Effect of Intensive Training on Mood With No Effect on Brain-Derived Neurotrophic Factor

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Context: Monitoring mood state is a useful tool for avoiding nonfunctional overreaching. Brain-derived neurotrophic factor (BDNF) is implicated in stress-related mood disorders. Purpose: To investigate the impact of intensified training-induced mood disturbance on plasma BDNF concentrations at rest and in response to exercise. Methods: Eight cyclists performed 1 wk of normal (NT), 1 wk of intensified (INT), and 1 wk of recovery (REC) training. Fasted blood samples were collected before and after exercise on day 7 of each training week and 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 = .01) and REC (P = .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 < .001). Conclusion: Consistent with a state of functional overreaching (FOR), impairments in performance and mood state with INT were restored after 1 wk of REC. These results support evidence for 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

Endurance athletes routinely schedule training sessions of increased volume (intensity and duration) in carefully planned training programs. As a result, they often experience acute feelings of fatigue that subsequently manifest a temporary decrement in performance known as functional overreaching (FOR). When FOR is followed by a period of reduced training volume, performance is improved. In contrast, nonfunctional overreaching (NFOR) describes a prolonged decrease in performance after an intensified period of training that is followed by an insufficient period of recovery. 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. The transition between FOR and NFOR is gradual and includes a state of overreaching necessary to improve performance. Several other factors such as psychological and social disturbances are thought to trigger training distress. 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. 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. 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. BDNF is a crucial player in adaptive responses of the brain and the body to metabolic challenges. BDNF and different neurotransmitters such as serotonin, dopamine, and noradrenaline are released in various brain nuclei. Voluntary exercise has been shown to increase levels of BDNF mRNA and protein content in the hippocampus and other brain regions. Although the exact mechanisms underlying the increased release of BDNF with acute exercise remains unknown, a potential mediator is neurotransmission. 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.

Chronic stress is one of the most robust negative regulators of adult neurogenesis. The increase in glucocorticoids during both acute and chronic stress may negatively affect brain function and contribute to the pathophysiology of mood disorders by decreasing the expression of BDNF in the hippocampus. Whereas the modulation of BDNF by chronic stress is well established, recent
work suggests a role for BDNF in stress-related mood disorders.14 Therefore, the purpose of the current 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 al15 performance level 3 classification) (age 27 ± 8 y, body mass 73 ± 7 kg, maximal oxygen uptake [VO2max] 64.2 ± 6.5 mL · kg⁻¹ · min⁻¹) 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 and psychological mood state on time-trial performance, each participant completed a 3-week period of quantified training. Sequentially, training periods were divided into 1 week of normal training (NT), 1 week of intensified training (INT), and 1 week of recovery training (REC). Maximal aerobic capacity was assessed by a VO2max 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,16–18 exercise trials in the current study were performed in a fasted state.

Preliminary Exercise Testing

Preliminary testing included a VO2max test for the assessment of maximal aerobic capacity and a familiarization time trial, as described previously.16,17 Maximum-power data generated from the VO2max test were used to customize the workload to be completed during the time trial, also described previously.16,17

Performance Assessment

Time Trials. To determine endurance performance, a preload (120 min of submaximal exercise at 50% Wmax) time trial lasting ~45 minutes was performed on day 7 of each training condition. On arrival at the laboratory, participants were fitted with a heart-rate (HR) monitor, and a Teflon catheter was inserted into a forearm vein. After a 10-minute rest period, a baseline blood sample was drawn. Participants cycled for 120 minutes at 50% maximum power (Wmax) on an 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. On cessation of submaximal exercise, participants performed a 45-minute 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 but received no feedback on cadence, power output, and elapsed time. Blood samples (15 mL) were collected at baseline (BL), after 120 minutes of the preloaded time trial (SM), and immediately (MAX) and 1 hour after exercise completion (1h-POST). Measurements of HR and rating of perceived exertion (using the modified Borg scale19) were collected at 20-minute intervals. Time trials were performed in the morning (start of exercise between 6:30 and 8:00 AM) after an overnight fast.

