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High-intensity interval training evokes larger serum BDNF levels compared with intense continuous exercise

Cinthia Maria Saucedo Marquez,[1] Bart Vanaudenaerde,[4] Thierry Troosters,[3,4] and Nicole Wenderoth[1,2]

[1]*KU Leuven, Department of Kinesiology and Rehabilitation Sciences, Research Center for Movement Control and Neuroplasticity, Heverlee, Belgium;* [2]*ETH Zurich, Department of Health Sciences and Technology, Neural Control of Movement, Zurich, Switzerland;* [3]*KU Leuven, Department of Rehabilitation Sciences and Respiratory Division, University Hospital, Leuven, Belgium; and* [4]*KU Leuven, Pneumology Division, University Hospital, Leuven, Belgium*

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Saucedo Marquez CM, Vanaudenaerde B, Troosters T, Wenderoth N. High-intensity interval training evokes larger serum BDNF levels compared with intense continuous exercise. *J Appl Physiol* 119: 1363–1373, 2015. First published October 15, 2015; doi:10.1152/japplphysiol.00126.2015.—Exercise can have a positive effect on the brain by activating brain-derived neurotrophic factor (BDNF)-related processes. In healthy humans there appears to be a linear relationship between exercise intensity and the positive short-term effect of acute exercise on BDNF levels (i.e., the highest BDNF levels are reported after high-intensity exercise protocols). Here we performed two experiments to test the effectiveness of two high-intensity exercise protocols, both known to improve cardiovascular health, to determine whether they have a similar efficacy in affecting BDNF levels. Participants performed a continuous exercise (CON) protocol at 70% of maximal work rate and a high-intensity interval-training (HIT) protocol at 90% of maximal work rate for periods of 1 min alternating with 1 min of rest (both protocols lasted 20 min). We observed similar BDNF kinetics in both protocols, with maximal BDNF concentrations being reached toward the end of training (*experiment 1*). We then showed that both exercise protocols significantly increase BDNF levels compared with a rest condition (CON $P = 0.04$; HIT $P < 0.001$), with HIT reaching higher BDNF levels than CON ($P = 0.035$) (*experiment 2*). These results suggest that shorter bouts of high intensity exercise are slightly more effective than continuous high-intensity exercise for elevating serum BDNF. Additionally, 73% of the participants preferred the HIT protocol ($P = 0.02$). Therefore, we suggest that the HIT protocol might represent an effective and preferred intervention for elevating BDNF levels and potentially promoting brain health.

acute-exercise; BDNF; high-intensity interval training; neuroplasticity; brain health

IT IS WELL KNOWN THAT PHYSICAL exercise improves cardiovascular health (29, 33, 37). Additionally, recent evidence shows that exercise can have a positive effect on brain health by activating specific processes that promote synaptic plasticity, growth, and the survival of neurons (12, 44, 85, 86, 89).

One mechanism through which exercise might facilitate brain health is by increasing the expression of brain-derived neurotrophic factor (BDNF), a natural protein found mainly in the brain (51). BDNF is a neurotrophin that regulates crucial functions of the central nervous system such as neurogenesis, neuroprotection, neuroregeneration, cell survival, and the development and maintenance of synaptic connections between neurons (34, 87, 89). In rodent models it has been repeatedly demonstrated that physical exercise elevates BDNF mRNA expression and protein concentration in hippocampus, striatum, and various cortical regions (13, 45, 51, 58, 88, 95), even if only one bout of exercise is performed (17, 26, 37, 51). Remarkably, these BDNF increases in the brain are positively associated with improved cognition, especially in learning and memory tasks that are hippocampus-dependent such as the Morris water maze task (26, 89) or the object recognition task (49).

Also, in humans it is believed that acute physical exercise increases BDNF levels in the brain, providing an interesting paradigm for enhancing cognitive functions in healthy individuals and patients (43, 62, 63). Unlike in animals, this conclusion is based on indirect evidence derived from either measuring BDNF levels in the blood or from data in cohorts that differed according to their BDNF genotype (8, 52). The current hypothesis is that BDNF is primarily produced in the brain, some of which crosses the blood-brain barrier (53) and travels to the periphery where it can be measured in plasma and serum (36). BDNF is stored in platelets and is released during clotting processes, which leads to concentrations of serum BDNF ($[BDNF]_{ser}$) that are approximately 200-fold higher relative to the concentration of plasma BDNF ($[BDNF]_{pla}$) (17, 31). Other potential loci of BDNF production are skeletal muscle cells (38, 50), but it is currently believed that BDNF cannot leave the cell (58), implying that BDNF produced in muscle does not contribute directly to BDNF levels measured in serum or plasma.

Several studies found that a single bout of exercise increases either $[BDNF]_{pla}$ (58, 72, 100), $[BDNF]_{ser}$ (15, 22, 64, 79), or both (9, 48). More specifically, in healthy individuals the positive effect of exercise on BDNF levels seems to be intensity dependent (15, 31, 96). This has important implications when exercise is used to positively influence BDNF expression to facilitate neural plasticity and cognition in patients and raises the question as to which type of training might be optimal for influencing BDNF synthesis. Two exercise protocols that are commonly used in clinical settings are continuous training (CON) performed at moderate to high intensities or high-intensity interval training (HIT). Both are equally effective at improving exercise performance and reducing the risk of cardiovascular disease (18, 27). Importantly, use of the HIT protocol over other high-intensity exercise protocols is preferred in patient populations due to its higher efficacy, tolerability, and adherence without compromising patient safety (19, 24, 94). However, whether or not HIT and CON protocols are also equally effective at increasing BDNF levels and poten-

Address for reprint requests and other correspondence: N. Wenderoth, ETH Zürich, Dept. of Health Sciences and Technology, Y36 M4, Winterhurerstrasse 190, Zürich, Switzerland (e-mail: nicole.wenderoth@hest.ethz.ch).

tially facilitating brain health to a similar extent is currently unknown.

Here we investigated the kinetics of $[BDNF]_{ser}$ by measuring it before, during, and after participants performed either a CON or HIT exercise protocol (*experiment 1*). We then tested and in a larger cohort whether the maximal increase in $[BDNF]_{ser}$ between these two protocols is different (*experiment 2*). Based on previous research (15, 23, 31, 64, 96) we hypothesize that both exercise protocols will elevate $[BDNF]_{ser}$ concentrations compared with a resting control condition. Furthermore, we assessed exercise-induced changes in lactate, cortisol, and range of perceived exhaustion using Borg CR-10 scale ratings for dyspnea and leg fatigue. We also asked participants which protocol they preferred to quantify potential adherence to an exercise routine. The results of this study might have important clinical implications because exercise is increasingly considered as an adjuvant therapy beneficial not just for cardiovascular health, but also for brain health in a large variety of patient populations.

MATERIALS AND METHODS

Participants

Eight active men (age 28 ± 5 yr) were recruited for *experiment 1* and 21 men (age 27 ± 4 yr) were recruited for *experiment 2*. All participants were required to complete all exercise sessions.

This study was approved by the Ethical Committee for Biomedical Research at the KU Leuven in agreement with the Code of Ethics of the World Medical Association Declaration of Helsinki (60). Participants were screened with the Physical Activity Readiness Questionnaire for any cardiac or respiratory problems that might have placed them at risk by participating in the study. Healthy men who were physically active for 3 or more days/wk with no contraindications to exercise, and who were currently not taking drugs, tobacco, or any other medications were included in this study. To minimize confounding effects, participants were asked to avoid consumption of alcohol or caffeine 24 h prior to testing. They were also instructed not to exercise on the same day of the experiment. At the beginning of every session, sleep hours, quality of sleep, smoking, alcohol and caffeine consumption, and physical activity performed before the experiment were documented. Written informed consent was obtained from all participants prior to participation.

Overall Experimental Design

We performed two separate experiments. In *experiment 1* we sampled $[BDNF]_{ser}$ multiple times during the exercise sessions to investigate the kinetics of $[BDNF]_{ser}$ in response to two different high-intensity exercise protocols. In particular, we were interested in testing whether $[BDNF]_{ser}$ might reach a plateau even earlier than after 20 min. On the basis of findings from *experiment 1* we designed an optimized protocol for *experiment 2* with the aim of accurately measuring the magnitude of BDNF changes induced by CON vs. HIT while limiting the discomfort of the participants.

Even though both experiments used a crossover design to test the effect of the CON and HIT exercise protocols on $[BDNF]_{ser}$ in healthy active men, some aspects of the overall methodology differed between the two experiments (i.e., number of sessions and blood measurements; for more details see Fig. 1, *A* and *C*).

