Progress in elucidating neuroimmune connections has created new opportunities for improving the treatment of Parkinson’s disease (PD). In recent years, mind-body therapies have been shown to have positive effects on the immune and nervous systems, but interactions at the molecular level have not been tested. Thus, the main aim of the present study was to investigate the effects of therapy based on visualization and suggestion on the concentrations of IL-6, CRP, DA, BDNF, CoQ10, and TAC in patients with advanced PD.

Eight patients with PD and 8 elderly healthy men (control group) were enrolled in the study. The therapy lasted 19 days and consisted of three parts: individual sessions with the therapist, mixed (therapist and audio-file), and self-training (audio-file). Blood samples were taken before training and at the end of each part.

The expected changes in the investigated markers was observed during therapy: the serum concentration of IL-6 and CRP decreased, whereas DA and BDNF increased, however, this change was observed only after the first part of intervention when the therapy was conducted by the therapist. In the subsequent stages, the levels returned to the baseline. Noteworthy, after the therapy, we observed a significant increase in the motor and intellectual skills of the PD patients. No such changes were observed in the control group.

Mind-body therapy based on visualization and suggestions aimed at changing the concentrations of signaling molecules, which are crucial in the development or treatment of PD, may be an effective element in supporting pharmacological therapy.

Keywords: mind-body therapy, visualization, inflammation, dopamine, BDNF Parkinson’s disease
INTRODUCTION

Many epidemiological, immunological, and genetic studies in recent years have demonstrated that peripheral inflammation may create or exacerbate an inflammatory response in the brain via its mediators, such as inflammatory cytokines (e.g., interleukin (IL)-6), leading to neurodegenerative disorders including Parkinson’s disease (PD) (Träger et al., 2013; Jenner, 2003; Nagatsu et al., 2000). In addition, the expression of pro-inflammatory cytokines in the immune system and microglial cells in the brain may potentiate the degradation processes within the dopaminergic neurons of the substantia nigra. As a result, a reduced level of dopamine (DA) has been observed (Qian, Flood, 2008). Pro-inflammatory cytokines are not only responsible for the escalation of inflammation in the brain but are also related to impaired cognitive function, memory, or mobility (Lindqvist et al., 2013; Scalzo et al., 2010). Moreover, studies have shown the reduced expression of the brain-derived neurotrophic factor (BDNF) in the substantia nigra in patients with PD, accompanied by the deterioration of dopaminergic neurons (Baquet et al., 2005). Scalzo et al. (2010) have suggested that low levels of BDNF in the early stages of the disease may be one of the causes responsible for the development of PD.

So far, no effective treatment has been developed for PD. The main challenge is overcoming the blood-brain barrier (BBB), which protects the brain from unwanted chemicals in the blood but also prevents most drugs from entering the brain from the blood, making it difficult to develop new treatments for brain diseases (Desai et al., 2007).

In recent years, increasing evidence has indicated two-way (top down and bottom up) communication between the nervous, endocrine, and immune systems. These interdependencies underlie the effectiveness of the placebo effect or psychical intervention, such as mind-body therapies (Exton et al., 2000). Since immune functions were proven to undergo classical conditioning, it has become possible to design targeted therapy to achieve desired changes within the immune system. The most commonly used strategies include deep relaxation, visualization, or hypnosis (self-hypnosis) combined with suitable suggestions. Metaphorical images have often been used in these therapies, making it easier to obtain the desired effect (Miller et al., 2001; Gregorson et al., 1996, Wahbeh et al., 2009). The goal is usually to obtain overall improvement in immune function observed as an increase in the number of white blood cells, NK cell activity or changes in the level of immunoglobulins or some hormones (e.g., cortisol) (Gruzelier, 2002a; Gruzelier, 2002b).

