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## MODERATE-INTENSITY INTERVAL TRAINING INCREASES SERUM BRAIN-DERIVED NEUROTROPHIC FACTOR LEVEL AND DECREASES INFLAMMATION IN PARKINSON'S DISEASE PATIENTS

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It has been demonstrated that physical training increases serum brain-derived neurotrophic factor (BDNF) in healthy people. The aim of this study was to establish the effect of physical training on the basal serum level of the BDNF in the Parkinson's disease patients (PD patients) in relation to their health status. Twelve PD patients (mean  $\pm$  S.E.M: age  $70 \pm 3$  years; body mass  $70 \pm 2$  kg; height  $163 \pm 3$  cm) performed a moderate-intensity interval training (three 1-hour training sessions weekly), lasting 8 weeks. Basal serum BDNF in the PD patients before training amounted to  $10,977 \pm 756$  pg  $\cdot$  mL<sup>-1</sup> and after 8 weeks of training it has increased to  $14,206 \pm 1256$  pg  $\cdot$  mL<sup>-1</sup> (*i.e.* by 34%,  $P=0.03$ ). This was accompanied by an attenuation of total Unified Parkinson's Disease Rating Scale (UPDRS) ( $P=0.01$ ). The training resulted also in a decrease of basal serum soluble vascular cell adhesion molecule 1 (sVCAM-1) ( $P=0.001$ ) and serum tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) ( $P=0.03$ ) levels. We have concluded that the improvement of health status of the Parkinson's disease patients after training could be related to the increase of serum BDNF level caused by the attenuated inflammation in those patients.

**Key words:** *brain-derived neurotrophic factor, training, Parkinson's disease, exercise, inflammation, oxidative stress, vascular cell adhesion molecule, tumor necrosis factor alpha*

### INTRODUCTION

It is well documented that regular physical activity has beneficial effect on health status of people and it is recommended in prevention and treatment of various pathological conditions especially cardio-pulmonary metabolic and locomotors insufficiencies (1). Beginning with the pioneer discovery by Nepper *et al.* (2) showing that physical exercise up-regulates the brain-derived neurotrophic factor (BDNF) gene expression in the brain the popularity of research concerning the effect of physical exercise on central nervous system has significantly increased. It has been shown that BDNF which is widely expressed in various parts of the human brain (3) is involved in several processes determining its functioning including: synapse development and plasticity, neuronal connectivity as well as promotes the development of immature neurons and enhances the survival of adult neurons (4, 5). Moreover, it has been shown that the exercise-induced enhancement of brain BDNF level in the hypothalamus was related to an improvement of cognitive function in rodents (6).

It has been demonstrated that BDNF can cross the blood-brain barrier in both directions *i.e.* from the side of brain to the periphery and from the blood to brain *via* the high capacity saturable transporter system (7). Therefore, it is considered that serum BDNF levels reflect the BDNF concentration in the brain (8, 9). It has been shown, that physical training can significantly increase plasma and/or serum BDNF level in healthy people (10, 11), although some researchers reported no effect of various kind of

physical exercises on basal BDNF level (12, 13). Little is known about the basal BDNF level in blood of patients suffering from neurodegenerative diseases such as Parkinson disease (PD) although it has been recently reported that basal serum BDNF level in the PD patients is significantly lower than in controls (14). PD is a neurodegenerative disorder particularly characterized by the loss of dopaminergic neurons in the substantia nigra (15) resulting in resting tremor rigidity, bradykinesia and gait disturbances in PD patients (16, 17). The background of the dopaminergic neurons loss in substantia nigra is poorly understood, however it is considered, that oxidative stress and inflammation are involved in the pathogenesis of Parkinson's disease (18, 19). The importance of the neuroinflammatory mechanisms has been confirmed in the post-mortem and *in vivo* studies (20). It has been demonstrated, that in the PD patients an increased expression of pro-inflammatory mediators is accompanied by a presence of activated microglial cells and T lymphocytes in substantia nigra, which clearly show an involvement of innate and adaptive immunity in the affected brain regions (21). As suggested the activated microglial cells through a release of pro-inflammatory cytokines and through a direct or indirect release of ROS have negative impact on the substantia nigra (22). Moreover, in the substantia nigra of PD patients higher level of biomarkers of reactive oxygen species oxidative stress such as 4-hydroxy-2,3-nonenal, 8-hydroksyguanosine and lower glutathione (GSH) levels has been found when compared to the controls (23). Based on the post mortem studies it is suggested that the earliest sign of Parkinson disease is the axon degeneration and

therefore the axon re-growth might be the most appropriate goal of the early therapy in PD (24).

