"Effect of Intensive Training on Mood With No Effect on Brain-Derived Neurotrophic Factor" by Piacentini MF et al.
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Abstract

Purpose Monitoring mood state is a useful tool for avoiding non-functional overreaching (NFOR). Brain derived neurotrophic factor (BDNF) is implicated in stress-related mood disorders. The purpose of the present study was to investigate the impact of intensified training-induced mood disturbance on plasma BDNF concentrations at rest and in response to exercise. M**ethods** Eight cyclists performed 1 week of normal (NT), 1 week of intensified (INT) and 1 week of recovery (REC) training. Fasted blood samples were collected before and after exercise, on day 7 of each training week and were analyzed for plasma BDNF and cortisol concentrations. A 24-item Profile Of Mood State questionnaire was administered on day 7 of each training week and global mood score (GMS) was calculated. **Results** Time trial performance was impaired during INT (p=0.01) and REC (p=0.02) compared with NT. Basal plasma cortisol (NT=153±16 ng/ml, INT=130±11 ng/ml, REC=150±14 ng/ml) and BDNF (NT=484±122 pg/ml, INT=488±122 pg/ml, REC=383 ±56 pg/ml) concentrations were similar between training conditions. Likewise, similar exercise-induced increases in cortisol and BDNF concentrations were observed between training conditions. GMS was 32% greater during INT *vs.* NT (P<0.001). **Conclusion** Consistent with a state of functional overreaching (FOR), impairments in performance and mood state with INT were restored after one week of REC. These results support evidence that mood changes before plasma BDNF concentrations as a biochemical marker of FOR and that cortisol is not a useful marker for predicting FOR.

Keywords: Functional overreaching; psychological mood state; neurotrophins; cortisol; trained cyclists

INTRODUCTION

Endurance athletes routinely schedule training sessions of increased volume (intensity and duration) into carefully planned training programs. As a result, endurance athletes often experience acute feelings of fatigue that subsequently manifest a temporary decrement in performance[1], known as 'functional overreaching (FOR)'. When FOR is followed by a period of reduced training volume, performance is improved[1]. In contrast, non-functional overreaching (NFOR) describes a prolonged decrease in performance after an intensified period of training that is followed by an insufficient period of recovery[1]. The ability to distinguish between adaptive FOR and maladaptive NFOR and/or diagnose impending NFOR is an important consideration when periodizing the training programs of endurance athletes.

To date, no single measurement exists to differentiate between adaptive and maladaptive training responses[1]. The transition between FOR and NFOR is gradual and includes a state of overreaching necessary to improve performance[2]. Several other factors, such as psychological and social disturbances, are thought to trigger training distress[1]. In particular, mood disturbance is recognized to be an early predictive marker of NFOR since mood state exhibits a predictable dose–response relationship with training volume[1]. In principle, identifying a physiological marker that predicts impending mood disturbance during periods of intensified training will provide a useful diagnostic tool for impending NFOR.

Brain derived neurotrophic factor (BDNF) is a neurotrophin shown to stimulate neuronal outgrowth, differentiation, synaptic connectivity and neuronal repair[3]. BDNF is the most abundantly expressed member of the nerve growth factor family and plays a fundamental role in the development, maintenance and plasticity of the central and peripheral nervous system[4,5]. BDNF is a crucial player in adaptive responses of the brain and the body to metabolic challenges[6]. BDNF and different neurotransmitters (NT) such as serotonin (5-

HT), dopamine and noradrenaline are released in various brain nuclei[7]. Voluntary exercise has been shown to increase levels of BDNF mRNA and protein content in the hippocampus and other brain regions [8]. Although the exact mechanisms underlying the increased release of BDNF with acute exercise remains unknown, one potential mediator is neurotransmission[9]. Whereas acute bouts of aerobic-type exercise have consistently been shown to modulate circulating BDNF levels, studies investigating the impact of chronic periods of exercise training on basal BDNF concentrations have provided inconsistent findings, potentially due to differences in the intensity of training and/or training status of participants [3].

