

# Physiological Research Pre-Press Article

**Title:** The importance of the training-induced decrease in basal cortisol concentration in the improvement in muscular performance in humans

**Short title:** Cortisol concentration and skeletal muscle performance

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## Summary

Acute exercise-induced changes in cortisol concentration (C) and training related adaptation within hypothalamic-pituitary-adrenal (HPA) axis has been widely examined, but their influence on muscle strength performance is at best uncertain. Twenty four young healthy men were randomly assigned to an endurance training group (ET, n=12) or to a non-exercising controls (CON, n=12). ET performed supervised endurance training on cycle ergometer for 20 weeks. Endurance training program improved exercise capacity (14% increase in power output generated at peak oxygen uptake –  $VO_{2peak}$ ), muscle strength performance (increase in MVC – maximal voluntary contraction – by 9% and in TTF 50% MVC – time to fatigue at 50% MVC – by 21%) and led to a decrease in basal serum C concentration ( $P=0.006$ ) and an increase in basal testosterone to cortisol (T/C) and free testosterone to cortisol (fT/C) ratios ( $P=0.01$  and  $P=0.02$ , respectively). It was found that the decrease in C concentration ( $\Delta C$ ) was positively correlated to the increase in local muscular performance ( $\Delta TTF$  50% MVC). No significant hormonal changes were seen in CON group. It is concluded that greater decrease in cortisol concentration after the endurance training is accompanied by poorer improvement in skeletal muscle performance in previously untrained subjects.

**Key Words:** glucocorticoids; testosterone; strength; local muscular endurance;

## Introduction

Activation of the hypothalamic-pituitary-adrenal (HPA) axis is indispensable for the appropriate regulation of the energetic, metabolic and immunological processes in response to exercise stress (Galbo 1986). Acute and training-induced changes in cortisol (C) concentration and its physiological role has been widely studied (see e.g. O'Leary and Hackney 2014), and it is known that exercise training leads to exposition to high glucocorticoids concentration, both in animal (de Araujo *et al.* 2014) and human studies (Hill *et al.* 2008). It is of great importance, especially in endurance type of training, because prolonged hypercortisolemia may have also adverse effects on different cell functions in humans, in e.g. leading to muscle proteolysis and protein degradation (Schakman *et al.* 2013)), impairment of insulin secretion of beta-cells (van Raalte *et al.* 2010) and suppression of the nitric oxide system in the endothelium (Liu *et al.* 2009). These can occur as a consequence of elevated circulating glucocorticoids, but also due to enhanced activity of the glucocorticoid receptor or the 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ HSD1), an enzyme that converts cortisone to active cortisol. Moreover, it was demonstrated that the repeated hypercortisolemia may disrupt the negative feedback loop of the HPA axis and results in both basal and exercise-induced modification in hormonal responses (Luger *et al.* 1987).

Training-induced changes in C concentration may also affect muscle performance as it is currently understood that glucocorticoids influence intracellular signaling pathways involved in training adaptation (Schakman *et al.* 2013). Decrease in the activity of p38 MAPK and PI3K/Akt/mTOR pathway by enhanced C concentration may have adverse effect on muscle strength performance and conversely, the diminished C level after a training program seems to create a favorable condition for intracellular pathways that augment muscle protein synthesis and muscle performance. Interestingly, there is no data in the literature on the

relationship between training-induced changes in C concentration and muscle strength performance. This issue is even more complex, because there is also no agreement on the direction of the C changes during long term training as well as on the mechanism(s) by which these adaptive changes occur.

Basal C concentration has been reported to either increase (Hoogeveen and Zonderland 1996, Kirwan *et al.* 1988, Seidman *et al.* 1990) or not change (Bresciani *et al.* 2011, Grandys *et al.* 2008, Lehmann *et al.* 1993) in response to endurance training. It was also demonstrated that heavy endurance training periods in competitive athletes may lead to decreased C concentration (Lucia *et al.* 2001) what has been linked to overtraining syndrome (Snyder *et al.* 1995). Similarly, different results were also presented when basal C concentrations were compared between endurance-trained athletes and untrained subjects. In some reports, the athletes had higher C concentration (Barron *et al.* 1985, Luger *et al.* 1987) and in others its concentration remained similar to the untrained controls (Duclos *et al.* 2003, Wittert *et al.* 1996).

In this study we have employed this type of training that is commonly used by millions of people worldwide who are taking part in recreational exercise training (i.e. moderate intensity endurance training). It is well known that high physiological demands of the long-term endurance training program, even at relatively moderate intensities, results in need for closely monitoring the responses of individuals to training load. Monitoring hormonal changes seems to be especially important in the case of people with subclinical hormonal disturbances who undertake physical exercise training as well as in the case of patients involved in endurance based rehabilitation programs. Therefore, we aimed in this study to determine cortisol concentration and its relationship to muscle performance during well controlled exercise training of moderate intensity. Moreover, we have also assessed other hormonal variables that influence anabolic-catabolic status of the body i.e. testosterone (T),

free testosterone (fT), sex hormone-binding globulin (SHBG) and T/C and fT/C ratios, as they may serve as an additional tool for monitoring training status.