It has been also reported that enhanced oxidative stress and inflammation can decrease BDNF level (25). Moreover, a relationship between the inflammation and BDNF level has been reported in varied experimental models (26-28). Therefore, it seems to be the case that physical activity potent to modify the level of oxidative stress and inflammation can influence serum BDNF level and the clinical picture of Parkinson's disease. It has been reported that various kinds of rehabilitation programs involving physical activities seem to have beneficial effect on PD patients (29-32), however, the underlining mechanism remains unknown. It is postulated, based on animal model, that neuroprotective effect of physical activity in PD is related to activation of BDNF signaling pathway (33). However, surprisingly the knowledge concerning the effect of physical exercise training on serum BDNF level in the PD patients is very poor. Therefore, in the present study we have aimed to evaluate the effect of 8 weeks of regular physical training in PD patients on serum BDNF level. Furthermore, we have hypothesized that the training-induced elevation of basal serum BDNF level will be accompanied by an improvement of health status and attenuation of inflammation and lipid peroxidation in the Parkinson's disease patients.

PATIENTS AND METHODS

Participants

The subjects were informed about the aim of the study, familiarized with training environment and gave their written

consent prior to the study that had been approved by the local Ethics Committee and according with the Declaration of Helsinki.

Twelve patients with idiopathic PD (women: n=5 men: n=7: mean ± S.E.M: age 70 ± 3 years; body mass 70 ± 2 kg; height 163 ± 3 cm, *Table 1*) participated in the present study. According to the Hoehn and Yahr scale (34) PD patients were mildly to moderately affected (score of 1–3) and one of them severely affected with score 4.0 (PD#7 during the post-training session). Detailed clinical characteristics of the PD patients are given in *Table 2*. The exclusion criteria for participation in the training program were: any cardiovascular and respiratory systems' symptoms (based on physician's subjects examination) and also motor deficits (related to PD stage or orthopedic and post-traumatic symptoms) that could limit the participation of PD patients in training sessions.

Clinical assessment of Parkinson's disease

PD patients were tested clinically by experienced neurologist blinded to other patients' results based on the Hoehn and Yahr scale and Unified Parkinson's Disease Rating Scale (UPDRS) (34).

Experimental procedure

The group of studied PD patients was tested twice: in April and in July (before and after 8 weeks of training). Before and after 8-weeks of training blood samples were taken from all subjects for an appropriate analysis. During both testing sessions the PD patients were in their medication off-phase (during intensification of PD symptoms after 12 hours (overnight) of

Table 1. Characteristics of the Parkinson's disease patients (PD, n=12).

Variable	mean ± SEM	Me	min ÷ max
Age (years)	70 ± 3	68	58 ÷ 88
Body mass (kg)	70 ± 2	67	61 ÷ 84
Height (cm)	163 ± 3	162	149 ÷ 182
Disease duration (years)	8.5 ± 1.3	7.5	2 ÷ 16
Hoehn and Yahr (points)	2.3 ± 0.2	2.5	1 ÷ 3

Data are presented as mean ± SEM, Me, min÷max. See: *Statistical analysis*.

Table 2. Clinical characteristics of the Parkinson's disease patients (PD, n=12).

PD patient (No.)	Sex (F/M)	Affected upper limb (R/L)	Affected lower limb (R/L)	Hoehn and Yahr (points)
PD#1	M	R	L	2.5
PD#2	M	R	R	1.0
PD#3	F	L	L	2.5
PD#4	M	R	R	2.5
PD#5	F	R	R	1.5
PD#6	M	R	R	3.0
PD#7	M	L	L	1.5
PD#8	M	L	L	2.5
PD#9	F	L	L	3.0
PD#10	M	R	R	3.0
PD#11	F	L	L	3.0
PD#12	F	R	L	2.0

Abbreviations: PD - Parkinson's disease; No. - number; F - female; M - male; R - right; L - left.

anti-parkinsonian drugs withdrawal), however, they were in their medication on-phase during training sessions (beneficial effect of anti-parkinsonian medication: mainly levodopa and within some patients piribedil, ropinirol). Medication doses for each PD patient remained the same as normally administered by their leading neurologist (disease stage-adjusted) and were constant throughout the whole 8-weeks training period.

