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The distribution of rest periods affects performance and adaptations of energy metabolism induced by high-intensity training in human muscle

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ABSTRACT

The effect of the distribution of rest periods on the efficacy of interval sprint training is analysed. Ten male subjects, divided at random into two groups, performed distinct incremental sprint training protocols, in which the muscle load was the same (14 sessions), but the distribution of rest periods was varied. The 'short programme' group (SP) trained every day for 2 weeks, while the 'long programme' group (LP) trained over a 6-week period with a 2-day rest period following each training session. The volunteers performed a 30-s supramaximal cycling test on a cycle ergometer before and after training. Muscle biopsies were obtained from the *vastus lateralis* before and after each test to examine metabolites and enzyme activities. Both training programmes led to a marked increase (all significant, $P < 0.05$) in enzymatic activities related to glycolysis (phosphofructokinase – SP 107%, LP 68% and aldolase – SP 46%, LP 28%) and aerobic metabolism (citrate synthase – SP 38%, LP 28.4% and 3-hydroxyacyl-CoA dehydrogenase – SP 60%, LP 38.7%). However, the activity of creatine kinase (44%), pyruvate kinase (35%) and lactate dehydrogenase (45%) rose significantly ($P < 0.05$) only in SP. At the end of the training programme, SP had suffered a significant decrease in anaerobic ATP consumption per gram muscle ($P < 0.05$) and glycogen degradation ($P < 0.05$) during the post-training test, and failed to improve performance. In contrast, LP showed a marked improvement in performance ($P < 0.05$) although without a significant increase in anaerobic ATP consumption, glycolysis or glycogenolysis rate. These results indicate that high-intensity cycling training in 14 sessions improves enzyme activities of anaerobic and aerobic metabolism. These changes are affected by the distribution of rest periods, hence shorter rest periods produce larger increase in pyruvate kinase, creatine kinase and lactate dehydrogenase. However, performance did not improve in a short training programme that did not include days for recovery, which suggests that muscle fibres suffer fatigue or injury.

Keywords anaerobic exercise, enzyme activities, glycogen, glycolysis, lactate, recovery, skeletal muscle metabolism, sprint training.

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Muscle adaptation owing to exercise or physical training seems to be correlated with the amount, intensity, distribution and duration of muscle loads (Dudley *et al.* 1982). The combination of these factors is especially important when interval high-intensity training is designed because biochemical responses depend on the protocol of contractile activity to which the muscle is subjected (summarized in MacDougall *et al.* 1998). Hence, a direct relationship between adaptations and the components of the sprint training has been hard to find. However, certain desirable biochemical adaptations and sprint performance improvement seem to be associated with high-intensity training. Increases in enzymatic activities related to glycolysis, including phosphofructokinase, lactate dehydrogenase or glycogen phosphorylase and changes in metabolite concentration, including phosphocreatine or glycogen, have been recorded, although the extent of these changes varies from one sprint training programme to another (Thorstensson *et al.* 1975, Costill *et al.* 1979,

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Roberts *et al.* 1982, Cadefau *et al.* 1990, Linossier *et al.* 1993, 1997, Dawson *et al.* 1998, MacDougall *et al.* 1998).

The duration of each bout affects the adaptations induced by interval high-intensity training, as described by Costill *et al.* (1979), comparing 6 s in the left leg with 30 s in the right leg of maximal isokinetic exercise of the same subject. Bouts lasting less than 10 s are considered more anaerobically dependent (Hellsten-Westing *et al.* 1993, Linossier *et al.* 1993) than longer bouts, which are more demanding and during which power output decreases before the end (Bogdanis *et al.* 1995, 1996).

The recovery periods between bouts are also decisive and the time ratio between recovery and exercise phases has to be taken into account (Linossier *et al.* 1997). Intense and brief muscle loads with long recovery periods are proposed to induce an adaptative response in phosphocreatine metabolism (Thorstensson *et al.* 1975), while an increased training load has no effect on this process, but seems to produce a greater adaptative response in lactate metabolism (Roberts *et al.* 1982, Cadefau *et al.* 1990). The distribution of rest periods between days of training is usually less carefully planned than the ratio of recovery and work in each session and its effects on the adaptations induced by sprint training have not been studied. The rest periods between sessions prevent fatigue, which could appear when rest periods are insufficient and/or muscle load is exhausting.