## Methods

### *Subjects*

Twenty four young healthy men of mean ( $\pm$ SD) age 22.58 ( $\pm$ 1.35) years, height 179.1 ( $\pm$ 6.91) cm, body mass 75.11 ( $\pm$ 12.47) kg and BMI 23.36 ( $\pm$ 3.16) kg  $\cdot$  m<sup>-2</sup>, voluntarily recruited students of physiotherapy and tourism and leisure, participated in this study. All students had normal medical histories and were untrained but physically active, non-smoking men (on average, less than 2 hours of physical activity weekly). After a standard medical evaluation and a routine blood tests (see Table 2), they were equally and randomly assigned to an endurance training group (ET, n = 12) or to a non-exercising control group (CON, n = 12). There were no differences between these two groups in age, body height, body mass (BM) and BMI (Table 1). ET group underwent all laboratory and physical performance tests (anthropometrical measurements, incremental exercise test, blood tests and isometric strength and endurance testing), whereas CON were subjected only to basic anthropometric measurements and blood testing in order to exclude a potential influence of seasonal variation on hormone concentrations examined in this study. Each group of subjects were tested twice, at 20-week interval, i.e. before and after endurance training in ET group and before and after control period in CON group.

### **Table 1 about here**

All subjects were fully informed about the aims and experimental procedures of this study and gave written consent to participate before entering the study. The authors obtained approval for this investigation from the Local Ethical Committee and the study protocol was conducted in accordance to the Declaration of Helsinki.

### *Incremental exercise test*

In order to examine pre- and post-training level of physical fitness, ET group (n = 12) performed a maximal incremental exercise test on a cycle ergometer. After a 6-min rest sitting on the cycle ergometer, the test started at 30 W and the power output was then gradually increased by 30 W every 3 min. When the subjects could not continue cycling at the required pedaling rate ( $60 \text{ rev} \cdot \text{min}^{-1}$ ) and power output, the test was stopped. Incremental exercise test was conducted on Ergometrics 800 S cycle ergometer (Ergoline GmbH, Bitz, Germany) and during the test, the peak oxygen uptake ( $\text{VO}_{2\text{peak}}$ ) was measured using breath-by-breath gas analysis system (Oxycon Champion Jaeger, Mijnhardt B.V., Bunnik, Netherlands) as previously described by Zoladz *et al.* (1995). Moreover, a blood samples were taken at rest and at the end of every 3 min stage to determine lactate threshold (LT), that was defined as the power output ( $\text{PO}_{\text{LT}}$ ) above which plasma lactate displayed a continuous increase of at least  $0.5 \text{ mmol} \cdot \text{l}^{-1}$  during each subsequent stage of incremental test (Majerczak *et al.* 2012, Zoladz *et al.* 2013, Zoladz *et al.* 1995). Heart rate was continuously monitored and recorded throughout all exercise test (Polar S810, Polar Electro Oy, Kempele, Finland).

### *Maximal voluntary contraction (MVC)*

Maximal voluntary isometric contraction (MVC) of the knee extensor muscle was performed in ET group on a prototype chair equipped with a strain gauge as described in detail in a previous paper (Zoladz *et al.* 2012). Briefly, the subjects were tested in a seated position with their pelvis and thigh stabilized to minimize unwanted movements. Hips and knees were fixed at  $90^\circ$  of flexion. Inextensible strap was placed around the subject's ankle at the front of the chair and attached to the strain gauge at the back of the chair. During forceful isometric contraction against the strap, the output signal from the strain gauge was amplified

and converted from analog to digital and monitored and stored on a computer by a purpose made software.

MVC testing session began with standard warm-up procedure including bicycling on the stationary ergometer, freely chosen lower extremity exercises as well as short submaximal isometric contraction after positioning on the testing chair. During measurement of the MVC of the knee extensors, the subjects were asked to push as forcefully as possible for about 3 s against the strap. This measurement was repeated three times with each leg with 2 min break in between and the MVC was determined as an average from the two trials that yielded the largest value for each leg. In addition to MVC data, the torque was calculated by multiplying the MVC by the lever arm that was kept constant before and after the training program.

#### *Time to fatigue at 50% of maximal voluntary contraction (TTF 50% MVC)*

After completing the last MVC trial a minimum 5 min of rest was applied and then the subjects underwent the measurement of TTF 50% MVC for each leg with at least 5 min rest in between. In this test, the subjects were instructed to maintain a target force level (50% MVC) until maximal exhaustion. The target as well as instantaneous force level were visibly displayed on the monitor screen in front of the subjects. TTF 50% MVC was measured in seconds from the onset of the test (subjects reached the target force in 1-2 seconds) to the point when the subjects were unable to maintain the required force level for 3 consecutive seconds. TTF 50% MVC was expressed as an average of two measurements (right and left leg). This time is regarded as an index of local muscular endurance (see Kraemer *et al.* 2006).