#### Training protocol

The PD patients performed 8-weeks training program which consisted of three 1-hour training sessions weekly (total 24 training sessions). This training was performed on a stationary cycle ergometer (MONARK Ergomedic 874E, Sweden) that allowed to measure cadence (rpm) and power output (W). Each 1-hour training session consisted of 10-minutes warm-up (at slow voluntary speed), 40-minutes of moderate-intensity interval exercise and 10-minutes of cool-down phase (with slow voluntary speed). The moderate-intensity interval training session (IT) consisted of 8 sets of 5 minute interval exercise including 3-minutes cycling at 80–90 rpm (fast phase of IT) and 2-minutes cycling less than 60 rpm (slow phase of IT). The heart rate (HR) was measured by Polar system (Polar Electro Oy, Kempele, Finland) cadence and power were monitored and collected during each training session (warm up, IT, cool down phases). Training supervisor adjusted the resistance at the cycle ergometer for each patient to ensure cycling at each patient's target heart rate (THR) and with appropriate cadence. The patients cycled at 60–75% of their individualized  $HR_{max}$ , which was predicted for each patient based on the formula developed by Tanaka *et al.* (35). The PD patients were encouraged to cycle faster (80–90 rpm or 30% faster than their freely chosen pedaling rate) during the fast part of the IT. Each patient increased its THR every 2 weeks by 5% (60% of the  $HR_{max}$  during the first two weeks, 65% during the third and fourth week, 70% during the fifth and sixth week and 75% of  $HR_{max}$  during the seventh and eighth week of training period). Since the  $HR_{peak}$  in the PD patients was reported to be by about 5% lower than the  $HR_{peak}$  in the healthy individuals at similar age (36), most likely our patients during the applied training exercised at slightly higher percentage of  $HR_{max}$  than that calculated from the formula developed by Tanaka *et al.* (35). Nevertheless, taking also into consideration the results reported by Protas *et al.* (36) the highest HR reached by our patients during this training was still by about 20% lower than their predicted  $HR_{max}$ .

The supervisor provided water and any additional help during training session. The eight intervals' averaged values of the HR, cadence and power of each training session for each subject during the fast and slow phases of IT were calculated. Then the average

value of the 24 training sessions for each of the parameter was calculated. Detailed information about moderate-intensity interval forced training parameters for each PD patient is given in Table 3. This interval training was well tolerated by the patients and it could be called interval training with moderate-intensity.

#### Blood sampling and measurements

In the studied groups blood samples for measurements of blood variables were taken from the antecubital vein at rest in the morning hours between 8:00–10:00 a.m. in the fasting state. Blood for plasma measurements was collected in tubes (SARSTEDT AG & Co, Germany) containing the appropriate anticoagulant solution (potassium EDTA) and centrifuged at  $435 \times g$  for 15 min at 4°C. Blood for serum measurements was collected in anticoagulant-free tubes with the clotting activator (SARSTEDT AG & Co, Germany) centrifuged at  $653 \times g$  for 10 minutes at 4°C. Plasma and serum were stored at  $-80^{\circ}C$  for further analysis.

#### Hematological parameters

The total and differential blood cell counts were analyzed using standard hematological procedures and a dedicated analyzer - model ADVIA 60 (Bayer Corporation, 511 Benedict AV, Tarrytown, New York, USA).

#### Serum brain-derived neurotrophic factor measurements

Blood for serum BDNF measurements [BDNF]<sub>s</sub> was collected in anticoagulant-free tubes with the clotting activator (SARSTEDT AG & Co, Germany) and kept for 1 hour on ice (at a temperature of about 4°C). After that blood was centrifuged to isolate the serum at  $653 \times g$  for 10 minutes at 4°C. The serum was assayed for BDNF with an enzyme-linked immunosorbent assay (ELISA) Kit (Promega, Wallisellen, Switzerland) after appropriate dilution with Block and Sample solution (provided with the kit). Amicroplate reader (BioTek Instruments, USA) set at 450 nm was used to determine BDNF values (intra-assay and interassay variation were less than 9% and 15% respectively) (37).