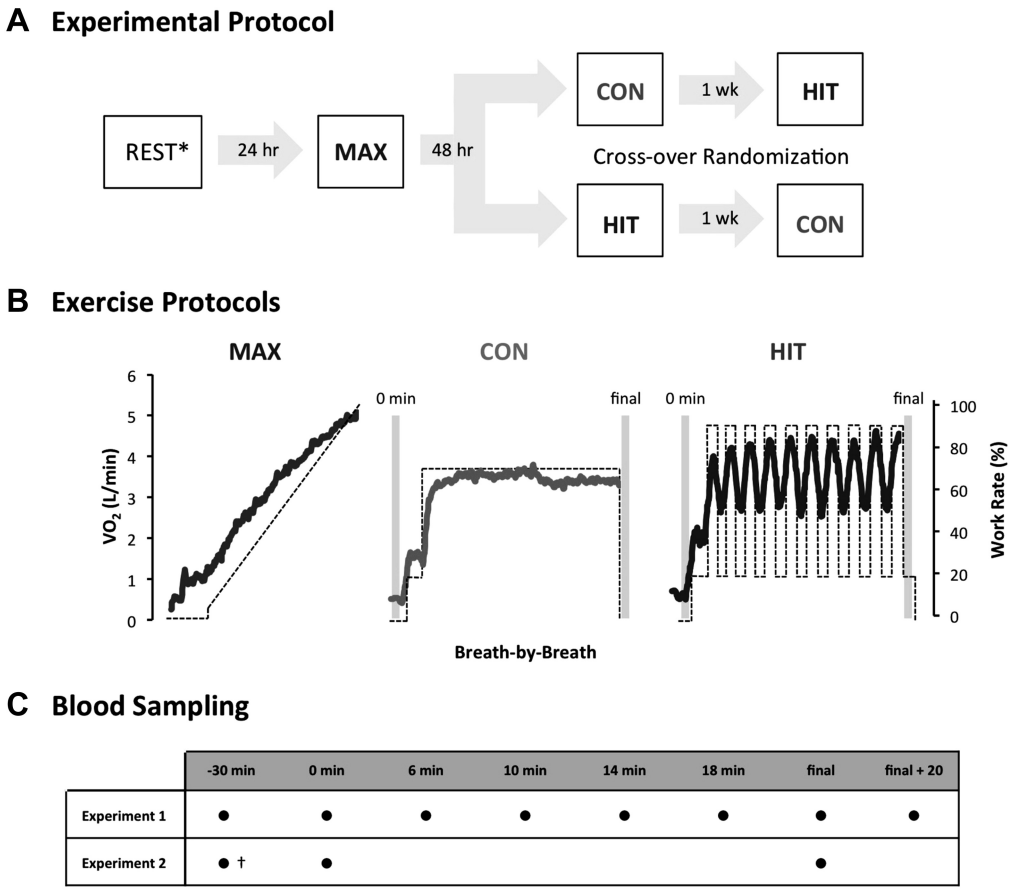

Fig. 1. Overall experimental design. *A*: experimental protocol. *The REST session was carried out on a separate day only in *experiment 1*. *B*: exemplary data of a representative participant showing breath by breath V_{O_2} values (see left axis for scale) during the three test conditions (maximal exercise text, MAX; continuous exercise, CON; high-intensity interval training, HIT). Work rate (see right axis for scale) for each exercise protocol is indicated by the dashed line. *C*: blood sampling. †This sample was taken in half of the participants during the CON protocol and in the other half during the HIT protocol.

All participants came to the laboratory on four (*experiment 1*) or three (*experiment 2*) different days. The first active session consisted of a maximal exercise test (MAX) to determine maximal oxygen consumption ($\dot{V}O_{2\,max}$) and maximal work rate output. Afterward, participants were randomly assigned to perform either the CON protocol followed by the HIT protocol or vice versa (the order counterbalanced across participants) as shown in Fig. 1*A*.

At the beginning of each session, participants were fully informed about the entire exercise test protocol and the upcoming procedures. All exercise tests were performed on a stationary cycle ergometer (Ergometrics 900; Ergoline, Bitz, Germany). Work rate, oxygen consumption ($\dot{V}O_2$), and heart rate (HR) were monitored breath by breath (Vmax series; SensorMedics, Anaheim, CA). For safety, oxygen saturation (Datex-Ohmeda), blood pressure, and electrocardiographic recordings were continuously monitored (Ergoline). Borg CR-10 scale ratings for dyspnea and leg fatigue were assessed before and immediately after all exercise tests to measure perceived exhaustion. At the end of the study we asked participants to select the protocol they preferred to estimate the possible adherence to an exercise routine. Each session lasted ~1 h.

Maximal Exercise Test

The MAX test started with a rest period of 2 min (no pedaling) followed by a warm-up period of 3 min (unloaded pedaling). Next, the resistance of the cycle ergometer started at 20 W and was increased by 10 W every minute until exhaustion. Participants were instructed to cycle at a constant speed (measured in crank revolutions per minute) throughout the entire test. Criteria to stop the MAX test included chest pain, dizziness, headache, nausea, or inability to maintain a constant speed throughout the test.

HIT and CON Exercise Protocols

After an initial resting period of 30 min, participants mounted the cycle ergometer. Both protocols started with a rest period of 2 min (no pedaling) and a warm-up period of 3 min at 60 W (corresponding to 20.7 ± 3.5% of the maximal work rate). For the HIT protocol participants performed intervals of 1 min at 90% of maximal work load, alternating with 1 min rest at 60 W for a total duration of 20 min. For the CON protocol the resistance was set at 70% of maximal work rate and participants cycled continuously at the same intensity for 20 min.

Even though both exercise protocols were considered intense, the HIT protocol was designed to reach higher $\dot{V}O_2$ levels in the short-interval training bouts than the CON protocol as illustrated in Fig. 1*B* (*right*, *y*-axis).

Blood Sampling

Experiment 1. A venous catheter was placed in the right forearm at the beginning of both exercise sessions. Immediately after placement of the catheter a blood sample was collected (i.e., −30 min). After a resting period of 30 min, participants were seated on the cycle ergometer while blood samples were collected at the beginning (0 min), during exercise (at 6, 10, 14, and 18 min), and at the end of exercise (*final*). This final measurement was collected immediately after the last minute of exercise, at 20 min for the CON protocol and at 19 min for the HIT protocol (immediately after the last sprint). Additionally, we collected a blood sample 20 min after completion of exercise (*final* + 20) (Fig. 1*C*). $[BDNF]_{ser}$ was determined for the samples taken at −30, 0, 6, 10, 14, 18 min; *final*; and *final* + 20 as shown in Fig. 2. Lactate and cortisol measurements were determined for 0 min and final samples. We measured the REST condition on a separate day (*experiment 1* only), which consisted of a nonactive session of 20 min sitting on the cycle ergometer without pedaling.

Experiment 2. We decreased the number of samples to reduce distress to participants and obtained venous blood only at the beginning (0 min) and at the end (*final*) of the CON and HIT exercise protocols (Fig. 1*C*). Serum BDNF, lactate, and cortisol concentrations were measured in all samples taken at 0 min and *final*. These time points were chosen on the basis of *experiment 1* because we wanted to estimate the maximal $[BDNF]_{ser}$ levels that could be obtained with each protocol. To determine REST values, a third blood sample was taken 30 min before the exercise protocol started (−30 min). In half of the participants, resting $[BDNF]_{ser}$ was determined before the CON session and in the other half before the HIT session.

Coagulant-free serum separation tubes (SSTTube, 5 ml; BD Vacutainer) were used to collect blood samples. SST tubes were left to clot at room temperature for 30 min after collection. They were then centrifuged (Capricorn) for 15 min at 4,800 *g*. The serum was separated and stored in Eppendorf tubes at −20°C for a week, after which they were stored at −80°C until analysis.

Biochemical Analysis

Samples to determine $[BDNF]_{ser}$ were analyzed using the Quantikine ELISA kit from R&D Systems (Minneapolis, MN). The minimum sensitivity of BDNF in this kit is <20 pg/ml. The analysis was conducted according to manufacturer's guidelines. All samples were diluted 20-fold with a calibrator diluent prior to assay.

Cortisol and lactate concentrations were analyzed by the central laboratory of the University Hospital Leuven using a radioimmunoassay (IM1841 Immunotech cortisol assay kit; Beckman Coulter) for cortisol and ADVIA 1650 (Bayer, Tarrytown, NY) for lactate.

Statistical Analysis

All statistical analyses were performed using STATISTICA 10 (StatSoft). The alpha level was set to 0.05, and data are shown as means ± SE.

Values that were 3 SD below or above the mean were considered as outliers and were removed from further analysis. Following these criteria, two participants were removed from the change in lactate analysis, and one from the BDNF analysis that compared the percent change in $[BDNF]_{ser}$ between exercise protocols.

Due to the small sample size in *experiment 1* we analyzed the data using nonparametric statistics. First, we tested the effect of time (−30, 0, 6, 10, 14, 18 min; *final*; and *final* + 20) on $[BDNF]_{ser}$ separately for the CON and the HIT protocols with a Friedman's two-way ANOVA by ranks. Post hoc Wilcoxon matched-pairs signed-rank tests were performed where necessary. Separate Wilcoxon matched-pairs signed-rank tests were calculated for each time point comparing HIT and CON $[BDNF]_{ser}$ values to test whether the $[BDNF]_{ser}$ kinetics differed between the two exercise protocols.

