Salivary Steroid Changes and Physical Performance in Highly Trained Cyclists

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Abstract


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The purpose of this work was to study the seasonal salivary cortisol and testosterone changes, and their relationships with lean body mass variations, in highly trained cyclists. Physical fitness, body composition (6 skinfolds) and basal salivary testosterone were evaluated in 7 male cyclists, on two separate occasions. The first assessment was made at the onset of the competitive season and the second 6 months later. Two kinds of exercise tests were carried out. The first test was an incremental exercise test to determine the maximum O2 consumption (VO2max) and the maximum workload (Wmax). We also measured the VO2 and workload (W) attained at the first and second ventilatory thresholds (VO2VT1, WVT1, VO2VT2, WVT2). During the tests the VO2 was recorded every 30 seconds (Oxycon-5, Mijhardt BV, Odijk). As a second test two days later, we assessed the anaerobic capacity expressed as the maximal accumulated O2 deficit (MAOD). Briefly, each subject underwent five submaximal exercises each lasting 6 min at an intensity of 200, 220, 240, 260 and 280 W. We estimated individually the O2 demand by extrapolating the linear relationship between the power and the O2 demand previously established. Afterwards the subjects performed a supramaximal bout at an intensity producing exhaustion between 2 and 4 minutes. The accumulated O2 demand was calculated by multiplying the O2 demand by the supramaximal test duration. The MAOD was computed as the difference between the accumulated O2 demand and the O2 consumed during the supramaximal rides. We found a significant increase in some physical fitness parameters related to aerobic capacity. The Wmax increased from 5.7±0.5 to 6.1±0.3 W·kg⁻¹ (p<0.05); the WVT2 increased from 3.6±0.5 to 4.0±0.5 W·kg⁻¹ (p<0.05); the WVT2 increased from 4.4±0.6 to 4.9±0.4 W·kg⁻¹ (p<0.05). The VO2max (from 75.7±4.8 to 75.3±3.5 ml·kg⁻¹·min⁻¹, p = NS), the VO2VT1 (from 51.7±6.2 to 54.1±5.3 ml·kg⁻¹·min⁻¹, p = NS), and the VO2VT2 (from 62.5±7.2 to 65.1±3.4, p = NS) showed a non-significant increasing pattern. Nevertheless, the anaerobic capacity (expressed as the MAOD) decreased from 75.8±13.9 to 53.3±16.6 ml·kg⁻¹ (p<0.05). The cycling economy, as reflected by the steady state VO2 at 240 W, ameliorated slightly (from 55.5±5.3 to 53.5±3.2 ml·kg⁻¹·min⁻¹, p = NS), but the changes were non-significant. The subjects showed a significant decrease in weight (from 64.3±3.6 to 62.5±3.6 kg, p<0.05) and in lean body mass (from 60.4±3.5 to 58.4±3.0 kg, p<0.05). Salivary testosterone showed a non-significant decreasing pattern (from 0.45±0.17 to 0.39±0.18 nmol/l, p = 0.15). The testosterone/cortisol ratio decreased by 29% (from 0.022±0.010 to 0.016±0.005, p<0.01), but this change did not reach statistical significance. A firm correlation was found between increment of testosterone and increment of lean body mass (r=0.87, p<0.05). Our cyclists increased their aerobic capacity, but they showed a deterioration of the anaerobic capacity. In spite of the high volume of exercise accumulated by these cyclists, the diminishing effect of endurance training on testosterone was not significantly evident. Our findings suggest that basal bioavailable testosterone changes are related to lean body mass variations.

Key words

Salivary cortisol, salivary testosterone, aerobic capacity, cycling economy, endurance training

Introduction

In recent years it has been shown that intensive training, specially endurance training, may affect the normal function of the reproductive system. Exercise induced-amennorrhea is conspicuous and easily distinguishable, so women have been studied more often. It seems that chronic exercise may cause an inhibition of the hypothalamic-pituitary-ovarian axis that has been associated with anovulation and other forms of menstrual dysfunction, anovulation, inadequate luteal phase, as well as decreased estradiol levels (8,16).