Exercise Training. In an attempt to accurately monitor training, we had each participant complete a detailed logbook, 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 to Z5 (according to the British cycling guidelines20) was increased. During INT, participants performed 1 or 2 training sessions each day. Training sessions consisted of a combination of long, continuous rides, usually 4 to 5 hours in duration, or high-intensity interval sessions above lactate threshold lasting 2 to 3 hours. During REC, training volume was reduced by 60% compared with NT.

Blood Samples. Blood samples were collected into prechilled Vacutainers containing K3EDTA or lithium heparin (Becton Dickinson, Franklin Lakes, NJ). Whole blood was immediately placed on ice until centrifugation at 3000 rpm for 10 minutes at 4°C, within 2 hour of collection. Plasma was stored at ~80°C until further analysis.

Commercially available sandwich ELISA kits were used to determine plasma cortisol concentrations (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%.

The expression of plasma BDNF concentrations was 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) and 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-hour intervals, respectively. Color reaction started with the TMB/E solution and was stopped 15 minutes later. Absorbance was measured using a Bio-Rad microplate reader at a wavelength of 450 nm.

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

Profile of Mood States

On waking, on day 7 of each training week, participants completed a 24-item version of the 24-item Profile of Mood State (POMS) questionnaire.22 The POMS has 3 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 1-sample Kolmogorov-Smirnov test with scatter plots was used to test the normality of data, and sphericity was verified by Mauchly 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) 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 responses of plasma BDNF and cortisol concentrations to exercise over time were compared using a doubly multivariate repeated-measures ANOVA. Finally, post hoc Bonferroni analysis was performed to detect significant main effects. Statistical significance was set at an α-level of .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 ~1.3-fold increase) compared with NT. During REC there was a 60% decrease in training volume and intensity (Z1 8%, Z2 3%, Z3 9%, Z4 <1%, Z5 <1%) compared with NT.

Performance

As reported previously,16 a decrement in time-trial performance was observed during INT (48:12 ± 7:30 min:s, P < .01) and REC (43:36 ± 6:54 min:s, P < .01) compared with NT (42:36 ± 5:12 min:s). Subsequent univariate tests revealed a significant increase in performance during INT (48:12 ± 7:30 min:s, P = .02) compared with NT (42:36 ± 5:12 min:s). During REC there was a 60% decrease in training volume and intensity (Z1 1%, Z2 <1%, Z3 3%, Z4 <1%, Z5 <1%) compared with NT.

Plasma Cortisol Concentrations

Subsequent to the significant MANOVA (3 training conditions, 3 dependent variables: CORT, BDNF, GMS) (F3,38 = 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 (F2,6 = 17.108, P < .001), but no time × training-condition interaction effect was detected (F2,6 = 1.312, P = .2). Subsequent univariate tests revealed that plasma cortisol concentrations increased in response to exercise (F2 = 28.386, P < .001). Adjustments for multiple comparisons were made through post hoc Bonferroni corrections and demonstrated a significant increase in cortisol concentrations at MAX and 1h-POST compared with BL and SM. Cortisol concentrations were significantly higher after 1h-POST compared with SM (P = .047).

Plasma BDNF Concentrations

Subsequent to the significant MANOVA (3 training conditions, 3 dependent variables: CORT, BDNF, GMS) (F3,38 = 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 (F2,6 = 17.108, P < .001), but no time × training-condition interaction effect was detected (F2,6 = 1.312, P = .2). Subsequent univariate tests showed that plasma BDNF concentrations increased over time in response to exercise (F2 = 18.537, P < .001). Adjustments for multiple comparisons were made through post hoc Bonferroni corrections and demonstrated a significant increase in plasma BDNF concentrations at SM (P < .001) and MAX (P < .001) compared with BL, whereas no difference in plasma BDNF concentration was observed between MAX and 1h-POST.