#### Serum soluble vascular cell adhesion molecule 1 and tumor necrosis factor- $\alpha$

Soluble vascular cell adhesion molecule 1 (sVCAM-1, human Quantikine ELISA kit, DVC00, R&D System) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ , human Quantikine HS ELISA, HSTA00D, R&D Systems) concentrations were assayed in serum according to manufacturer's instructions.

Table 3. Parameters of the moderate-intensity interval training (IT) of the studied Parkinson's disease patients (n=12).

PD patient (No.)	Fast phase of IT			Slow phase of IT			All (fast and slow) phases of IT		
	Cadence (rpm)	PO (W)	HR <sub>max</sub> (%)	Cadence (rpm)	PO (W)	HR <sub>max</sub> (%)	Cadence (rpm)	PO (W)	HR <sub>max</sub> (%)
PD#1	77	44	64	44	23	57	61	34	60
PD#2	81	93	74	45	54	65	63	74	70
PD#3	48	2	54	35	1	51	42	2	52
PD#4	80	75	77	47	42	69	64	59	73
PD#5	79	67	80	45	35	71	62	51	75
PD#6	83	96	76	50	57	72	66	77	74
PD#7	58	6	58	40	5	57	49	6	57
PD#8	70	13	72	43	6	63	56	10	68
PD#9	53	2	54	36	1	49	44	2	52
PD#10	77	45	78	44	24	73	60	34	75
PD#11	43	0	61	34	0	58	39	0	60
PD#12	61	32	68	38	22	59	50	27	63
<b>x ± SEM</b>	<b>68 ± 4</b>	<b>40 ± 10</b>	<b>68 ± 3</b>	<b>42 ± 2</b>	<b>23 ± 6</b>	<b>62 ± 2</b>	<b>55 ± 3</b>	<b>31 ± 8</b>	<b>65 ± 3</b>

Abbreviations: PD - Parkinson's disease; No. - number; PO - power output; %HR<sub>max</sub> - percentage of maximal heart rate

### Serum cortisol

Serum cortisol level was measured by means of electrochemiluminescent immunoassay (ECLIA) by Roche Diagnostics Ltd (Switzerland) using cobas e411 Roche Diagnostics Ltd analyzer (Switzerland).

### Plasma 8-epi-prostaglandin $F_{2\alpha}$ ( $F_2$ isoprostanes) $_{pl}$

8-epi-prostaglandin  $F_{2\alpha}$  concentration in plasma samples [ $F_2$ isoprostanes] $_{pl}$  was assayed by 8-Isoprostane EIA Kit 516351, Cayman Chemical according to the manufacturer's instructions.

### Plasma syndecan-1

Syndecan-1 concentration in plasma samples was assayed using human CD138/Syndecan ELISA kit 950.640.192, Diaclone according to manufacturer's instructions.

### Statistical analysis

The results are presented as means  $\pm$  S.E.M., median (Me), minimum  $\div$  maximum (min $\div$ max). The significance was set at  $P < 0.05$ . Statistical significance of the differences for paired samples was tested using the non-parametric Wilcoxon-signed-rank test (before vs. after the training). Non-asymptotic exact two-sided  $P$  - values are presented. The statistics were done using the statistical packet StatXact 9 (Cytel software, Corporation Cambridge, MA, USA) and STATISTICA 10.0 (StatSoft, Tulsa, OK, USA).

## RESULTS

### Total Unified Parkinson's Disease Rating Scale in Parkinson's disease patients group before and after the training

Unified Parkinson's Disease Rating Scale (UPDRS) total score in patient before training amounted to  $48.9 \pm 4.3$  points

and it decreased significantly after the training to  $38.1 \pm 3.9$  points ( $P=0.01$ ) (Fig. 1).

### Brain-derived neurotrophic factor

In the group of patients ( $n=12$ ) basal serum BDNF level [ $BDNF$ ] $_s$  before training amounted to  $10\,977 \pm 756$  pg  $\cdot$  mL $^{-1}$  and after 8 weeks of training it has increased significantly ( $P=0.03$ ) by about 34% to  $14\,206 \pm 1\,256$  pg  $\cdot$  mL $^{-1}$  (Fig. 2).

