Peripheral vascular reactivity and serum BDNF responses to aerobic training are impaired by the BDNF Val66Met polymorphism

José R. Lemos Jr.,1,2 Cleber R. Alves,3 Sílvia B. C. de Souza,2 Julia D. C. Marsiglia,2 Michelle S. M. Silva,2 Alexandre C. Pereira,2 Antônio L. Teixeira,4 Erica L. M. Vieira,4 José E. Krieger,2 Carlos E. Negrão,2,3 Guilherme B. Alves,2 Edilmar M. de Oliveira,3 Vladimir Bolani,2 Rodrigo G. Dias,2 and Ivani C. Trombetta2,5

1School of Physical Education, Military Police of São Paulo State, São Paulo, Brazil; 2Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil; 3School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil; 4Federal University of Minas Gerais, Minas Gerais, Brazil; and 5Universidade Nove de Julho (UNINOVE), São Paulo, Brazil

Submitted 19 August 2015; accepted in final form 13 November 2015

Lemos JR Jr, Alves CR, de Souza SBC, Marsiglia JDC, Silva MSM, Pereira AC, Teixeira AL, Vieira ELM, Krieger JE, Negrão CE, Alves GB, de Oliveira EM, Bolani W, Dias RG, Trombetta IC. Peripheral vascular reactivity and serum BDNF responses to aerobic training are impaired by the BDNF Val66Met polymorphism. *Physiol Genomics* 48: 116–123, 2016. First published November 24, 2015; doi:10.1152/physiolgenomics.00086.2015.—Besides neuronal plasticity, the neurotrophin brain-derived neurotrophic factor (BDNF) is also important in vascular function. The BDNF has been associated with angiogenesis through its specific receptor tropomyosin-related kinase B (TrkB). Additionally, Val66Met polymorphism decreases activity-induced BDNF. Since BDNF and TrkB are expressed in vascular endothelial cells and aerobic exercise training can increase serum BDNF, this study aimed to test the hypotheses: 1) Serum BDNF levels modulate peripheral blood flow; 2) The Val66Met BDNF polymorphism impairs exercise training-induced vasodilation. We genotyped 304 healthy male volunteers (Val66Val, n = 221; Val66Met, n = 83) who underwent intense aerobic exercise training on a running track three times/wk for 4 mo. We evaluated pre- and post-exercise training serum BDNF and proBDNF concentration, heart rate (HR), mean blood pressure (MBP), forearm blood flow (FFB), and forearm vascular resistance (FVR). In the pre-exercise training, BDNF, proBDNF, BDNF/proBDNF ratio, FBF, and FVR were similar between genotypes. After exercise training, functional capacity (V02peak) increased and HR decreased similarly in both groups. Val66Val, but not Val66Met, increased BDNF (interaction, P = 0.04) and BDNF/proBDNF ratio (interaction, P < 0.001). Interestingly, FBF (interaction, P = 0.04) and the FVR (interaction, P = 0.01) responses during handgrip exercise (HG) improved in Val66Val compared with Val66Met, even with similar responses of HR and MBP. There were association between BDNF/proBDNF ratio and FBF (r = 0.64, P < 0.001) and FVR (r = −0.58, P < 0.001) during HG exercise. These results show that peripheral vascular reactivity and serum BDNF responses to exercise training are impaired by the BDNF Val66Met polymorphism and such responsiveness is associated with serum BDNF concentrations in healthy subjects.

**BDNF Val66Met polymorphism; exercise training; vascular reactivity**

**EXERCISE TRAINING HAS BEEN considered a key element in the improvement in brain-derived neurotrophic factor (BDNF) levels** (39), which is the strongest factor linking exercise with cognitive benefits. However, the variability of individual responses may be linked to genetic differences.

While BDNF promotes neuronal survival and enhanced synaptic plasticity by activating the tropomyosin kinase B (TrkB) receptor, the action of its precursor proBDNF results in apoptosis by interacting with the p75 neurotrophin receptor (p75NTR), and both are significantly involved in different physiological functions (15, 53).

