Professional road cycling is an extreme endurance sport. Approximately 30,000–35,000 km are cycled each year and the racing season of professional riders includes 90 competition days. In addition, despite the long duration of cycling events such as 3-week stage races, the relative contribution of intense exercise is surprisingly high during the more physically taxing events (mountain passes, time trials, sprints, “breakaways” etc.) [1].

Several investigators have analyzed a number of physiological variables in professional cyclists, both in laboratory [2–7] and field settings [1, 8–10]. However, no prospective, long-term investigation has established the specific physiological adaptations which occur in professional cyclists as a response to training and competition during a typical sports season (generally including different periods in terms of training volume and/or intensity; i.e., precompetition or training, competition, and postcompetition or “active” rest periods). In a recent study (unpublished data), we found no overall training-effect on the ventilatory response (i.e., pulmonary ventilation, tidal volume, ventilatory equivalents, “timing” of respiration, etc.) in the same 13 subjects who formed the present study population. It was therefore considered of interest to extend this investigation to determine whether meta-

Abstract: The aim of this longitudinal study was to analyze the changes in several metabolic and neuromuscular variables in response to endurance training during three defined periods of a full sports season (rest, precompetition and competition). The study population was formed by thirteen professional cyclists (age ± SEM: 24 ± 1 years; mean VO2max ~ 74 ml · kg⁻¹ · min⁻¹). In each testing session, subjects performed a ramp test until exhaustion on a cycle ergometer (workload increases of 25 W · min⁻¹). The following variables were recorded every 100 W until the tests: oxygen consumption (VO2 in l · min⁻¹), respiratory exchange ratio (RER in VCO2 · VO2⁻¹) and blood lactate, pH and bicarbonate concentration [HCO3⁻]. Surface electromyography (EMG) recordings were also obtained from the vastus lateralis to determine the variables: root mean square voltage (rms-EMG) and mean power frequency (MPF). RER and lactate values both showed a decrease (p < 0.05) throughout the season at exercise intensities corresponding to submaximal workloads. In contrast, no significant differences were found in mean pH or [HCO3⁻]. Finally, rms-EMG tended to increase during the season, with significant differences (p < 0.05) observed mainly between the competition and rest periods at most workloads. In contrast, precompetition MPF values increased (p < 0.05) with respect to resting values at most submaximal workloads but fell (p < 0.05) during the competition period. Our findings suggest that endurance conditioning induces the following general adaptations in elite athletes: (1) lower circulating lactate and increased reliance on aerobic metabolism at a given submaximal intensity, and possibly (2) an enhanced recruitment of motor units in active muscles, as suggested by rms-EMG data. [Japanese Journal of Physiology, 50, 381–388, 2000]

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bolic or neuromuscular mechanisms might be involved in the adaptation to training/competition in this type of athlete. The prevailing specific adaptation to training incurred by well-trained endurance athletes is, in general, a controversial issue. Weston et al. [11] reported that a specific training program (~2 months duration) increased racing performance in well-trained amateur cyclists mainly as the result of improved buffering capacity in muscle with no concomitant change in muscle oxidative enzyme capacity or \( V\dot{O}_2 \max \). Such findings, however, may not be directly extrapolated to professional cyclists. It was recently shown in our laboratory that professional cyclists show remarkable physiological characteristics with respect to elite, amateur cyclists [5, 6]. Such characteristics may at least partly explain their superior performance. It may be generalized that professional athletes show (1) a characteristic breathing pattern [6], (2) the capacity to perform at high workloads (~90% of \( V\dot{O}_2 \max \)) for long periods of time (i.e., 60 min) [1], (3) a considerable reliance on fat metabolism even at high power outputs (determined by the blood lactate response and respiratory exchange ratio), and (4) several neuromuscular adaptations shown by surface electromyography (EMG) [5].

The aim of this longitudinal study was to analyze the changes in metabolic and neuromuscular variables recorded in elite athletes (professional cyclists) induced by strenuous endurance training during a full sports season. It was hypothesized that these variables might be significantly modified in response to training and competition.

MATERIALS AND METHODS

Subjects. Thirteen professional road cyclists participated in the study. Subjects’ mean (±SEM) age, height, and weight (at the beginning of the study) were: 24±1 years, 180.2±1.6 cm and 70.8±1.5 kg respectively. A previous physical examination (including ECG and echocardiographic evaluation within the same year) ensured that each participant was in good health. The subjects had professional competition experience of 4±1 year and had covered 32,000±2,500 km (mean figure including training and competition) in the last season. Some of them are among the best road cyclists in the world, and had won several major races of the International Cycling Union.

