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


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**Running Head:** Exercise training and Val66Met BDNF polymorphism

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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 3 times/week for 4 months. We evaluated pre and post-exercise training serum BDNF and proBDNF concentration, heart rate (HR), mean blood pressure (MBP), forearm blood flow (FBF), 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 (VO2peak) 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=-0.56, 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.

**Keywords:** BDNF Val66Met polymorphism, exercise training, vascular reactivity.
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INTRODUCTION

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 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 has implications in the health of other tissues. Indeed, besides the hippocampus, the circulating BDNF is produced by a number of peripheral non-neuronal 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 release (8). The increase in vasodilation after exercise training is endothelium-dependent (42) primarily by the shear stress stimulation (12) and by other mechanisms like enhanced acetylcholine (35) and circulating catecholamines (43). Nevertheless, among several genetic variations, it is not known whether the Val66Met polymorphism could affect an individual’s vascular response to exercise training. Nevertheless, among several genetic variations, it is not known whether the Val66Met polymorphism could affect an individual’s vascular response to aerobic exercise training.

To our knowledge, to date there has been no prospective or clinical study that has examined the effects of exercise on the BDNF/proBDNF ratio. Moreover, it is not known whether the BDNF/proBDNF response would impact on peripheral vascular reactivity in human beings. Thus, we aimed to test the hypotheses that the serum BDNF levels would modulate peripheral blood flow, and that the presence of the allele Met in the BDNF Val66Met polymorphism would impair the gain in vasodilation achieved by aerobic exercise training.
MATERIALS AND METHODS

Subjects
The study overview, participant flow chart, study visits, and evaluations are demonstrated in Figure 1. There were 317 pre-selected 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 years). 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 months prior to enrollment in the study, and abstained from consuming caffeine and alcohol one 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, pro-BDNF) by enzyme-linked immunosorbent assay (ELISA), sandwich, using kits R & D Systems (Minneapolis, MN, USA). Into each well of the ELISA plate was added 100 uL of a solution containing monoclonal antibody to BDNF, pro-BDNF diluted in PBS solution (capture antibody). The plates were incubated for at least 12
hours at 4°C. The plates with nonadherent antibodies were discarded by inversion and washing in PBS-0.1% Tween (Sigma-Aldrich, MO, USA). Then, the plates were blocked with a solution (200 uL/well) containing bovine serum albumin (BSA) 1% (Sigma-Aldrich) for 2 hours at room temperature. After the plates were washed further (0.1% Tween-PBS), 100 uL of sample or standard protein was added to each well. The plates were incubated for at least 12 hours at 4°C and then washed (PBS-Tween 0.1%). After lavage, the plates were incubated with biotin-conjugated antibodies diluted in 0.1% BSA for 2 hours at room temperature. Then, after further washing (PBS-Tween 0.1%), 100 uL/well of peroxidase conjugated streptavidin was added to the plates, which were incubated for 30 minutes at room temperature. Finally, after further washing (PBS-Tween 0.1%), the chromogen o-phenylene-diamine (OPD) (Sigma-Aldrich) was added to the plates, in the absence of light. The reaction was stopped with 1M 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 single nucleotide polymorphism was to be amplified by PCR with the primers 5' - TGATGACCATCCTTTTCCTT- 3' and 5' CACTGGGAGTCCAATGC - 3'. Each reaction uses 10 ng of genomic DNA, 0.5 pM of each primer oligonucleotide, 1 x uL 10 PCR buffer, 250 uM dNTPs, 3 mM MgCl2, and 0:25 i-Star unit taq DNA polymerase and Milli-Q water to a total volume of 10 uL reaction. The amplification protocol consisted of a denaturing step at 95°C for 5 minutes followed by a cycle of denaturation at 95°C for 30 seconds, annealing at 55°C for 1 minute and extension at 72°C for 1 minute repeated 30 times and a final extension at 72°C for 7 minutes. The Val66Met
BDNF gene polymorphism (rs6265) was genotyped with the 2-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 Company, 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 VO₂ at the end of the exercise period in which the subject could no longer maintain the running exercise. This method is considered the gold standard for assessing patients’ exercise capacity (45). Anaerobic threshold and respiratory compensation point were determined as previously described (1). The reproducibility of the peak VO₂ measured at a different time interval in the same individual expressed as mL/kg/min in our laboratory was r = 0.95. Heart rate was continuously monitored by ECG, and blood pressure was measured with a sphygmomanometer. These measurements were repeated after 16 weeks 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 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 wrist and upper arm. At 15-sec intervals, the upper cuff was
inflated above venous pressure for 7-8 sec. Forearm blood flow (mL/min/100mL tissue) was determined on the basis of a minimum of 4 separate readings. Forearm vascular resistance (mm Hg/mL/min) was calculated by dividing forearm blood flow by mean arterial pressure. The reproducibility of forearm blood flow 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 (Dixtal, DX 2710; 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 was performed by heart rate 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, Finland), 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 3 tracks, the components of the frequency band of from 0.04 to 0.15 Hz are considered low frequency (LF), 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 (VLF) was compensated by software features (44).

**Experimental protocol.** At least 2h after a light meal without caffeine, the subjects were positioned supine and electrocardiogram leads were placed on the chest. Cuffs for forearm blood flow 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 forearm blood flow, blood pressure, and heart rate were recorded for 3 min. Moderate static handgrip exercise at 30% of maximal voluntary contraction (MVC) was then performed for 3 min, followed by a recovery period of 3 min (Figure 2). Forearm blood flow, blood pressure, and heart rate were recorded continuously during handgrip exercise. Mean blood pressure was measured every minute, and forearm blood flow was measured every 15 secs (1). All studies were performed in a temperature-controlled (21°C) room, between 8:00-10:00 AM (45).