Given the complex etiology of PD, which is associated with the limited possibility of treatment or prevention, a properly constructed therapy can be an extremely helpful tool to affect the functioning of the immune and nervous system, and may positively affect the health of patients with PD. Few studies have evaluated the effect of mind-body therapies on people with Parkinsonism and these were concerned mostly with the improvement of balance and motor skills after
movement imagery. Notably, imaginative techniques have minimal cost, no side effects, and can be easily introduced into therapy (Morris et al., 2001; Brain et al., 2001; Tamir et al., 2007).

The therapy used in the present study was constructed on deep relaxation and visualization with elements of suggestions. To the best of our knowledge, this is the first time in this type of psychical intervention that suggestions were focused on specific signaling molecules, some being responsible for the regulation of inflammation (e.g., IL-6), others being extremely important in the treatment of PD (e.g., DA, BDNF, or coenzyme Q10 (CoQ10)).

The aim of the study was to evaluate the effect of mind-body therapy focused on inducing desired changes in the concentration of IL-6, c-reactive protein (CRP) (decrease), and DA, BDNF, and CoQ10, total antioxidant capacity (TAC) (increase) in PD patients and the healthy control.

**MATERIAL AND METHODS**

**Study population**

Eight men diagnosed with PD participated in the study (mean ± SD: age 65.5 ± 5.5 years; height 168 ± 0.05 cm; weight 81.5 ± 8.1 kg; BMI 28.7 ± 3.4). PD patients were recruited through advertising at the Neurological Department of the Hospital in Gorzow Wlkp. Moreover, an eight-person group of healthy older people (age 63.4 ± 4.2 years; height 169 ± 2.1 cm; weight 77.2 ± 7.1 kg; BMI 26.1 ± 8.4) comprised the control group. All patients had blood routine, and they were tested by a physician in the order to exclude severe accompanying conditions before trial entrance. None of the participants (both PD and control) were treated with NSAIDs or corticosteroids or had any acute inflammatory disease. Fulfillment of this condition was extremely difficult, hence this study had a limited number of participants.

**Ethics statement**

The Bioethical Committee at the Poznan University of Medical Science, Poland approved this study before trial commencement. All study participants provided written consent for participation in the study, which was performed in accordance with the provisions of the Helsinki Declaration.

**Motor and cognitive skills**

For the purposes of characterizing patients with PD, a questionnaire was constructed based on questions about general health from the Medical Outcome Study Short Form 20, Unified Parkinson’s Disease Rating Scale, and Weschler’s intelligence test (WAIS-R). A set of selected questions from these tests directly concerned aspects of PD, such as the ability to walk unaided, memory, intelligence, and well-being. The use of such compilation testing was necessary because of the need to shorten the time of additional studies in order to avoid
unnecessary fatigue or irritation with protracted tests. The questionnaire was com-
pleted by PD patients and the control group before and after the therapy. During
the experiment, all participants were asked not to make any changes in their habits,
especially physical activity, which could affect the results of the experiment.

**Intervention (training)**

Mind-body therapy was conducted for 19 days in three parts. The first part con-
sisted of individual therapy sessions led by a specialist in psychological inter-
ventions. The second part was mixed therapy consisting of individual training sessions
and listening to audio files with the training recorded for practice at home. In the
last part of the therapy, participants only listened to the recorded training.

The model of training used had a verbal character, but its main part regarding
suggestion was reinforced with animations showing desired changes in the levels
of the markers selected for the study. Prepared animations showed decreasing
levels of negative particles (i.e., IL-6) and increasing levels of positive particles
(i.e., DA, BDNF, and CoQ10). Before the therapy, participants were lectured
about the role of peptides chosen for the experiment in the etiology and treatment
of PD and were asked to watch the animations 3 days prior to training whenever
possible. Changes in the concentrations of the selected particles were shown in
the animation as tubes with increased or decreased content as appropriate. Each
of the particles undergoing suggestion was assigned a different color to facilitate
their verbal imagery. Participants were also informed about the changes that PD
causes in the substantia nigra and shown animations depicting the process of
restoring the substantia nigra to the correct state. The entire training lasted ap-
proximately 26 minutes.