#### *Endurance training program*

Half of the volunteers (ET group), i.e. 12 subjects, underwent a supervised endurance training program on a cycle ergometer (Monark 874 E, Monark Exercise AB, Vansbro,



Sweden), four times a week for 20 weeks. The training program started at the beginning of December and ended in the middle of March. Training consisted of two different exercise protocols (each lasting 40 minutes): a moderate-intensity continuous cycling (CC) and a high-intensity intermittent cycling (IC). CC was performed at the power output corresponding to 90% of the previously determined lactate threshold (90% LT), whereas IC consisted of 6 minutes of unloaded cycling followed by a 3 minutes exercise bout at the power output corresponding to 50% $\Delta$ , repeated four times and finished with 4 minutes of unloaded cycling. The power output corresponding to 50% $\Delta$  was calculated as the difference between the power output reached at  $\text{Vo}_{2\text{peak}}$  ( $\text{PO}_{\text{max}}$ ) and the power output obtained at the LT ( $\text{PO}_{\text{LT}}$ ) [ $50\%\Delta = \text{PO}_{\text{LT}} + 0.5 (\text{PO}_{\text{max}} - \text{PO}_{\text{LT}})$ ]. To maintain an adequate training stimulus, the power output was adjusted after first 5 weeks of training and then after each of the five weeks period of the study. From the beginning of the 6<sup>th</sup> week until the end of the 10<sup>th</sup> week, the unloaded cycling in the IC sessions was replaced by cycling at the 90% LT. During the next five weeks, the power output corresponding to 90% LT was increased by 5% both in CC and IC protocols (from week 11<sup>th</sup> to 15<sup>th</sup>) and by 15% during the last five weeks of the training program (from week 16<sup>th</sup> to 20<sup>th</sup>). The CC was performed on Tuesdays and Fridays, and IC on Mondays and Thursdays. Each training session was monitored using a heart rate monitor (Polar S810, Polar Electro Oy, Kempele, Finland) and supervised by one of the authors. The average training heart rate during the 20 week training period amounted to  $125 \pm 10 \text{ bt} \cdot \text{min}^{-1}$  (67% of maximum heart rate) and  $133 \pm 9 \text{ bt} \cdot \text{min}^{-1}$  (71% of maximum heart rate), for CC and IC exercise protocols respectively. Moreover, the session RPE ratings was taken within 15 min after the completion of each exercise bout. During the training period subjects were asked to keep their normal mixed diet and to avoid taking any nutritional supplements or consuming alcohol.

### *Blood collection*

Fasting (overnight) blood samples were taken at rest between 7:30 and 8:30 a.m. from both group of subjects (ET and CON). Samples were drawn from the antecubital vein. Blood for hemoglobin [Hb], erythrocyte [E], and leukocyte [L] concentrations as well as for hematocrit value (Hct) was collected into plain tubes containing EDTA. Blood for serum sodium [Na<sup>+</sup>], potassium [K<sup>+</sup>], creatinine [Cr], albumin (Alb), T, C and SHBG concentrations was collected into plain tubes and left to clot for a minimum of 30 minutes at room temperature and then centrifuged at 4000 rev · min<sup>-1</sup> for 5 min. Serum was stored at -80°C until analysis.

Moreover, in order to determine the lactate threshold (LT) in ET subjects, the blood samples were also collected during incremental exercise test (see above) via an Abbot Int-Catheter, Ireland (18G/1.2 × 45 mm) inserted into an antecubital vein and connected to an extension set using a “T” Adapter (SL Abbot, Ireland) (the length of the tube was 10 cm). The blood samples (0.5 ml each) were placed in 1.8 ml Eppendorf tubes containing 1 mg ammonium oxalate and 5 mg sodium fluoride, mixed for 20 seconds and then centrifuged at 4000 rev · min<sup>-1</sup> for 4 min. The obtained samples of plasma were stored at -80°C until analysis.

### *Blood analysis*

Hemoglobin (Hb), hematocrit (Hct), erythrocyte (E) and leukocyte count (L) were analyzed by optical method with an Advia 2120 automated hematological analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Serum sodium [Na<sup>+</sup>], potassium [K<sup>+</sup>] and creatinine [Cr] concentrations were determined according to the manufacturer's protocol using the Cobas c501 analyzer (Roche Diagnostics, Mannheim, Germany). Plasma lactate concentration was measured using an automatic analyser Vitros 250 Dry Chemistry System,

Kodak (Rochester, NY, USA). Moreover, in order to further calculate the fT, serum [Alb] was quantified by capillary electrophoresis (Minicap, Sebia, Lisses, France).

All hormone measurements were performed in duplicate and serum T, C and SHBG were determined by electrochemiluminescence immunoassay using the Cobas e411 analyzer (Roche Diagnostics, Mannheim, Germany) with detection limits of 0.09, 1.0 and 0.8 nmol · L<sup>-1</sup> for T, C and SHBG, respectively. The intra and interassay coefficient of variation (CV) for these assays was 3.4% and 5.9%, 1.2% and 1.6%, 2.4% and 3.7% for T, C and SHBG respectively. Moreover, fT was calculated using the assumption-free empirical equations (Ly and Handelsman 2005), as it was showed that this method gives fT concentrations closer to the precise laboratory measurements (equilibrium dialysis and centrifugal ultrafiltration) than other commonly used standard calculated fT estimates (Ly and Handelsman 2005).