Chronic stress is one of the most robust negative regulators of adult neurogenesis[10]. The increase in glucocorticoids during both acute and chronic stress, may negatively affect brain function and contribute to the pathophysiology of mood disorders[11] by decreasing the expression of BDNF in the hippocampus [12]. Whereas the modulation of BDNF by chronic stress is well established[13], recent work suggests a role for BDNF in stress-related mood disorders[14]. Therefore, the purpose of the present study was to investigate the relationship between intensified training-induced mood disturbance and plasma BDNF concentrations. We hypothesized that intensified training would induce mood disturbances and decrease plasma BDNF concentrations at rest and in response to exercise.

Materials and methods

Participants

Eight well-trained cyclists (according to De Pauw et al. 2013[15] Performance Level 3 classification) (age 27 ± 8 yrs, BM 73 ± 7 kg, VO_2max 64.2 ± 6.5 ml/kg/min) were recruited to participate in this study, as previously described[16.] All procedures were approved by the Research Ethics Committee of the School of Sport and Exercise Science, University of Birmingham, United Kingdom and written information of the potential risks and benefits associated with participation and oral instructions were provided to the participants who

signed a written informed consent form. Before participation, the health status of each participant was assessed using a general health questionnaire.

Experimental Design

To evaluate the impact of intensified training on plasma cortisol and BDNF concentrations, psychological mood state and time trial performance, each participant completed a 3 wk period of quantified training. Sequentially, training periods were divided into 1 wk of normal training (NT), 1 wk of intensified training (INT), and 1 wk of recovery training (REC). Maximal aerobic capacity was assessed by a VO_2max test and endurance performance was assessed by a preloaded time trial on days 6 and 7, respectively of each training week. To eliminate the potential, albeit unknown, acute effects of nutrition on plasma BDNF concentrations, and in accordance with the study design of other similar investigations[16, 17, 18], exercise trials in the present study were performed in a fasted state.

Preliminary Exercise Testing

Preliminary testing included a VO_2max test for the assessment of maximal aerobic capacity and a familiarization time trial, as described previously [16,17]. Maximum power data generated from the VO_2max test was used to customize the workload to be completed during the time trial, as described previously [16,17.]

Performance Assessment

Time Trials

To determine endurance performance, a pre- loaded (120 min of submaximal exercise at 50% Wmax) time trial lasting ~45 min was performed on day 7 of each training condition. On arrival at the laboratory, participants were fitted with a HR monitor, and a Teflon catheter was inserted into a forearm vein. After a 10-min rest period, a baseline blood sample was drawn. Participants cycled for 120 min at 50% Wmax with the electromagnetically braked

ergometer (Lode Excalibur Sportv. 2.0, Groningen, Netherlands) set in the hyperbolic (cadence-independent) mode so that work rate was independent of pedaling rate. Upon cessation of submaximal exercise, participants performed a 45-min time trial. After adjusting the ergometer to the cadence-dependent (linear) mode, participants were required to complete a set amount of work (670 ± 52 kJ) as fast as possible[18]. Participants could monitor task progress, however received no feedback on cadence, power output and elapsed time. Blood samples (15 mL) were collected at baseline (BL) after 120 min of the pre-loaded time trial (SM), immediately (MAX)- and 1h- after exercise (1h-POST) completion (45-min time trial). Measurements of HR and RPE (using the modified Borg scale[19]) were collected at 20 min intervals. Time trials were performed in the morning (start of exercise between 06:30 and 08:00 a.m.) after an overnight fast.

Exercise training

In an attempt to accurately monitor training, each participant completed a detailed log-book, as detailed previously [16]. Briefly, athletes were equipped with a downloadable HR monitor (Polar Vantage NV, Kempele, Finland) for the duration of each trial to monitor individual training sessions. During NT, participants continued with their normal training volume. During INT, training volume was increased by ~70% and time spent in training zones Z3-Z5 (according to the British cycling guidelines[20]) was increased. During INT, participants performed 1 or 2 training sessions each day. Training sessions consisted of a combination of long, continuous rides, usually between 4 and 5 h in duration or high-intensity interval sessions above lactate threshold lasting between 2 and 3 h. During REC, training volume was reduced by 60% compared to NT.

Blood Samples

Blood samples were collected into pre-chilled vacutainers containing K_3EDTA or lithium heparin (Becton Dickinson, Franklin Lakes, NJ). Whole blood was immediately

placed on ice until centrifugation at 3000 rpm for 10 min at 4°C, within 2 h of collection. Plasma was stored at -80°C until further analysis.

