our group, namely that an increase in the duration of training of up to 4 wk appeared to be ineffective in potentiating the metabolic adaptations observed after 5–7 days of training, when the muscle's mitochondrial content was unchanged (9). However, this comparison was made based on cross-sectional data where the two participant groups differed by ~10 ml·kg^{-1}·min^{-1} in their pretraining $\dot{V}O_{2peak}$ (8, 9). The possibility remains that differences in initial $\dot{V}O_{2peak}$ may reflect differences in initial training status, resulting in a bias in our previous comparisons (9).

The discrepancy between ATP supply and demand (%imbalance) in an active muscle is reflected by changes in ATP concentration, an increase in [IMP], and/or the hydrolysis of PCr (14). The imbalance between ATP supply and demand has been represented by Hochachka and Matheson (14) as

$$\%\text{imbalance} - \frac{\Delta[\text{ATP}] \times 100}{\text{total ATP turnover}}$$

Hence, our findings suggest that the current training protocol resulted in a smaller imbalance between ATP supply and demand after 5 days and a further reduction of this imbalance by 31 days. Because any contribution to ATP supply from glycolysis was probably lower after both 5 and 31 days, it is plausible that the smaller percent imbalance was due to an increased supply of ATP from oxidative metabolism (3, 8, 9). There is a stoichiometric relationship between ATP depletion and IMP accumulation (6, 22). In the present study, given the increase in IMP that occurred with exercise duration, one would have expected to see a decrease of ~3% in the concentration of ATP. That we did not observe such a reduction may have been due both to the variability in the HPLC method for determining ATP, and the relatively small reduction in ATP expected (~1.2 mmol/kg dry wt) compared with the total concentration (~25 mmol/kg dry wt). We have observed similar discrepancies previously (8, 9, 14).

Exercise resulted in an increase in the calculated concentration of ADP_f and AMP_f, whereas training resulted in a reduction in the exercise-induced increase in both metabolites at 5 days and a further reduction at 31 days. The smaller levels of both ADP_f and AMP_f after training, at each exercise time point, were accompanied by a lower accumulation of IMP at each corresponding time point. This is not surprising since increases in the concentrations of ADP_f and AMP_f and reductions in pH would activate AMP deaminase (4).

A tighter metabolic control as reflected in lower estimated concentrations of ADP_f, AMP_f, and H^+ has also been postulated to result in a depression of glycogenolysis and glycolysis via PHOS and PFK regulation (3, 5, 15). Our results are consistent with this notion. Lactate, as an example, was progressively reduced at 15 min of exercise at 5 and 31 days of training. The fact that similar effects were not noted at 90 min of exercise may simply reflect differences between lactate production and removal. Early in exercise, lactate production would be expected to predominate, whereas later in exercise removal would be more emphasized.

At present, it is unclear whether increases in exercise intramuscular lactate and L/P and a decrease in estimated NAD^+/NADH are due to an insufficiency of oxygen within the cell as postulated (18) or whether the increase occurs simply to support mitochondrial respiration by supplying reducing equivalents (3). In a companion study using the same subjects, we have found that 4 days of training accelerated $\dot{V}O_2$ kinetics during the nonsteady adjustment to acute exercise (20). In addition, findings from another study have suggested that changes in muscle high-energy phosphate metabolism are a stable function of time after the

initial 3-min non-steady-state adjustment to moderate-intensity exercise and that the metabolic adaptations to short-term training are expressed during the same time period (7). It is entirely possible that the tighter control observed between ATP synthetic and utilization rates consequent to training is primarily expressed during this non-steady-state period and is secondary to increases in oxidative phosphorylation. Moreover, the putative depression in glycolysis that occurs early in exercise also may be coupled to increased oxidative phosphorylation. It is clear that the increase in ATP synthesis generated from oxidative phosphorylation during the nonsteady state is not dependent on an increased respiratory capacity at least early in training.

It should be emphasized that we and others (5, 13, 15, 17) have only measured the maximal activities of a number of representative enzymes to provide a measure of muscle mitochondrial respiratory capacity. This has enabled us to investigate whether the increase in active enzyme concentration measured "in vitro" can be implicated in the constant oxidative phosphorylation observed during the steady state at a higher phosphorylation potential after training.