**Exercise training protocol.**
Individuals participated in 90-min moderate/intense aerobic exercise training sessions, 3 times weekly, beginning at 10h00 AM, supervised by an exercise specialist for 4 months. Exercise intensity was individually graded according to heart rate based on the aerobic/anaerobic thresholds obtained in the cardiopulmonary exercise testing. Exercise training consisted of 2 months of moderate to intense aerobic training, using heart rate levels at the anaerobic threshold
toward the heart rate achieved at the respiratory compensation point. In the last 2 months of the protocol, the exercise training intensity remained mostly at heart rate 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).

Statistical Analysis

Data are presented as mean ± SE. The distribution of all absolute measures of BDNF and proBDNF was normalized using a logarithmic transformation. BDNF and proBDNF concentrations, BDNF/proBDNF ratio, cardiopulmonary exercise test analyses and FBF and FVR pre and post-aerobic exercise training were analyzed using 2-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 FVC data are expressed as area under the curve (AUC).

RESULTS

The study overview, participant flow chart, study visits, and evaluations are described in Figure 1. The pre-selected volunteers were submitted for 2 visits. In the pre-exercise training visit 1 the blood sample was collected and stored for future measures of BDNF levels. In the post-exercise 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 5 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 (Figure 3, Panel B), MBP, HRV, FBF and FVR at rest (Table 2) and in response to handgrip exercise (Figure 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 months of aerobic training, the subjects had an increase of at least 8% in peak VO$_2$ (mL/kg/min) and a similar trend toward a fall in HR, thus the 2 genotype groups had a significant, similar increase in functional capacity (Figure 3, Panel A) and a significant, similar decrease in resting heart rate (Figure 3, Panel B). No differences occurred in the post-training 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) (Figure 5). In addition, in the post-training 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, Figure 4, Panel A) and decreased FVR (Interaction, P=0.01, Figure 4, Panel B) in response to the handgrip exercise test (as AUC). However, after endurance training, no changes occurred in HR (Figure 3, Panel B) 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 pre-intervention values) between variables, and the individuals were matched in pre and post-training 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, Figure 6, Panel A). In addition, there was a negative association between BDNF/proBDNF and FVR as AUC (r = -0.58, P<0.001, Figure 6, Panel B).
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 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 metallo-proteinases (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 (LDL) to the intima, which causes decrease in the vasodilation endothelium-dependent (37). Some recent studies have shown that the increases in BDNF levels resulting by exercise are accompanied by reductions in basal serum vascular cell adhesion molecule 1 (VCAM-1) and serum tumor necrosis factor α (TNF-α), which produces attenuation of inflammation and prevents atherosclerosis (52).

Interestingly, despite the BDNF Val66Met polymorphism, exercise training caused similar improvement in the functional capacity (VO₂peak) and a decrease in resting HR in both groups (Figure 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 (Figure 5), increased significantly only in Val66Val individuals, when 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 FBR and FVR expressed as area under the curve increased significantly, whereas the same response was not found in Val66Met individuals (Figure 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 endothelium vascular wall structures that produce vasoactive substances, like nitric oxide (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 occurs the release of ATP in erythrocytes and adenosine formation, which vasodilatory effect is prostacyclin and NO-depends (26). A connection has been established between the functional capacity and vascular prostacyclin release (51), being prostacyclin production accompanied by increase in NO. However, since the shear stress-induced NO production leads to a suppressant effect on prostacyclin production in physiological conditions (33), in the present study NO is in fact the main vasoactive agent.

Our results demonstrate that probably 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 ANS. 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 (Figure 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.

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Disclosure

Funding sources had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.
REFERENCES


Table 1. Baseline characteristics

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Values are mean±SE. BMI, body mass index.
Table 2. Hemodynamic and BDNF data

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Values are mean±SE. 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 pre training period.
Legends

Figure 1. Study overview. Participant flow chart, study visits, and evaluations.

Figure 2. Timeline of experimental protocols (see Experimental Protocol for explanation).
FBF=forearm blood flow. BP=blood pressure. HR=heart rate.

Figure 3. A: Aerobic fitness gains represented by the VO$_2$peak. *$P<0.05$ vs. pre.
B: Improvements in cardiovascular capacity represented by HR. *$P<0.05$ vs. pre.

Figure 4. A: The FBF AUC responsiveness mediated by physical exercises. *$P<0.05$ vs. pre.
B: The FVR AUC responsiveness mediated by physical exercises. *$P<0.05$ vs. pre.

Figure 5. BDNF/proBDNF ratio behavior ($\log_{10}$ transformed values) in response to physical exercises. *$P<0.05$ vs. pre; †$P<0.05$ vs. Val66Met.

Figure 6. A: Positive correlation between BDND/proBDNF ratio and FBF AUC amplifications, modulated by physical exercises. R=0.64; $P<0.001$. B: Negative correlation between BDND/proBDNF ratio and FVC AUC amplifications, modulated by physical exercises. R=0.58; $P<0.001$. 


Pre-selected sample = 317

Visit 1
Blood test
Plethysmography
Visit 2 (one week later)
Cardiopulmonary exercise test
Exercise training protocol
Visit 1
Blood test
Plethysmography
Visit 2 (one week later)
Cardiopulmonary exercise test
Final sample = 304

Genotypic frequencies

Val66Val
N=221
Sample% = 72.7

Val66Met
N=78
Sample% = 25.7

Met66Met
N=5
Sample% = 1.6

Val66Val
N=221
Sample% = 72.7

Val66Met
N=83
Sample% = 27.3