Plasma cortisol: Commercially available sandwich ELISA kits were used to determine plasma cortisol concentrations (cortisol; IDS, Tyne and Wear, UK). Plates were read in duplicate on a Labsystems Original Multiskan MS at selected wavelengths (450 nm). The reported sensitivity of the ELISA kit was 2.5 ng/mL. Intra- assay variations were calculated as 7%.

Plasma BDNF: The expression of plasma BDNF concentrations were estimated using an ELISA kit (CYT306, ChemiKine®, Millipore®, Billerica, MA, USA). Plasma was diluted 20 times with sample diluent (PBS, 1% BSA, 0.05% tween-20). All samples were added in duplicate to the plate together with a standard series (7.8-500 $pg.mL^{-1}$) and were incubated overnight. The next day, biotinylated anti-BDNF monoclonal antibody and streptavidin-HRP conjugate solution were added, with incubation and washing steps at 1 and 3 h intervals, respectively. Color reaction started with the TMB/E solution and was stopped 15 min later. Absorbance was measured using a Bio-Rad® microplate reader at a wavelength of 450nm.

Given that no significant % change in plasma volume between NT and IT (or NT vs. REC or IT vs. REC) was observed at any timepoint (data not shown) and previous work has shown that plasma volume during exercise has negligible impact on results[21], we did not adjust plasma concentrations of BDNF or cortisol for plasma volume.

Profile of Mood States

Upon waking, on day 7 of each training week, participants completed a 24-item version of the POMS-24 questionnaire[22]. The POMS has three subscales: tension, vigor and fatigue. Global mood state (GMS) was calculated as the sum of all negative categories minus the score for vigor, plus 100. Given that vigor and fatigue are the scores that show the

greatest changes in response to training[1], the "energy index" (vigor – fatigue) was used to monitor these changes throughout the study.

Data Presentation and Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 22 software. A one-sample Kolmogorov-Smirnov test with scatter plots was used to test the normality of data, and sphericity was verified by Mauchly's test. When the assumption of sphericity was not met, the significance of F-ratios was adjusted using the Greenhouse-Geisser procedure. A Multivariate Analysis of Variance (MANOVA) (condition × time with 3 factors) test was chosen to reduce the likelihood of type I error. In case of significant condition effect, a subsequent univariate ANOVA was conducted to determine differences in baseline plasma cortisol concentrations, plasma BDNF concentrations and mood state values between training conditions. The response of plasma BDNF and cortisol concentrations to exercise over time was compared using a doubly multivariate analysis of repeated measures ANOVA. Finally, post-hoc Bonferroni analysis was performed to detect significant main effects. Statistical significance was set at an α-level of 0.05.

RESULTS

Training:

Time spent in each training intensity zone during each training condition is reported in Figure 1. A significant increase in training volume and intensity was observed during INT (Z1: 1%, Z2: <1%, Z3: 2%, Z4: 5%, Z5: about 1.3-fold increase) compared to NT. During REC there was a 60% decrease in training volume and intensity (Z1: 8%, Z2: 3%, Z3: 9%, Z4: < 1%, Z5: < 1%) compared to NT.

Performance

As reported previously[16], a decrement in time trial performance was observed during INT (48:12±7:30 min:sec; p=0.01) and REC (43:36±6:54 min:sec sec; p=0.02) compared with NT (42:36±5:12 min:sec). No statistical difference in time trial performance was observed between NT and REC.

Plasma cortisol concentrations

Subsequent to the significant MANOVA (3 training conditions; 3 dependent variables (CORT, BDNF, GMS)) ($F(8,38) = 3.65$ $p < .001$), the univariate ANOVA revealed no difference in baseline plasma cortisol concentrations between training conditions (Figure 2). In response to exercise, doubly multivariate repeated measures ANOVA showed a significant time main effect ($F(2,6) = 17.108$; $p < 0.001$), however no time × training condition interaction effect was detected ($F(2,6) = 1.312$; $p = 0.2$). Subsequent univariate tests revealed plasma cortisol concentrations increased in response to exercise ($F(4)= 28.386$; $p < 0.001$). Adjustments for multiple comparisons were made through post-hoc Bonferroni corrections and demonstrated a significant increase in cortisol concentrations at MAX and 1 h- POST compared with BL and SM. Cortisol concentrations were significantly higher after 1 h-POST compared with SM ($p =0.047$).