Considering the fact that the BDNF gene and its TrkB receptor are expressed in several tissues, such as brain, heart, lungs, and endothelial cells (12, 28), besides neuronal plasticity, it is possible that the neurotrophin BDNF also is involved in the health of other tissues. Indeed, besides the hippocampus, the circulating BDNF is produced by a number of peripheral nonneuronal tissues, including vascular endothelial cells (28, 53). Moreover, the neurotrophin BDNF has been associated with angiogenesis through its TrkB receptor (46). In this context, recent studies have indicated that expression of BDNF correlates with oxidative stress resulting from ROS generation and correlates with VEGF expression, contributing to the regulation of angiogenesis (20, 46).

Currently, Val66Met, a variant in the human BDNF gene that occurs in 20–30% of the Caucasian population (34, 40), has been highlighted (9, 18). Val66Met, a single nucleotide polymorphism (SNP) at nucleotide 196 (G/A) that encodes an amino acid substitution, a valine (Val) to methionine (Met) at codon 66 in the prodomain of the BDNF gene, results in a decreased activity-induced BDNF response (4). Met66Met and Val66Met individuals have less BDNF neurotrophic support for plasticity, as well as impaired intracellular trafficking and regulated secretion of BDNF decreased in neurons, whereas Val66Val individuals experience the inverse (5, 9). These factors together result in less BDNF being produced in the central nervous system, decreasing the circulating amount of this neurotrophin in Val66Met and Met66Met individuals (18). Therefore, the Val66Met polymorphism may affect the BDNF concentrations in plasma and activity in all peripheral tissues containing TrkB receptors, such as vascular endothelial cells.

Several previous studies consistently demonstrated that aerobic exercise training improves vasodilation in response to exercise. Acutely, the vasodilation during exercise depends upon the equilibrium between vasoconstrictor factors, mediated basically by α1-adrenergic and vasodilator forces, mediated by β2-adrenergic receptors (10, 36), and local nitric oxide...
**MATERIALS AND METHODS**

**Subjects**

The study overview, participant flow chart, study visits, and evaluations are demonstrated in Fig. 1. There were 317 preselected healthy male Brazilian policemen recruits who were invited to participate in the study. Of these, 304 recruits completed the full training protocol and after this were genotyped for BDNF Val66Met gene polymorphism. To avoid the confounding factors of sex, age, and disease in our study, the eligibility for participating in the study was determined by sex (only males), health status (no known history of medical conditions), tobacco consumption (nonsmokers only), and age (between 19 and 36 yr). The individuals were screened for cardiovascular, endocrine, and metabolic disorders. They had no apparent cardiovascular disease. Clinical examination, laboratory testing, and a cardiopulmonary exercise test determined their health status. Individuals included in the study took no medication 3 mo prior to enrollment in the study and abstained from consuming caffeine and alcohol 1 day before study measurements were taken. The Human Subject Protection Committees of the Heart Institute (InCor) and Clinical Hospital, University of São Paulo Medical School, approved the study protocol. Each subject gave written consent.

**SERUM DOSAGE CIRCULATING BDNF, PROBDNF**

Plasma samples obtained from subjects who participated in this study were stored in a freezer at −80°C and later thawed for joint evaluation (before and after physical training) of the levels of brain derived neurotrophic factors (BDNF, proBDNF) by enzyme-linked immunosorbent assay (ELISA), sandwich, using kits from R & D Systems (Minneapolis, MN). Into each well of the ELISA plate was added 100 μl of a solution containing monoclonal antibody to BDNF, proBDNF diluted in PBS solution (capture antibody). The plates were incubated for at least 12 h at 4°C. The plates with nonadherent antibodies were discarded by inversion and washing in PBS-0.1% Tween (Sigma-Aldrich, St. Louis, MO). Then, the plates were blocked with a solution (200 μl/well) containing bovine serum albumin (BSA) 1% (Sigma-Aldrich) for 2 h at room temperature. After the plates were washed further (0.1% Tween-PBS), 100 μl of sample or standard protein was added to each well. The plates were incubated for at least 12 h at 4°C and then washed (PBS-Tween 0.1%). After lavelage, the plates were incubated with biotin-conjugated antibodies diluted in 0.1% BSA for 2 h at room temperature. Then, after further washing (PBS-Tween 0.1%), we added 100 μl/well of peroxidase conjugated streptavidin to the plates, which were incubated for 30 min at room temperature. Finally, after further washing (PBS-Tween 0.1%), we added the chromogen o-phenylene-diamine (Sigma-Aldrich) to the plates, in the absence of light. The reaction was stopped with 1 M solution containing sulfuric acid. Reporting the intensity reading was performed on the ELISA reader at λ 490 nM (SOFTmax Pro - version 2.2.1) (11, 24).