In order to analyse the effect of rest distribution on muscle adaptations, we designed two high-intensity training cycle protocols with identical daily muscle loads but different distribution of rest periods. Enzymatic activities related to glycolysis, glycogen and creatine metabolism and aerobic metabolism were measured together with adenine nucleotides, glycolytic intermediates and creatine concentrations. We also evaluated the effect of the two training protocols on performance and muscle metabolic response by a 30-s all-out cycling test before and after training.

METHODS

Subjects

Ten healthy male student volunteers agreed to take part in this study. Their age, height and body mass were (mean ± SD) 23.6 ± 2.4 years, 171.1 ± 3.4 cm and 70.2 ± 4.8 kg, respectively. All were active, but none was currently participating in a regular training programme. During the experiment, all volunteers stopped their normal physical activity and only exercised within the experiment. Before the commencement of the experiment the volunteers underwent a medical check-up to verify that they were healthy and fit. They were divided at random into two groups called 'short programme' (SP) and 'long programme' (LP).

The experiment was conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and approval was given by the Ethical Committee of Human Experimentation from the Pi i Sunyer Biomedicine Research Institute of Barcelona (Hospital Clínic i Provincial – University of Barcelona). All subjects were informed before recruitment about the purpose of the study, known risks, and possible hazards associated with the experimental protocol and each gave his written consent.

Performance test

In order to evaluate the anaerobic capacity of volunteers and possible improvement owing to training, subjects were required to perform a supramaximal cycling test (30 s) the day before the initiation of training and 48 h after finishing 14 training sessions. The test was performed on a friction-loaded cycle ergometer (Monark 814 E, Varberg, Sweden). A microprocessor, interfaced with the cycle ergometer, counted flywheel revolutions every second for 30 s of supramaximal cycling sprinting against a constant resistance of 0.075 kg × (kg body mass)$^{-1}$. With the flywheel progression per pedal revolution and the elapsed time, the following variables were calculated: peak power (the highest power output) and mean power (the average power output during the 30 s). The subjects were comfortably seated with feet secured to the pedals by toe clips. They were requested to pedal as fast as possible from the start and were encouraged to maintain maximum pedalling speed throughout the 30-s period.

Training protocol

Familiarization with the equipment, sprint cycling and testing procedures took place before the experiment started, until there was complete confidence in reaching an all-out effort from a stationary start. Each group (SP and LP) participated in a different high-intensity programme designed to improve performance in high-intensity tests. These programmes involved the same 14 training sessions but with different duration of rest. The SP group trained every day for 2 weeks while the LP group trained for 6 weeks resting for 2 days between each session. The sessions comprised a number of warm-up repetitions of 15 s maximal cycling with 45 s rest-periods and a number of training repetitions of 30 s maximal cycling with 12 min rest-periods. The number of repetitions was modified and the total muscle load increased during training. The first three

sessions comprised of two bouts of 15 s sprints and two bouts of 30 s supramaximal cycling sprints. In the following sessions, the number of 15- and 30-s bouts were increased by one every two training sessions. The last three sessions consisted of seven bouts of 15 s and seven bouts of 30 s. As in the performance tests, subjects were instructed to remain seated during the cycle sprints in the training period. The flywheel tension was set at 0.075 kg (kg body mass)$^{-1}$ and remained constant for the duration of the training programme. The maximum number of pedal revolutions reached by each volunteer in every 30-s bout was recorded. All subjects were highly motivated and verbally encouraged during training and instructed to cycle with maximum effort in every session.

Muscle biopsies

The needle biopsy technique was used to sample muscle tissue. Muscle biopsy samples (30–50 mg) were taken under local anaesthesia (mepivacaine 2%) from the mid-region of the *quadriceps femoris* muscle (*vastus lateralis*) from both legs, 15 cm above the top edge of the patella on the first day and 5 cm above it next day (48 h after finishing training). On the day of the performance test, volunteers reported to the laboratory at least 3 h after their last meal. After a light warm-up, they sat quietly on an examination couch while small incisions were made in both legs through the skin and fascia and the first muscle biopsy was obtained from left leg (rest). Subjects then performed the test and the second biopsy from right leg (30 s) was taken immediately after, while they were still seated on the cycle ergometer. The same protocol was performed 1 day before (pre) and 2 days after (post) training. The samples were directly frozen, removed from the biopsy needles under liquid nitrogen and stored at –80 °C until they were lyophilized and analysed.