For *experiment 2*, we compared how $[BDNF]_{ser}$ changed in response to CON vs. HIT protocols. Analyzing relative change in BDNF ($\%\Delta\ [BDNF]_{ser}$) (i.e., expressing values obtained after exercise relative to baseline measurements prior to exercise) is a commonly reported measurement mainly because there is high variability in absolute BDNF concentrations across studies due to methodological differences in BDNF analyses (31). In our data, however, we observed high variability among baseline BDNF levels (ranging from 3,256 to 14,263 pg/ml with a mean of 8,019 ± 268 pg/ml) of which only 21% was explained by individual differences (Pearson's correlations $r = 0.46$, $P = 0.02$). To reduce the risk that our data were biased due to baseline variability between sessions, we report three different approaches to test $[BDNF]_{ser}$ changes after exercise. First, we compared absolute $[BDNF]_{ser}$ after 20 min (*final*) between conditions (REST/CON/HIT). $[BDNF]_{ser}$ data were normally distributed, thus a repeated-measures ANOVA was used. Post hoc tests were conducted using Tukey's honestly significant difference test to further analyze a significant main effect. Second, we calculated a Pearson's correlation between the final $[BDNF]_{ser}$ values of the CON and HIT protocols. Third, we determined one common $[BDNF]_{ser}$ baseline by averaging an individual's rest values determined at 0 min across

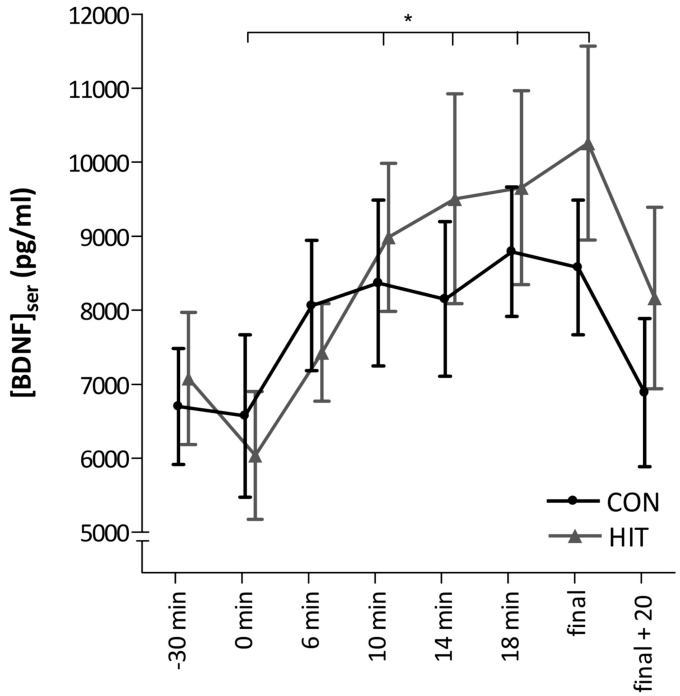

Fig. 2. Brain-derived neurotrophic factor (BDNF) kinetics during rest, exercise, and recovery for both exercise protocols. Bars indicate SEM. *$P < 0.05$.

conditions, and calculated the %Δ $[BDNF]_{ser}$ for each exercise protocol relative to this common baseline. Data for %Δ $[BDNF]_{ser}$ were normally distributed and subjected to a paired *t*-test to compare exercise protocols.

Paired *t*-tests were also used to compare the maximal values of the following exercise parameters between exercise protocols: work rate, $\dot{V}O_2$, HR, lactate, and cortisol. We also investigated whether any of these exercise parameters would be a significant predictor of $[BDNF]_{ser}$ changes in response to exercise. Pearson's correlations were calculated between maximal values of work rate, $\dot{V}O_2$, HR, lactate (final), cortisol (final), and final $[BDNF]_{ser}$. Borg CR-10 scale ratings for dyspnea in the CON protocol and leg fatigue in the HIT protocol deviated from normality (Shapiro-Wilks test, $P \leq 0.04$). Thus Wilcoxon matched-pairs signed-rank tests were used to compare these parameters between CON and HIT protocols, and the extent to which they were correlated with final $[BDNF]_{ser}$ was calculated with Spearman correlation coefficients.

All exercise parameters were entered as regressors into a multiple stepwise regression analysis to test the cumulative effect of exercise measurements in final $[BDNF]_{ser}$. All correlation analyses and the stepwise regression model were calculated separately for each protocol.

Finally, goodness-of-fit χ^2 tests were performed to determine whether the two exercise protocols were equally preferred, and to determine whether the proportion of participants who showed higher final $[BDNF]_{ser}$ levels in each protocol was equal.

RESULTS

Participants

In *experiment 1*, one individual dropped out due to needle anxiety. In *experiment 2*, two individuals were unable to complete both sessions. As a result, the analysis of *experiment 2* consisted of 26 participants (including 7 from *experiment 1*). Their age ranged from 22 to 35 yr (28 ± 1 yr), with a mean body mass index of 22.5 ± 1 kg/m^2 and a mean $\dot{V}O_{2\,max}$ of 56.6 ± 2 ml·kg^{-1}·min^{-1}, indicating an overall fitness level that was above average based on their gender and age.

Experiment 1

$[BDNF]_{ser}$ kinetics in response to exercise are shown in Fig. 2, which revealed that $[BDNF]_{ser}$ increased gradually during exercise in both protocols, reaching maximum concentrations toward the end of exercise. After the exercise was finished, $[BDNF]_{ser}$ returned quickly to baseline such that the postexercise measurement at *final* + 20 was not significantly different from that at rest levels. During the HIT protocol there were statistically significant increase in $[BDNF]_{ser}$ over time ($\chi^2_{7,\,n\,=\,7} = 26.4$, $P < 0.001$). Wilcoxon matched-pairs signed-rank tests showed significant differences between 0, 10, 14, and 18 min, and *final* ($P \leq 0.02$).

The CON protocol evoked a similar increase in $[BDNF]_{ser}$ even though the response was somewhat weaker than for the HIT protocol. Statistics yielded only a trend toward a significant increase in $[BDNF]_{ser}$ levels across time for the CON protocol ($\chi^2_{\,7,\,n\,=\,7} = 12.5$, $P = 0.08$). Wilcoxon matched-pairs signed-rank tests conducted between CON and HIT at each time point revealed no significant differences between the two protocols ($Z \geq -1.521$, $P \geq 0.13$).

Experiment 2

Table 1 summarizes the maximal values of the exercise parameters obtained throughout each exercise protocol (CON vs. HIT), which in most cases, corresponded to the values recorded during the final measurements. Both protocols show values for lactate of 8 mmol/l and above confirming that both exercise protocols were of high intensity.

The effect of REST, CON, and HIT protocols on final $[BDNF]_{ser}$ values is illustrated in Fig. 3. A repeated-measures ANOVA revealed a significant main effect of condition (REST 7,958 ± 448, CON 9,806 ± 581, HIT 11,049 ± 588; $F_{2,50} = 20.56$, $P < 0.001$). Tukey's honestly significant difference post hoc test revealed significant differences between CON and

Table 1. *Maximal values of exercise parameters reached in each exercise protocol*

	CON		HIT		
	Mean	SD	Mean	SD	*P*
Work rate, W	198	28	269	47	<0.001*
$\dot{V}O_2$, kg·ml^{-1}·min^{-1}	50.5	8.4	51.1	9.0	0.71*
HR, bpm	175.9	10.7	176.3	9.2	0.80*
Lactate, mM	8.4	3.5	11.1	4.1	<0.001*
Cortisol, μg/dl	22.6	7.7	20.7	7.1	0.21†
	Median	IQR	Median	IQR	
Borg CR-10 scale for dyspnea	7.0	2.0	7.0	3.0	0.28†
Borg CR-10 scale for leg fatigue	6.0	2.0	7.0	4.0	0.38†

CON, continuous exercise; HIT, high-intensity interval training; HR, heart rate; bpm, beats per minute; IQR, interquartile range; SD, standard deviation. *P* refers to the comparison between CON and HIT. Most measurements were numerically higher for the HIT protocol, however, only differences in lactate and work rate reached statistical significance. The difference in work rate was expected given the design of the exercise protocols. *Parametric paired *t*-tests. †Nonparametric Wilcoxon signed-rank tests).

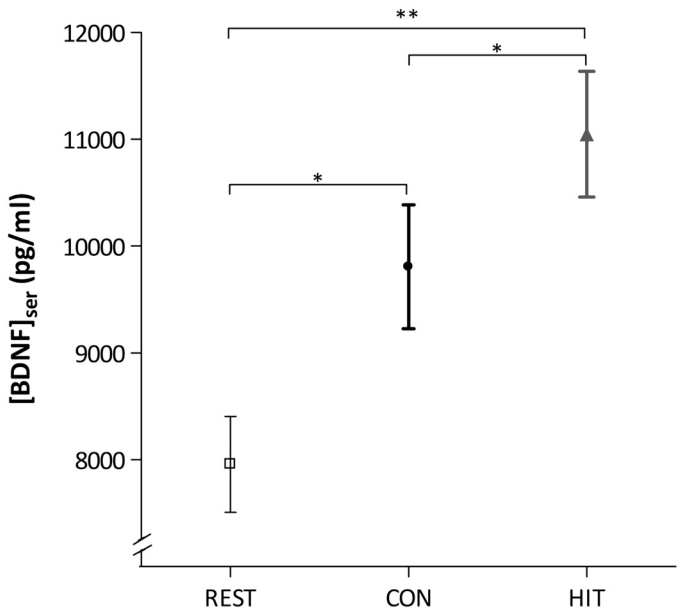

Fig. 3. BDNF serum concentration levels at *final* for the three test conditions. Both CON and HIT protocols showed higher $[BDNF]_{ser}$ levels compared with the REST condition, with the HIT protocol reaching higher $[BDNF]_{ser}$ levels than the CON protocol. Bars indicate SEM. $^{*}P < 0.05$, $^{**}P < 0.001$.

REST ($P = 0.001$), HIT, and REST ($P < 0.001$) and between CON and HIT ($P = 0.035$).

Also, we found that final $[BDNF]_{ser}$ was significantly correlated between CON and HIT protocols ($r = 0.62$, $P = 0.001$; Fig. 4). Visual inspection of the data revealed that 18 of 26 participants reached higher $[BDNF]_{ser}$ concentrations after performing the HIT protocol (black squares above the diagonal in Fig. 4). Furthermore, this proportion was not equally distributed in our sample ($\chi^2_{1,\, n\,=\,26} = 3.85$, $P = 0.05$).

Figure 5 shows $\%\Delta$ $[BDNF]_{ser}$ data (relative to a common baseline). A paired *t*-test revealed a significantly larger increase for the HIT protocol than for the CON protocol (CON 23.77 ± 6.37 vs. HIT 37.72 ± 4.78; $t_{24} = -2.16$, $P = 0.04$).