Study protocol. Informed consent was obtained from each participant in accordance with the guidelines of the “Complutense” University. Each subject reported to the laboratory three times during the study. In each of these visits, subjects performed an exercise test. Each test corresponded to the “active” rest (fall: November), precompetition (winter: January) and competition periods (spring: May) of the sports season.

Training. For each of three periods, training volume was expressed in both average number of kilometers cycled during a “typical” training week of each of the three periods (rest, precompetition or competition) and in total number of kilometers accumulated during the season (from the beginning of November) prior to each laboratory evaluation. Subjects adopted an almost sedentary lifestyle (with no cycling exercise at all) during the first 2–3 week of the “active” rest period. The intensity of training, on the other hand, was determined by estimating, for each period, the mean percentage of weekly training performed (1) “below the first ventilatory threshold” (VT1) [12] (i.e., at heart rates lower than ~150 beats·min\(^{-1}\), “low-intensity” training), (2) between VT1 and the “second ventilatory threshold” (VT2) [12] (i.e., at heart rates ranging between 150 and 175 beats·min\(^{-1}\), “moderate-intensity” training), and (3) above VT2 (i.e., at heart rates higher than ~175 beats·min\(^{-1}\), “high-intensity” training). All of the subjects wore a heart rate telemeter (Polar Vantage NV, Polar Electro OY, Finland) to continuously record heart rates during the training sessions. Because of the specific characteristics of professional cycling, which includes numerous competition days (~100 d) during the season, heart rate recordings during races were also included in the training log corresponding to the competition period.

Exercise tests. Each test was performed on a cycle ergometer (Ergometrics 900; Ergoline; Barcelona, Spain) following a ramp protocol until exhaustion. This type of protocol has been used for the physiological evaluation of professional cyclists in several previous studies conducted in our laboratory [1, 2, 5, 6]. Starting at 0 W, the workload was increased by 25 W·min\(^{-1}\) and pedalling cadence was kept between 70 to 90 rev·min\(^{-1}\). A pedal-frequency meter was used by the subject to maintain this cadence. Each exercise test was terminated either: (1) voluntarily by the subject, (2) when pedalling cadence could not be maintained at 70 rev·min\(^{-1}\) (at least); or (3) when established criteria of test termination were met [13]. During the tests, subjects adopted the conventional (upright) cycling posture. This posture was characterized by a trunk inclination of ~75° and by the subject placing his hands on the handlebars with elbows slightly bent (~10° of flexion). Tests were performed under similar environmental conditions (21 to 24°C, 45 to 55% relative humidity). Gas exchange, blood and EMG variables were determined during the
tests as detailed below.

Gas exchange variables. Gas exchange data were obtained during the exercise tests using an automated breath-by-breath system (CPX; Medical Graphics; St. Paul, MN). The instruments were calibrated before each test with the necessary environmental adjustments. The oxygen consumption \( \left( \dot{V}O_2 \right) \) and respiratory exchange ratio (RER in \( \dot{V}CO_2/\dot{V}O_2 \)) of each subject was recorded (average of the last 30 s) every 100 W during the tests until exhaustion.

Blood sampling and analysis. Prior to the start of the experimental protocol, a 21-gauge butterfly needle was inserted into the antecubital vein of each subject. The catheter was kept patent by periodic flushing with heparinized saline. Blood samples were collected every 100 W during the tests until exhaustion. During each sampling period (~15 s), a 1-ml sample was initially withdrawn to clear the catheter and a 1.5 ml blood sample was subsequently collected using a heparinized syringe. A portion of each sample was taken for: (1) the immediate estimation of \( PCO_2 \) and pH using an automated blood gas analyzer (ABL5, Radiometer, Copenhagen, Denmark), and (2) the immediate determination of lactate concentration using an automated lactate analyzer (YSI 1500; Yellow Springs Instruments; Yellow Springs, OH). The blood bicarbonate concentration \( [HCO_3^-] \) was calculated using the pH and \( PCO_2 \) values.