### *Statistical analysis*

In this study, non-parametric tests were employed because of the relatively small sample size (n = 12 in each group of subjects). With this amount of data points, it is not possible to reliably verify the assumption of normality that is needed for an application of standard parametric tests. It should be also noted that the use of non-parametric tests is a much more conservative approach to testing the significance of the differences than parametric testing. From these reasons, intragroup comparisons were performed with the use of the Wilcoxon signed rank test and intergroup comparisons with the use of the Mann-Whitney U test. The correlations between hormonal and TTF 50% MVC data were evaluated using the Spearman's correlation coefficient. The significance level was set at  $P < 0.05$  and all data are presented as mean  $\pm$  SD. The analyses were performed using STATISTICA software, version 10 (StatSoft, Inc., 2011, [www.statsoft.com](http://www.statsoft.com)).

## Results

### *Physical characteristics of the subjects*

The subjects from ET and CON group did not differ significantly in mean age, body height, BM and BMI at inclusion to the study (see Table 1). 20 weeks of endurance training program did not change significantly BM and BMI in the ET group ( $76.8 \pm 14.4$  vs.  $77.1 \pm 12.8$  kg and  $23.58 \pm 3.82$  vs.  $23.67 \pm 3.41$  kg · m<sup>-2</sup> for pre- and post-training BM and BMI, respectively,  $P > 0.7$ ). Similarly, the same period of time did not change BM and BMI in the CON group ( $73.4 \pm 10.5$  vs.  $73.5 \pm 11.0$  kg and  $23.14 \pm 2.48$  vs.  $23.17 \pm 2.69$  kg · m<sup>-2</sup> for before and after control period BM and BMI, respectively,  $P > 0.6$ ). As it could be expected, body mass and BMI measured after this 20 weeks of intervention were not different between ET and CON groups and changes in these two variables ( $\Delta$ BM and  $\Delta$ BMI) were also non-significant.

### *Blood morphology and biochemistry*

Selected blood morphology and biochemistry parameters are given in Table 2. Besides significantly higher pre-training E concentration in the ET group there were no other significant differences between ET and CON group at the beginning as well as at the end of the study (i.e. after 20 weeks of the endurance training in ET subjects and after 20 weeks of control period in CON subjects). Within group comparisons revealed significant decrease in Cr concentration in ET group after 20 weeks of training ( $P < 0.01$ ) and albumin concentration in CON group after 20 weeks of control period ( $P < 0.05$ ), see Table 2.

**Table 2 about here**

### *Exercise capacity*

Before training the mean  $\text{VO}_{2\text{peak}}$  was  $3198 \pm 458 \text{ mL} \cdot \text{min}^{-1}$  ( $42.27 \pm 5.69 \text{ mL} \cdot \text{kg} \cdot \text{min}^{-1}$ ),  $\text{PO}_{\text{LT}}$  amounted to  $120 \pm 29 \text{ W}$  and  $\text{PO}_{\text{max}}$  to  $232 \pm 35 \text{ W}$  in ET group. Although, 20 weeks of endurance training did not change  $\text{VO}_{2\text{peak}}$  ( $P > 0.05$ ), the  $\text{PO}_{\text{LT}}$  and  $\text{PO}_{\text{max}}$  increased significantly by 19% ( $P = 0.01$ ) and by 14% ( $P = 0.003$ ), respectively. Power output reached at  $\text{VO}_{2\text{peak}}$  was also increased when expressed in relative terms ( $3.1 \pm 0.4 \text{ W} \cdot \text{kg}^{-1}$  before and  $3.5 \pm 0.3 \text{ W} \cdot \text{kg}^{-1}$  after the training,  $P = 0.002$ ).

### *Isometric strength and endurance*

Isometric strength and endurance of the knee extensor muscles were significantly augmented after 20 weeks of endurance exercise training on a cycle ergometer. MVC increased from  $672 \pm 117$  to  $732 \pm 126 \text{ N}$  (before and after training respectively,  $P = 0.002$ ), torque from  $202 \pm 35$  to  $220 \pm 38 \text{ Nm}$  (before and after training respectively,  $P = 0.002$ ) and TTF 50% MVC from  $61 \pm 19$  to  $74 \pm 25 \text{ s}$  (before and after training respectively,  $P = 0.004$ ). Additionally, it was found that change in MVC ( $\Delta\text{MVC}$ ) after endurance training was positively correlated with change in  $\text{PO}_{\text{max}}$  ( $\Delta\text{PO}_{\text{max}}$ ) ( $r \text{ Spearman} = 0.68$ ,  $P = 0.014$ ).

### *Hormonal responses*

Hormone concentrations for both groups are given in Table 3. Endurance training in ET group did not lead to basal changes in T, fT and SHBG concentrations, but significantly reduced basal C concentration ( $P = 0.006$ , Figure 1, panel A). The decreased basal C concentration after training in ET group was clearly observed in a majority of subjects (see Figure 1, panel B). This substantial drop in C concentration resulted in significantly higher T/C and fT/C ratio after 20 weeks of the endurance training.

**Figure 1 about here**

On the other hand, 20 weeks of control period in CON group did not alter significantly any of the hormone concentrations determined in this study (see Table 3 and Figure 1, panel A).