**DNA AMPLIFICATION AND GENOTYPING OF THE POLYMORPHISM VAL66MET**

Genomic DNA was obtained from whole blood following a standard salting-out protocol (22, 25). The genomic DNA flanking the SNP was to be amplified by PCR with the primers 5′-TGATGAC-CATCCTTTTCTT-3′ and 5′-CACTGGGAAGCTTCTTGAC-3′. Each reaction uses 10 ng of genomic DNA, 0.5 μM of each primer oligonucleotide, 1× μl 10 PCR buffer, 250 μM dNTPs, 3 mM MgCl2, and 0.25 i-Star unit tag DNA polymerase and Milli-Q water to a total volume of 10 μl reaction. The amplification protocol consisted of a denaturing step at 95°C for 5 min followed by a cycle of denaturation at 95°C for 30 s, annealing at 55°C for 1 min and extension at 72°C for 1 min and a final extension at 72°C for 7 min. The Val66Met BDNF gene polymorphism (rs6265) was genotyped with the two-way direct sequencing method, preceded by a product purification step PCR with ExoSAP - IT enzyme.

**Maximal Cardiopulmonary Exercise Test**

Maximal exercise capacity was determined during a maximal progressive exercise test on a treadmill (Quinton Instruments, Seattle, WA), with workload increments of 1 MET every minute until exhaustion. A breath-by-breath gas exchange analyzer (Vmax 29; Sensor Medics, Buena Vista, CA) was used to measure VO2 and carbon dioxide production. Peak VO2 was defined as the maximum attained during a maximal exercise test on a treadmill (Quinton Instruments, Seattle, WA), with workload increments of 1 MET every minute until exhaustion. The reproducibility of the peak VO2 measured at a different time interval in the same individual expressed as ml·kg−1·min−1 in our laboratory was r = 0.95. Heart rate was

![Fig. 2. Timeline of experimental protocols (see Experimental protocol for explanation). FBF, forearm blood flow; BP, blood pressure; HR, heart rate.](http://physiolgenomics.physiology.org/doi/10.1152/physiolgenomics.00086.2015)
EXERCISE TRAINING AND Val66Met BDNF POLYMORPHISM

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Val66 Val</th>
<th>Val66 Met</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>221</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>24 ± 0.4</td>
<td>25 ± 0.7</td>
<td>0.37</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>76 ± 0.7</td>
<td>77 ± 1.6</td>
<td>0.90</td>
</tr>
<tr>
<td>Height, cm</td>
<td>175 ± 0</td>
<td>176 ± 0</td>
<td>0.40</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.8 ± 0.2</td>
<td>24.6 ± 0.4</td>
<td>0.42</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>88.4 ± 0.9</td>
<td>92.9 ± 2.5</td>
<td>0.65</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>168 ± 2.5</td>
<td>171 ± 5.7</td>
<td>0.67</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dl</td>
<td>118 ± 2.2</td>
<td>125 ± 5.2</td>
<td>0.21</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dl</td>
<td>41 ± 1.7</td>
<td>38 ± 1.3</td>
<td>0.19</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>106 ± 5.1</td>
<td>118 ± 7.4</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index.

continuously monitored by ECG, and blood pressure was measured with a sphygmonanometer. These measurements were repeated after 16 wk of exercise training.

Peripheral Vascular Reactivity Evaluation

The purpose of this protocol was to study the hemodynamic and vascular responses to handgrip exercise stimulation in individuals with BDNF Val66Val and Val66Met polymorphisms.