Comparisons of Exercise Parameters for CON and HIT Protocols

Comparing the exercise parameters between both exercise protocols revealed significant differences in work rate ($t_{25} = -7.98$, $P < 0.001$) and lactate ($t_{25} = -4.25$, $P < 0.001$), which were both higher for HIT than for CON. None of the other parameters reached significance (see Table 1).

Figure 6 shows changes in lactate and cortisol concentrations confirming the higher lactate increases for the HIT than the CON protocol (CON 6.8 ± 0.77 vs. HIT 9.5 ± 0.83; $t_{23} = -4.04$, $P = 0.001$), whereas the increase in cortisol tended to be numerically higher in CON than HIT even though significance was not reached (CON 5.3 ± 1.2 vs. HIT 3.7 ± 0.82; $t_{25} = 1.29$, $P = 0.21$).

Parameters Predicting Change in $[BDNF]_{ser}$ After Exercise

Correlations between exercise parameters and final $[BDNF]_{ser}$ levels were not significant for the CON protocol ($r \leq 0.12$, $P \geq 0.24$). The stepwise regression model did not identify a statistically significant predictor of $[BDNF]_{ser}$ measured in the last minute of exercise (*final*) ($F_{8,11} = 0.39$, $P = 0.91$, $r^2 = 0.35$).

For the HIT protocol, only the correlation between final $[BDNF]_{ser}$ levels and Borg CR-10 scale ratings for leg fatigue reached significance ($r = 0.446$, $P = 0.033$; Fig. 7). All other parameters exhibited insignificant correlations ($r \leq 0.16$, $P \geq 0.096$). The stepwise multiple regression revealed no other significant predictors ($F_{1,21} = 5.23$, $P = 0.033$, $r^2 = 0.199$).

Finally, preference for the two protocols was not equally distributed in our sample, with 19 of 26 participants preferring the HIT protocol ($\chi^2_{1,\, n\,=\,26} = 5.538$, $P = 0.02$).

DISCUSSION

In this study we investigated the effectiveness of two types of intense exercise training to increase $[BDNF]_{ser}$ concentrations in young active men. Both protocols resulted in significantly increased $[BDNF]_{ser}$ levels after exercise compared with rest. However, this increase was slightly higher for the HIT protocol, suggesting that HIT exercise could be an effective strategy for promoting brain health via BDNF-related mechanisms, which makes it an interesting intervention for clinical applications in patients with neurological or cognitive challenges.

Effect of Both Exercise Protocols on $[BDNF]_{ser}$

We observed that $[BDNF]_{ser}$ levels increase relative to resting levels when participants perform intensive exercise, which is consistent with previous work in humans (21-23, 31, 64, 70, 71, 79). Investigating the underlying kinetics revealed that $[BDNF]_{ser}$ rose gradually during the training session, with maximum concentrations being measured toward the end of

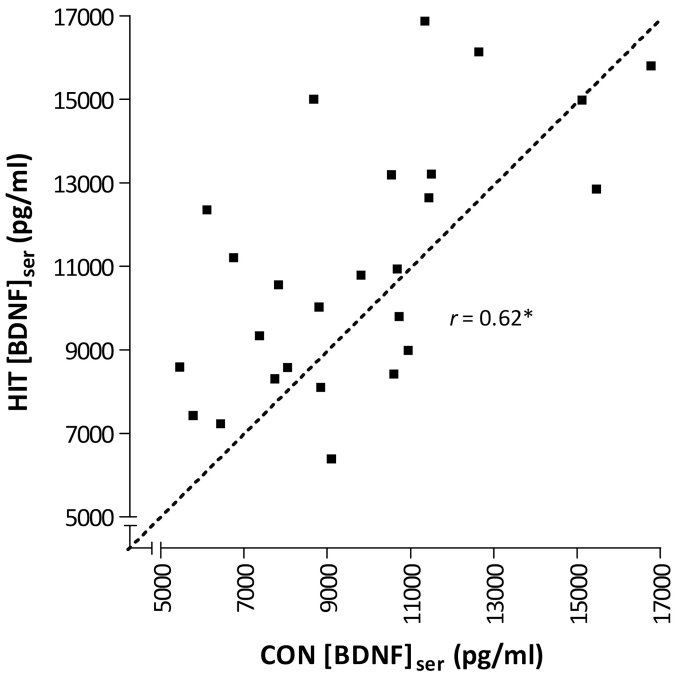

Fig. 4. Correlation between BDNF concentration levels from CON and HIT protocols immediately after exercise (*final*). This figure supports the validity of our within-participants values for $[BDNF]_{ser}$ by showing a linear relationship. Note that it has also shifted toward HIT, indicating higher values were reached during this exercise protocol. $^{*}P < 0.05$.

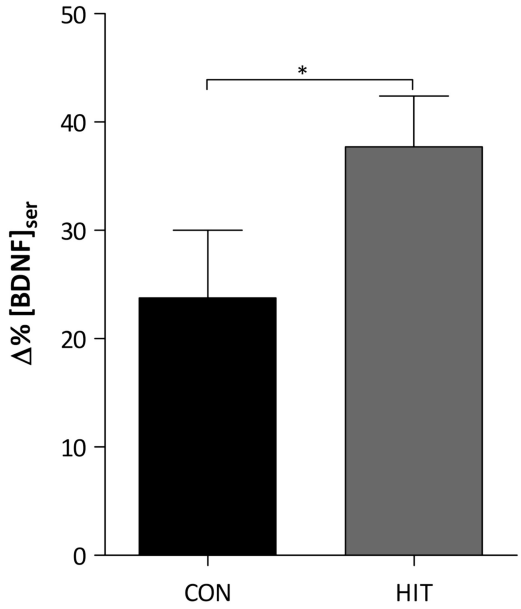

Fig. 5. Difference between CON and HIT expressed as percentage change. Bars indicate SEM. *$P < 0.05$.

training. After the exercise was finished $[BDNF]_{ser}$ returned quickly to baseline levels such that the postexercise measurement at *final* + 20 was not significantly different from levels at 0 min. These results are very much in line with results reported by Schmidt-Kassow et al. (70), who showed that BDNF levels gradually increased over time, reaching maximal levels after 20 min of exercise and returned to baseline values after 10 min of recovery. Our data extend these previous results because we defined exercise intensity levels on the basis of $\dot{V}O_2$ measurements, whereas Schmidt-Kassow et al. (70) used a subjective measurement of intensity. Moreover, we show that $[BDNF]_{ser}$ kinetics are similar for both exercise training protocols, whereas they tested CON exercise protocols only. Our data suggest that HIT exercise of at least 20 min is needed to increase $[BDNF]_{ser}$ by 37.7%. In literature, the $\%\Delta$ $[BDNF]_{ser}$ concentrations after performing exercise vary from 11.7 to 410% (in both healthy and clinical populations) (31). The lowest increase in $[BDNF]_{ser}$ that appeared to influence behavior was reported by Winter et al. (96), who found that a 12% increase in $[BDNF]_{ser}$ induced a 20% increase in novel vocabulary learning. Others showed that a 10–30% increase in $[BDNF]_{ser}$ improved stroop task (15) and face-name recognition task performance (23). Hence, the relative change in $[BDNF]_{ser}$ observed in the present study falls within the values that have previously shown improvements in cognition.

Several lines of evidence suggest that BDNF concentration measured in blood serum is reflective of BDNF expression in the brain. Klein et al. (30) demonstrated in rats that BDNF levels in brain tissue correlate with BDNF concentrations in the blood, and Sartorius et al. (67) also reported a positive correlation between $[BDNF]_{ser}$ and brain tissue BDNF after electroconvulsive treatment in rats. More importantly, in humans it has been confirmed that most of the BDNF transported in the blood and measured in the periphery during exercise, and during rest comes from the brain (58), which was demonstrated by measuring changes in BDNF arterial-to-internal jugular venous differences.

Potential Mechanism Mediating the Higher $[BDNF]_{ser}$ Response to the HIT Protocol

Our data showed that both protocols were able to increase $[BDNF]_{ser}$ compared with rest, but the HIT protocol induced slightly larger effects than the CON protocol. The mechanism leading to this result is currently unclear. Nevertheless, possible BDNF modulating factors such as lactate (15, 69), cortisol (74), and intensity (31) have been previously proposed.

Several studies have assessed the relationship between lactate and BDNF, but results are not consistent. Schiffer et al. (69) discovered that a sodium-lactate infusion at rest was able to induce an increase in BDNF concentrations in the blood of human volunteers. However, the authors pointed out that a sodium-lactate infusion may differ from lactic-acid infusion in causing alkalosis rather than acidosis in the blood. The increase in lactate during a lactate clamp procedure as used by Schiffer et al. (69) at rest might therefore differ markedly from the lactate increase during high intensity exercise. Up until now, Ferris et al. (15) published the only study in which a positive correlation between BDNF and lactate was presented, whereas no such correlation was found in other studies (64). In our study we did not find any significant correlation between $[BDNF]_{ser}$ and lactate for either exercise protocol, although we observed greater lactate increases after the HIT protocol than after the CON protocol at the group level.

