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Research article

Effects of a Single-Session Cognitive Enhancement Fitness Program on Serum Brain-Derived Neurotrophic Factor Levels and Cognitive Function in Middle-Aged Women

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Abstract
Few studies have been undertaken to develop cognitive functional improvement-focused exercise programs and determine their effect. The objectives of this study were to evaluate the effects of a cognitive enhancement fitness program (CEFP) on short-term memory and serum brain-derived neurotrophic factor (BDNF) levels according to the cognitive state in middle-aged women. A total of 30 healthy volunteers aged 40–59 years were divided into two groups, that is, a mild cognitive impairment (MCI) group and a non-MCI group based on results from the Korean Dementia Screening Questionnaire. A single-session CEFP was conducted over 50 min and consisted of four parts: warm-up, low intensity interval circulation dance exercises, moderate intensity resistance exercises using elastic bands, and cool-down. Serum BDNF levels were measured by ELISA and short-term memory determined by forward digit/word span test was assessed before and after CEFP. After CEFP, forward digit/word span test scores and BDNF levels increased to median 119.2%/115.1% and 118.7%, respectively. After CEFP, the MCI and non-MCI groups produced higher forward digit span test scores (from 6.7 ± 1.5 to 7.5 ± 1.4 points, p = 0.023 and from 6.2 ± 2.0 to 7.0 ± 2.1 points, *P*=0.011, respectively). After CEFP, forward word span scores and BDNF levels increased (from 3.5 ± 1.7 to 4.6 ± 1.8 points, p = 0.029 and from 610.8 ± 221.1 to 757.9 ± 267.9 pg/ml, p = 0.017, respectively) in non-MCI group only. No group differences were observed between change in short-term memory and change in BDNF. Short-term memory and BDNF levels after CEFP were found to be negatively correlated with age, but pre- to post-intervention changes in short-term memory and BDNF were not. The present study shows that a single, 50-minute CEFP improved short-term memory and increased serum BDNF levels in healthy middle-aged women, especially those without MCI.

Key words: Exercise, dance, cognition, memory, brain-derived neurotrophic factor.

Introduction

Dementia is a major public health concern and its incidence among the elderly is rising rapidly. The prevalence of dementia in individuals aged > 60 depends on geographic location, but is 5-7% in most countries, and this implies tremendous healthcare and social burdens, thus, dementia is becoming an issue of considerable concern (Park et al., 2013; Prince et al., 2013). To prevent dementia, it is critical to detect progressive decline such as mild cognitive impairment (MCI) from middle age. Most causes of dementia are irreversible and reduced cognitive function is a known powerful risk factor of dementia (Baumgart et al., 2015). Non-drug treatments to improve cognitive function include physical exercise, dance (Kim et al., 2011), meditation (Gard et al., 2014; Kaufman et al., 2007), music therapy, cognitive training, and brain stimulation (Sachdeva et al., 2015; Vaughan et al., 2014, Vedovelli et al., 2017). Physical exercise is known to be effective in preventing decline in cognitive function by improving systemic physical health (Forbes et al., 2015). Previous studies have reported low physical activity influences cognitive function, impaired growth and differentiation of neurons and synapses, lowered secretion of neurotrophic factors, brain function deterioration, and the risk of mental illness (Forbes et al., 2015; Gomez-Pinilla and Hillman, 2013; Laske et al., 2010). Serum brain-derived neurotrophic factor (BDNF) concentration is widely used as an index to verify the beneficial effect of exercise on preservation of existing brain cells and stimulation of new neuronal growth. BDNF levels were increased after long-term aerobic exercise or even a single aerobic exercise session (Huang et al., 2014; Laske et al., 2010). However, the effects of resistance training on BDNF levels are less clear, and remain controversial (Chang et al., 2012; Goekint et al, 2010; Knaepen et al., 2010; Ruiz et al., 2015). These inconsistencies could be explained by personal characteristics, existing co-morbidities, body composition, and types or intensities of resistance exercise (Chang et al., 2012; Knaepen et al., 2010).