Biochemical studies

Freeze-dried samples were dissected free of blood and connective tissue and powdered. A part (20 mg) of the dry tissue was treated with 0.5 M $HClO_4$ and centrifuged at 13 000 × *g* at 4 °C for 15 min. The supernatant was neutralized with 2.1 M $KHCO_3$. The neutralized extract was assayed for phosphocreatine (PCr), ATP, creatine (Cr), free glucose, glucose 1-phosphate (G-1-P), glucose 6-phosphate (G-6-P), fructose 6-phosphate (F-6-P), fructose 1,6-bisphosphate (F-1,6-P_2), pyruvate (Pyr) and lactate (Lac). All muscle metabolites were assayed enzymatically by fluorometric analysis (Lowry & Passonneau 1972). Glycogen concentration was measured both in the neutralized extract and in the pellet, by prior 1 M HCl hydrolysis extraction. Then the free glucose produced was determined by the method described above. From the neutralized extraction, IMP, ATP, ADP and AMP were measured using the HPLC method (Ingebretsen *et al.* 1982). Muscle metabolite concentrations were adjusted to the individual mean total creatine (PCr + Cr) because this mean should be kept constant during exercise (Harris *et al.* 1976). The adjustment to total creatine content enabled any variability in solid non-muscle constituents of the biopsies to be corrected.

For the enzymatic analyses, a portion of the muscle biopsies (10 mg) taken before the tests was homogenized in 30 volumes of ice-cooled extraction medium. The extraction medium contained 50 mM HCl–Tris (pH 7), 4 mM EDTA, 50 mM KF and 30 mM β-mercaptoethanol. The preparation was centrifuged at 15 000 × *g* at 4 °C for 15 min. The following activities were immediately measured in the supernatant: glycogen synthase (GS), glycogen phosphorylase (GPh), creatine kinase (CK), phosphofructokinase (PFK), aldolase (ALD), lactate dehydrogenase (LDH) and pyruvate kinase (PK), as described in Cadefau *et al.* (1990); hexokinase (HK), citrate synthase (CS), phosphoglucoseisomerase (PGI) and 3-hydroxyacyl-CoA dehydrogenase (HAD) as described in Essen-Gustavsson & Henriksson (1984) and myokinase (MK) as described in Oliver (1955).

Calculations

Values of pH in the muscle, before and after the test, were calculated from changes in lactate and pyruvate concentration [expressed as mmol (kg dry tissue)$^{-1}$] as reported by Sahlin (1978):

$$\mathrm{pH} = 7.06 - 0.00413 \cdot ([\mathrm{Lac}] + [\mathrm{Pyr}])$$

ATP consumption [mmol (kg dry tissue)$^{-1}$] during the tests, before and after training, was also calculated using a specific equation (Katz *et al.* 1986), where increments in ATP, ADP, PCr, Lac and Pyr were obtained from each value before and after the 30 s all-out cycling test.

$$\text{ATP consumption} = 2(-\Delta\mathrm{ATP}) - \Delta\mathrm{ADP} - \Delta\mathrm{PCr} + 1.5\Delta(\mathrm{Lac}) + 1.5(\Delta\mathrm{Pyr})$$

No corrections were made for lactate or pyruvate efflux during sprints or for anaerobic-produced ATP as a result of pyruvate oxidation.

The flux in glycogenolytic and glycolytic pathways [mmol glucosyl units (kg dry tissue)$^{-1}$] was calculated from a combination of the variation in concentration of several metabolites, as described by Spriet *et al.* (1987):

$$\text{Glycogenolytic rate} = \Delta\text{G-1-P} + \Delta\text{G-6-P} + \Delta\text{F-6-P} + 0.5(\Delta\mathrm{Lac} + \Delta\mathrm{Pyr})$$

$$\text{Glycolytic rate} = 0.5(\Delta\mathrm{Lac} + \Delta\mathrm{Pyr})$$

Table 1 Nucleotides and creatine concentration in muscle biopsies of LP and SP groups at rest and after the 30-s cycle ergometer sprint test before (pre) and after (post) training