**Table 3 about here***Correlations between muscle performance and hormonal variables*

There was a significant positive correlation between the change in C concentration ( $\Delta C$ ) and change in TTF 50% MVC ( $\Delta TTF$  50% MVC) (Figure 2). As it could be expected a negative correlations were also found between the changes in T/C ratio and fT/C ratio ( $\Delta T/C$  and  $\Delta fT/C$  ratio, respectively) and  $\Delta TTF$  50% MVC (Figure 3, panel A and B). Moreover, there was also a trend for negative correlations between change in MVC and change in fT/C ratio, although this correlation did not reach statistical significance ( $r$  Spearman = -0.48,  $P$  = 0.11).

**Figure 2 about here****Figure 3 about here**

## Discussion

The main and original finding of this study is that the long-term endurance training program of moderate intensity led to an increase in muscle strength (9% increase in MVC) and local muscle endurance (21% increase in TTF 50% MVC), what was accompanied by a significant decrease in C concentration (Figure 1) and a significant increase in T/C and fT/C ratio in ET group (Table 3). Interestingly, however, we have observed that training induced change in TTF 50% MVC was positively correlated with change in C concentration (Figure 2) and negatively correlated with changes in T/C and fT/C ratio (Figure 3). From these findings, one may conclude that skeletal muscle performance was more improved in subjects with the smallest changes in hormonal variable i.e. no or only small drop in C concentration and the least increase in T/C and fT/C ratio (see Figure 2 and 5).

Our data demonstrating increased muscle strength and local muscular endurance confirms previously reported suggestions (Bell *et al.* 2000, Grandys *et al.* 2008) that endurance cycling training may improve muscular strength performance. Although, the exact mechanism(s) of this improvement in our subjects remains unknown, it is well accepted that muscle strength could be enhanced due to increase in muscle mass and / or neural control of movement (in e.g. increase in the number of motor unit recruited or greater motoneuron firing frequency) (see Sale 1988, Zoladz *et al.* 2012). The lack of changes observed in BM and BMI after 20 weeks of endurance training could reflect hidden changes in body composition in ET group i.e. decrease in body fat and increase in muscle mass that may be responsible for the observed improvement in muscle strength performance. We have also shown that this improvement is important for the endurance cycling performance, because change in MVC was correlated to change in  $PO_{max}$  (see Results). However, to our best knowledge, there is no study showing that the greater improvement in muscle performance was found in the subjects with the smallest changes in C concentration (i.e. smallest decrease) and T/C and fT/C ratio

(i.e. smallest increase). In previously published studies, where changes in basal T, C and their ratio were linked with muscle strength improvement (usually strength training programs), the opposite findings were presented and an increase in muscle strength performance was related to an increase in T/C ratio and a decrease in C concentration (Hakkinen *et al.* 1985, Staron *et al.* 1994). However, we are inclined to think that basal changes in anabolic-catabolic hormone balance during exercise training programs are above all an outcome of the applied training load (Flynn *et al.* 1994, Grandys *et al.* 2011, Kraemer *et al.* 2004) and have rather little influence on the skeletal muscle performance (Grandys *et al.* 2012). From this point of view the observed decrease in basal C concentration in this study as a consequence of 20 weeks of endurance cycling training is very intriguing.

Endurance training programs generally result in no change (Bresciani *et al.* 2011, Grandys *et al.* 2008, Lehmann *et al.* 1993) or an increase in basal C concentration as a consequence of increased physiological stress related to high training loads (Hoogeveen and Zonderland 1996, Kirwan *et al.* 1988, Seidman *et al.* 1990). To our best knowledge, endurance training-induced decrease in serum basal C concentration in previously untrained men was only once reported (Wheeler *et al.* 1991), but this pattern of response was often demonstrated in endurance trained elite athletes who were exposed to excessive training loads (Banfi *et al.* 1993, Lucia *et al.* 2001) or diagnosed as overtrained (Lehmann *et al.* 1992, Snyder *et al.* 1995). The decreased basal C concentration after prolonged period of heavy load training could be a result of two probably interdependent mechanisms that have been implicated in the literature in training-induced alteration in the hypothalamic-pituitary-adrenal axis (HPA): decreased pituitary sensitivity to C negative feedback (Duclos *et al.* 2001) and a reduced adrenal responsiveness to ACTH stimulation (Lehmann *et al.* 1993, Wittert *et al.* 1996).



Despite relatively moderate training intensity used in this study (~85% of total training time was performed at the intensity below LT) we cannot rule out the possibility of overreaching (for discussion of the overreaching and its connection to overtraining see Meeusen *et al.* 2013) after nearly 5 months of progressively increasing training loads, at least in these subjects from ET group that revealed the smallest training-induced increase in MVC and TTF 50% MVC. An individual analysis of our data and the positive correlation between the changes in C concentration and the changes in TTF 50% MVC (Figure 2) revealed that subjects with the greatest decrease in serum C concentration had the smallest increase in local muscular performance. On the other hand, the direct consequence of the overtraining syndrome and its main symptom, i.e. decline in physical fitness, was not present in any of the subjects. Conversely, our ET group increased their mean endurance performance during incremental exercise test and mean muscle strength performance (see Results). Similar findings were presented in the aforementioned paper by Wheeler *et al.* (1991), where a decrease in C concentration was accompanied by an increase in  $V_{O_{2max}}$ . Moreover, decrease in basal C concentration that occurred concomitantly with significant improvement in strength performance in a previously untrained men was also noted after resistance training programs (Arazi *et al.* 2013, Staron *et al.* 1994). These data suggest that decreased basal C concentration after training period cannot be interpreted as a reliable sign of overtraining syndrome, and what is more, there are also studies where basal C concentration did not change (Urhausen *et al.* 1998) or even increased (Barron *et al.* 1985) in overtrained subjects. Taking into account all these data, it seems that a large variability in the C response to training load and its relationship with exercise capacity depends on the specific time-point in the training-induced alteration of the HPA axis.