**The blood samples**

Blood samples were taken from the antecubital veins of all participants before
the training (controls and PD-baseline). Blood samples were also taken at the
end of each part of the training: after 3 days of individual training (PD T-1, C T-
1), after 6 days of mixed training (PD T-2, C T-2), and after another 6 days of
self-training (PD T-3, C-T3). The samples were left at room temperature until the
formation of a clot and then centrifuged at 2500g for 10 min at 4°C. The sepa-
rated serum was frozen and stored at -80°C until analyzed.

**Biochemical parameters**

The total level of serum inflammation markers (IL-6 and CRP) was determined
by immunoenzymatic assay methods using diagnostic kits (DRG International
Inc, USA). The detection limits for IL-6 and CRP were 2 pg/mL (CV 4.4%) and
0.1 mg/L (CV <20%), respectively.

Immmunoenzymatic assay was also used to measure the serum levels of DA
(Sunred, Shanghai) and BDNF (Labs Inc., London). The detection limits were
7.043 nmol/L (CV <12%) and <2 pg/mL, respectively.
Total antioxidant capacity (TAC) was analyzed using a set of fast track TAC (LDN Labor Diagnostica Nord GmbH & Co.KG) with a detection limit of 0.08 mmol/L (CV 3.33%). CoQ10 levels were determined based on a set (Sunred, Shanghai) with a detection limit of 0.751 ng/mL (CV <12%).

**Statistical analysis**

Changes in the serum concentrations of the measured blood parameters in PD patients and control were calculated as the percentage change from the corresponding individual’s baseline concentration. The data were presented as the mean percentage changes ± SD. The ANOVA test and Tukey HSD post hoc test were used to compare the percentage changes in blood markers concentration within the subjects. The ANOVA and Tukey HSD post hoc test were also used to compare the results between pre-and post-training in motor and cognitive skills.

Associations among the measured parameters were analyzed based on the absolute value of measured parameters using Pearson’s linear regression (coefficient, r). Significance was set at P<0.05 in all tests. Statistical analyzes were performed using STATISTICA 9.

**RESULTS**

**Cognitive and motor abilities**

Table 1 presents the variables present in the questionnaire for the pre-training and post-training comparison. As a result of the good health state of the participants enrolled to the control group, we did not observe any changes in measured parameters, however, a significant improvement in the area of intelligence (WAIS-R) was observed. But in the PD patients, the changes in measured parameters were significant almost in all the investigated areas. Significant improvement was observed in the intelligence area, in both subjective and objective assessment. Significant improvement was also observed in the motor aspect, both in terms of self-bathing, toilet, dressing, and walking. The greatest improvement was observed in moving along a straight line and in descending the stairs. The improvement was also observed in terms of well-being (lack of a sense of hopelessness and mood). Inflammatory and neuroimmune markers.

The baseline levels of investigated markers have shown changes specific to Parkinsonism, including elevated levels of IL-6 and CRP and reduced levels of DA and BDNF in relation to the control group, but the differences were not significant (Fig. 1).

Only the CRP serum concentration in PD patients, as a percent change from the baseline, was significantly lower after the first part of training (P<0.05). During subsequent stages the low concentration was still observed, however, the changes were not significant. Furthermore, during the training a decreasing trend in the concentration of IL-6 in PD patients was observed, but the changes were not significant. However, the significant differences were detected in the CRP con-
Table 1. Effect of therapy on motor and cognitive skills