### Blood platelets

Basal PLT level in PD patients before the training has amounted to  $184 \pm 13$  K  $\cdot$  L $^{-1}$  and after 8 weeks of training its value amounted to  $197 \pm 12$  K  $\cdot$  L $^{-1}$  and was not significantly different from pre-training level ( $P=0.91$ ).

### Serum cortisol level

No effect of the 8 weeks of training on the serum cortisol level ( $P=0.30$ ) in the group of patients was found ( $514 \pm 47$  nmol  $\cdot$  L $^{-1}$  vs.  $480 \pm 43$  nmol  $\cdot$  L $^{-1}$ , respectively before and after training).

### Plasma $F_2$ isoprostanes level

Basal [ $F_2$  isoprostanes] $_{pl}$  after the training was not significantly different ( $P=0.38$ ) than before training ( $39.8 \pm 2.6$  vs.  $40.3 \pm 2.9$  pg  $\cdot$  mL $^{-1}$ , respectively before and after the training).

### Plasma syndecan-1 level

The 8 weeks of training did not affect ( $P=0.91$ ) the basal plasma syndecan-1 level ( $22.28 \pm 1.86$  vs.  $22.24 \pm 2.23$  ng  $\cdot$  mL $^{-1}$ , respectively before and after the training) in the group of patients.

### Serum vascular cell adhesion molecule 1 level

The 8 weeks of training resulted in a decrease in [ $sVCAM$ ] $_{ss}$  level ( $P=0.001$ ) in the studied group of patients by about 21%

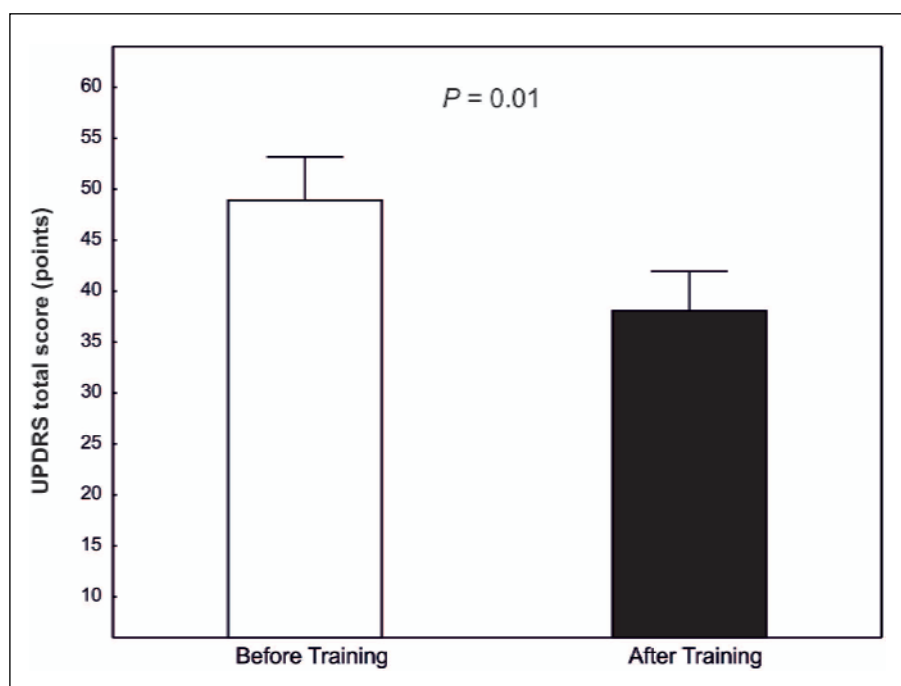


Fig. 1. Total Unified Parkinson's Disease Rating Scale (UPDRS) score in the PD patients ( $n=12$ ) before and after 8 weeks of training.