EMG. EMG recordings were taken from the vastus lateralis muscle (at approximately one-third of the perpendicular distance from the superior border of the patella to the greater trochanter). Pairs of surface electrodes (Bluesensor Medicotest Ag/AgCl electrodes; Rugmarken, Denmark) were attached to the skin 4 cm apart. The electrodes were placed longitudinally with respect to the underlying muscle fibre arrangement. A reference electrode was placed over the anterior superior spine of the iliac crest. Prior to electrode placement, the skin was shaved, abraded using sandpaper and cleaned with alcohol to minimize source impedance. A saline EMG electrode gel was placed between the electrode and the underlying skin to enhance conductivity. The wires used to measure myoelectrical activity (connected to the electrodes) were well attached with tape to minimize artefacts from leg movements. Myoelectrical activity was recorded with the aid of a ME3000P analyzer (ME3000P; Mega Electronics Ltd; Kuopio, Finland). The measurement sensitivity of the instrument is \( \pm 1 \mu \text{V} \) and its range for bipolar EMG signals is \( \pm 5,000 \mu \text{V} \). The raw EMG signals were band-pass filtered from 20 to 480 Hz, amplified and analogue-to-digital converted at a sampling rate of 1 kHz. An EMG power spectral density was then computed for 2-s sampling periods at fixed intervals throughout the tests. The root mean square voltage (rms-EMG) [14] and the mean power frequency (MPF) [15] were calculated (in \( \mu \text{V} \)) for each 2-s spectrum. The rms-EMG was used as an indicator of the "total myoelectric activity" of the exercising muscle since it has been previously shown that this computation is: (1) an accurate measure of electromyographic amplitude, and (2) highly correlated with the number of active motor units (fibre recruitment) [14, 16]. In contrast, MPF was used as an indication of the firing rate of motor units since it is linearly related to the action potential conduction velocity of the muscle fibres [17]. MPF and rms-EMG (average of the last 30 s) were recorded every 100 W during the tests until exhaustion to statistically compare the three periods.

Statistical analysis. One way, repeated-measures analysis of variance (ANOVA) was used to compare the mean variables (i.e., blood lactate, rms-EMG, MPF, etc.) recorded over the three periods. When the ANOVA tests indicated a significant difference, post hoc Scheffé test was applied. In addition, a two-way factor (period, power output) ANOVA with repeated measures on the second factor (power output) was used to determine whether there was an interactive effect between the factors (period \( \times \) power output) for any of the variables measured. In this way, we could determine whether the factor period (i.e., training) could significantly influence the overall response of each variable (i.e., blood lactate, rms-EMG, MPF, etc.) during the tests.

Results are expressed as means±standard error of the mean (SEM). The level of significance was set at 0.05.

RESULTS

Training

Training characteristics are shown in Tables 1 and 2. Both the average and accumulated training volumes were significantly higher \((p<0.05\) and \(p<0.01\), respectively) in competition than in the other two periods and in precompetition than in rest \((p<0.05)\) (Table 1). Concerning training intensity, significant differences were found in: (1) percentage of low and moderate intensity training (rest vs. both precompetition and competition, \(p<0.05\)), and (2) percentage of hard intensity training (rest vs. both precompetition and competition, \(p<0.05\); precompetition vs. competition, \(p<0.05\)) (Table 2).

Briefly, both training volume and intensity increased in the following order: rest<precompetition<competition.
The mean variables recorded every 100 W until the maximal power output are shown in Figs. 1–4. The average maximal power output attained by the subjects averaged 514.2±10.8, 520.0±9.0 and 512.7±12.3 W, respectively.

Gas exchange parameters

No significant differences were found in mean \( \dot{V}O_2 \) recorded for the three periods (Fig. 1). Similarly, the \( \dot{V}O_2 \) max of the subjects did not vary and averaged \( 74 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) throughout the study (72.6±1.5, 74.4±1.3 and 75.2±1.6 ml·kg⁻¹·min⁻¹ for the rest, precompetition and competition periods, respectively). In contrast, RER values tended to decrease throughout the season. The decrease in RER was statistically significant (\( p<0.05 \)) at 100 and 200 W.

Blood parameters

Lactate values decreased (\( p<0.05 \)) throughout the season at each submaximal workload (Fig. 2). No significant differences were found in pH or [HCO₃⁻] at any exercise intensity (Fig. 3).

### Table 1. Training volume of the subjects during each of the three periods.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Precompetition</th>
<th>Competition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average weekly training (km)</td>
<td>267±30</td>
<td>713±28†</td>
<td>810±15†</td>
</tr>
<tr>
<td>Accumulated training volume (km)</td>
<td>500±100</td>
<td>7,433±491†</td>
<td>12,767±1027†</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. †\( p<0.01 \) for Competition vs. Remaining periods. ††\( p<0.05 \) for Precompetition vs. Rest.