Plasma BDNF concentrations

Subsequent to the significant MANOVA (3 training conditions; 3 dependent variables (CORT, BDNF, GMS)) ($F(8,38) = 3.65$ $p < .001$), the univariate ANOVA revealed no difference in baseline plasma BDNF concentrations between training conditions (Figure 3). In response to exercise, doubly multivariate repeated measures ANOVA showed a significant main time effect ($F(2,6) = 17.108$; $p < 0.001$), however no time × training condition interaction effect was detected ($F(2,6) = 1.312$; $p = 0.2$). Subsequent univariate tests showed

that plasma BDNF concentrations increased over time in response to exercise ($F(2)=18.537$; $p < 0.001$). Adjustments for multiple comparisons were made through post-hoc Bonferroni corrections and demonstrated a significant increase in plasma BDNF concentrations at SM ($p<0.001$) and MAX ($p < 0.001$) compared with BL, whereas no difference in plasma BDNF concentration was observed between MAX and 1-h POST.

Mood state

Subsequent to the significant MANOVA (3 training conditions; 3 dependent variables (CORT, BDNF, GMS)) ($F(8,38) = 3.65$ $p < .001$), the univariate ANOVA revealed a significant difference in GMS between training conditions ($F(2)=13.3$, $p < 0.001$). A 32% increase GMS score was reported during INT (120.7±4.0) compared with NT (91.1±5.0) (post-hoc $p < 0.001$) and REC (93.2±5.0) (post-hoc $p = 0.001$) (Figure 4). For the subscales, tension was higher during INT (12.1±2.6) compared with NT (5.3±2.0, $p=0.01$) and REC (5.4±1.8, $P<0.001$), vigor was lower during INT (9.3±1.2) compared with NT (19.0±3.0, $p=0.02$), and fatigue was higher during INT (17.8±1.5) compared with NT (4.9±1.2, $P<0.001$) and REC (3.6±1.1, $P<0.001$). The energy index decreased during INT (-8.65±2.0) compared with NT (14.12±3.7, $p<0.001$).

DISCUSSION

The purpose of the present study was to investigate the impact of intensified training-induced mood disturbance on plasma BDNF concentrations at rest and in response to exercise. Consistent with previous literature[3,8], these data demonstrate that acute exercise increases plasma BDNF concentrations and the magnitude of increase is intensity dependent[23]. Refuting the original hypothesis, we demonstrated that the intensified training-induced disturbance in psychological mood state was not associated with a change in plasma BDNF concentrations at rest and in response to exercise.

To our knowledge, the present study is the first to investigate the impact of training intensity on mood state and BDNF release in well-trained athletes. We report no differences in the baseline (rested and fasted) or exercise-induced response of plasma BDNF concentrations between NT, IT and REC. Previous studies that have investigated the impact of training intensity on BDNF concentrations recruited moderately trained or sedentary volunteers and reported inconsistent findings. For instance, Zoladz et al[24] reported an increase in baseline plasma BDNF concentrations after 5 weeks of moderate endurance training in previously untrained volunteers. In addition, the same authors reported an increase in exercise-induced BDNF concentrations after chronic exercise training [24]. Moreover, whereas Seifert et al[25] reported an increased release of BDNF at rest after 3 months of endurance training in previously sedentary overweight males, Schiffer et al[26] reported no significant increase in baseline plasma BDNF concentrations after strength or endurance training in moderately trained individuals. Taken together, these past [24,25,26] and present data suggest that training intensity and training status may influence the baseline and exercise-induced response of plasma BDNF concentrations to a period of exercise training. Since serum BDNF levels are ~200 fold higher than plasma BDNF levels (for review see[3]), we acknowledge this interpretation must be tempered to plasma measurements of BDNF, rather than generalized to both plasma and serum measurements.