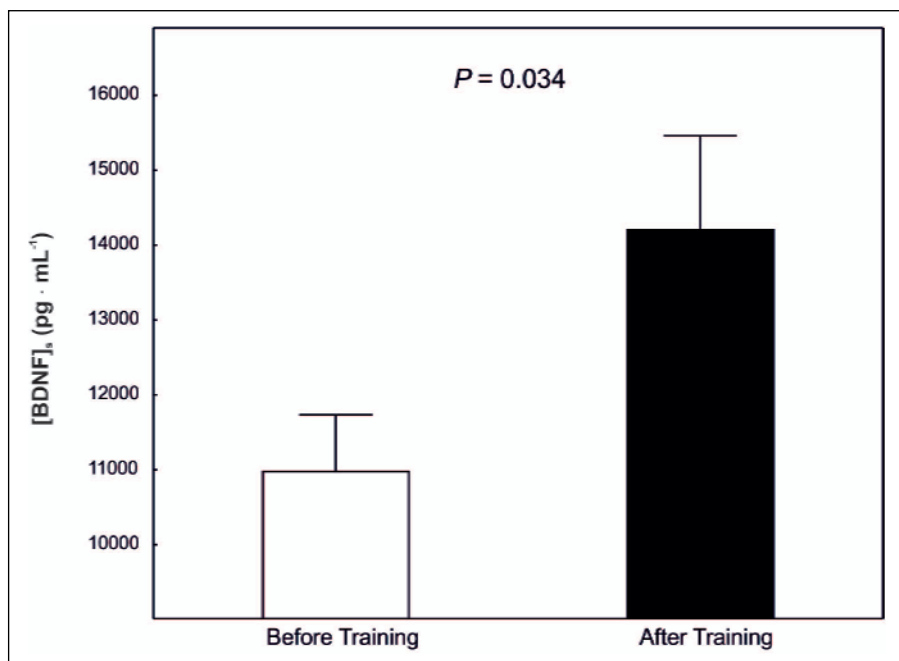


Fig. 2. Serum brain-derived neurotrophic factor level [BDNF]<sub>s</sub> in the PD patients (n=12) before and after 8 weeks of training.

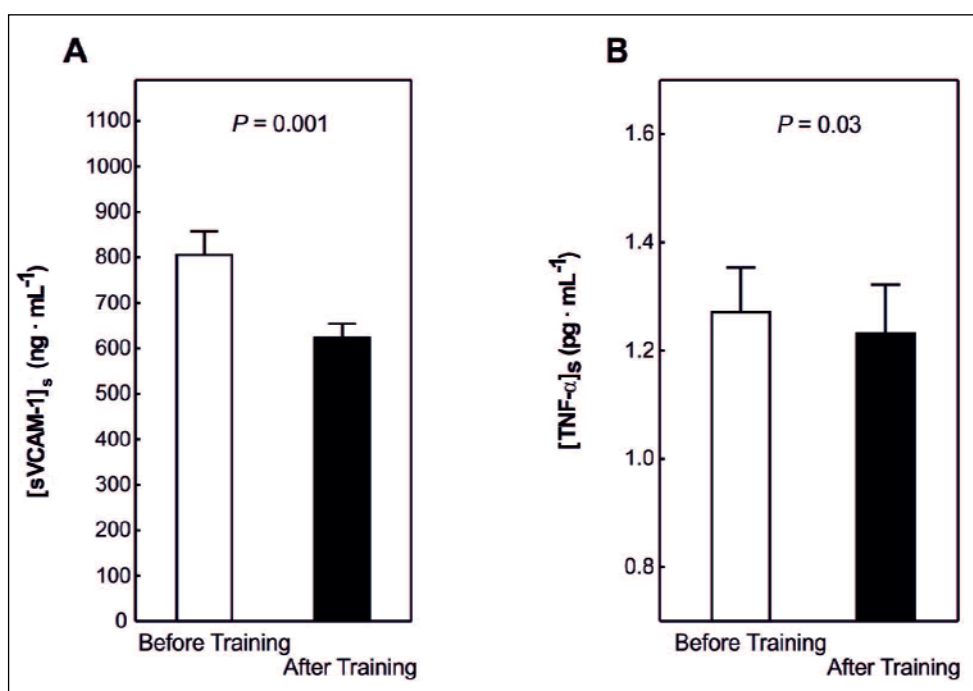


Fig. 3. Basal serum soluble vascular cell adhesion molecule-1 level [sVCAM-1]<sub>s</sub> in the PD patients before and after 8 weeks of training (panel A) and basal serum tumor necrosis factor level [TNF-α]<sub>s</sub> in the PD patients before and after 8 weeks of training (panel B).