Cortisol is commonly known as a stress hormone (66). It has been shown that chronically elevated cortisol levels inhibit neurogenesis and neuronal plasticity. More specifically, exposure to corticosterone decreases BDNF expression in the brain of rats, which suggests a negative relationship between cortisol and BDNF (74). However, in humans there is no evidence of

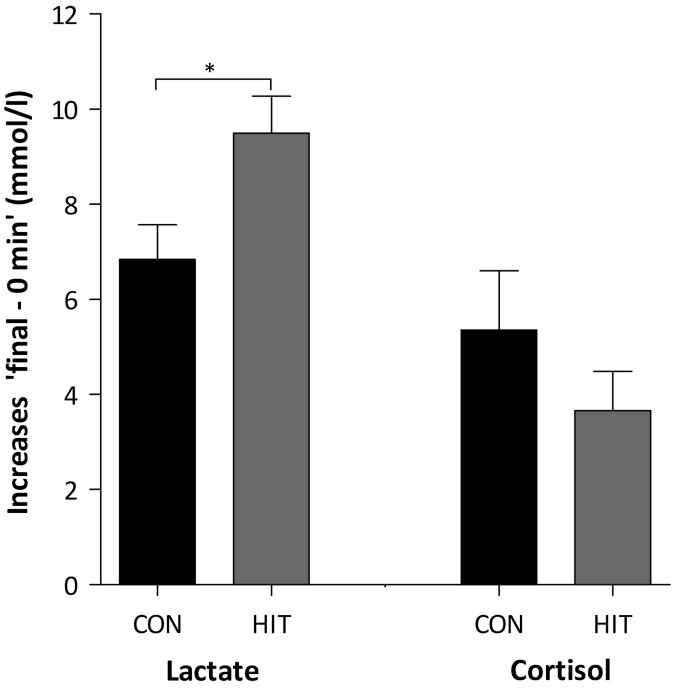

Fig. 6. Lactate and cortisol increases from *0 min* to *final*. Average exercise-induced changes in lactate and cortisol when concentrations were compared from *0 min* to *final* for the HIT and CON exercise protocols. Error bars indicate SEM. *$P < 0.05$.

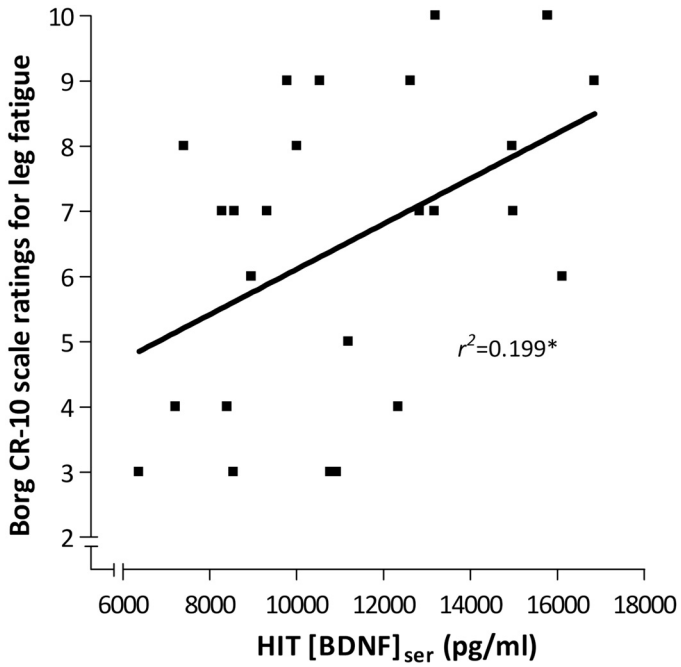

Fig. 7. Relationship between Borg CR-10 scale ratings for leg fatigue and absolute $[BDNF]_{ser}$ levels at the end of the HIT exercise protocol (*final*). *$P < 0.05$.

such a correlation between cortisol and BDNF levels, and our data do not support a simple linear relationship between $[BDNF]_{ser}$ and cortisol concentrations in the blood. Several studies have investigated the relationship between cortisol response and exercise intensity. Rojas and colleagues (64) observed no change in cortisol levels after 10 min of moderate exercise, whereas they were significantly elevated 10 and 15 min after an incremental exercise test. This finding is further supported by Van Bruggen et al. (84) who found that serum cortisol levels increased significantly only in response to high intensity exercise. Moreover, Tauler et al. (80) demonstrated that the magnitude of the increase in cortisol depends on the intensity and duration of physical activity. In our data, both exercise protocols significantly increased cortisol levels, supporting again that both protocols were physically demanding. Nonetheless, this difference did not reach significance and there was no correlation between the cortisol concentration and $[BDNF]_{ser}$ for either exercise protocol.

In exercise testing, Borg scale ratings have been widely used to assess exercise intensity (14, 46). In our data, numerically higher Borg CR-10 scale ratings for leg fatigue were reported during the HIT exercise condition, but the median difference did not reach significance. However, from all possible mediating factors that we tested, Borg CR-10 scale rating for leg fatigue was the only variable exhibiting a significant correlation such that 19.9% of the variance in $[BDNF]_{ser}$ measured at the end of the HIT exercise protocol could be explained by Borg CR-10 scale ratings for leg fatigue. It is tempting to speculate that skeletal muscle cells might be involved in mediating changes in $[BDNF]_{ser}$. It has been shown that muscle contractions elevate BDNF concentration in the muscle (38), however, BDNF produced in muscle cells does not appear to be released into circulation, indicating that elevated $[BDNF]_{ser}$ in the periphery must come from other sources (38). Very recently, Wrann et al. (98) proposed a novel biochemical pathway linking an exercise-induced secreted factor from skeletal muscle to BDNF gene expression in the brain. They suggested a model in which activation of peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α is induced by exercise in skeletal muscle cells, and that when PGC-1α binds with the transcription factor Errα it could activate FNDC5 gene expression, which is a positive regulator of BDNF levels in the brain, especially in the hippocampus. On the basis of this model we speculate that skeletal muscle contractions during high-intensity exercise might be a possible trigger of this biochemical pathway to induce elevated BDNF levels in the brain.

An alternative source of $[BDNF]_{ser}$ is platelets, which have the ability to store BDNF and release it upon agonist stimulation depending on the specific need of BDNF in certain tissues (17). Although it remains unknown how exercise influences the platelets, one potential use of BDNF stored in platelets is thought to be in the repair of exercise-induced muscle damage (48). It is unclear, however, the extent to which physical fiber disruptions may be associated with stimuli that lead to BDNF release. Recent evidence has suggested that the exercise itself, rather than the tissue damage or fatigue, is essential for BDNF increase (91). Wahl and colleagues (91) showed that cycling exercises combined with electrical stimulation (the latter leading to more marked muscle damage) did not increase BDNF levels more than cycling exercises only. Cycling combined with electrical stimulation did increase other markers of damage such as creatine kinase, interleukin-6, and myoglobin more than cycling only.

Interestingly, however, if isolated muscle activation was the main source of BDNF upregulation, then resistance training would be the ideal protocol for elevating BDNF levels. Yet evidence to support this notion is inconclusive. Few studies have investigated the influence of resistance training on the upregulation of BDNF (11, 20, 68, 77, 99), and three out of five resistance training studies show negative effect sizes on BDNF upregulation (11, 68, 77). This low number of studies and the considerable variation in the direction of the observed effects make it difficult to draw a clear conclusion on the beneficial effect of this type of exercise on upregulating BDNF levels in humans. Importantly, the low response of BDNF upregulation to this type of training might stem from differences in exercise protocols used, as well as the time and methods of blood sampling. For instance, in most of these studies, basal/resting BDNF levels were tested; these are the changes in BDNF observed when the acute exercise-induced changes have been washed out and therefore are a less sensitive measurement of long-lasting change in BDNF levels after exercise (31). Additionally, most of the resistance protocols used were very short with long resting periods in between that, as a whole, consisted of fewer muscle contractions compared with our cycling protocols that involved 2,000 or 3,000 (10–20 min times $\approx$70–80 revolutions per minute) contractions and therefore placed a greater total load on the muscle. Hence, more studies with longer resistance training protocols that place a greater load to the muscle following appropriate methodological blood sampling and that include measurement of transient and long-term changes in BDNF

would be necessary to better characterize changes in the concentration of BDNF after resistance training.

An additional factor that also has the potential to elevate BDNF levels in the brain is tissue hypoxia (4, 93). It has been shown that BDNF is released by the cerebral vascular endothelium following hypoxic stresses (92). Similar to muscle contractions, hypoxia stimulates signaling pathways involved in mitochondrial biogenesis (25). Accordingly, successive bouts of HIT have been shown to cause transient increases in mRNA, which lead to sustained increases in the content of transcription and metabolic proteins, which also lead to greater mitochondrial protein content and enzyme activity (54). More specifically, HIT activates several kinases and phosphatases involved in signal transduction, including the AMPK and MAPK cascades and increases the expression of PGC-1α (19). Furthermore, studies show that HIT protocols elevate PGC-1α levels more than CON protocols (81, 94, 97). As mentioned previously, PGC-1α could activate FNDC5 gene expression, which is a positive regulator of BDNF levels in the brain (67). Hence, higher expression of this important coactivator after a HIT protocol may be one important factor that could explain why this type of exercise training showed greater BDNF levels.

Based on animal work, other factors have been proposed. A recent study compared the effects of high-intensity interval and continuous training regimes on BDNF levels in the rat brain (1). Similarly to our study Afzalpour et al. found that the HIT protocol resulted in greater BDNF increases in the brain compared with the CON protocol. They suggest that these differences might be related to the higher concentrations of hydrogen peroxide (H_2O_2) and tumor necrosis factor alpha (TNF-α) in the brain after HIT. They argued that intensive interval and continuous training regimens may differentially activate stress oxidative resources and antioxidant systems and thereby produce different levels of H_2O_2 (1). Even though we did not measure H_2O_2 levels, it has been shown that production of H_2O_2 causes loss of muscle contractility (59), which is in line with our observation that the perceived muscle fatigue (measured by Borg CR-10 scale ratings for leg fatigue) was the only predictor of $[BDNF]_{ser}$ changes after exercise. Furthermore, it has been shown that H_2O_2 and TNF-α induce the transport of p65:p50 subunits of the nuclear factor-κB complex from the cytoplasm to the nucleus, where it binds to the target sites in the DNA, thereby inducing BDNF expression (16, 65, 73).