Recently, endocrinologists have begun to study the effects of physical activity on the male endocrine system. Some authors have reported an association between high volume endurance training and reduced levels of serum testosterone, both free and bioavailable (11,13,30,33,36).
Beyond the apparent banality of these hormonal changes lies the possibility of developing some pathological conditions, like, for example, osteoporosis in women (9). However, in males few data on the pathophysiological effects of a slight hormonal changes associated with endurance training are available.

The maintenance of muscle mass depends on the balance between the anabolic and the catabolic hormones. It is well known that castration in males produces a dramatic diminution of testosterone and lean body mass, specially the muscle mass. Testosterone administration, particularly in the course of strength training, in contrast, may increase muscle mass (12). Some investigators support the concept that the plasma testosterone/cortisol ratio plays an important role in trainability and the maintenance of performance during prolonged training periods (1, 27, 34).

Usually, endurance training provokes fat body mass to decrease, in previously sedentary subjects, but the changes of these parameters are less apparent in top athletes. Since the evolution of the body mass components is dependent on the influence of the hormonal milieu, it seems appropriate to analyze both aspects simultaneously.

The present work is a prospective study on the effects of endurance training on the levels of cortisol and testosterone in saliva, and the relationships between the hormonal changes and body mass variations in top level cyclists analyzed on two separate occasions during one season.

Material and Methods

Subjects

Seven male cyclists aged 21 ± 3 years and weighing 64.8 ± 3.7 kg, served as test subjects. After a full description of the procedures, benefits and risk involved in this investigation, they agreed to participate in the study. None had clinically evident cardiovascular disease, acute illness or active chronic systemic disease.

All of them were active cyclists participating in amateur competitions at national level with the exception of one professional. The overall distance covered (races and trainings considered altogether) during the 6 month period was 12,414 ± 4,204 km, the weekly schedule ranged between 300 and 750 km. They usually took part in two races each weekend, ranging between 100 and 150 km. During the 6 months they accumulated a races volume of 4528 ± 2803 km. No reliable information was available about the relative intensity of each training season. In addition, they participated in 5 to 8 competitions lasting from 3 to 7 days.

The subjects were instructed to follow similar diets and abstain from sexual activities (i.e. sexual intercourse or masturbation) and lessen physical activities for 48 hours prior to the evaluations.

Procedures

Body composition, physical fitness, and basal salivary steroids were evaluated on two separate occasions. The first assessment was made at the onset of the competitive season in February, and the second, 6 months later, in August.

Body composition was assessed by anthropometric procedures. Skinfold thickness was measured with the test subjects standing according to Wilmore and Behnke (37). The value used for analysis was the average of three measurements obtained, on the right side of the body, with a Harpenden skinfold caliper at the subscapular, triceps, abdominal, suprailiac, midhigh and calf sites. The Yuhasz equation (4) was used for determining body density. The percentage of body fat was calculated from body density by means of the Siri’s equation (28). The lean body weight was obtained by subtracting body fat from body weight.

Physical fitness was analyzed by means of two kinds of test. Firstly, we evaluated the aerobic capacity by using an incremental bicycle ergometer exercise test (Monark 818, Varberg, Sweden). The initial load was set at 100 watts and increased by 20 watts every minute until exhaustion. The subjects were instructed to keep a fixed pedaling rate of 90 revolutions per minute (rpm). During the tests, ventilatory and gas exchange parameters were monitored every 30 seconds by an open-circuit sampling system (Oxycon-5, Mijhardt BV, Odijk, The Netherlands). We considered as maximum workload (Wmax) the last load sustained immediately before the pedaling rate decrease due to exhaustion. The highest oxygen consumption attained, in the 30 seconds exercise schedule, was taken as maximal oxygen consumption (VO2max).