On the other hand, decreased C concentration, as it was present in our subjects after endurance training, also appear to have health related consequences. We may suggest that this

adaptive hormonal response may bring benefits for cardiovascular health, since it was recently shown that dysregulation of the HPA axis and increased C concentration is related to higher risk of a cardiovascular event (Vogelzangs *et al.* 2010), probably due to suppression of the nitric oxide system (Liu *et al.* 2009) and endothelial dysfunction (Akaza *et al.* 2010) what accelerates the process of atherosclerosis. Moreover, unfavorable blood lipid profile that is related to higher C concentration (Reynolds *et al.* 2010), allow us to speculate that heavy endurance training resulting in increased C concentration may create a potential risk for vascular disease, whereas moderate intensity endurance training enhances so called “metabolic fitness” (Despres and Lamarche 1994) that is related to improved metabolic variables responsible for the cardiovascular risk.

In this study, the decreased C concentration in ET group resulted also in an increased T/C and fT/C ratio (there were no changes in T and fT concentrations). We are not aware of any studies that showed an increased T/C and fT/C ratio after endurance training program that can be attributed to the training effect *per se* and not to restoration of these indices as a result of decreased training loads after heavy training period (see e.g. Banfi *et al.* 1993). In the studies, where strength or sprint training programs were employed, the enhanced anabolic-catabolic balance expressed as an increase in T/C ratio was often observed (Farzad *et al.* 2011, Hakkinen *et al.* 1985), but in the endurance training programs usually no changes (Flynn *et al.* 1994, Grandys *et al.* 2009) or a decrease in fT/C and T/C ratio was demonstrated (Hoogeveen and Zonderland 1996, Seidman *et al.* 1990). Recently however, it was suggested (Grandys *et al.* 2009) that endurance training program can also lead to increase in this indices if the appropriate training load and sufficiently long training period is applied. In this study, we have observed an increase in T/C and fT/C ratios after 20-week long endurance training program, but as it was mentioned earlier, the decrease in C concentration was responsible for these changes. These findings demonstrate that training induced changes in T/C and fT/C

should be used with caution in monitoring training loads. In some circumstances increased T/C and fT/C ratios may be inappropriately interpreted as a positive adaptation in anabolic-catabolic balance, whereas their changes resulted from markedly decreased C concentration without T and fT changes (as it was presented in this paper).

In conclusion, we have demonstrated that 20 weeks of endurance cycling training of moderate intensity increased maximal isometric muscle strength and local muscular endurance and that these changes were most likely beneficial for the observed improvement in the maximal power output generated during incremental cycling exercise test. Moreover, endurance training led to a decrease in basal C concentration and as a result to observed increase in T/C and fT/C ratios (T and fT concentration did not change). The novel finding of this study is that the improvement in the muscle strength performance was reduced in subjects with the most noticeable decrease in basal cortisol concentration after endurance training. Because, the other components of the HPA axis were not investigated in this study, we cannot fully explain this result. However, based on available data, it could be supposed that in some of our subjects the nearly 5 months of endurance training resulted in overreaching, that led to diminished C concentration and reduced gain in muscle strength performance. From this finding one may also infer that a decrease in C concentration might be indeed an early marker of overreaching, because this study shows that its decrease during endurance training precedes the decline in physical performance. However, in order to precisely establish the usefulness of cortisol measurement in diagnosis of overreaching, the further studies are needed to follow simultaneously the changes in muscle performance and C level during heavy training leading to overreaching.

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## Tables

Table 1. Basic anthropometric characteristics of the students from endurance training group (ET) and control group (CON) at inclusion to the study.

	Age, yr	Body height, cm	BM, kg	BMI, kg · m <sup>-2</sup>
ET (n=12)	22.33 ± 1.44	180.3 ± 7.98	76.84 ± 14.40	23.58 ± 3.82
CON (n=12)	22.83 ± 1.27	177.8 ± 5.72	73.38 ± 10.54	23.14 ± 2.48

BM, body mass. There were no significant differences ( $P > 0.2$ ).

Table 2. Basic blood variable at the beginning of the intervention period in both groups (ET, n = 12 and CON, n = 12).