To conclude, even though it is unclear which are the main pathways that mediate exercise-induced BDNF expression in the brain, we tentatively speculate that the higher levels of $[BDNF]_{ser}$ during the HIT protocol resulted from the combined effect of various factors most likely including high muscle activation (measured by Borg CR-10 scale ratings for leg fatigue) and high intensity (as indicated by high lactate levels), as well as a shorter total exercise duration compared with the CON protocol, which could have induced optimal short-term periods of oxidative stress leading to a rise in H_2O_2 and TNF-α. As a result, the appropriate signaling cascade (most likely PGC-1α) might have been activated leading to higher BDNF expression in the brain.

Adherence and Safety of the HIT Protocol

Seventy-three percent of our participants preferred the HIT protocol. Therefore, when it comes to incorporating these exercise-training protocols into a daily exercise routine, it is important that participants are able to tolerate the exercise to maximize adherence. This is an additional advantage of the HIT protocol.

Numerous studies show that HIT can be used effectively even in less-fit populations and patients, including older adults (55); overweight adolescents (82); individuals with paraplegia (83); and in persons with diabetes (35), metabolic syndrome (81), chronic obstructive pulmonary disease (75, 90), stable angina (39), and heart failure (28, 97); those undergoing cardiac rehabilitation (10, 24, 61); or even after coronary artery bypass surgery (40) and heart transplant (57). In fact, in more-frail patients, HIT (tailored at the maximal tolerance of a patient) is often the preferred and better tolerated mode of exercise training. More importantly, the above-mentioned studies have demonstrated the safety and effectiveness of this type of training (5, 10, 24). In a review of studies of cardiac rehabilitation, no adverse or other significant clinical, hemodynamic, electrical, or biological signs of ischemia or arrhythmia events secondary to participation in HIT protocols were observed (24). These findings suggest that HIT is safe, beneficial, and tolerable for many populations, including patients. Good tolerability of HIT exercise has received attention from within the scientific community because many patients are not only interested in improving their cardiovascular health but would also potentially benefit from the elevation of BDNF levels that might result in improved brain health and cognition.

Interpretational Issues

Low basal $[BDNF]_{ser}$ levels of participants. The baseline $[BDNF]_{ser}$ levels we found in our participants ranged from 3,256.5 to 14,263.2 pg/ml, which fall in the lower range of baseline BDNF values reported in the literature (31). One explanation for our low BDNF values could be attributed to the characteristics of our participants. We tested young, healthy, and active participants whose maximal $\dot{V}O_2$ suggested good to excellent fitness levels. Several studies have examined the difference in basal $[BDNF]_{ser}$ between trained and untrained participants. Chan et al. (7) and Nofuji et al. (47) found that rest $[BDNF]_{ser}$ values were lower in athletes and trained participants compared with untrained participants, a finding that was confirmed by subsequent studies (2). A possible explanation for lower rest $[BDNF]_{ser}$ in physically active persons is that they have a more effective clearance, leading to less stored and circulating basal BDNF levels.

Another explanation for the relatively low $[BDNF]_{ser}$ in our study is the time when the exercise tests were carried out. In the majority of studies investigating increases in BDNF levels after acute physical exercise, blood collection times took place early in the morning and some even after an overnight fasting state (6, 9, 47, 64, 76, 78, 100). In the present study, we were limited to testing after 2:00 P.M., which according to Begliuomini et al. (3), could have had an impact on BDNF levels measured due to diurnal variations in the circadian rhythm of cortisol resulting in higher BDNF values in the morning compared with later in the day (3, 56). Another influencing factor is that our participants were not tested in the fasted state,

which could have added variability to the $[BDNF]_{ser}$ measurements at rest due to the confounding effect of sugar levels in the blood. For example, in animals, a high-sugar diet caused lower BDNF levels (41). Nonetheless, even if testing in the afternoon had influenced $[BDNF]_{ser}$, it is unlikely that it can explain the differential response of $[BDNF]_{ser}$ to HIT vs. CON exercise.

Limitations of the Study

In this study we tested healthy active men, who represent a small part of the general population, making it difficult to transfer our results to other groups such as children, women, elderly, or patients. The reasoning behind selecting this specific group was to eliminate other confounding variables in our experiment. For example, Monteleone et al. (42) reported a high positive correlation between estrogen and plasma BDNF levels in women. Although our study provides evidence of a positive influence of HIT on $[BDNF]_{ser}$ only in physically active healthy young men, it certainly justifies future research in patients, elderly, or sedentary populations.

Moreover, we did not control for the BDNF polymorphism, which could have provided more specific information on how the polymorphism might modulate BDNF secretion in response to exercise.

Conclusion

In summary, we demonstrated that $[BDNF]_{ser}$ levels gradually increase during both types of intense exercise, CON and HIT, and that this increase is temporary, with BDNF levels returning to baseline values 20 min after the end of exercise. Moreover, we showed that $[BDNF]_{ser}$ levels after our HIT protocol were slightly higher than after our CON protocol when they were tested in healthy, active young men using a crossover design.

We detected a correlation between HIT exercise-induced increases in $[BDNF]_{ser}$ and Borg CR-10 scale ratings for leg fatigue, which might suggest that muscle fatigue caused by higher intensity protocols could coincide with activation of a signaling cascade that triggers the release of BDNF in the brain. Because HIT induced greater $[BDNF]_{ser}$ levels than CON, and because most of our participants preferred the HIT protocol, we recommend HIT as a potential intervention for increasing BDNF levels in the brain, which might promote neural plasticity and good cognitive function. However, further studies are needed to reproduce this effect in elderly and patient populations and to yield further insights into the underlying mechanisms.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.M.S.M., T.T., and N.W. conception and design of research; C.M.S.M. performed experiments; C.M.S.M. and B.V. analyzed data; C.M.S.M., T.T., and N.W. interpreted results of experiments; C.M.S.M. prepared figures; C.M.S.M. drafted manuscript; C.M.S.M., B.V., T.T., and N.W. edited and revised manuscript; N.W. approved final version of manuscript.

REFERENCES

1. **Afzalpour ME, Chadorneshin HT, Foadoddini M, Eivari HA.** Comparing interval and continuous exercise training regimens on neurotrophic factors in rat brain. *Physiol Behav* 147: 78–83, 2015.
2. **Babaei P, Damirchi A, Mehdipoor M, Tehrani BS.** Long term habitual exercise is associated with lower resting level of serum BDNF. *Neurosci Lett* 566C: 304–308, 2014.
3. **Begliuomini S, Lenzi E, Ninni F, Casarosa E, Merlini S, Pluchino N, Valentino V, Luisi S, Luisi M, Genazzani AR.** Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. *J Endocrinol* 197: 429–435, 2008.
4. **Behrens MM, Strasser U, Lobner D, Dugan LL.** Neurotrophin-mediated potentiation of neuronal injury. *Microsc Res Tech* 45: 276–284, 1999.
5. **Boutcher SH.** High-intensity intermittent exercise and fat loss. *J Obes* 2011: 868305, 2011.
6. **Castellano V, White LJ.** Serum brain-derived neurotrophic factor response to aerobic exercise in multiple sclerosis. *J Neurol Sci* 269: 85–91, 2008.
7. **Chan KL, Tong KY, Yip SP.** Relationship of serum brain-derived neurotrophic factor (BDNF) and health-related lifestyle in healthy human subjects. *Neurosci Lett* 447: 124–128, 2008.
8. **Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, Houlden H, Bhatia K, Greenwood R, Rothwell JC.** A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. *J Physiol* 586: 5717–5725, 2008.
9. **Cho HC, Kim J, Kim S, Son YH, Lee N, Jung SH.** The concentrations of serum, plasma and platelet BDNF are all increased by treadmill VO(2)max performance in healthy college men. *Neurosci Lett* 519: 78–83, 2012.
10. **Cornish AK, Broadbent S, Cheema BS.** Interval training for patients with coronary artery disease: a systematic review. *Eur J Appl Physiol* 111: 579–589, 2011.
11. **Correia PR, Pansani A, Machado F, Andrade M, Silva AC, Scorza FA, Cavalheiro EA, Arida RM.** Acute strength exercise and the involvement of small or large muscle mass on plasma brain-derived neurotrophic factor levels. *Clinics (Sao Paulo)* 65: 1123–1126, 2010.
12. **Cotman CW, Berchtold NC.** Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci* 25: 295–301, 2002.
13. **Ding Y, Li J, Luan X, Ding YH, Lai Q, Rafols JA, Phillis JW, Clark JC, Diaz FG.** Exercise pre-conditioning reduces brain damage in ischemic rats that may be associated with regional angiogenesis and cellular overexpression of neurotrophin. *Neuroscience* 124: 583–591, 2004.
14. **Eston RG, Davies BL, Williams JG.** Use of perceived effort ratings to control exercise intensity in young healthy adults. *Eur J Appl Physiol Occup Physiol* 56: 222–224, 1987.
15. **Ferris LT, Williams JS, Shen CL.** The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. *Med Sci Sports Exerc* 39: 728–734, 2007.
16. **Figiel I.** Pro-inflammatory cytokine TNF-alpha as a neuroprotective agent in the brain. *Acta Neurobiol Exp (Warsz)* 68: 526–534, 2008.
17. **Fujimura H, Altar CA, Chen R, Nakamura T, Nakahashi T, Kambayashi J, Sun B, Tandon NN.** Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thromb Haemost* 87: 728–734, 2002.
18. **Gibala MJ, Little JP, van Essen M, Wilkin GP, Burgomaster KA, Safdar A, Raha S, Tarnopolsky MA.** Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *J Physiol* 575: 901–911, 2006.
19. **Gibala MJ, McGee SL, Garnham AP, Howlett KF, Snow RJ, Hargreaves M.** Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. *J Appl Physiol* 106: 929–934, 2009.
20. **Goekint M, De Pauw K, Roelands B, Njemini R, Bautmans I, Mets T, Meeusen R.** Strength training does not influence serum brain-derived neurotrophic factor. *Eur J Appl Physiol* 110: 285–293, 2010.