The majority of previous studies have examined the effects of aerobic, dance, and resistance exercises separately on cognitive function, however, few studies have sought to devise a cognitive functional improvement-focused combined exercise program and then examined its effects on cognitive function. Although there are some previous studies that combined exercise improved cognitive function, the exercise was a simple repetitive exercise under supervision. In order to stimulate not only peripheral nerves but also the central nervous system, it is necessary to develop an exercise program to memorize, stimulate and concentrate on each exercise, as if playing a game. It is necessary to develop an exercise program that allows each exercise movement to be recalled so that it is more focused compared with a combination of randomly arranged exercise movements, which are simply performed rather than repeated. In the present study, we developed a cognitive enhancement fitness program (CEFP) based on the princi-

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ple of exercise-induced improvements in cognitive performance. In addition, we evaluated changes in short-term memory and serum BDNF levels before and after a single session CEFP according to cognitive state in healthy middle-aged women.

Methods

Participants

The study was approved by the Institutional Review Board of Kyungnam University (2014-023-HR-03) and performed in accordance with the principles laid down in the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment in the study. Thirty women aged 40 to 59 years residing in Y city, Korea were enrolled in the study. All thirty were healthy and had not taken any supplements or medications, including antidiabetic drugs, antihypertensive drugs or, cognitive-enhancing drugs during the previous four weeks. In addition, no participant had any physical or emotional impairment nor participated in any regular exercise program during the previous 6 months. The Canadian Physical Activity Readiness Questionnaire was used to screen candidates beforehand for study eligibility. After enrollment, all intervention and measurements were performed on the same day.

Intervention

CEFP is a cognitive functional improvement-focused exercise program and was developed using integrated functional physical exercise as a basis to induce brain cell activation and neurogenesis. To target brain cell proliferation and neurogenesis using dance, the program was developed using existing integrated functional physical exercise programs (Cassilhas et al., 2007; Chang et al., 2012; Gates et al., 2010; Hogan et al., 2013; Kim et al., 2011). The single 50-minute CEFP comprised as the following: 5 minutes of general warm up and mat stretching, 20 minutes of low intensity (Rating of Perceived Exertion, RPE 11-12) activities with 8 dance exercises (Bounce, Side-to-Side, Twist, Swan lake, Lady's kick, Jump, Sweety, and Shuffle dances), another 20 minutes of moderate intensity (RPE 13-15) activities including 8 resistance exercises (biceps curl, chest press, single arm press, lateral raise, overhead pull, good mornings, squat, kicks) using elastic bands (Thera-Band®, Hygenic Corporation, Akron, OH, USA), and finally a 5-minute general cool down with yoga. Thus, the main exercise routine was composed of 8 dance exercises and 8 resistance exercises. First, after we taught subjects to memorize numbers allocated to each dance, subjects were requested to perform the dance exercise chosen by randomly calling numbers (from 1 to 8). Second, in a similar manner subjects were requested to perform a resistance exercise by number (also from 1 to 8). All subjects were taught to perform these movements before the start of the study, which took about 20 minutes.

Measurements

Physical activity, total energy expenditure, mean metabolic equivalents (METs), and exercise intensity were measured using Armband® (Bodymedia, Co. USA). The band was worn on the upper portion (triceps) of the right arm. A 5 ml blood sample was obtained from the antecubital vein of each participant at rest 1 hour before and again 1 hour after the program. Blood samples were drawn into chilled tubes containing Na2EDTA (1 mg/ml) and aprotinin (500 U/ml) and were used to determine serum BDNF levels. Serum was separated immediately by centrifugation (2,000×g) at 4ºC and stored at -70ºC until assayed. Serum BDNF levels were measured using commercial quantitative sandwich ELISA kits (Abfrontier, Seoul, Korea), which had a detection limit of 2 pg/ml and inter- and intra-assay coefficients of variation of 5.4 % and 4.1 %, respectively. The samples were collected under the same conditions before and after CEFP, and the results were obtained by running the ELISA measure twice.