We also determined the VO2 and workload (W) attained at the first and second ventilatory thresholds (VO2VT1, WVT1, O2VT2, WVT2). The first ventilatory threshold (VT1) was localized using the criteria described by Wasserman (35) for the identification of the ventilatory threshold i.e., W or VO2 at which (1) V E began to increase nonlinearly compared to VO2, (2) carbon dioxide output (VCO2) began to increase nonlinearly compared to VO2, (3) V E/VO2 exhibited a systematic increase without a concomitant increase in FeCO2, (4) expiratory oxygen fraction (FeO2) exhibited a systematic increase without a concomitant increase in FeCO2. The second ventilatory threshold (VT2) was defined as the W or the VO2 at which (1) V E showed a second loss of its linear relationship with VO2, (2) V E/VO2 began systematically to increase, (3) FeCO2 began to decrease.

Secondly, we assessed the anaerobic capacity (AnC) expressed as the maximal accumulated O2 deficit (MAOD). Two days later, the subjects came back to the laboratory and underwent five submaximal exercises at an intensity of 200, 220, 240, 260 and 280 W. All the tests were carried out at a fixed pedaling rate of 90 rpm. The duration of each bout was set at 6 minutes and we took the mean of the last three minutes as VO2 for each load. In all cases, our subjects reached steady state conditions after the third minute of exercise. We estimated individually the O2 demand by extrapolating the linear relationship between the power and the O2 demand previously established. One hour after ending the last submaximal load the subjects performed a supramaximal bout at an intensity producing exhaustion between 2 and 4 minutes. The accumulated O2 demand was calculated by multiplying the O2 demand by the supramaximal test duration. The MAOD was computed as the difference between the accumulated O2 demand and the O2 consumed during the supramaximal ride.

Between 7 and 10 days after the exercise tests, the subjects came back to our laboratory, under fasting condi-
tions, for collection of saliva. After 30 minutes of rest, each subject was provided with a plastic tube and rinsed their oral cavity with water. Afterwards, at 9 a.m., they began to deposit saliva into the tubes until a volume of 3 ml was reached. The time spent in the collection procedure ranged between 5 and 10 minutes. The saliva samples were centrifuged and preserved at −70 °C until analyzed. Salivary testosterone concentrations were assessed by a radioimmunoassay method based on a Farmos Diagnostica kit (90460 Oulunsalo, Finland). The intra and inter assay imprecision coefficients were 8.9% and 12% respectively at physiological concentrations. The reference values obtained in 25 males, aged between 20 and 40 years, were 0.37±0.13 nmol/l. Increment of testosterone (ΔT) was defined as the individual difference between the second determination and the first one. Salivary cortisol concentration were analyzed by a radioimmunoassay method using a Clinical Assays kit (Stillwater MN 55082-0285). The intra and inter assay imprecision coefficients were 6.5% and 9.4% respectively at physiological concentrations. Increment of cortisol (ΔC) was also defined as the individual difference between the second determination and the first one.

The reference values for salivary cortisol, obtained in 20 males aged between 18 and 50 years, were 18.9±7.8 nmol/l.

Statistical analysis

The results are given as mean±one standard deviation. The changes in physical fitness parameters and hormone levels were verified by means of the Wilcoxon signed-ranks test. The steady state relationship between VO2 and workload was obtained by linear regression. The longitudinal differences in cycling economy (VO2 at 200, 220, 240, 260 and 280 W) were determined by analysis of variance for repeated measures. When these F test were significant, individual comparisons were made by the Wilcoxon signed-ranks test. The relationship between lean body mass and delta-testosterone was assessed by using the Pearson correlation test. The statistical level of significance was set at 0.05.

Results

The physical characteristics and training changes of the subjects are described in Table 1. We found a significant increase in their aerobic physical fitness as assessed by the augment in their Wmax, WVT1 and WVT2. However, the variables VO2VT1, VO2VT2 and VO2max showed a non-significant increasing pattern. After training, the VO2-workload relationship was shifted to the right, nevertheless this shift was only significant when the VO2 was expressed as absolute value, i.e. ml·min⁻¹, but not when it was adjusted for body weight (see Fig. 1).

On the other hand, the anaerobic capacity, as reflected by the MAOD, decreased from 75.8±13.9 to 53.3±16.6 ml·kg⁻¹ (p<0.05).

The subjects also underwent a significant decrease in weight (from 64.8±3.7 to 62.5±2.8 kg, p<0.05) and in lean body mass (from 60.6±3.5 to 58.5±3.0 kg, p<0.05).