	SP Group				LP Group			
	Pre-training		Post-training		Pre-training		Post-training	
	Rest	30 s	Rest	30 s	Rest	30 s	Rest	30 s
ATP	24.4 ± 0.9	16.8 ± 0.9*	22.6 ± 0.7	19.4 ± 1.0	24.2 ± 0.9	16.6 ± 0.7*	22.6 ± 0.8	19.8 ± 0.7
ADP	2.38 ± 0.20	2.76 ± 0.60	1.96 ± 0.20	2.66 ± 0.20	2.34 ± 0.49	2.85 ± 0.32	2.08 ± 0.27	2.30 ± 0.26
AMP	0.25 ± 0.05	0.42 ± 0.03*	0.30 ± 0.07	0.40 ± 0.03	0.26 ± 0.04	0.42 ± 0.08*	0.24 ± 0.05	0.34 ± 0.03*
IMP	0.63 ± 0.10	6.65 ± 1.10**	0.57 ± 0.02	1.03 ± 0.20[a]	0.76 ± 0.12	7.15 ± 1.21**	0.52 ± 0.13	2.55 ± 0.53*[a]#
TAN	27.3 ± 0.8	19.8 ± 0.9*	24.9 ± 1.0	22.5 ± 1.0	26.8 ± 0.8	20.9 ± 0.9*	24.9 ± 0.8	22.4 ± 0.8
TAN + IMP	27.9 ± 0.9	26.5 ± 1.3	25.5 ± 1.2	23.5 ± 1.3	27.6 ± 0.7	27.0 ± 0.8	25.4 ± 0.9	25.0 ± 0.7
PCr	55.9 ± 6.6	19.0 ± 8.1**	68.4 ± 2.0[a]	30.2 ± 8.9**	64.8 ± 8.1	35.5 ± 7.9*	64.2 ± 7.0	19.9 ± 4.8**[a]
Cr	52.1 ± 10.7	88.1 ± 9.8*	53.7 ± 5.2	93.1 ± 12.5*	44.8 ± 8.7	74.3 ± 11.7*	46.7 ± 4.9	91.1 ± 16.5**[a]
Total creatine	107.9 ± 13.2	107.9 ± 14.9	123.0 ± 10.2[a]	123.0 ± 11.5[a]	109.8 ± 11.4	109.8 ± 12.7	111.0 ± 8.7	111.0 ± 8.7

Values are means ± SD for five subjects in each group expressed in mmol (kg dry tissue)$^{-1}$. *,** Significant difference ($P < 0.05$, $P < 0.01$) between the rest and after the test in the same training status. [a] Significant difference ($P < 0.05$) between values of the same parameter before and after training. # Significant difference ($P < 0.05$) between values of the same parameter on different group.

The mean rate was calculated by dividing the absolute values by the time of the test (30 s).

Statistics

Differences in the same groups before and after training were analysed by non-parametric Wilcoxon test for paired values. Differences between the two groups were evaluated by non-parametric Mann–Whitney test for unpaired values. Differences were considered significant at $P < 0.05$ and values were expressed as means ± SD.

RESULTS

Muscle metabolites in the pre-training test

One day before the beginning of the training period, subjects undertook a supramaximal test (pre-training test). Several metabolites were measured from biopsies taken immediately before and after the test for both groups (Tables 1, 2). Before training, neither group showed any significant differences in metabolite concentration at rest and the changes produced owing to the test were similar. ATP and PCr concentration decreased significantly in both groups (in all cases $P < 0.05$). IMP increased significantly during the test in both groups. Despite the extent of changes in total adenine nucleotide (TAN = ATP + ADP + AMP), the amount of TAN + IMP remained unchanged after 30 s of supramaximal cycling. Glycogen concentration decreased significantly in both groups ($P < 0.05$) and to a similar extent (SP 29%; LP 26%). As a result of glycogen degradation, G-1-P concentration increased significantly, about 3–4-fold ($P < 0.01$ both groups). G-6-P concentration increased more than 10-fold (both groups $P < 0.01$). F-6-P increased (both groups $P < 0.01$) in a similar manner to G-6-P. Although glucose concentration increased in both groups, no significant differences were found. The increase in lactate concentration was higher than 10-fold in both groups and the increase in pyruvate reached 5-fold ($P < 0.01$ in all cases). As lactate and pyruvate concentrations increased, the muscle fibres became acidic. The calculated pH fell sharply after the 30-s-test in both groups.

Enzymatic adaptations to sprint training

There were no significant differences in enzymatic activities between the SP and LP groups before the start of the training programmes. However several enzymatic activities were modified in response to training (Table 3). Myokinase, glycogen synthase and glycogen phosphorylase did not vary significantly in either group, but the percentage of change was different between groups ($P < 0.05$).

Creatine kinase activity showed significant increase in the SP group (44%, $P < 0.05$), but only a slight variation in the LP group (9%). Pyruvate kinase and lactate dehydrogenase increased significantly ($P < 0.05$) and to a considerable extent (35 and 45%, respectively) in the SP group, but not in the LP group. All these enzyme activities (CK, PK and LDH) showed a different ($P < 0.05$) percentage of change between both SP and LP group.