21. **Goekint M, Heyman E, Roelands B, Njemini R, Bautmans I, Mets T, Meeusen R.** No influence of noradrenaline manipulation on acute exercise-induced increase of brain-derived neurotrophic factor. *Med Sci Sports Exerc* 40: 1990–1996, 2008.
22. **Gold SM, Schulz KH, Hartmann S, Mladek M, Lang UE, Hellweg R, Reer R, Braumann KM, Heesen C.** Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. *J Neuroimmunol* 138: 99–105, 2003.
23. **Griffin ÉW, Mulally S, Foley C, Warmington SA, O'Mara SM, Kelly AM.** Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males. *Physiol Behav* 104: 934–941, 2011.
24. **Guiraud T, Nigam A, Gremeaux V, Meyer P, Juneau M, Bosquet L.** High-intensity interval training in cardiac rehabilitation. *Sports Med* 42: 587–605, 2012.
25. **Gutsaeva DR, Carraway MS, Suliman HB, Demchenko IT, Shitara H, Yonekawa H, Piantadosi CA.** Transient hypoxia stimulates mitochondrial biogenesis in brain subcortex by a neuronal nitric oxide synthase-dependent mechanism. *J Neurosci* 28: 2015–2024, 2008.
26. **Huang AM, Jen CJ, Chen HF, Yu L, Kuo YM, Chen HI.** Compulsive exercise acutely upregulates rat hippocampal brain-derived neurotrophic factor. *J Neural Transm* 113: 803–811, 2006.
27. **Iellamo F, Manzi V, Caminiti G, Vitale C, Castagna C, Massaro M, Franchini A, Rosano G, Volterrani M.** Matched dose interval and continuous exercise training induce similar cardiorespiratory and metabolic adaptations in patients with heart failure. *Int J Cardiol* 167: 2561–2565, 2013.
28. **Isaksen K, Munk PS, Valborgland T, Larsen AI.** Aerobic interval training in patients with heart failure and an implantable cardioverter defibrillator: a controlled study evaluating feasibility and effect. *Eur J Prev Cardiol* 22: 296–303, 2015.
29. **Kavanagh T.** Exercise and the heart. *Ann Acad Med Singapore* 12: 331–337, 1983.
30. **Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, Knudsen GM, Aznar S.** Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *Int J Neuropsychopharmacol* 14: 347–353, 2011.
31. **Knaepen K, Goekint M, Heyman EM, Meeusen R.** Neuroplasticity - exercise-induced response of peripheral brain-derived neurotrophic factor: a systematic review of experimental studies in human subjects. *Sports Med* 40: 765–801, 2010.
33. **Lee DC, Sui X, Ortega FB, Kim YS, Church TS, Winett RA, Ekelund U, Katzmarzyk PT, Blair SN.** Comparisons of leisure-time physical activity and cardiorespiratory fitness as predictors of all-cause mortality in men and women. *Br J Sports Med* 45: 504–510, 2011.
34. **Lipsky RH, Marini AM.** Brain-derived neurotrophic factor in neuronal survival and behavior-related plasticity. *Ann NY Acad Sci* 1122: 130–143, 2007.
35. **Little JP, Gillen JB, Percival ME, Safdar A, Tarnopolsky MA, Punthakee Z, Jung ME, Gibala MJ.** Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J Appl Physiol* 111: 1554–1560, 2011.
36. **Lommatzsch M, Zingler D, Schuhbaeck K, Schloetcke K, Zingler C, Schuff-Werner P, Virchow JC.** The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiol Aging* 26: 115–123, 2005.
37. **Lowensteyn I, Coupal L, Zowall H, Grover SA.** The cost-effectiveness of exercise training for the primary and secondary prevention of cardiovascular disease. *J Cardiopulm Rehabil* 20: 147–155, 2000.
38. **Matthews VB, Aström MB, Chan MH, Bruce CR, Krabbe KS, Prelovsek O, Akerström T, Yfanti C, Broholm C, Mortensen OH, Penkowa M, Hojman P, Zankari A, Watt MJ, Bruunsgaard H, Pedersen BK, Febbraio MA.** Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia* 52: 1409–1418, 2009.
39. **Meyer P, Guiraud T, Gayda M, Juneau M, Bosquet L, Nigam A.** High-intensity aerobic interval training in a patient with stable angina pectoris. *Am J Phys Med Rehabil* 89: 83–86, 2010.
40. **Moholdt TT, Amundsen BH, Rustad LA, Wahba A, Lovo KT, Gullikstad LR, Bye A, Skogvoll E, Wisloff U, Slordahl SA.** Aerobic interval training versus continuous moderate exercise after coronary artery bypass surgery: a randomized study of cardiovascular effects and quality of life. *Am Heart J* 158: 1031–1037, 2009.
41. **Molteni R, Wu A, Vaynman S, Ying Z, Barnard RJ, Gómez-Pinilla F.** Exercise reverses the harmful effects of consumption of a high-fat diet on synaptic and behavioral plasticity associated to the action of brain-derived neurotrophic factor. *Neuroscience* 123: 429–440, 2004.
42. **Monteleone P, Artini PG, Simi G, Cela V, Casarosa E, Begliuomini S, Ninni F, Pluchino N, Luisi M, Genazzani AR.** Brain derived neurotrophic factor circulating levels in patients undergoing IVF. *J Assist Reprod Genet* 24: 477–480, 2007.
43. **Nagamatsu LS, Flicker L, Kramer AF, Voss MW, Erickson KI, Hsu CL, Liu-Ambrose T.** Exercise is medicine, for the body and the brain. *Br J Sports Med* 48: 943–944, 2014.
44. **Neeper SA, Gómez-Pinilla F, Choi J, Cotman C.** Exercise and brain neurotrophins. *Nature* 373: 109, 1995.
45. **Neeper SA, Gómez-Pinilla F, Choi J, Cotman CW.** Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res* 726: 49–56, 1996.
46. **Noble BJ.** Clinical applications of perceived exertion. *Med Sci Sports Exerc* 14: 406–411, 1982.
47. **Nofuji Y, Suwa M, Moriyama Y, Nakano H, Ichimiya A, Nishichi R, Sasaki H, Radak Z, Kumagai S.** Decreased serum brain-derived neurotrophic factor in trained men. *Neurosci Lett* 437: 29–32, 2008.
48. **Nofuji Y, Suwa M, Sasaki H, Ichimiya A, Nishichi R, Kumagai S.** Different circulating brain-derived neurotrophic factor responses to acute exercise between physically active and sedentary subjects. *J Sports Sci Med* 11: 83–88, 2012.
49. **O'Callaghan RM, Ohle R, Kelly AM.** The effects of forced exercise on hippocampal plasticity in the rat: a comparison of LTP, spatial- and non-spatial learning. *Behav Brain Res* 176: 362–366, 2007.
50. **Ogborn DI, Gardiner PF.** Effects of exercise and muscle type on BDNF, NT-4/5, and TrKB expression in skeletal muscle. *Muscle Nerve* 41: 385–391, 2010.
51. **Oliff HS, Berchtold NC, Isackson P, Cotman CW.** Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. *Brain Res Mol Brain Res* 61: 147–153, 1998.
52. **Ozan E, Okur H, Eker C, Eker OD, Gonul AS, Akarsu N.** The effect of depression, BDNF gene val66met polymorphism and gender on serum BDNF levels. *Brain Res Bull* 81: 61–65, 2010.
53. **Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ.** Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology* 37: 1553–1561, 1998.
54. **Perry CG, Lally J, Holloway GP, Heigenhauser GJ, Bonen A, Spriet LL.** Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J Physiol* 588: 4795–4810, 2010.
55. **Pichot V, Roche F, Denis C, Garet M, Duverney D, Costes F, Barthelemy JC.** Interval training in elderly men increases both heart rate variability and baroreflex activity. *Clin Auton Res* 15: 107–115, 2005.
56. **Pluchino N, Cubeddu A, Begliuomini S, Merlini S, Giannini A, Bucci F, Casarosa E, Luisi M, Cela V, Genazzani AR.** Daily variation of brain-derived neurotrophic factor and cortisol in women with normal menstrual cycles, undergoing oral contraception and in postmenopause. *Hum Reprod* 24: 2303–2309, 2009.
57. **Pokan R, Von Duvillard SP, Ludwig J, Rohrer A, Hofmann P, Wonisch M, Smekal G, Schmid P, Pacher R, Bachl N.** Effect of high-volume and -intensity endurance training in heart transplant recipients. *Med Sci Sports Exerc* 36: 2011–2016, 2004.
58. **Rasmussen P, Brassard P, Adser H, Pedersen MV, Leick L, Hart E, Secher NH, Pedersen BK, Pilegaard H.** Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Exp Physiol* 94: 1062–1069, 2009.
59. **Reid MB, Stokic DS, Koch SM, Khawli FA, Leis AA.** N-acetylcysteine inhibits muscle fatigue in humans. *J Clin Invest* 94: 2468–2474, 1994.
60. **Rickham PP.** Human experimentation code of ethics of the world medical association. Declaration of Helsinki. *Br Med J* 2: 177, 1964.
61. **Rognmo O, Moholdt T, Bakken H, Hole T, Molstad P, Myhr NE, Grimsmo J, Wisloff U.** Cardiovascular risk of high- versus moderate-intensity aerobic exercise in coronary heart disease patients. *Circulation* 126: 1436–1440, 2012.
62. **Roig M, Nordbrandt S, Geertsen SS, Nielsen JB.** The effects of cardiovascular exercise on human memory: a review with meta-analysis. *Neurosci Biobehav Rev* 37: 1645–1666, 2013.