Forearm blood flow. Forearm blood flow (FFB) was measured by venous occlusion plethysmography (30). The nondominant arm was elevated above heart level to ensure adequate venous drainage. A mercury-filled silastic tube attached to a low-pressure transducer was placed around the forearm and connected to a plethysmograph (Hokanson, Bellevue, WA). Sphygmomanometer cuffs were placed around the forearm and connected to a plethysmograph (Hokanson, Bellevue, WA). Sphygmomanometer cuffs were placed around the wrist and upper arm. At 15 s intervals, the upper cuff was inflated above venous pressure for 7–8 s. FBF (ml·min⁻¹·100 ml tissue⁻¹) was determined on the basis of a minimum of four separate readings. Forearm vascular resistance (FVR, mmHg·ml⁻¹·min⁻¹) was calculated by dividing FBF by mean arterial pressure. The reproducibility of FBF measured at different time intervals in the same individual expressed as milliliters per minute per 100 milliliters of tissue in our laboratory had a correlation coefficient of 0.93.

Blood pressure and heart rate. During the handgrip exercise, blood pressure was monitored noninvasively and intermittently with the use of an automatic oscillometric cuff (DX 2710; Dixtal, Manaus, Brazil) placed on the ankle with cuff width adjusted to ankle circumference (30, 31). Heart rate was monitored continuously through lead II of the ECG.

Handgrip exercise. After the maximal voluntary contraction (MVC; average of 3 trials) was obtained, handgrip isometric exercise was performed at 30% of MVC with the dominant arm using a handgrip dynamometer. The individuals were instructed to breathe normally during exercise and to avoid inadvertent performance of a Valsalva maneuver (1).

Spectral Analysis of Heart Rate Variability

To control for differences in the parasympathetic or sympathetic changes that may occur with exercise training, spectral analysis of heart rate variability (HRV) was performed by HR analysis, obtained through electrocardiographic recording in lead II. The ECG signal was recorded in a computer sampling frequency of 500 Hz using the Windaq acquisition system of biological signals. The RR interval time series was analyzed by Kubios (HRV software version 2.0), by the time domain method, and the following indices are used: RMSSD (square root of the mean squared differences between successive RR intervals) and pNN50 (percentage of the difference between successive RR intervals that are >50 ms). The RMSSD and pNN50 were related to vagal activity. For the frequency domain method, the power spectrum was analyzed on three tracks, the components of the frequency band of from 0.04 to 0.15 Hz are considered low frequency, which reflect both sympathetic and parasympathetic modulation here (8). The components of between 0.15 and 0.40 Hz are considered high frequency (HF), and reflect the parasympathetic modulation (23). The interference of very low frequency was compensated by software features (44).

Experimental protocol. At least 2 h after a light meal without caffeine, the subjects were positioned supine and electrocardiogram leads were placed on the chest. Cuffs for FBF measurement were placed on the nondominant arm, and a cuff for blood pressure measurement was placed on the ankle. After a 15 min rest period, baseline values for FBF, blood pressure, and HR were recorded for 3 min. Moderate static handgrip exercise at 30% of MVC was then performed for 3 min, followed by a recovery period of 3 min (Fig. 2). FBF, blood pressure, and HR were recorded continuously during handgrip exercise. Mean blood pressure (MBP) was measured every minute, and FBF was measured every 15 s (1). All studies were performed in a temperature-controlled (21°C) room, between 8:00 and 10:00 AM (45).

Exercise Training Protocol

Individuals participated in 90 min moderate/intense aerobic exercise training sessions, three times weekly, beginning at 10:00 AM, supervised by an exercise specialist for 4 mo. Exercise intensity was individually graded according to HR based on the aerobic/anaerobic thresholds obtained in the cardiopulmonary exercise testing. Exercise training consisted of 2 mo of moderate to intense aerobic training, using HR levels at the anaerobic threshold toward the HR achieved at the respiratory compensation point. In the last 2 mo of the protocol, the exercise training intensity remained mostly at HR levels achieved at the respiratory compensation point. Exercise training sessions consisted of a 5 min warm-up, followed by 60 min of jogging or running and 25 min of local strength training (sit-ups, push-ups, and pull-ups).