Table 2 Muscle metabolite concentration in biopsies of LP and SP groups at rest and after the 30-s cycle ergometer sprint test before (pre) and after (post) training

	SP Group				LP Group			
	Pre-training		Post-training		Pre-training		Post-training	
	Rest	30 s	Rest	30 s	Rest	30 s	Rest	30 s
Glycogen	251 ± 19	178 ± 26*	332 ± 22[a]	281 ± 25*[a]	246 ± 25	181 ± 16*	321 ± 29[a]	242 ± 19*[a]
G-1-P	0.07 ± 0.01	0.35 ± 0.07**	0.11 ± 0.02	0.29 ± 0.08	0.06 ± 0.02	0.35 ± 0.17**	0.06 ± 0.01	0.36 ± 0.07*
Glucose	3.84 ± 0.98	6.47 ± 1.49	2.87 ± 0.14	4.81 ± 0.91	3.68 ± 0.49	5.78 ± 0.88	3.44 ± 0.76	4.73 ± 0.54
G-6-P	0.72 ± 0.24	15.9 ± 1.8**	1.82 ± 0.39	6.77 ± 3.40[a]	1.08 ± 0.26	12.67 ± 3.65**	0.73 ± 0.09	19.20 ± 1.85**#
F-6-P	0.48 ± 0.05	3.40 ± 0.35**	0.73 ± 0.07	2.41 ± 0.53*	0.62 ± 0.14	3.23 ± 0.93**	0.75 ± 0.14	3.42 ± 0.69**
F-1,6-P_2	0.20 ± 0.05	0.28 ± 0.07	0.15 ± 0.03	0.33 ± 0.04*	0.18 ± 0.06	0.27 ± 0.07	0.17 ± 0.02	0.27 ± 0.06
Pyr	0.30 ± 0.03	1.49 ± 0.70**	0.58 ± 0.16	1.29 ± 0.33*	0.37 ± 0.11	1.52 ± 0.68**	0.29 ± 0.06	2.27 ± 0.33**
Lac	8.7 ± 0.8	103.5 ± 15.2**	9.4 ± 2.2	87.0 ± 17.3**	9.0 ± 1.8	105.2 ± 16.6**	6.9 ± 1.0	102.5 ± 19.7**
pH	7.02 ± 0.02	6.63 ± 0.04*	7.02 ± 0.03	6.70 ± 0.04*	7.02 ± 0.02	6.62 ± 0.04*	7.03 ± 0.02	6.63 ± 0.05*

Values are means ± SD for five subjects in each group expressed in mmol (kg dry tissue)$^{-1}$. *,** Significant difference ($P < 0.05$, $P < 0.01$) between the rest and after the test in the same training status. [a] Significant difference ($P < 0.05$) between values of the same parameter before and after training. # Significant difference ($P < 0.05$) between values of the different group.

Phosphofructokinase, aldolase, citrate synthase and 3-hydroxyacyl-CoA dehydrogenase increased in both groups (all cases $P < 0.05$). Although for the PFK and HAD activities, the increases were more extended in SP group ($P < 0.05$).

Hexokinase and phosphoglucose isomerase activities remained unchanged or showed only slight variations.

Muscle metabolites in the post-training test

After training and before the post-training test, both groups showed similar metabolite concentration at rest. However, the SP training caused a slight variation in total creatine (14%), together with increases in PCr (39%, $P < 0.05$) and glycogen (32%, $P < 0.05$) concentration, while only glycogen (30%, $P < 0.05$) increased in the LP group. In contrast, ATP and TAN concentration showed a slight change after 14 training sessions in both groups (not significant).

After the post-training test, IMP and AMP varied as during the pre-training test in both groups. Whereas the change was smaller and not significant in the SP group, the increase in the LP group was significant ($P < 0.05$). In both cases, the variation was less pronounced than that previously produced in the pre-training test. ATP and PCr concentration changed in both groups, but in this second test only PCr decreased significantly ($P < 0.01$, in both groups). Glycogen concentration decreased significantly in both groups ($P < 0.05$). The increase in lactate concentration after the post-training test was more than 9-fold in both groups ($P < 0.01$). Long programme group attained values similar to those in the pre-training test, while the SP group showed a slightly lower lactate concentration than in the pre-training test.

Performance evaluation

The test gave a different result for the two groups after training (Fig. 1). The LP group significantly improved their maximum peak power (20%) and mean power (14%), while the variation in the same parameters of the SP group was smaller and not significant (3 and 3%, respectively). The difference in performance between SP and LP groups was significant ($P < 0.05$).