Mood State

Subsequent to the significant MANOVA (3 training conditions, 3 dependent variables: CORT, BDNF, GMS) (F3,38 = 3.65, P < .001), the univariate ANOVA revealed a significant difference in GMS between training conditions (F2 = 13.3, P < .001). A 32% increase in GMS score was reported during INT (120.7 ± 4.0) compared with NT (91.1 ± 5.0; post hoc P < .001) and REC (93.2 ± 5.0; post hoc P = .001) (Figure 4). For the subscales, tension was higher during INT (19.0 ± 3.0, P = .01) than during NT (14.9 ± 2.3, P < .001), and fatigue was higher during INT (17.8 ± 1.5) than during NT (9.3 ± 1.2) than during NT (9.3 ± 1.2) and REC (5.4 ± 1.8, P < .001). The energy index decreased during INT (~8.65 ± 2.0) compared with NT (14.12 ± 3.7, P < .001).

Discussion

The purpose of the current 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 current 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 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.
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 3 training conditions: normal (NT), intensified (INT), and recovery (REC) training. No difference was observed between the 3 training conditions. *Statistically different from BL ($P < .05$). #Statistically different from SM ($P < .05$).

Figure 3 — Plasma brain-derived neurotrophic factor 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 3 training conditions: normal (NT), intensified (INT), and recovery (REC) training. No difference was observed between the 3 training conditions. *Statistically different from BL ($P < .05$). #Statistically different from SM ($P < .05$).

Figure 4 — Global mood score (GMS) during normal (NT), intensified (INT), and recovery training (REC). *Statistically different from INT ($P < .05$).
concentrations between NT, INT, and REC. Previous studies that 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.\(^2\)\(^4\) Moreover, whereas Seifert et al.\(^2\)\(^5\) reported an increased release of BDNF at rest after 3 months of endurance training in previously sedentary overweight males, Schiffer et al.\(^2\)\(^2\) reported no significant increase in baseline plasma BDNF concentrations after strength or endurance training in moderately trained individuals. Taken together, these past\(^2\)\(^4\)–\(^2\)\(^6\) 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 Knaepen et al.), we acknowledge that this interpretation must be limited 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.\(^2\)\(^7\) Although the mechanisms responsible for this down-regulation of BDNF are not fully understood, elevated levels of adrenal glucocorticoids appear to play a significant role.\(^2\)\(^7\) Yau et al.\(^1\)\(^0\) 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.\(^2\)\(^8\) 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.\(^2\)\(^9\) Notably, performing exercise before a stressful event has been shown to counteract this down-regulation.\(^3\)\(^0\) Moreover, Goekint et al.\(^3\)\(^1\) 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\(^1\)\(^1\)). A recent review\(^3\)\(^2\) articulates how glucocorticoids can either increase or decrease adult neurogenesis depending on the topology 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 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 current 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 (impaired time-trial performance, psychological mood state) suggest that the training stress (increased duration and intensity of training) imposed by the current study design was severe, it could be argued that this training stress was predictable, particularly in the cohort of well-trained cyclists recruited. It is possible that the predictability of intensified training resulted in different central responses, while most animal studies instead used 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.\(^3\)\(^3\)–\(^3\)\(^5\) For example, Halson et al.\(^3\)\(^3\) reported a 28% increase in total mood disturbance after 2 weeks of intensified training. In the current study, mood disturbance increased by 32% after only 1 week of intensified training and was restored after 1 week of recovery. Therefore, monitoring mood is a simple and effective method for preventing NFOR.\(^3\)\(^6\)

Variations in fatigue and vigor are normally observed during training camps with an overall decrease in the energy index, leading to a negative psychological state.\(^3\)\(^7\) In the current 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.\(^3\)\(^7\),\(^3\)\(^8\) 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\(^3\)\(^9\) 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 nonexecutive functions.\(^3\)\(^8\),\(^4\)\(^0\) This observation could be caused by a decrease in cerebral oxygenation,\(^3\)\(^8\) an up-regulation of brain neurotransmitters, and a deregulation of BDNF,\(^3\)\(^6\) even without symptoms of NFOR. However, none of the previously 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.\(^1\)\(^2\) Each of these components is 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 current 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 3 mood subscales (vigor, 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 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.\(^3\)\(^6\) 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 that this conclusion is based on a small sample size that may affect statistical power, assumes that 1 week of intensified training caused similar levels of FOR in all athletes, and is based on a study design that may not mimic real-world practice (ie, 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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References