(805 ± 52 ng · mL<sup>-1</sup> vs. 623 ± 31 ng · mL<sup>-1</sup>, respectively before and after training) (Fig. 3A).

#### Serum tumor necrosis factor

Basal serum TNF-α ( $P=0.03$ ) significantly decreased after 8 weeks of training in the group of patients (by about 7%, Fig. 3B).

## DISCUSSION

In this study we have found that moderate-intensity interval training (IT) performed 3 times per week for about an hour involving cycling at high pedaling rates improves the clinical

status of Parkinson's disease patients as judged by a decrease in the UPDRS total score (Fig. 1). The main and original findings of this study are as follows: a) this training resulted in an increase of the basal serum BDNF level by about 34% ( $P=0.03$ ) (Fig. 2) in PD patients; b) attenuated inflammation (a decrease in serum sVCAM-1 and serum TNF-α levels, Fig. 3) was not harmful to the endothelial glycocalyx (no changes in plasma syndecan-1 level) and did not result in enhanced oxidative stress as judged by no changes in [F<sub>2</sub> isoprostanes]<sub>pl</sub>.

Training-induced increase in serum or plasma BDNF levels in healthy subjects has been published before (12, 13). However, the training-induced increase in serum BDNF level in the PD patients shown in this study to our best knowledge is the first reported finding in this area of research. The mechanism by

which training increases serum BDNF level in humans remain unknown. Since the BDNF in blood is mainly stored in blood platelets (38) one could speculate that the training-induced increase in the serum BDNF level in the PD patients is simply caused by an increase in their blood platelets count. However, in the present study, blood platelets count in the PD patients before as well as after training did not change ( $P=0.91$ ). Therefore, we can exclude an increase in blood platelets count as the explanation of the observed increase in serum BDNF level in the PD patients. More likely explanation of the training-induced increase in serum BDNF level in our patients is an increase in its production in the brain as well as in periphery. It has been recently shown by Seifert *et al.* (39) that endurance training lasting 3 months resulted in enhanced resting release of BDNF from the brain in young, healthy men, which suggest, that the observed in our study training-induced elevation of the basal serum BDNF level in the PD patients could be also caused by an increase of its release from the brain. Moreover, we cannot exclude an increase of BDNF release after training from other peripheral tissues including endothelium as reported recently by Prigent-Tessier *et al.* (40).

In view of literature data the training-induced increase in serum BDNF level might contribute to the improvement of patients health status in several ways. First of all, it is well documented that BDNF plays an important role in functioning of the brain (5, 41, 42). Moreover, a recent studies have shown that BDNF stimulates synthesis of prostacyline ( $\text{PGI}_2$ ) in cerebral arteries (43). Prostacyline plays an important role in endothelium-dependent relaxation and also possess anti-platelet, anti-atherogenic, vasculoprotective and cardioprotective properties (44). Indeed, it was reported that patients with acute coronary syndromes have reduced levels of BDNF in plasma (45). It has been also recently postulated that the already established role of BDNF in synaptic plasticity and synaptic growth could be a challenge for clinical therapies in neurodegenerative disorders (5). Therefore, the training-induced increase in the serum BDNF level in the PD patients (*Fig. 2*) could be directly linked to the observed improvement of health status of our patients (*Fig. 1*). Interestingly, Ziebel *et al.* (46) recently reported that serum BDNF levels in patients with parkinsonism correlates positively with striatal dopamine transporter (DAT) availability. These authors concluded that in patients with striatal dopaminergic neurodegeneration serum BDNF levels decrease along with loss in striatal DAT binding. This suggest that the training-induced increase in serum BDNF level might have protective effect on dopaminergic neurons in the PD patients.