Fig. 3. A: aerobic fitness gains represented by the VO₂ peak. *P < 0.05 vs. pre. B: improvements in cardiovascular capacity represented by HR. *P < 0.05 vs. pre.
Table 2. *Hemodynamic and BDNF data*

<table>
<thead>
<tr>
<th></th>
<th>Val66Val (n = 221)</th>
<th>Val66Met (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>91 ± 0.81</td>
<td>90 ± 0.93</td>
</tr>
<tr>
<td>FBF, ml/min</td>
<td>2.56 ± 0.08</td>
<td>2.62 ± 0.11</td>
</tr>
<tr>
<td>FVR, mmHg·ml⁻¹·min⁻¹</td>
<td>44.27 ± 1.57</td>
<td>40.18 ± 1.49</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>52.01 ± 1.74</td>
<td>56.27 ± 2.11</td>
</tr>
<tr>
<td>HF, n.u.</td>
<td>45.44 ± 1.22</td>
<td>42.43 ± 1.38</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.51 ± 0.09</td>
<td>1.62 ± 0.09</td>
</tr>
<tr>
<td>BDNF, pg/ml</td>
<td>3.65 ± 0.05</td>
<td>3.88 ± 0.03*</td>
</tr>
<tr>
<td>proBDNF, pg/ml</td>
<td>3.08 ± 0.06</td>
<td>3.03 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE. BDNF, brain-derived neurotrophic factor; MBP, mean blood pressure; FBF, forearm blood flow at rest; FVR, forearm vascular resistance at rest; RMSSD, root mean square successive difference between adjacent normal interbeat intervals; HF, high-frequency power; LF/HF, low-frequency/high-frequency ratio. *P < 0.05 vs. pretraining period.

**Statistical Analysis**

Data are presented as means ± SE. The distribution of all absolute measures of BDNF and proBDNF was normalized by a logarithmic transformation. BDNF and proBDNF concentrations, BDNF/proBDNF ratio, cardiopulmonary exercise test analyses, and FBF and FVR pre- and postaerobic exercise training were analyzed by two-way ANOVA with repeated measures. When significance was found, Scheffé’s post hoc comparisons were performed. Probability values of ≤0.05 were considered statistically significant. The FBF and FVR data are expressed as area under the curve (AUC).

**RESULTS**

The study overview, participant flow chart, study visits, and evaluations are described in Fig. 1. The prescreened volunteers were submitted for two visits. In the pre-exercise training visit 1 the blood sample was collected and stored for future measures of BDNF levels. In the postexercise training visit 1 the collected blood sample was used to DNA amplification and genotyping of the polymorphism Val66Met as well as the BDNF measures.

**Baseline Measurements**

The demographic characteristics and metabolic measurements of the 304 individuals according to genetic characteristics are shown in Table 1. The genotyping results showed 221 Val66Val (72.7%), 78 Val66Met (25.7%), and five Met66Met individuals (1.6%), in Hardy Weinberg equilibrium (P = 0.555). This frequency corroborates data presented in the literature (34, 40). Due to reduced frequency of allele Met in the population, a small number of Met66Met individuals were available and, thus, we adopted a strategy used by other authors (14), joining both (Val66Met and Met66Met) into a single group called Val66Met. Our final sample comprised 221 Val66Val and 83 Val66Met subjects.

There were no significant differences between Val66Val and Val66Met groups, regarding physical and metabolic characteristics at baseline (Table 1). In addition, there were no differences in the pretraining period on the HR (Fig. 3B), MBP, HRV, FBF, and FVR at rest (Table 2) and in response to handgrip exercise (Fig. 4). During the pretraining period, the BDNF and proBDNF concentrations in blood were similar between Val66Val and Val66Met groups (Table 2).