We hypothesized that a period of intensified training would decrease resting plasma BDNF concentrations. Several different physiological and psychological stressors, such as immobilization and chronic unpredictable stress, have been shown to decrease the expression of BDNF[27]. Although the mechanisms responsible for this downregulation of BDNF are not fully understood, elevated levels of adrenal glucocorticoids, appear to play a significant role[27]. Yau et al[10] demonstrated that chronic cortisol treatment significantly impaired spatial learning and hippocampal BDNF in animals, a condition that was reversed with running.

Similarly, Schaaf et al[28] reported that corticosterone suppresses BDNF expression several hours after administration. However, after training animals in the water maze test, they reported no suppression of BDNF in any hippocampal subfield, despite a significant rise in corticosterone[29]. Interestingly, performing exercise prior to a stressful event has been shown to counteract this downregulation[30]. Moreover, Goekint et al[31] reported no correlation between peripheral cortisol and BDNF levels, suggesting that cortisol does not acutely regulate peripheral BDNF levels (as opposed to a central suppressive effect previously reported in the hippocampus[13]). A recent review[32] articulates how glucocorticoids can either increase or decrease adult neurogenesis depending on the typology of stress (controllable/uncontrollable). In particular, a controllable amount of stress (such as physical exercise or enriched environment) will increase glucocorticoid concentrations and BDNF while a suppression of glucocorticoid and BDNF activity is reported after periods of uncontrollable stress (chronic) and low stimulating factors. Based on these data, we can speculate that well-trained participants in the present study exhibited the required mechanisms to cope with the stress of intensified training, thus preventing intensive training-induced changes in plasma BDNF and cortisol concentrations. Moreover, whereas performance- and psychology-related markers (psychological mood state, impaired time trial performance) suggest the training stress (increased duration and intensity of training) imposed by the present study design was severe, it could be argued that this training stress was predictable, particularly in the cohort of well-trained cyclists recruited in the present study. It is possible that the predictability of intensified training resulted in different central responses while most animal studies instead utilized unpredictable models of chronic stress.

Although intensified periods of training are necessary for athletes to induce FOR and improve performance, it is already well known that increased training load may cause mood disturbances [33,34, 35]. For example, Halson et al.[33] reported a 28% increase in total mood

disturbance after 2 weeks of intensified training. In the present study, mood disturbance increased by 32% after only one week of intensified training and was restored after 1 week of recovery. Therefore, monitoring mood is a simple and effective method for preventing NFOR[36].

Variations in fatigue and vigour are normally observed during training camps with an overall decrease in the energy index, leading to a negative psychological state[37]. In the present study, the decrease in the energy index is consistent with previous reports during training camps and periods of intensified training that resulted in the FOR of athletes[37, 38]. Mood disturbances, changes in emotional behavior, neuroendocrine dysfunction, sleep disturbances and cognitive performance decrements are associated with NFOR, indicating that changes in the regulation and coordinative function of the hypothalamus are prevalent. The hypothalamus is regulated by higher brain centers and different neurotransmitter systems and there is a reciprocal feedback between central serotonergic neurotransmission and glucocorticoids[39] that seems to be disrupted during periods of intensive stress (which include training).

It has been shown that periods of FOR in endurance trained athletes induce changes in executive and non-executive functions[38,40]. This observation could be caused by a decrease in cerebral oxygenation[38], an upregulation of brain neurotransmitters and a deregulation of BDNF[38] even without symptoms of NFOR. However, none of the previous mentioned studies directly measured BDNF levels, and thus this supposition remains speculative. The hippocampus is a limbic structure rich in serotonin that is actively involved in mood disorders, the control of learning and memory and also the regulation of the HPA axis[12]. Each of these components are altered with depression and during periods of chronic stress and NFOR. Therefore, the role of BDNF in stress-related mood disorders has been acknowledged since BDNF is highly concentrated in the hippocampus.

Practical applications

All participants in the present study demonstrated a decrement in performance and mood state after intensified training. This mood disturbance is consistent with the definition of functionally overreached athletes because performance and mood were both restored after 1 week of recovery training[1]. According to the POMS-24 profile, all three mood subscales

(vigour, fatigue and tension) were modified by intensified training. These data imply that the first visible markers of FOR include an initial disturbance of psychological mood state, followed by an impaired endurance performance, with no lasting negative symptoms. Furthermore, these data support the notion that plasma BDNF concentrations respond acutely to fatiguing exercise typical of FOR, and that the psychological disturbances were not severe enough to disrupt the HPA axis and higher brain centers. Although performance decrements are often considered early signals for FOR or NFOR, it is plausible that monitoring alternative, and more responsive, markers of NFOR is required to prevent sustained

decrements in performance that necessitate extended periods of recuperation[36]. As previously suggested[1], the measurement of basal cortisol concentration is not a useful tool for monitoring training stress.