63. **Roig M, Skriver K, Lundbye-Jensen J, Kiens B, Nielsen JB.** A single bout of exercise improves motor memory. *PLoS One* 7: e44594, 2012.
64. **Rojas VS, Struder HK, Vera WB, Schmidt A, Bloch W, Hollmann W.** Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. *Brain Res* 1121: 59–65, 2006.
65. **Saha RN, Liu X, Pahan K.** Up-regulation of BDNF in astrocytes by TNF-alpha: a case for the neuroprotective role of cytokine. *J Neuroimmune Pharmacol* 1: 212–222, 2006.
66. **Sapolsky RM, Romero LM, Munck AU.** How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 21: 55–89, 2000.
67. **Sartorius A, Hellweg R, Litzke J, Vogt M, Dormann C, Vollmayr B, Danker-Hopfe H, Gass P.** Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats. *Pharmacopsychiatry* 42: 270–276, 2009.
68. **Schiffer T, Schulte S, Hollmann W, Bloch W, Struder HK.** Effects of strength and endurance training on brain-derived neurotrophic factor and insulin-like growth factor 1 in humans. *Horm Metab Res* 41: 250–254, 2009.
69. **Schiffer T, Schulte S, Sperlich B, Achtzehn S, Fricke H, Struder HK.** Lactate infusion at rest increases BDNF blood concentration in humans. *Neurosci Lett* 488: 234–237, 2011.
70. **Schmidt-Kassow M, Schädle S, Otterbein S, Thiel C, Doehring A, Lötsch J, Kaiser J.** Kinetics of serum brain-derived neurotrophic factor following low-intensity versus high-intensity exercise in men and women. *Neuroreport* 23: 889–893, 2012.
71. **Schmolesky MT, Webb DL, Hansen RA.** The effects of aerobic exercise intensity and duration on levels of brain-derived neurotrophic factor in healthy men. *J Sports Sci Med* 12: 502–511, 2013.
72. **Seifert T, Brassard P, Wissenberg M, Rasmussen P, Nordby P, Stallknecht B, Adser H, Jakobsen AH, Pilegaard H, Nielsen HB, Secher NH.** Endurance training enhances BDNF release from the human brain. *Am J Physiol Regul Integr Comp Physiol* 298: R372–R377, 2010.
73. **Siamilis S, Jakus J, Nyakas C, Costa A, Mihalik B, Falus A, Radak Z.** The effect of exercise and oxidant-antioxidant intervention on the levels of neurotrophins and free radicals in spinal cord of rats. *Spinal Cord* 47: 453–457, 2009.
74. **Smith MA, Makino S, Kvetnansky R, Post RM.** Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* 15: 1768–1777, 1995.
75. **Spruit MA, Singh SJ, Garvey C, ZuWallack R, Nici L, Rochester C, Hill K, Holland AE, Lareau SC, Man WD, Pitta F, Sewell L, Raskin J, Bourbeau J, Crouch R, Franssen FM, Casaburi R, Vercoulen JH, Vogiatzis I, Gosselink R, Clini EM, Effing TW, Maltais F, van der Palen J, Troosters T, Janssen DJ, Collins E, Garcia-Aymerich J, Brooks D, Fahy BF, Puhan MA, Hoogendoorn M, Garrod R, Schols AM, Carlin B, Benzo R, Meek P, Morgan M, Rutten-van Mölken MP, Ries AL, Make B, Goldstein RS, Dowson CA, Brozek JL, Donner CF, Wouters EF; ATS/ERS** Task force on Pulmonary Rehabilitation. An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation. *Am J Respir Crit Care Med* 188: e13–e64, 2013.
76. **Strohle A, Stoy M, Graetz B, Scheel M, Wittmann A, Gallinat J, Lang UE, Dimeo F, Hellweg R.** Acute exercise ameliorates reduced brain-derived neurotrophic factor in patients with panic disorder. *Psychoneuroendocrinology* 35: 364–368, 2010.
77. **Swift DL, Johannsen NM, Myers VH, Earnest CP, Smits JA, Blair SN, Church TS.** The effect of exercise training modality on serum brain derived neurotrophic factor levels in individuals with type 2 diabetes. *PLoS One* 7: e42785, 2012.
78. **Tang A, Sibley KM, Thomas SG, Bayley MT, Richardson D, McIlroy WE, Brooks D.** Effects of an aerobic exercise program on aerobic capacity, spatiotemporal gait parameters, and functional capacity in subacute stroke. *Neurorehabil Neural Repair* 23: 398–406, 2009.
79. **Tang SW, Chu E, Hui T, Helmeste D, Law C.** Influence of exercise on serum brain-derived neurotrophic factor concentrations in healthy human subjects. *Neurosci Lett* 431: 62–65, 2008.
80. **Tauler P, Martinez S, Moreno C, Martinez P, Aguilo A.** Changes in salivary hormones, immunoglobulin A, and C-reactive protein in response to ultra-endurance exercises. *Appl Physiol Nutr Metab* 39: 560–565, 2014.
81. **Tjønna AE, Lee SJ, Rognmo Ø, Stølen TO, Bye A, Haram PM, Loennechen JP, Al-Share QY, Skogvoll E, Slørdahl SA, Kemi OJ, Najjar SM, Wisløff U.** Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation* 118: 346–354, 2008.
82. **Tjønna AE, Stølen TO, Bye A, Volden M, Slørdahl SA, Odegård R, Skogvoll E, Wisløff U.** Aerobic interval training reduces cardiovascular risk factors more than a multitreatment approach in overweight adolescents. *Clin Sci (Lond)* 116: 317–326, 2009.
83. **Tordi N, Dugue B, Klupzinski D, Rasseneur L, Rouillon JD, Lonsdorfer J.** Interval training program on a wheelchair ergometer for paraplegic subjects. *Spinal Cord* 39: 532–537, 2001.
84. **VanBruggen MD, Hackney AC, McMurray RG, Ondrak KS.** The relationship between serum and salivary cortisol levels in response to different intensities of exercise. *Int J Sports Physiol Perform* 6: 396–407, 2011.
85. **Vaynman S, Gomez-Pinilla F.** License to run: exercise impacts functional plasticity in the intact and injured central nervous system by using neurotrophins. *Neurorehabil Neural Repair* 19: 283–295, 2005.
86. **Vaynman S, Gomez-Pinilla F.** Revenge of the "sit": how lifestyle impacts neuronal and cognitive health through molecular systems that interface energy metabolism with neuronal plasticity. *J Neurosci Res* 84: 699–715, 2006.
87. **Vaynman S, Ying Z, Gomez-Pinilla F.** Interplay between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. *Neuroscience* 122: 647–657, 2003.
88. **Vaynman S, Ying Z, Gomez-Pinilla F.** Exercise induces BDNF and synapsin I to specific hippocampal subfields. *J Neurosci Res* 76: 356–362, 2004.
89. **Vaynman S, Ying Z, Gomez-Pinilla F.** Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *Eur J Neurosci* 20: 2580–2590, 2004.
90. **Vogiatzis I.** Strategies of muscle training in very severe COPD patients. *Eur Respir J* 38: 971–975, 2011.
91. **Wahl P, Hein M, Achtzehn S, Bloch W, Mester J.** Acute effects of superimposed electromyostimulation during cycling on myokines and markers of muscle damage. *J Musculoskelet Neuronal Interact* 15: 53–59, 2015.
92. **Wang H, Ward N, Boswell M, Katz DM.** Secretion of brain-derived neurotrophic factor from brain microvascular endothelial cells. *Eur J Neurosci* 23: 1665–1670, 2006.
93. **Wang H, Yuan G, Prabhakar NR, Boswell M, Katz DM.** Secretion of brain-derived neurotrophic factor from PC12 cells in response to oxidative stress requires autocrine dopamine signaling. *J Neurochem* 96: 694–705, 2006.
94. **Weston KS, Wisloff U, Coombes JS.** High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br J Sports Med* 48: 1227–1234, 2014.
95. **Widenfalk J, Olson L, Thoren P.** Deprived of habitual running, rats downregulate BDNF and TrkB messages in the brain. *Neurosci Res* 34: 125–132, 1999.
96. **Winter B, Breitenstein C, Mooren FC, Voelker K, Fobker M, Lechtermann A, Krueger K, Fromme A, Korsukewitz C, Floel A, Knecht S.** High impact running improves learning. *Neurobiol Learn Mem* 87: 597–609, 2007.
97. **Wisløff U, Støylen A, Loennechen JP, Bruvold M, Rognmo Ø, Haram PM, Tjønna AE, Helgerud J, Slørdahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen Ø, Skjaerpe T.** Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* 115: 3086–3094, 2007.
98. **Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, Lin JD, Greenberg ME, Spiegelman BM.** Exercise induces hippocampal BDNF through a PGC-1alpha/FNDC5 pathway. *Cell Metab* 18: 649–659, 2013.
99. **Yarrow JF, White LJ, McCoy SC, Borst SE.** Training augments resistance exercise induced elevation of circulating brain derived neurotrophic factor (BDNF). *Neurosci Lett* 479: 161–165, 2010.
100. **Zoladz JA, Pilc A, Majerczak J, Grandys M, Zapart-Bukowska J, Duda K.** Endurance training increases plasma brain-derived neurotrophic factor concentration in young healthy men. *J Physiol Pharmacol* 59, *Suppl* 7: 119–132, 2008.