All participants were assessed before CEFP using the Korean Dementia Screening Questionnaire Cognition (KDSQ-C). The KDSQ-C is a self-administered questionnaire using 15 cognitive dysfunction items, each rated on a three-point scale: 0 (no change), 1(sometimes/occasional change) and 2 (often/frequent change). The KDSQ-C is not influenced by age or educational level, and its optimum cut-off value was 6 points with sensitivity of 79% and specificity of 86% to detect MCI (Choi and Park, 2016). According to a baseline KDSQ-C cutoff score of 6, subjects were divided into two groups; that is, ≥ 6 points (the MCI group, n = 11) and < 6 points (the non-MCI group, n = 19). Before and after CEFP implementation, short-term memory was tested using forward digit/word spans (Rabinowitz and Lavner, 2014). The examinee read a sequence of digits, then recalled the digits in the same forward sequence. Novel sequences were provided after CEFP in order to prevent any familiarity with prior exposure to digit sequences. Participants were asked to write down the digit list they recalled from level 1 to a higher level (see Table 1) after five seconds of memorization. Digit span scores were calculated using the longest list length correctly recalled and ranged 0 to 10. For word span, participants were asked to speak the word they recalled after each word list from level 1 to a higher level (see Table 1) after five seconds of memorization. Word span scores were calculated using the total number of items (word lists) correctly recalled and ranged from 0 to 10. Although different sequences were provided immediately just before and immediately after CEFP, all subjects were tested using the same sets of digits or words before or after CEFP (Table 1). No practice trials were included.

Statistical analyses

Power analysis was carried out using G*Power 3.1, and for a two-tailed test at the 0.05 level and 85% power, a total sample size of 30 participants would be needed to detect a desirable effect size of 0.5. A Shapiro-Wilk test verified a normal distribution for all parameters. Parametric variables that were normally distributed were expressed as mean ± SDs while changes of parameters that were not normally distributed were expressed as median and 95% CIs. A paired *t*-test was used to compare differences in BDNF levels and memory scores between post-CEFP and pre-CEFP, called 'change' scores or 'gain' scores. Gain scores were calculated by subtracting pre-CEFP scores from post-CEFP scores. Pearson correlation coefficient analysis was

Table 1. Forward memory span task before brain fitness program (an example).

Level	Digits	Words*
1	2-3-8	cat-airplane
2	1-9-7-2	shoes-cabbage-watch
3	8-7-3-2-4	colored paper-pencil-monkey-scissors
4	6-8-1-2-3-9	fishbowl-apartment-dyestuff-triangle-farmer
5	2-8-4-9-1-1-4	doll-classroom-spinach-pumpkin-goldfish-grape
6	5-8-6-4-3-8-9-7	sea-fox-spinach-pencil-goldfish-banana-pumpkin
7	7-4-9-6-8-5-3-1-8	doll-crayon-kettle-notebook-fox-mathematics-grape-sea
8	6-8-7-9-5-2-3-4-1-5	spinach-pig-doll-tiger-language-fisher-desk-scissors-potato
9	4-7-5-1-3-6-4-9-6-8-2	doll-crayon-kettle-lion-chair-gym-notebook-desk- mathematics-picture
10	7-6-1-8-2-5-7-6-2-1-9-4	doll-jumping rope-kettle-lion-chair-cucumber-ball-desk- mathematics-cup-doll

* used Korean language in the field.

Table 2. Basal characteristics of the study subjects. Data are means (±SD).

Variables	MCI group (n=11)	Non-MCI group (n=19)
Age (years)	47.6 (6.7)	51.8 (6.5)
KDSQ-C*	7.4 (1.7)	2.7 (1.7)
Forward digit span scores (points)	6.7 (1.5)	6.2 (2.0)
Forward word span scores (points)	4.1 (1.2)	3.5 (1.8)
Pre-BDNF	787.0 (258.5)	610.8 (221.0)

*p < 0.001 by two sample *t*-test. KDSQ-C, Korean Dementia Screening Questionnaire Cognition; BDNF, brain-derived neurotrophic factor