As in other studies (1, 8–10), we observed that training resulted in a reduced rate of glycogen utilization after 5 days and a further reduction in glycogen depletion at 31 days. Hence, the rate of glycogenolysis appears to be controlled both by mechanisms that are dependent and independent of muscle mitochondrial content. We have previously hypothesized that there is a coupling between the phosphorylation potential and glycolysis, notably early in exercise (1, 8, 9). These conclusions were based on the fact that smaller decrements in PCr and smaller increases in Cr concentration, P_i concentration ($[P_i]$), and [IMP] were associated with smaller increases in muscle and blood [lactate] and muscle glycogen degradation (8, 10). The present results support this conclusion, since the reduction in PCr (and the associated increases in both Cr and P_i) occurred in conjunction with the increase in intramuscular [lactate]. The mechanistic basis for this relationship may be due to the control that P_i, pH, and AMP_f have on both PHOS and PFK (3, 21). The lower intramuscular $[P_i]$ at both 5 and 31 days vs. PRE would result in less substrate available for utilization by PHOS in the breakdown of glycogen. Moreover, a decrease in intramuscular $[P_i]$, and a smaller decrease in intracellular pH, would result in less allosteric relief of the inhibition of PFK by ATP (3, 15). The result would be a decrease in both glycogenolytic and glycolytic rates.

The lower respiratory exchange ratio has been taken as an indication of increased fat oxidation after extended training (2, 15–17, 19). The sources of the extra substrates that were oxidized cannot be directly ascertained from the results reported here; however, some inferences can be drawn from the data. It has been postulated that training enhances the glucose-fatty acid cycle (15, 17). However, we found no evidence for this mechanism either from the concentrations of either intramuscular glucose or G-6-*P* that the glucose-fatty acid cycle was operating to a greater degree, after training. Further, the levels of intramuscular G-6-*P* were actually lower early in exercise after prolonged training, a finding that we (Table 4) and others have observed (2). In contrast, however, we found that, after 31 days, intramuscular citrate concentration was significantly increased vs. both PRE and 5 days at 90 min of exercise. It is possible that the elevated intramuscular citrate concentration that we and others have observed (17) is exclusive to the mitochondrial space and does not inhibit PFK. In this case, intramuscular citrate concentration is simply a function of the increased mitochondrial mass (Table 5) induced by the prolonged training protocol (2). In support of this hypothesis, we found that the concentration of intramuscular citrate was correlated with maximal muscle oxidative enzyme (citrate synthase and SDH) activity ($r = 0.71$ and 0.73, respectively; $P < 0.01$).

A number of studies have shown that an increasing proportion of fats are oxidized after training from intramuscular stores (16, 17, 19). Our results support this conclusion, since the depletion of IMTG was greatest during the exercise at 31 days vs. both PRE and 5 days. We made measures of IMTG concentration at 15 and 90 min during exercise, although the variability of the assay prevented us from ascertaining whether significant triglyceride oxidation had occurred between rest and 15 min. Depletion of IMTG during the exercise was not different during PRE and 5 days. Interestingly, muscle mitochondrial content was also not significantly different at these times. Hence, it appears that an increase in muscle mitochondrial mass may be necessary for an increase in IMTG oxidation to occur.

In summary, 5 days of training resulted in a change in muscle metabolism reflective of a tighter metabolic coupling in high-energy phosphate metabolism, glycolysis, and glycogenolysis (glycogen depletion). However, at 5 days, muscle oxidative capacity remained unchanged from PRE, suggesting that alternative mechanisms, other than muscle mitochondrial potential, were responsible for these adaptations. After a further 25 days of training, however, all of the adaptations in muscle metabolism were potentiated. Because muscle mitochondrial potential was increased at this time, it is hypothesized that the changes seen at 31 days were due, to some degree, to the increased muscle mitochondrial volume. Training also resulted in an increase in IMTG oxidation, but only after 31 days of training, suggesting that an increase in muscle mitochondrial capacity might be necessary for an increase in IMTG oxidation.

We thank George Obminsky for technical assistance in the conduct of the experiments.

This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

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Received 1 May 1995; accepted in final form 7 September 1995.

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