	Hct [L/L]	[Hb] [g · dL <sup>-1</sup> ]	L [×10 <sup>9</sup> · L <sup>-1</sup> ]	E [×10 <sup>12</sup> · L <sup>-1</sup> ]	[Na <sup>+</sup> ] [mmol · L <sup>-1</sup> ]	[K <sup>+</sup> ] [mmol · L <sup>-1</sup> ]	[Cr] [μmol · L <sup>-1</sup> ]	[Alb] [g · L <sup>-1</sup> ]
BEFORE 20 weeks of endurance training (ET) / control period (CON)								
ET	0.47 ± 0.23	16.0 ± 0.9	6.00 ± 0.87	5.42 ± 0.30	140.1 ± 1.8	4.34 ± 0.35	90.2 ± 8.1	44.6 ± 2.1
CON	0.45 ± 0.20	15.3 ± 0.8	5.49 ± 0.81	5.06 ± 0.22	140.1 ± 1.2	4.39 ± 0.33	89.0 ± 11.9	46.0 ± 2.1
<i>P</i>	.089	.052	.178	.002	.410	.671	.976	.128

Hct, hematocrit value; Hb, hemoglobin concentration; E, erythrocyte concentration; L, leukocyte concentration; [Na<sup>+</sup>], sodium concentration; [K<sup>+</sup>], potassium concentration; [Cr], creatinine concentration; [Alb], albumin concentration.

Table 3. Hormonal data in ET and CON group before and after 20 weeks of the intervention period.

	Training	ET (n = 12)	CON (n = 12)	<i>P level</i> <i>ET vs. CON</i>
T, nmol · L <sup>-1</sup>	before	20.25 ± 5.01	21.09 ± 4.08	.630
	after	20.54 ± 4.19	20.60 ± 4.71	.755
	<i>P level</i>	.754	.530	
fT, pmol · L <sup>-1</sup>	before	344 ± 70	362 ± 61	.630
	after	345 ± 60	349 ± 80	.630
	<i>P level</i>	.814	.480	
SHBG, nmol · L <sup>-1</sup>	before	29.46 ± 13.07	29.99 ± 14.61	.932
	after	32.54 ± 15.01	31.49 ± 10.37	.799
	<i>P level</i>	.084	.136	
C, nmol · L <sup>-1</sup>	before	560 ± 91	529 ± 140	.410
	after	455 ± 127	487 ± 143	.551
	<i>P level</i>	.006	.530	
T/C, ×10 <sup>3</sup>	before	37.03 ± 10.63	41.95 ± 10.97	.291
	after	47.61 ± 12.55	45.27 ± 15.33	.551
	<i>P level</i>	.010	.638	
fT/C, ×10 <sup>-3</sup>	before	0.63 ± 0.17	0.72 ± 0.19	.143
	after	0.81 ± 0.24	0.76 ± 0.24	.755
	<i>P level</i>	.015	.754	

T, testosterone concentration; fT, free testosterone concentration; SHBG, sex hormone-binding globulin concentration; C, cortisol concentration; T/C, testosterone to cortisol ratio; fT/C, free testosterone to cortisol ratio.

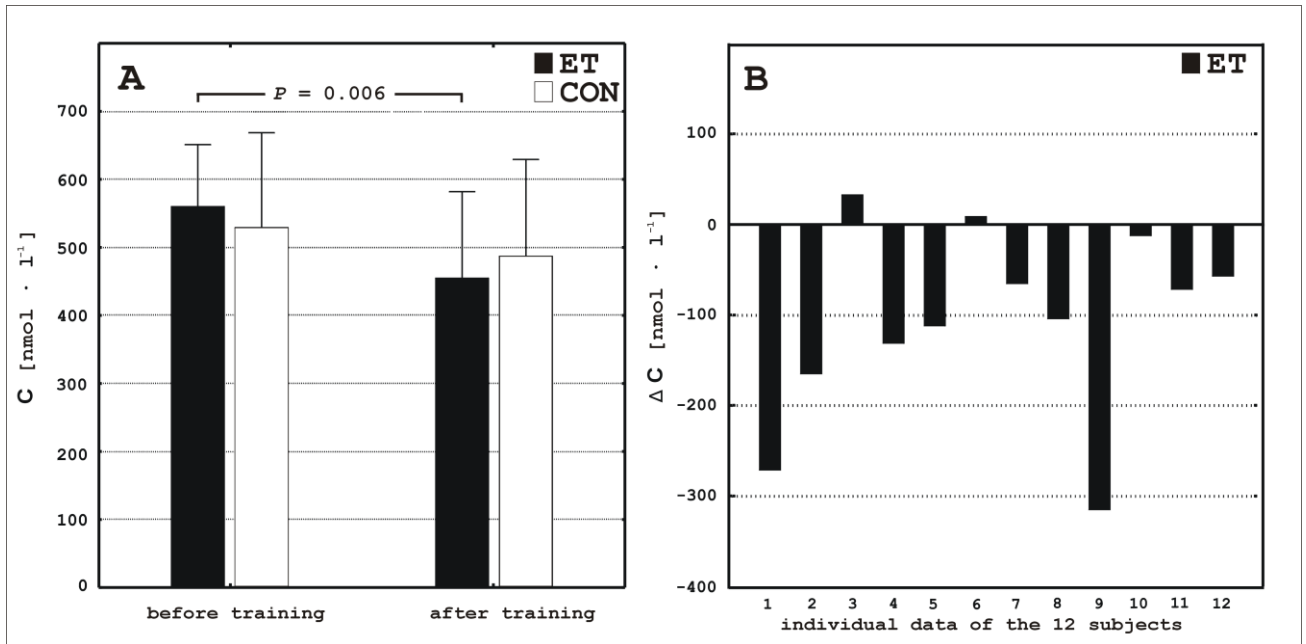


Figure 1. Basal mean serum cortisol concentrations before and after 20 weeks of the study period in ET and CON group (panel A) and individual changes in basal C concentration ( $\Delta C$ ) in each of the 12 subjects from ET group in response to endurance training (panel B).