The individual relationships between the exercise done during the season and both the concomitant changes in the hormonal values and in the most relevant physical fitness parameters assessed are shown in Table 2. Salivary testosterone showed a non-significant decreasing pattern (from 0.45±0.17 to 0.39±0.18 nmol/l, p = 0.15). The individual values remained within the normal range, but decreased in 5 of the 7 subjects studied. Salivary cortisol showed a non-significant tendency to
Table 2  Individual changes in salivary steroids and physical fitness when comparing the values at the end of the study with those found at first evaluation.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Overall V. (km)</th>
<th>Races V. (km)</th>
<th>Δ Weight (kg)</th>
<th>Δ LBN (kg)</th>
<th>Δ Cortisol (nmol·L⁻¹)</th>
<th>Δ Testosterone (nmol·L⁻¹)</th>
<th>Δ T/C</th>
<th>Δ Wmax (W)</th>
<th>Δ MAOD (ml·kg⁻¹)</th>
<th>Δ VO₂ 240 W (ml·kg⁻¹·min⁻¹)</th>
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<tr>
<td>1</td>
<td>9000</td>
<td>3000</td>
<td>-6.3</td>
<td>-5.8</td>
<td>4.8</td>
<td>-0.18</td>
<td>-0.016</td>
<td>0.25</td>
<td>-9.7</td>
<td>2.7</td>
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<tr>
<td>2</td>
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<td>2200</td>
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<td>-1.7</td>
<td>-8.8</td>
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<td>0.84</td>
<td>-9.9</td>
<td>-8.9</td>
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<tr>
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<td>0.0</td>
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<td>4100</td>
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<tr>
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<td>8500</td>
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<td>4.7</td>
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<tr>
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<tr>
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<td>0.009</td>
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<td>-3.3</td>
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Overall V. is the amount of kilometers covered, considering training seasons and competitions altogether; Races V. is the amount of kilometers covered in competitions; Δ Weight, Δ LBN, Δ Cortisol, Δ Testosterone, Δ T/C, Δ VO₂ 240 W are the differences, between the first and the second assessment, in weight, lean body mass, salivary cortisol, salivary testosterone, salivary testosterone to cortisol ratio, maximal accumulated O₂ deficit and the steady state O₂ at 240 W, respectively.

Fig. 3  Relationship between changes of testosterone with both the changes of body weight and the changes of lean body mass. Pearson correlation coefficient r = 0.87 in both cases (p < 0.05).

Fig. 2  Mean (± SD) salivary, cortisol and testosterone/cortisol ratio at the onset of the study (before) and at the height of the competitive period (after). The changes were not significant.

increase (from 23.6±12.0 nmol/L to 27.5±18.9 nmol/L). The individual values increased in 5 out of them and generally remained within the normal range. But in one subject, at the end of the study, we found a salivary cortisol value of 68.7 nmol/L, practically doubling the concentration found at the onset of the season.

The testosterone/cortisol ratio decreased by 29 % (from 0.022±0.010 to 0.016±0.005, p = 0.10), but this change did not reach significance (Fig. 2). As can be seen in Table 2, the subjects 1, 6 and 7 showed the greatest decreases in the salivary T/C index. All these cyclists experienced salivary testosterone decrease and salivary cortisol increase. In addition, subject 6, the one who covered most kilometers in training as well in competition, showed an accentuated decrease in both salivary testosterone and salivary T/C index.

The individual changes of testosterone concentration have correlated with increment of weight and increment of lean body mass (Pearson r = 0.87, p < 0.05, in both cases) (Fig. 3). Nevertheless, no other correlations were found among the changes in aerobic or anaerobic physical fitness parameters and hormonal changes. Neither did we find significant correlations between testosterone and cortisol changes.
Discussion

Recently some authors have reported the development of a certain degree of hypoandrogenism in endurance trained males (13,30,33,34), including cyclists (11). All the subjects participating in the aforementioned investigations were experienced sportsmen, who submitted themselves to very hard training programs. Other authors have carried out prospective studies on previously sedentary subjects. In contrast, they reported non-significant changes (10,27) or even an increase (22,23) of the basal plasma testosterone at the end of the training. It seems that the possibility of developing hypoandrogenism depends mainly on the intensity, volume and duration of the exercise program as well as the physical fitness of the individuals assessed.