<table>
<thead>
<tr>
<th>Test</th>
<th>Pre-training score</th>
<th>Post-training score</th>
<th>P-value</th>
<th>Pre-training score</th>
<th>Post-training score</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subjective</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Intelligent</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Memory</td>
<td>2.66 ± 0.47</td>
<td>3.83 ± 0.40</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness of thinking</td>
<td>2.83 ± 0.37</td>
<td>3.83 ± 0.40</td>
<td>n.s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planning behavior</td>
<td>3.0 ± 0.0</td>
<td>4.0 ± 0.0</td>
<td>n.s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Self-service</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Eating</td>
<td>3.1 ± 0.37</td>
<td>4.16 ± 0.40</td>
<td>n.s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bath and toilet</td>
<td>3.16 ± 0.68</td>
<td>4.33 ± 0.51</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dressing</td>
<td>2.66 ± 0.74</td>
<td>4.0 ± 0.0</td>
<td>0.01</td>
<td></td>
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<tr>
<td><strong>Walking</strong></td>
<td></td>
<td></td>
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<tr>
<td>Straight line</td>
<td>3.0 ± 1.0</td>
<td>4.1 ± 0.75</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking downstairs</td>
<td>2.83 ± 0.89</td>
<td>4.0 ± 0.63</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking upstairs</td>
<td>2.66 ± 0.74</td>
<td>3.66 ± 0.81</td>
<td>n.s.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Frame of mind</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Happiness</td>
<td>2.66 ± 0.47</td>
<td>3.66 ± 0.51</td>
<td>n.s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of sense of hopelessness</td>
<td>2.66 ± 0.74</td>
<td>3.8 ± 0.40</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mood</td>
<td>2.66 ± 0.74</td>
<td>4 ± 0.40</td>
<td>0.001</td>
<td></td>
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<td></td>
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<tr>
<td><strong>Objective examination of intelligence (WAIS-R)</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Repeating numbers Directly+backwards</td>
<td>10.66 ± 2.06</td>
<td>14.5 ± 2.25</td>
<td>0.000</td>
<td>11.0 ± 0.9</td>
<td>13.7 ± 1.60</td>
<td>0.01</td>
</tr>
<tr>
<td>Similarities</td>
<td>14.33 ± 0.81</td>
<td>18.66 ± 1.03</td>
<td>0.000</td>
<td>10.3 ± 0.81</td>
<td>14.3 ± 3.77</td>
<td>0.01</td>
</tr>
<tr>
<td>Knowledge test</td>
<td>17.15 ± 1.32</td>
<td>19.5 ± 1.22</td>
<td>0.000</td>
<td>19.8 ± 1.72</td>
<td>19.8 ± 1.72</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

There were no changes observed in pre- and post values

Figure 1. Serum levels of circulating markers at baseline in PD patients and control
centration between PD patients and the control group after the first (T-1 PD vs. T-1 C; P<0.005) and the second (T-2 PD vs. T-1 C; P<0.05) part of training. No significant changes in the levels of CRP and Il-6 were observed in the control group over the course of the study. (Fig. 2).

Dopamine and BDNF

In the PD group, the significant increase in the serum concentration of DA and BDNF compared to the baseline was observed only after the individual training (P<0.05). In the next stages of the investigation, a slight increase in DA and BDNF was noted. In the control group, there were no changes observed in the DA and BDNF concentration. After the individual training (T-1), the significant differences in the concentration of DA and BDNF between PD patients and the control were observed (T-1PD vs. T-1 C; P<0.05) (Fig. 3).

TAC and CoQ10

In the concentration of CoQ10, an increase was observed only in T-2 compared to the baseline (P<0.05), as well as the significant differences between PD patients and the control at the same time (T-2 PD vs. T-2 C; P<0.05). No significant changes were observed in TAC activity in both groups (Fig. 4).

Correlations

Among the investigated immune and the nervous system markers and antioxidant capacity, only in PD patients were significant correlations found between
DA and TAC (P < 0.000), DA and BDNF (P < 0.000) and between DA and CoQ10 (P<0.044) (Fig. 5).