CONCLUSION

To conclude, the results of this study highlight the capacity for well-trained endurance athletes to adapt to short periods of intensified training. Furthermore, these data support the notion that mood remains one of the best markers to monitor adaptation to training. We acknowledge this conclusion (i) is based on a small sample size that may affect statistical power, (ii) assumes that one week of intensified training caused similar levels of FOR in all athletes and (iii) is based on a study design that may not be considered to mimic real-world practice (i.e., cyclists performed exercise in a fasted, rather than fed, state). Future studies are warranted to investigate changes in both plasma and serum BDNF and cortisol concentrations

during periods of unpredictable stress that lead to NFOR and investigate the role of an increased inflammatory state and oxidative stress in mediating these changes; admittedly a difficult proposition under controlled laboratory conditions.

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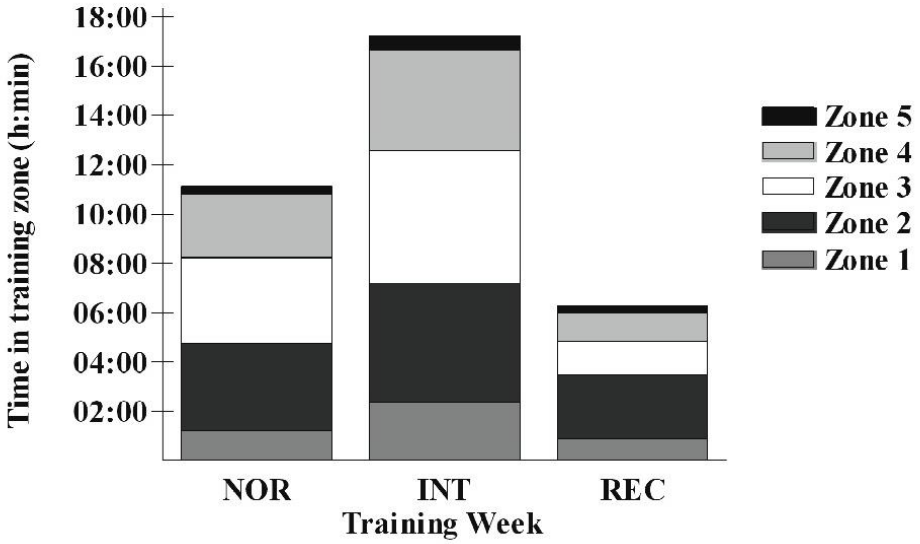

Figure 1: Time spent in each training zone during each training condition. Z1 = < 70%HRmax, Z2 = 70%–80%HRmax, Z3 = 80%–90%HRmax, Z4 = 90%–95%HRmax, and Z5 = > 95%Hrmax

INT training showed a significant increase in volume and intensity (time spent in Z4 and Z5).