It should be noticed that not all single bouts of exercise or training programs applied to varied groups of subjects resulted in a significant increase of serum or plasma BDNF levels in humans (12, 13). The reason for this is unknown, but it seems to be very likely that the intensity and training work load applied seem to play an important role in the magnitude of BDNF response to training (11-13, 47, 48). It seems to be the case that there is a threshold level of exercise intensity below which no effect of physical exercise on serum or plasma BDNF level can be found. Interestingly, it has been reported that in case of PD patients training programs involving high velocity cycling exercise is more effective than a program involving cycling at low pedaling rate only (49). In the present study, we have applied interval training composed of both low and high velocity cycling which for the PD patients was not always easy to follow. The choices of the high pedaling rate program was based on the earlier findings (49) showing that the physical training involving high pedaling rate is more beneficial to the PD patients than that based on low pedaling rates. Indeed, the previous human studies (40, 49) have suggested that physical activity exerts beneficial

neuroplastic effects in PD patients' central nervous system. The study by Ridgel *et al.* (32) in which a period of eight weeks of training (3 session per week) involving pedaling on a tandem cycle ergometer applied in PD patients showed that an improvement in motor PD symptoms and upper extremities manual dexterity occurred only in these patients who pedaled with higher than voluntary rate (about 90 rpm) but it did not occurred in voluntary pedaling patients (about 60 rpm). Interestingly, the training program involving cycling at high pedaling rates applied in the present study, was indeed effective in increasing basal serum BDNF level and it resulted in an improvement of clinical picture of our patients, as judged by an attenuation of UPDRS total score (*Fig. 1*). However, it should be noticed that the pedaling rate developed by our patients (*Table 3*) was clearly lower than in case of the study by Ridgel *et al.* (32) but even so it was difficult to follow by several of our patients. It should be also noticed that this rather demanding training program had no effect on the basal serum cortisol level in the studied patients ( $P=0.30$ ).

It is still not clear why in case of the PD patients the high velocity cycling is more effective than the slow one. There could be simple explanation that cycling at a given power output using high pedaling rate requires more energy than cycling at low frequency (50). Moreover, while cycling at high vs. slow pedaling rates at a given power outputs the PD patients could generate the required power outputs at greater reserves of muscle force generating capabilities (50). This strategy could allow the PD patients to perform the training program for a longer period of time at relatively high metabolic rates without exhaustion, as in the present study, and could provide sufficient stimulus for adaptation to the applied training.

It has been shown that Parkinson disease is accompanied by inflammation (18, 20-22) and oxidative stress (19, 23). As recently shown by Goldberg *et al.* (51) mitochondrial oxidative stress plays a key role in neurodegeneration in Parkinson disease. In the group of our PD patients training had no impact on the oxidative stress as judged by no changes in  $[\text{F}_2\text{isoprostanes}]_{\text{pl}}$ , ( $P=0.38$ ). However, it should be pointed out that the applied in this study training program resulted in a decrease of basal serum sVCAM-1 and plasma  $\text{TNF-}\alpha$  levels (*Fig. 3*). This indicates that this training did induce some anti-inflammatory responses in the PD patients. We postulate, that the attenuation of the inflammatory responses after training could be at least partly responsible for the observed increase of serum BDNF level after the training (*Fig. 2*) and improvement of clinical picture of our patients (*Fig. 1*). Therefore, for deeper understanding of the potential link between the training-induced attenuation of inflammation with an increase of BDNF level it would be interesting to examine, on animal model, the effect of PPAR- $\gamma$  agonists (rosiglitazone and troglitazone) administration, which mimic the effect of regular exercise (52) and possess anti-inflammatory activity (53, 54), on the BDNF level.

Interestingly, the applied in the present study high pedaling rate endurance training program in PD patients had no harmful effect on the endothelial glycocalyx, as judged by unchanged serum syndecan-1 level ( $P=0.91$ ), considered as a marker of endothelial glycocalyx damage (55).

In conclusion, the moderate-intensity interval training performed 3 times per week (each session lasting 60 min) involving cycling at high pedaling rates improves the clinical status of Parkinson's disease patients as judged by a decrease in the UPDRS total score. This was accompanied by a significant increase of the basal serum BDNF level in the studied patients. The training resulted also in a decrease of serum sVCAM-1 and  $\text{TNF-}\alpha$  levels indicating that this training program attenuated the inflammation in the PD patients. We have concluded that the improvement of health status of the

Parkinson's disease patients after training could be related to the increase of serum BDNF level caused by the attenuated inflammation in those patients.

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