The SP group improved the maximum peak power during the first session of training, reaching 10% on the 10th day of training (data not shown). However, in the last three sessions, their performance

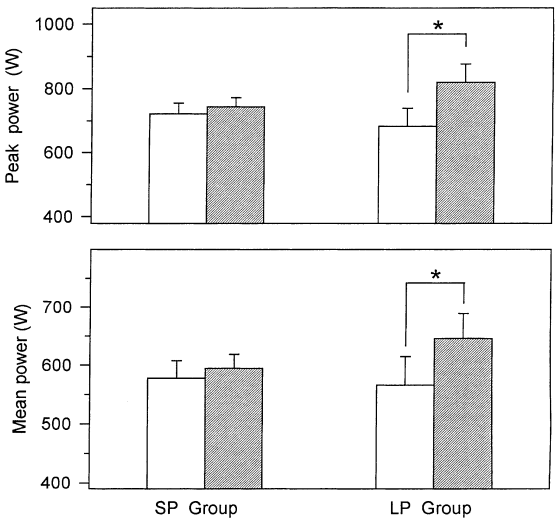

Figure 1 Mean and peak power values of SP and LP group during the 30 s tests, pre- (open bars) and post-training (striped bars). Values are means ± SD for five subjects each group. * Significant difference ($P < 0.05$) between values pre- and post-training.

Table 3 Enzymatic activities in muscle biopsies of SP and LP groups before (pre) and after (post) training

	SP Group			LP Group		
	Pre-training	Post-training	% of change	Pre-training	Post-training	% of change
MK	2788 ± 454	2711 ± 377	−2.8 ± 1.4	2463 ± 323	2910 ± 445	+18.1 ± 3.5#
CK	10847 ± 1686	15608 ± 1873*	+43.9 ± 4.8	12434 ± 1823	13571 ± 1990	+9.1 ± 2.1#
GS	7.42 ± 0.72	6.80 ± 0.62	−8.4 ± 1.7	8.41 ± 1.21	8.81 ± 0.60	+4.8 ± 0.9#
GPh	108.9 ± 8.3	99 ± 13.8	−9.1 ± 2.7	117 ± 19.5	120.2 ± 19.3	+2.7 ± 1.1#
HK	21.2 ± 0.8	22.3 ± 0.7	+5.2 ± 0.4	21.7 ± 0.8	23.3 ± 0.6	+7.4 ± 1.1
PGI	841 ± 30	976 ± 90	+16.0 ± 2.1	814 ± 95	861 ± 79	+5.8 ± 2.1
PFK	75.3 ± 6.6	155.5 ± 12.4**	+106.5 ± 8.2	89.9 ± 15.6	150.7 ± 14.9**	+67.6 ± 6.2#
ALD	317 ± 27	463 ± 52*	+46.1 ± 3.8	411 ± 41	526 ± 84*	+27.9 ± 4.1
PK	1384 ± 45	1872 ± 101*	+35.3 ± 2.7	1587 ± 117	1719 ± 156	+8.3 ± 1.9#
LDH	886 ± 89	1283 ± 124*	+44.8 ± 3.1	807 ± 97	876 ± 109#	+8.6 ± 1.6#
CS	28.1 ± 2.4	38.8 ± 1.6*	+38.1 ± 2.0	33.1 ± 3.6	42.5 ± 2.8*	+28.4 ± 2.1
HAD	19.3 ± 2.7	30.9 ± 3.1*	+60.1 ± 4.3	25.3 ± 3.1	35.1 ± 1.9*	+38.7 ± 3.3#

Values are means ± SD for five subjects in each group expressed in U (g dry tissue)$^{-1}$. *,** Significant difference ($P < 0.05$, $P < 0.01$) between values before and after training. (Unit = μmol min^{-1}). # Significant difference ($P < 0.05$) between values of the same parameter on different group.

deteriorated to the same extent as in the post-training test.

ATP consumption and glycogenolysis and glycolysis rates during test

ATP consumption and glycogenolysis and glycolysis rates during the pre-training test was similar in both groups, however, differences in those rates were observed after training (Table 4). Short programme showed a decrease in ATP consumption (16%, $P < 0.05$) as a consequence of a reduction in the glycolysis (18%, $P < 0.05$) and glycogenolysis (30%, $P < 0.05$) rates. Long programme showed a slight but non-significant variation in ATP consumption, probably produced by an increase in the glycogenolytic rate (11%).

The SP group showed lower values of ATP consumption and glycogenolysis and glycolysis rates than the LP group. However only the glycogenolysis rate was significantly different ($P < 0.05$) between groups after training.