The test subjects participating in our study were highly trained cyclists, as evidenced by the elevated VO2max attained in the first ergometric test (75.7±4.8 ml/kg/min), which is similar to that reported for top level athletes (6) and cyclists (29).

Although we did not observe any seasonal increase in VO2max, we must take into account that only two determinations were made. This VO2max behaviour has been confirmed in professional cyclists (29) and in other sport disciplines when examining previously trained athletes repeatedly during the season (34). Nevertheless, the amount of km covered by our cyclists and the numerous competitions in which they participated guarantee a great degree of stress on their oxygen transport system and, probably, on their endocrine system. Although VO2max remained at the same level, all the subjects reported an amelioration in their field performances. In addition, the results obtained in the laboratory demonstrate an increase in their aerobic performance since the workload attained at first and second ventilatory thresholds increased as well as Wmax. An increase in Wmax, without a concomitant augment of VO2max, could be justified by a greater degree of motivation when performing the second incremental exercise test, also by a more economical cycling and by an increase in anaerobic capacity. Anyway, for two reasons we think the cyclists were similarly motivated when they were tested. Firstly they were habituated to exercise testing procedures. Secondly, if they had manifested an increased level of motivation when performing the second test the anaerobic contribution to energy expenditure would have had to increase in the final step of the incremental exercise test and a VO2-plateauing effect would have been seen. Indeed, none of the already mentioned phenomena were apparent. Since anaerobic capacity decreased, we consider that the increase in WVT1, WVT2 and Wmax was mainly related to more economical cycling during the second control. Nevertheless, we must remember that the economy changes were only significant when assessed as absolute values, but not when adjusted for body weight.

To our knowledge, this is the first time that the MAOD has been assessed prospectively in high level cyclists. Our MAOD results are similar to those reported in cross-sectional designed studies by others, both in active subjects (ççmedbo y tabata) and in cyclists (38).

On the other hand, cycling economy has usually been reported unchanged as a result of training (26,31).

It has been suggested that the sustained increase in the plasma concentration of testosterone that accompanies prolonged exercise may disturb the normal hypothalamic GnRH releasing pattern (17). This, in turn, could lead to a pituitary malfunction causing inappropriate pulsatile release of LH and, consequently, a decrease in the basal plasma testosterone concentration (19). In contrast to MacConnie and colleagues (17), Hackney et al. (13) did not observe any effect of endurance training on the pulsatile release of LH in long-distance runners. Furthermore, in a recent prospective work, Wheeler et al. (36) have reported, in spite of a substantial decrease of the plasma testosterone and prolactin concentrations, a lack of alteration in the LH pulsatile release after 6 months of intense training in previously sedentary males. Obviously, the training intensity and volumes achieved by top athletes are so stressful that they could not be attained by sedentary people beginning a training program. That may be the reason why an impairment of the LH pulsatile release may contribute, at least in ultraendurance runners and elite athletes, to a diminution of plasma testosterone concentrations.

In spite of the high volume accomplished by our cyclists, we only found a non-significant tendency of both salivary testosterone and salivary T/C index to be lower at the second control. Nevertheless, the size of the sample studied and the number of determinations done does not allow us to come to definitive conclusions. Furthermore, the cyclists showed a great individual variability in cycling volume and races. Like the majority of competitive cyclists in our country, their training program was poorly controlled, and sometimes self-planned. In addition, we decided not to modify their habits and modes of training. Considering these limitations, we will emphasize two important issues. First, our subjects who were not professionals were submitted to a high load of exercise during the season. Perhaps the volume and the intensity of exercise were not enough to provoke hypoandrogenism. However, as can be seen in Table 2, generally the subjects who showed the higher decreases in salivary testosterone and in salivary T/C index were those who covered the most kilometers (for example, subject number 6). Also we did not find any effect of salivary testosterone and salivary T/C index decreases on the changes in aerobic or anaerobic performance.