**Effects of Exercise Training**

Exercise training was begun in 317 volunteers, and 304 completed the full training protocol. The adherence to the sessions was 95.9%. In response to the 4 mo of aerobic training, the subjects had an increase of at least 8% in peak VO₂ (ml·kg⁻¹·min⁻¹) and a similar trend toward a fall in HR; thus the two genotype groups had a significant, similar increase in functional capacity (Fig. 3A) and a significant, similar decrease in resting HR (Fig. 3B). No differences occurred in the posttraining period in MBP, HRV, FBF, and FVR at rest (Table 2). BDNF and proBDNF serum concentration. Exercise training increased BDNF serum levels only in the Val66Val group (3.65 ± 0.05 vs. 3.88 ± 0.04 pg/ml; P < 0.001; interaction, P = 0.04) (Table 2). On the other hand, there were no changes in proBDNF concentrations for both groups (Table 2). The BDNF/proBDNF ratio increased in Val66Val (1.17 ± 0.03 vs. 1.44 ± 0.05 pg/ml; P < 0.001), and there was no significant
difference in Val66Met individuals (1.20 ± 0.05 vs. 1.19 ± 0.06 pg/ml; \( P = 0.99 \), interaction, \( P = 0.001 \)) (Fig. 5). In addition, in the posttraining measures Val66Val individuals had a higher BDNF/proBDNF ratio compared with Val66Met individuals (1.44 ± 0.06 vs. 1.19 ± 0.05 pg/ml; \( P = 0.02 \)).

**Peripheral vascular reactivity evaluation.** In the Val66Val, but not the Val66Met, group, aerobic exercise training increased FBF (interaction, \( P = 0.04 \), Fig. 4A) and decreased FVR (interaction, \( P = 0.01 \), Fig. 4B) in response to the handgrip exercise test (as AUC). However, after endurance training, no changes occurred in HR (Fig. 3B) and MBP responses to the handgrip exercise test for both groups (Table 2).

**Association between BDNF/proBDNF ratio and peripheral vascular reactivity.** The model used to verify the association considered the delta (post- minus preintervention values) between variables, and the individuals were matched in pre- and posttraining periods. There was a positive association between the delta of BDNF/proBDNF ratio and the delta of FBF as AUC (\( r = 0.64, P < 0.001 \), Fig. 6A). In addition, there was a negative association between BDNF/proBDNF and FVR as AUC (\( r = -0.58, P < 0.001 \), Fig. 6B).

**DISCUSSION**

The main and new evidence of the present study is that the Val66Met BDNF polymorphism impairs peripheral vascular reactivity in response to exercise, and such responsiveness is associated with serum BDNF concentrations, represented by the ratio BDNF/proBDNF in healthy subjects.

Contrariwise is the expected increase in BDNF by endurance training in young healthy men (17, 54); in the present study only Val66Val men increased BDNF after aerobic exercise training (Table 2). Our results reinforce the idea that BDNF has a role in Val66Met polymorphism greater than the exercise training effect in modulating BDNF. Similar to our results, a recent study in elderly individuals with mild cognitive impairment verified that only wild-type genotypes (BDNF-Val66Val) exhibited significant improvement in peripheral BDNF levels after physical exercise (29). In fact, most studies of Val66Met polymorphism mediation on BDNF gene expression report an impairment in the activity regulated, due to the interference of the Met allele in intracellular proBDNF cleavage (5, 21).

Interestingly, it has been reported that inhibition of intracellular cleavage of proBDNF does not interfere significantly with circulating BDNF expression, but on the other hand, when the extracellular cleavage is prevented, a decrease occurs in circulating mature BDNF concentrations (27). Therefore, it is possible that this augmentation in resting BDNF concentration is linked to an increase in enzymatic profile, which is responsible for extracellular cleavage of BDNF, and physical exercise influences this constitutive context. It has been found that chronic exercise seems to be closely related to activation of matrix metalloproteinases (38) and increased plasmin (16), which is mainly responsible for extracellular cleavage of BDNF (27, 41), which are related to angiogenesis processes mediated by increases in serum BDNF concentration (19, 20, 41).

BDNF has an important anti-inflammatory effect. Vascular inflammation can be the first step to an endothelial dysfunction and an impaired blood flow that will originate the enhancement of the endothelial layer permeability, in turn facilitating the diffusion of low-density lipoproteins to the intima, which causes a decrease in the vasodilation endothelium dependently (37). Some recent studies have shown that the increases in BDNF levels resulting from exercise are accompanied by reductions in basal serum vascular cell adhesion molecule-1 and serum tumor necrosis factor-\( \alpha \), which produces attenuation of inflammation and prevents atherosclerosis (52).