Figure 3. Percentage changes in the serum levels of DA and BDNF during training compared to baseline
* significantly different from baseline (P<0.05); # significant differences between groups (P<0.05)

Figure 4. Percentage changes in the serum levels of CoQ10 and TAC activity during training compared to baseline
* significantly different from baseline (P<0.05); # significant differences between groups (P<0.05)
Figure 5. Correlations between coenzyme Q10 (CoQ10), dopamine (DA), total antioxidant capacity (TAC) and brain-derived neurotrophic factor (BDNF) in PD patients
DISCUSSION

Currently, one of the most important challenges in improving the effectiveness of the drug treatment of neurodegenerative diseases is crossing the BBB. Thus, alternative therapies that have the ability to affect the brain directly may increase the efficiency of treatment (Träger et al., 2013; Nagatsu et al., 2000). Therefore, in the present study, we attempted to verify the effectiveness of visualization and suggestion based mind-body interventions as a potential therapy supporting the treatment of Parkinson’s disease.

In our study, we did not expect spectacular results, but for patients with advanced Parkinson’s disease, even the smallest changes are fundamental. As it was not a clinical trial, as shown in similar subject literature, we decided to show our results as percentage changes from the baseline, as these far better illustrate the observed changes (Innes, 2010).

Since subject literature data suggested that inflammatory processes may be responsible for a lower concentration of DA and BDNF in PD patients, we can suppose, that a higher level of inflammatory markers may be responsible for the similar situation observed in our study (Träger et al., 2013; Nagatsu et al., 2000). Thus, the main finding of this investigation concern the changes of those crucial signal molecules in etiology and the healing of PD. During the training, we observed that the serum pro-inflammatory markers decreased while the level of DA and BDNF increased. The strongest response was observed after individual sessions with the therapist, over subsequentl stages the changes were not so strong, however, the decreasing trend was maintained.

Inflammation is also indicated as a factor responsible for the negative changes associated with cognitive function and dementia, as well as with motor status, non-motor symptoms, and disease severity (Lindqvist et al., 2013). The increased level of IL-6 and CRP with reduced levels of DA and BDNF in the PD participants of our study may have been manifested as weaker results from their cognitive and motor skills tests taken before therapy. According to these findings, the major strength of our study is also its observation of a significant improvement in the psychological aspects, ones taken in parallel with increased walking efficiency, as well as an improved ability to perform everyday activities in PD patients. Because participants had stability in behavior and a constant dose of L-dopa over the course of the whole training, we can suppose that the observed increase in DA and BDNF levels may be in part responsible for these results.

Furthermore, this result is interesting because the motor and cognitive skill assessment tests that were performed after the last stage of training showed a significant improvement, whereas blood concentrations of DA and BDNF had already returned to baseline levels. Because no similar studies have been carried out, it is unknown how long the positive effect of such therapy can last for. However, in the case of individuals with advanced PD, attempts should be made to use any intervention that may affect their performance and well-being.
Despite the wide disparity of research results that questions the effectiveness of mind-body therapies, one common advantage can be observed. Regardless of the form of psychological intervention, its duration, or the aims, a significant improvement in well-being, and often increased cognitive abilities have been observed (Gregorson et al., 1996; Braun et al., 2001; Tamir et al., 2007). Because there was no body-mind therapy targeted precisely at the specific signaling molecules responsible for the inducing (healing effect) or blanking (inflammation) processes in the body, the changes in the signaling peptides observed during training in our study exhibited interesting trends. According to suggestion, the levels of IL-6 and CRP should decrease and this change was observed. In addition, DA, BDNF, and CoQ10 concentrations were increased according to the given suggestions. However, these changes occurred after individual training, which was conducted under the guidance of the therapist. In the next stages, when mixed and self-training were conducted, the concentrations of all markers returned to baseline levels. Information about any training directly conducted by a trainer is rarely found in the subject literature, as audio file listening is used more often. But the results of other research are strongly inconclusive. Some enthusiastic reports have shown that the immune and endocrine function, and even muscle strength, have improved, whereas other studies have reported only changes in some of the selected test parameters or no changes at all (Wahbeh et al., 2009; Gruzelier 2002a; Gruzelier 2002b, Ranganathan et al., 2004). The results of mind-body interventions are dependent on many variables, including the aim of the study, the group being investigated, the duration of the study, and the components used in the intervention.