The plasma concentration of hormones should be regarded as a function of a dynamic equilibrium between production and clearance. It is known that plasma testosterone increases during prolonged exercise of moderate to high intensity. As testosterone is mainly removed by the liver, it has been suggested that the mechanism responsible for the plasma testosterone increase is the reduction in splanchnic blood flow during exercise (3). After exercise, splanchnic flow is reestablished enabling further uptake of steroids by the liver and, as consequence, plasma testosterone decreases. In addition, plasma testosterone may also decrease due to an impairment of Leydig cell function following exercise (7). Cortisol has been shown to influence testosterone production at the Leydig cell site. It has been reported that there is a correlation between the serum testosterone decrease and cortisol increase following stressful conditions (7).

The present data showed non-significant correlation between salivary testosterone and salivary cortisol changes. However, we must remember that the changes ob-
served in salivary steroids were not significant, and that we only had a single value of cortisol by determination.

Wheeler et al. (36) have speculated that the chronic exposure to high levels of testosterone during exercise could prompt tissue uptake by an unknown mechanism. If this hypothetical effect remains after exercise, it will be necessary to increase testosterone production to avoid hypoandrogenism. One of the most interesting findings of Wheeler et al.'s study (36) is that sedentary subjects, engaged in an exercise program, may develop a slight hypoandrogenism.

Salivary cortisol and testosterone are a good index for evaluating the values of free serum testosterone nad cortisol, and also allow sampling at frequent intervals, avoiding the interference produced by the stress of venipunctures (21,24,32). Since the determinations of salivary steroids are a good estimate of the bioavailability of both steroids, we can address the influence of salivary steroids changes upon androgen dependent tissues, like skeletal muscles. It is noteworthy to point out that we have observed a correlation between the basal salivary testosterone changes and lean body mass changes. Therefore, the cyclists showing the most accentuated descent in salivary testosterone presented the greatest decrease in lean body mass. Muscles can be regarded as a pool of amino acids that is mobilized in response to the systemic metabolic needs. Cortisol and testosterone play a crucial role in the regulation of protein metabolism and muscle mass. Cortisol promotes proteolysis, Type II fibers being affected most (2). Testosterone, in contrast, increases the muscular uptake of amino acids and the rate of protein synthesis. Thus, the balance between the cortisol proteolytic action and the ant$catabolic activity of testosterone seems to determine the increase or diminution of the muscle mass. Although we did not find any relationship between the changes in physical fitness and the hormonal variations, it is recognized that a diminution of the muscle cross-sectional area may lead to decreased maximal force production (18,25). Moreover, a decrease of the free androgen index may further reduce the maximal force reached per unit of muscle cross-sectional area (14,15). Unfortunately, we did not measure force production in our cyclists, but we found a decrease in anaerobic capacity. Costill (5) reported that leg power (as expressed by vertical jumping height) was lower in trained long-distance runners than in matched sedentary control subjects. Strikingly, these runners increased their leg power after detraining. The performance in both qualities, vertical jumping and anaerobic capacity, is mainly related to Type II muscle fibers.

As far as salivary testosterone/cortisol ratio is concerned, it was lower than that reported for total plasma values (27,34). This parameter showed a tendency (p = 0.10) to be lower at the second measurement. A reduced anabolic/catabolic ratio would perhaps permit an increased proteolysis affecting primarily type II fibers, and by this mechanism lessen maximal force production and anaerobic capacity. We think that metabolic and hormonal adaptations to high level endurance training could promote metabolic and neuromuscular changes that might be beneficial for endurance performance, but that are detrimental for anaerobic energy yield. Obviously, further studies are necessary to verify this hypothesis.

In summary, this study reveals that high level non-professional cyclists increase their aerobic physical fitness, but their anaerobic capacity deteriorates as a result of six months of combined training and competition. A decrease in their body weight as the consequence of lean body mass loss was evident, though its effect on cycling performance remains unknown. We also have detected a non-significant decrease of both salivary testosterone levels and salivary testosterone/cortisol ratio. The salivary testosterone decrease correlates with a loss of lean body mass.

References


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