DISCUSSION

Muscle metabolic response to the pre-training test

The muscle concentration of metabolites in the volunteers was within the same range reported in the literature (Bogdanis *et al.* 1995). Likewise, muscle metabolite response during the pre-training 30 s all-out test was in line with that described previously for normal active volunteers (Nevill *et al.* 1989, Bogdanis *et al.* 1995), with no differences being recorded between groups. However, it is worth noting the variability in glycolytic intermediate concentrations depending upon the experimental protocol (with or without warm-up) and the effect of previous training status of the volunteers.

The pre-training test led to a sharp reduction in ATP and PCr concentration after 30 s supramaximal cycling, which is consistent with other findings (Stathis *et al.* 1994, Bogdanis *et al.* 1995, 1996). A large increase in IMP concentration was found in muscle after 30 s of supramaximal exercise in both groups. This increase in IMP muscle concentration is a consequence of a greater

Table 4 ATP consumption and glycolgenolysis and glycolysis rates of SP and LP groups during tests, before (pre) and after (post) training

	SP Group			LP Group		
	Pre-training	Post-training	% Variation	Pre-training	Post-training	% Variation
ATP consumption	193.0 ± 14.7	161.6 ± 11.5*	−16.3 ± 4.8	188.6 ± 17.4	194.4 ± 17.1	+3.1 ± 1.1#
Glycogenolysis rate	65.8 ± 5.5	45.6 ± 5.1*	−30.7 ± 4.2	62.6 ± 5.8	69.2 ± 5.9#	+10.5 ± 2.8#
Glycolysis rate	47.4 ± 3.8	38.8 ± 3.9*	−18.1 ± 3.1	48.1 ± 4.1	47.8 ± 4.1	−0.6 ± 0.2#

Values are means ± SD for five subjects in each group expressed in mmol (kg dry tissue)$^{-1}$. * Significant difference ($P < 0.05$) between values before and after training. # Significant difference ($P < 0.05$) between values of the same parameter on different group.

requirement of the adenine nucleotide metabolism, often described after high intensity exercise (Sahlin *et al.* 1978, Stathis *et al.* 1994).

Although changes in ATP, ADP and IMP were considerable, the addition of TAN + IMP remained constant in both groups after the test, which suggests that IMP was not dephosphorylated during the 30 s of sprint cycling and there was no loss of purine nucleotides.

During the 30-s test, approximately 30% of the glycogen was broken down. We found an average rate of 1.6 mmol glucosyl units (kg dry muscle)$^{-1}$ s^{-1} after 30 s of sprint cycling, while Gaitanos *et al.* (1993) reported a glycolysis rate of 2.2 mmol glucosyl units (kg dry muscle)$^{-1}$ s^{-1} after the first 6 s and Jacobs *et al.* (1983) reported that after 10 s of maximal cycling the production of lactate was 60% of the total lactate produced during 30 s. All these results suggest that the expenditure of glucose via anaerobic glycolysis is not constant during a 30-s all-out cycling test.

Biochemical changes caused by sprint training

Sprint training produced changes in muscle metabolite concentration which seemed unaffected by rest period distribution, as variations were similar in both groups. Resting values of glycogen concentration increased after training (30%, $P < 0.05$) in both groups. Increases in glycogen storage after high-intensity training protocols has been described, although the amount depends on the programme design, as found elsewhere (Boobis *et al.* 1983, Cadefau *et al.* 1990).

TAN and ATP concentration at rest were lower (although not significantly) after training than before in both groups. Such a reduction of TAN owing to high-intensity muscle contractile activity has been previously reported (Hellsten-Westing *et al.* 1993, Stathis *et al.* 1994). Moreover, this fall has been related to an insufficient resting-time between bouts for PCr resynthesis and to a parallel loss of muscle IMP by catabolism (Stathis *et al.* 1994). In our experiment, the recovery periods between bouts were 12 min, long enough for total PCr resynthesis (Bogdanis *et al.* 1995), but not long enough for the resynthesis of IMP to AMP. However, the later sessions probably generated sustained high IMP concentration because of the intensity of training (seven bouts of 30 s) and IMP could then be catabolized. Indeed, increased catabolism of IMP has been described after intense exercise repetition (Bangsbo *et al.* 1992). Thus, the recovery periods between bouts and the exercise intensity seem to be important in the adaptation of ATP and IMP metabolism.