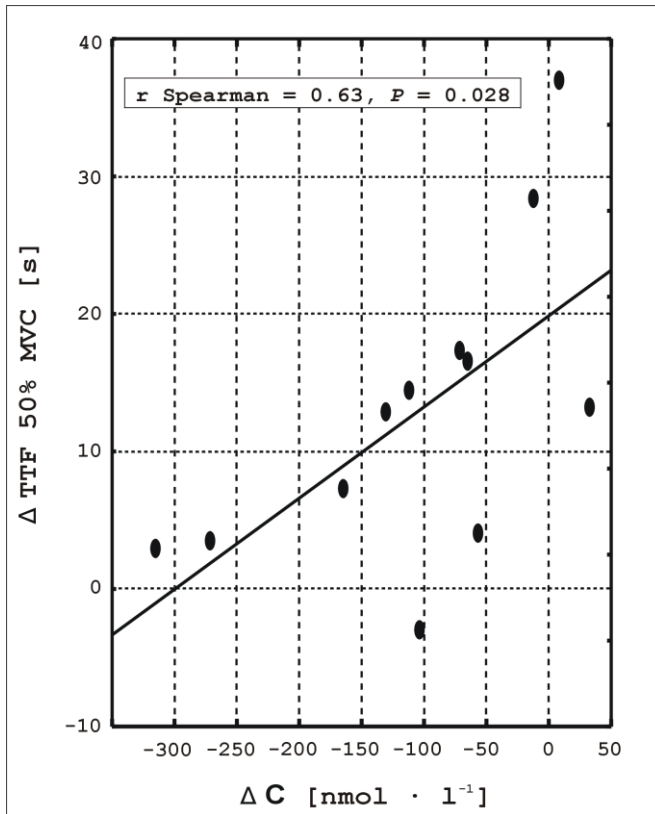


Figure 2. Correlation between the change in TTF 50% MVC ( $\Delta \text{TTF } 50\% \text{ MVC}$ ) and change in C concentration ( $\Delta C$ ) in ET group.



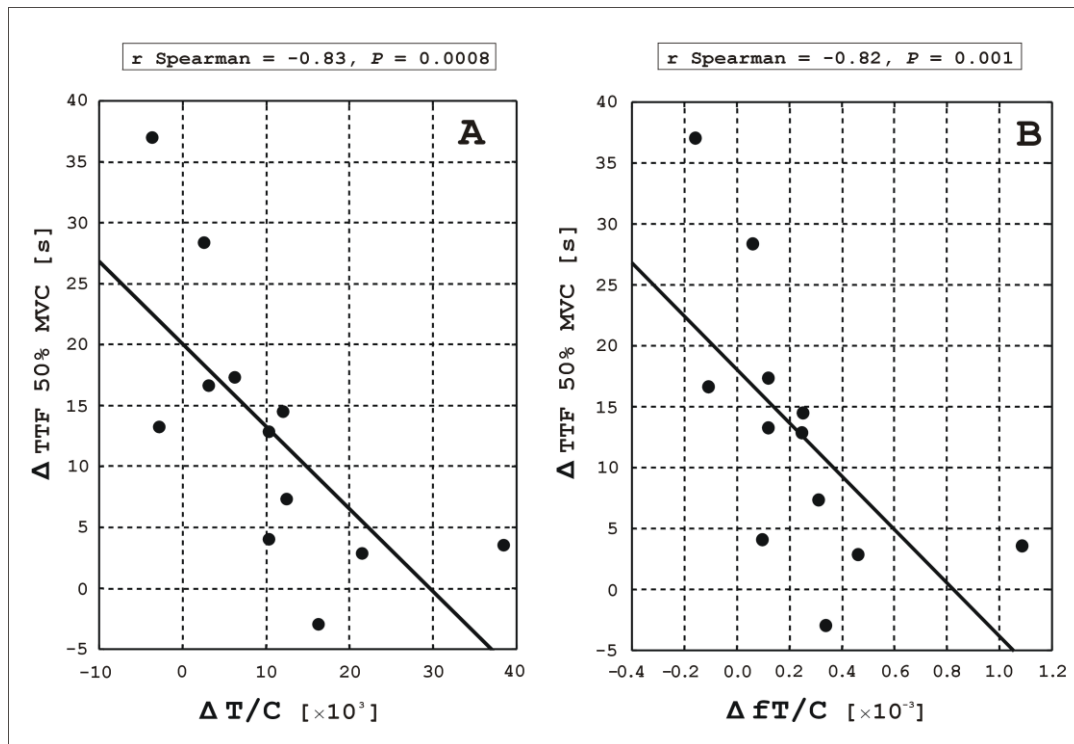


Figure 3. Correlations between the changes in TTF 50% MVC ( $\Delta\text{TTF } 50\% \text{ MVC}$ ) and changes in T/C ratio ( $\Delta\text{T/C}$ ) (panel A) and fT/C ratio ( $\Delta\text{fT/C}$ ) (panel B) in ET group.