We cannot indicate any direct mechanism responsible for the changes observed both in signaling peptides concentrations as well as for changes in the motor and cognitive abilities and functions, however, we can exclude the healing effect of levodopa (according to medical reviews, due to the progress of Parkinson’s disease, participants did not respond to this treatment). Although based on subject literature data, we can suppose that the type of mental training we used, based on systematically repeated therapy, affected the participants mostly via top-down mechanisms, which are initiated via mental processing at the level of the cerebral cortex (Ranganathan et al., 2004). However, we also cannot exclude the bottom up regulation, since the effect of the type of training used in therapy stimulated also some visceral activities (e.g. muscle relaxation). According to the subject literature, the bidirectional autonomic and neuroendocrine pathways responsible for transmitting information between the central nervous system and the periphery may play a crucial role in the expression of an affective, autonomic, hormonal and immune response (Taylor et al., 2010; Galper et al., 2010).

In our study, strong relationships were found between the markers important in therapy. Significant positive correlations were observed between TAC and CoQ10 and between TAC and DA. Oxidative stress present in cells, mainly due to malfunctioning mitochondria, is responsible for reducing the level of many peptides involved in the proper functioning of the nervous system. Studies in an-
imal models of PD suggest that an efficient system of antioxidants has neuroprotective properties, which may increase the production of DA and CoQ10 (Tulon et al., 2012; Venkateshappa et al., 2012). A positive correlation was also observed between DA and BDNF. However, the relationship between DA and BDNF is not fully explained and the results appear inconsistent. Guilllin et al. (2001) have suggested that BDNF plays a key role in the regulation of cell responses to DA by regulating the expression of DA receptors, whereas Küppers and Beyer (2001) showed that DA regulates the expression of BDNF and may act on BDNF through GABAergic cells. Regardless of the regulatory mechanisms, both particles are strongly interlinked. The positive correlation between CoQ10 and DA may indicate the protective effect of CoQ10 on dopaminergic neurons. Due to its ability to cross BBB, CoQ10 is proposed as a therapeutic option in patients with neurodegenerative diseases, including patients with PD (Cleren et al., 2008; Sharma et al., 2013).

CONCLUSIONS

As inflammation and reduced antioxidant defenses could be the cause of both decreased serum and brain concentrations of DA or BDNF, finding new ways to increase their levels may be crucial in healing processes in PD patients. The mind-body intervention used in this study affected the concentration of the tested peptides, especially pro-inflammatory cytokine, as expected, and probably these changes were reflected not only in a better well-being but also in patient’s mobility, which is a key aspect of all PD therapies.

IMPLICATIONS, STUDY LIMITATIONS, AND FUTURE DIRECTIONS

The results indicate the potential of mind-body therapy based on visualization and suggestion in the treatment of various aspects of PD by interfering directly with the peptides responsible for the control of disease processes. A limitation in the current study was the small size of the subject group. The sample size used in this study was based on the pragmatic approach adopted in recruiting those patients most suitable for our research. This was caused by the rigorous criteria which the participants had to meet, such as a lack of NSAID or corticosteroids treatment. Among PD patients these are very rare conditions, thus, our investigation group was small. Due to the small sample size our study could be underpowered to detect more differences. Nonetheless, these results indicate that further research is worthwhile to clarify the type of training at the highest possible efficiency, such as reducing the number of suggested markers or developing group sessions with a trainer, to support hitherto pharmacological therapy, yet control studies are needed to confirm these results.
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Trial registration

This trial was registered with the Chinese Clinical Trial Register (ChiCTR), ChiCTR-BIN-16009354 (retrospective). Registered October 10, 2016.

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