Interestingly, despite the BDNF Val66Met polymorphism, exercise training caused similar improvement in the functional capacity (\( \text{VO}_2\text{peak} \)) and a decrease in resting HR in both groups (Fig. 3). Even so, the exercise training effects on serum BDNF and peripheral vascular responsiveness did not follow the same
trend. After exercise training, the BDNF concentration, represented by the BDNF/proBDNF ratio (Fig. 5), increased significantly only in Val66Val individuals, where there were no significant changes in the circulating amounts of proBDNF in both groups.

The enhanced vascular peripheral response to handgrip exercise after exercise training was found only in Val66Val individuals, in which the FBF and FVR expressed as AUC increased significantly, whereas the same response was not found in Val66Met individuals (Fig. 4). No significant alterations occurred in MBP or HRV (Table 2) in either group; consequently the increase in the peripheral vascular reactivity in the Val66Val group was not significantly influenced by adaptations in the parasympathetic or sympathetic drives. During exercise, the adjustments in peripheral blood flow can be modulated by the sympathoadrenal system acting through bioavailable catecholamines (50) or by endothelial vascular wall structures that produce vasoactive substances, like NO (32), adenosine (13), and prostacyclin (51), under the influence of specific physiological circumstances, such as oxidative and shear stress. The role together of these vasodilators is controversial.

The metabolic demand required by skeletal muscle cells during exercise results in hyperemia and reduction in oxygen tension; consequently ATP is released in erythrocytes and adenosine is formed, whose vasodilatory effect is prostacyclin and NO dependents (26). A connection has been established between the functional capacity and vascular prostacyclin release (51), as being prostacyclin production accompanied by an increase in NO. However, since the shear stress-induced NO production leads to a suppressant effect on prostacyclin production in the endothelium in physiological conditions (33), in the present study NO is in fact the main vasoactive agent.

Our results demonstrate that it is likely that the axis of this vasodilation in Val66Val, but not in Val66Met, is mainly endothelial dependent, influenced by the enhancement in serum BDNF, not by the autonomic nervous system. Some recent studies have shown that BDNF concentrations influence angiogenesis and endothelial vasodilation (20, 46) in human cells. All these studies have been successful in demonstrating in vitro adaptations in the endothelium influenced by BDNF, but none has studied this response in vivo.

Furthermore, we performed a linear regression between the delta of BDNF/proBDNF augmentation after exercise training and the FBF and FVR adaptations (Fig. 6). Our results show that increases in BDNF/proBDNF ratio are positively associated with improvement in blood flow responsiveness and decreased peripheral vascular resistance in response to exercise training.

Thus, these results suggest that the BDNF Val66Met polymorphism can be one of the mechanisms that enhance the endothelium-dependent vascular tone in response to exercise. The trigger might be initiated by the shear stress stimulation, which induces BDNF release by activating platelets (12). There is a virtual circle where BDNF is secreted by the endothelial cells (53); in turn BDNF increases VEGF expression, which enhances angiogenesis (3, 20), and VEGF induces a marked increase in endothelial cell production of NO (49). On the other hand, NO regulates BDNF expression (6), and NO is known to be widely produced in endothelial tissue during oxidative stress (46).

With this scenario, it is reasonable to deduce that the increased production of BDNF influenced by exercise training triggers a positive feedback loop of paracrine manner, which induces benefits to peripheral vasculature. According to data obtained in our study, in Val66Val individuals, this set of factors contributed decisively to the improvements in peripheral vascular flow in response to exercise training mediated by serum BDNF concentrations.

ACKNOWLEDGMENTS

We thank the participating volunteers and acknowledge all clinical study site personnel who contributed to this trial and Cléber Rene Alves for helpful discussion.

We thank the Military Police of São Paulo State for absolute cooperation and diligence. The department provided the participants and coordinated the exercise training protocol. The authors state no conflicts of interest.

REFERENCES


EXERCISE TRAINING AND Val66Met BDNF POLYMORPHISM