### Table 2. Training intensity of the subjects during each of the three periods.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Precompetition</th>
<th>Competition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low intensity (%)</td>
<td>87.8±3.0*</td>
<td>77.8±1.4</td>
<td>76.8±0.4</td>
</tr>
<tr>
<td>Moderate intensity (%)</td>
<td>10.7±2.3*</td>
<td>17.3±1.2</td>
<td>15.1±1.5</td>
</tr>
<tr>
<td>High intensity (%)</td>
<td>1.5±1.0*</td>
<td>4.9±0.6†</td>
<td>8.1±1.6</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. * \( p<0.05 \) for Rest vs. Remaining periods. † \( p<0.05 \) for Precompetition vs. Competition.

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**Fig. 1. \( \dot{V}O_2 \) and RER response during the tests.** Data are shown as mean±SEM. Exh, point of exhaustion. * \( p<0.05 \) for Rest vs. Competition. † \( p<0.05 \) for Competition vs. Remaining periods.

**Fig. 2. Blood lactate response.** Data are shown as mean±SEM. Exh, point of exhaustion. * \( p<0.05 \) for Rest vs. Competition; † \( p<0.05 \) for Competition vs. Remaining periods; †† \( p<0.05 \) for Precompetition vs. Rest.
EMG
rms-EMG tended to increase over the season. Significant differences ($p<0.05$) were mainly observed between the competition and rest periods at most power outputs (Fig. 4). In contrast, MPF values tended to decrease as follows: precompetition $>$ rest $>$ competition. Further significant differences ($p<0.05$) were also found in this variable at several power outputs.

Finally, no interactive effect (period $\times$ power output) was observed between any of the measured variables ($p>0.05$).

DISCUSSION
To our knowledge, this is the first prospective, long-term (~7 months) study which evaluates the specific effects of strenuous endurance training on both metabolic and neuromuscular variables in elite athletes with a solid training background. The training loads of the present subjects (i.e., ~800 km·week$^{-1}$ during the competition period in which they had accumulated ~15,000 km) coupled with their racing performance background clearly reflects their high fitness level. The novel finding of our investigation was that endurance conditioning (i.e., considerable increases in terms of the volume and/or intensity of the training load during the season) induces the following main adaptations: (1) lower circulating lactate at submaximal intensities and possibly increased reliance on oxidative metabolism, and (2) an enhanced recruitment of motor units in active muscles, as explained below.

Gas exchange parameters. No significant differences were found in mean $\dot{V}O_2$ during each period. In addition, the $\dot{V}O_2_{max}$ of subjects did not differ in the three evaluations, averaging ~74 ml·kg$^{-1}$·min$^{-1}$ throughout the study. The average values of $\dot{V}O_2_{max}$ recorded in our study are among the highest reported for elite cyclists [10, 18, 19] and reflect the high fitness level and training status of our subjects. Although some authors have shown that the $\dot{V}O_2_{max}$ of elite cyclists increases slightly during the season [20, 21], our data are in line with most previous studies which show no significant effects of training intervention (i.e., considerable increases in training intensity) on $\dot{V}O_2_{max}$ and/or muscle oxidative enzyme capacity in well-trained athletes such as runners [22], swimmers...
Based on the present findings, it seems that once a certain training status is reached (i.e., professional cycling category), further increases in training intensity and volume are no longer associated with improvements in $\dot{V}O_2$ max. As discussed in our recent report involving the “Tour de France” [1], it would appear that other physiological characteristics, such as the ability to maintain high percentages (i.e., 90%) of $\dot{V}O_2$ max during prolonged periods (>30 min), play a more relevant role in successful endurance cycling. It is consequently felt that training programs should be designed to improve this ability.

In contrast, RER values tended to fall throughout the season at each submaximal workload. Further, the RER values recorded were below 1.00 at intensities less than 400 W during both the precompetition and competition periods. Such findings seem to be consistent with those of our previous investigation [5] in which it was concluded that one of the main adaptations to training and competition in the professional category was increased fat metabolism at any submaximal intensity (at least until 400 W or the $VT_2$, is reached). This adaptation appears to be crucial in some races such as long mountain stages (more than 6 h duration).