Before training, enzyme activities were similar in both groups. However, after training, enzyme activities were clearly varied. Creatine kinase activity showed a significant increase in the SP group. This enzyme usually shows only slight variations, probably because of its abundance (Cadefau *et al.* 1990). This study points out the possible importance of rest periods between sessions of training in order to produce an increase in the muscle CK activity.

In the glycolytic pathway, PFK and ALD activities increased in both groups. An increase in PFK activity is expected after sprint training, but its extent seems to depend on the training procedure (Cadefau *et al.* 1990, Linossier *et al.* 1993). PK and LDH activity increased significantly only in the SP group. These data indicate that the more concentrated the protocol, the greater the changes in glycolytic enzymes (PFK, PK and LDH).

Aerobic metabolism improvement, represented by CS and HAD activities, is an unusual adaptation to sprint training. However, high production of lactate following repeated bouts might induce an aerobic adaptation by improving the metabolism of the excess of pyruvate through pyruvate dehydrogenase (MacDougall *et al.* 1998). If we consider this possibility, both programmes were intensive enough to induce aerobic adaptation, although shorter rest periods induced larger increases in HAD activity ($P < 0.05$).

It is of particular interest to note the lack of variation in the HK activity of both groups, while increases of HK activity has been found after other sprint training protocols (Linossier *et al.* 1997, MacDougall *et al.* 1998). These authors described an increase in HK activity when recovery/work ratio was 8 (4 min of recovery between repetitions of 30-s bouts, MacDougall *et al.* 1998) or 11 (55 s of recovery between repetitions of 5 s bouts, Linossier *et al.* 1997). Our sessions had a recovery/work ratio of 24 in both groups. Thus, this ratio of recovery periods between bouts could be involved in the adaptation of the external glucose use through HK.

Muscle metabolic response to the post-training test

Muscle metabolic response to the post-training test was different in the two groups. Glycogen consumption during the post-training test reached 25% of the rest concentration in the LP group but only 15% in the SP group. Consequently, glycolysis and glycogenolysis rates during the post-training tests were significantly higher in LP group. However, part of this difference was a consequence of a significant decrease in rates of the SP group. These suggestions are proposed when related to the weight of muscle and they could differ in the case when related to the whole body because the recruited muscle mass may be increased after training.

The increase in performance of the 30 s all-out test in LP group was associated with an increase in

enzymatic activities related to muscle energy metabolism. The relationship between muscle enzymes and performance has been suggested by Linossier *et al.* (1997) and MacDougall *et al.* (1998). However, the lack of correlation between the improved performance (maximum power output appears at 5 s) in the 30-s test and the glycolytic rate averaged over the 30-s period is a question which invites further studies, specially focused on a better understanding of the muscle metabolism during the first few seconds of high-intensity exercise.

With shorter rest periods, the SP group consumed less glycogen and anaerobically generated ATP and produced less lactate during the post-training test than before training. In contrast, performance was similar to the pre-training value. Hence, we suggest a decreased involvement of anaerobic metabolism and an enhanced involvement of aerobic metabolism through the increase in CS and HAD activities.

However, the reduction in anaerobic ATP consumption and the failure to improve performance in the post-training 30 s test were unexpected, because changes of enzyme activities in SP group were greater than in the LP group. A possible explanation for this might be that their muscles were suffering fatigue or injury (Allemeier *et al.* 1994). Repeated exercise at high intensity may cause a loss of K^+ from the contracting muscle (McKenna *et al.* 1993) and a decrease in the gradient regulated by muscle Na^+-K^+-ATPase has been related to fatigue (Lindinger & Heigenhauser 1991). This K^+ homeostasis in exercising humans has been connected with rest duration (Kowalchuk *et al.* 1988). Another critical point to explain fatigue is the intracellular Ca^{2+} exchange (Williams & Klug 1995). Possible training-induced alterations in Ca^{2+} uptake and alterations in the sarcoplasmic reticulum could be rest-dependent, given the different responses of the two groups. More than 48 h of recovery, after the last training session, had in all probability avoided part of the remaining negative effects of the last training session.

It can be concluded that sprint cycling training may produce major enzyme activity changes in human muscle such as PFK, ALD, CS and HAD activities, together with increases in glycogen concentration. However, part of these modifications depend on rest distribution. We suggest that some adaptations are better induced by shorter rest periods, such as increases in PFK, HAD, PK and CK activities or in PCr concentration, although LDH activity is the most sensitive to rest distribution.

Furthermore, in spite of the fact that shorter rest periods during high-intensity training induce greater biochemical adaptation in human muscle than a more restful training programme with the same muscle load, less rest hinders the improvement of short-time performance, probably because of fatigue.

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