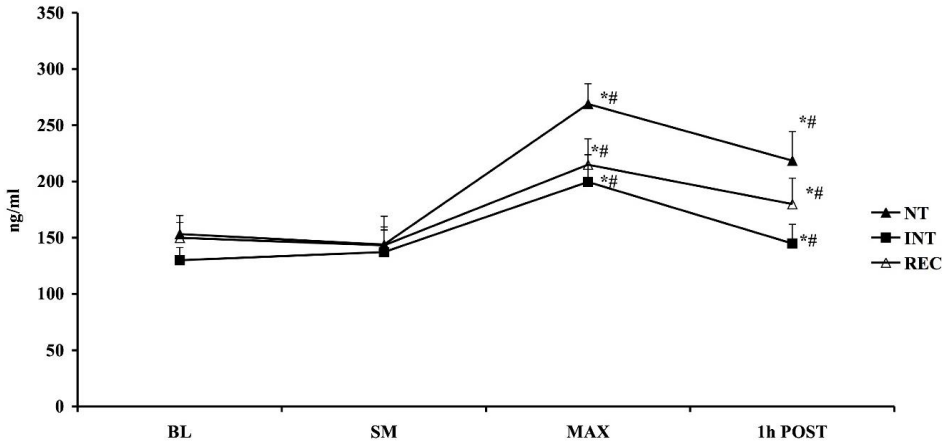

Figure 2. Plasma cortisol concentrations at baseline (BL), at the end of submaximal exercise (SM) at the end of the time trial (MAX) and after recovery (1h POST) in the three training conditions: normal (NT), intensified (INT) and recovery (REC) training. No difference was observed between the three training conditions.

* = statistically different from BL ($p<0.05$)

= statistically different from SM ($p<0.05$)

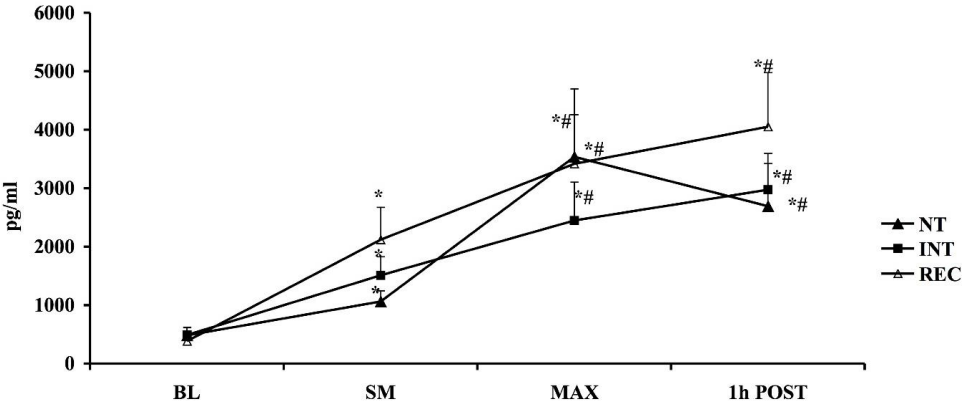

Figure 3. Plasma BDNF concentrations at baseline (BL), at the end of submaximal exercise (SM) at the end of the time trial (MAX) and after recovery (1h POST) in the three training conditions: during normal (NT), intensified (INT) and recovery (REC) training. No difference was observed between the three training conditions.

* = statistically different from BL ($p<0.05$)

= statistically different from SM ($p<0.05$)

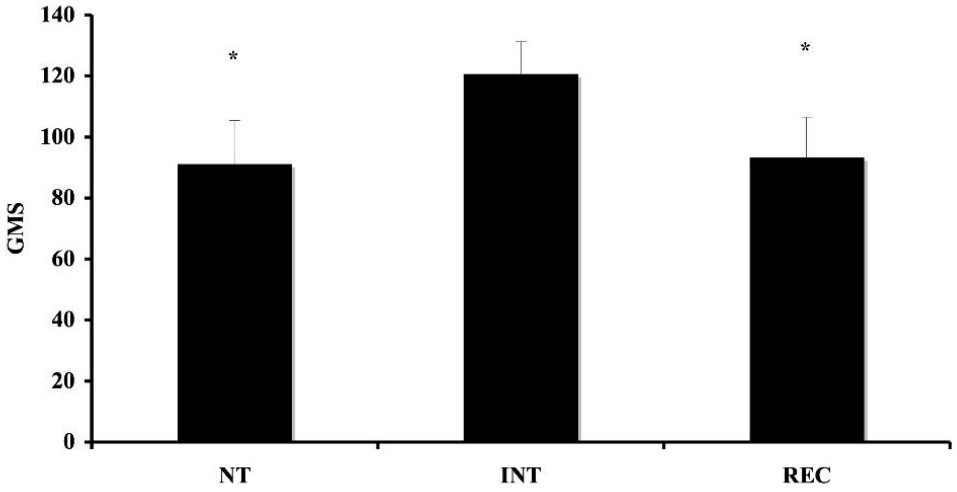

Figure 4. Global Mood Score (GMS) during normal (NT), intensified (INT) and recovery training (REC)

* = statistically different from INT ($p<0.05$)