**Blood parameters.** Lactate values decreased ($p<0.05$) throughout the season at each submaximal workload. These results are in line with the findings of a myriad of studies which have demonstrated by both cross-sectional and longitudinal comparisons that endurance training leads to decreased blood lactate accumulation at a given submaximal workload and that blood lactate variables are more related to performance than other variables such as $\dot{V}O_2$ max [24]. The lactate data, together with the RER values suggest, at least in part, that an important adaptation to endurance cycling may involve a lower reliance on anaerobic metabolism at any submaximal intensity (at least until 400 W or the $VT_2$, is reached). This adaptation appears to be crucial not only in terms of performance but also in terms of endurance. Indeed, Type I fibres are known (1) to use predominantly aerobic metabolism rather than anaerobic glycolysis, and (2) to be more efficient than fast (Type II) fibres (i.e., less $\dot{V}O_2$ for a given workload) [26]. The latter would explain the fact that $\dot{V}O_2$ values did not change throughout the season in spite of greater motor unit recruitment. Although EMG recordings of motor unit activity before and after resistance or an isometric training program have been used to evaluate neural adaptation [27, 28], data with regard to the effects of endurance training on the EMG response shown by elite athletes are surprisingly scarce. In a cross-sectional report, Jammes et al. [29] reported increased rms-EMG in trained cyclists (reflecting enhanced motor unit recruitment) compared to untrained individuals at similar workloads. However, these findings do not reflect the effects of training on the rms-EMG response of subjects with a solid training background. De Luca et al. [30] stated that during contraction, there appears to be a functional reserve of motor units. These motor units are presumably not readily available and part of the increased motor unit activity following training may be related to “learning” to fully activate some of the motor units that were not previously active.

In contrast to the rms-EMG values, in our investigation, MPF values tended to increase in the following order: competition<rest<precompetition. Considering both sets of data, it may be suggested that, as the volume of low-to-moderate intensity training increases (i.e., from rest to precompetition), a greater number of motor units firing at fast frequencies are recruited in the active muscles of elite endurance athletes. When further, more demanding training loads
are taken on during the spring and summer months (i.e., from the precompetition to the competition period), firing frequency, however, showed a considerable decrease. This finding (decrease in MPF during competition) might be attributed to a further improved ability to recruit additional slow motor units composed of Type I fibres at a particular submaximal intensity—given the lower conduction velocity of the action potential with respect to fast motor units. Such a hypothesis is corroborated by the lactate data, which show lower levels during the competition period suggesting reduced glycolytic metabolism (i.e., greater involvement of Type I fibres).

However, at high exercise intensities (400 W to exhaustion, or above the VT2), the MPF response during the rest period was different from that recorded during the remaining periods and mean MPF values tended to decrease at intensities in excess of 400 W during this rest. The tendency toward reduction, which can occur in MPF at high intensities during incremental exercise, appears to indicate the accumulation of glycolytic by-products such as lactate anions and hydrogen ions [31]. In vitro studies have indeed shown that a fall in pH may cause a reduction in MPF conduction velocity in skeletal muscle [32]. However, in the present study, MPF did not appear to decrease at the end of the tests during the precompetition and competition periods despite a substantial fall in blood pH once a certain power output (400 W) was reached. The lack of decrease in MPF observed at high intensities during the precompetition and competition periods may reflect the fact that the extent of intramuscular metabolite accumulation did not greatly affect the capacity of the muscle cell sarcolemma to conduct action potentials. Thus, an important adaptation to intense endurance training might be the ability to preserve the conduction velocity of fibre action potentials at high intensities when lactic acidosis is likely to occur. Such an adaptation may be highly relevant in professional cycling since to perform successfully, cyclists must sustain considerably high workloads (at or above the anaerobic threshold) for long periods of time during the decisive parts of a race (time trials, mountain passes, etc.) [1].

Finally, the interpretation of the EMG data is limited by the exercise protocol used. The results obtained in this progressive test performed on a cycle ergometer at a fixed cadence (70–90 rev · min⁻¹) cannot be easily extrapolated to real sport situations. A similar study, with no fixed cadence (i.e., field test) would be required to corroborate the implications of our EMG findings.

In conclusion, our findings suggest that endurance training in previously well-trained endurance athletes (professional cyclists) induces a decrease in circulating lactate at submaximal intensities. This response appears to be accompanied by a greater reliance on aerobic metabolism and possibly the enhanced recruitment of motor units (composed of Type I fibres) in active muscles.

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