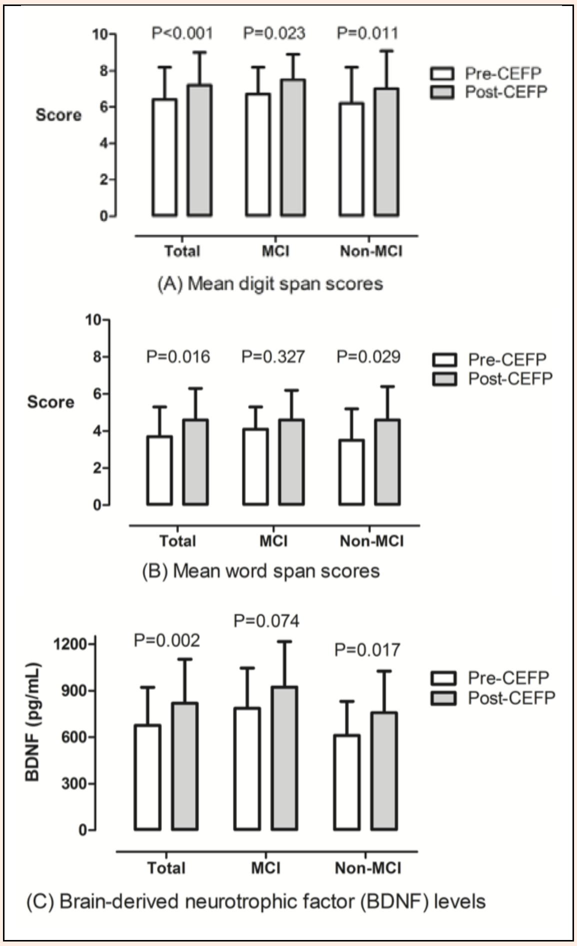

Figure 1. Mean primary outcomes changes before and after a cognitive enhancement fitness program in total (n = 30), the mild cognitive impairment (MCI, n = 11), and non-MCI groups (n = 19). Error bars indicate one standard deviation from mean values. (A) Mean digit span scores, (B) Mean word span scores, (C) Brain-derived neurotrophic factor (BDNF) levels.

used to assess the strength of the relationships between BDNF concentration and other variables. Furthermore, subgroup analysis was performed according to a baseline KDSQ-C cutoff score of 6 points. Statistical analysis was performed using the Statistical Package for Social Sciences ver. 13.0 for Windows (SPSS, Inc., Chicago, USA). Statistical significance was accepted for p values < 0.05.

Results

Basic characteristics of subjects

The mean age of total subjects was 50.3 ± 6.8 years, and mean KDSQ-C of the subjects was 4.4 ± 2.8 points. There was a significantly higher score of KDSQ-C in the MCI group than in the non-MCI group (7.4 ± 1.7 vs. 2.7 ± 1.7, p < 0.001), while there was no difference between the two groups in age (47.6 ± 6.7 vs. 51.8 ± 6.5, P = 0.1017, Table 2).

Energy expenditure and average exercise intensity during the CEFP session

Participants expended on average 147.2 ± 12.5 kcal per CEFP session. Energy expenditure per kg of body weight was determined by dividing total energy expenditure by body mass. Mean energy expenditure was 2.6 ± 0.4 kcal/kg and mean exercise intensity was 3.8 ± 0.6 METs.

Change in short-term memory

Forward digit span scores increased significantly from 6.4 ± 1.8 to 7.2 ± 1.8 points (p < 0.001) after the CEFP session, and averaged a median 119.2% (95% CI, 7.8-26.4) of baseline (left panel, Figure 1A). Forward word span scores increased significantly to a median value of 115.1% (95% CI, 0-27.1) of baseline, and increased from 3.7 ± 1.6 to 4.6 ± 1.7 points (p = 0.016) (left panel, Figure 1B).

Change in BDNF levels

Mean serum BDNF levels pre- and post-CEFP were 675.4 ± 246.6 pg/ml and 818.6 ± 284.0 pg/ml, respectively (*P* =

0.002), which represented a median value of 118.7% (95% CI, 0.7-37.3) of baseline (left panel, Figure 1C). BDNF levels were significantly increased; median value of 1.19-, 1.11-, and 1.23-fold after CEFP, for the whole group, the MCI, and non-MCI groups, respectively. Age was negatively associated with pre-BDNF (r = - 0.356, p = 0.053) and post-BDNF (r = - 0.481, p = 0.007).

Memory scores and BDNF levels in MCI and non-MCI groups

In the MCI and non-MCI groups forward digit span increased from 6.7 ± 1.5 to 7.5 ± 1.4 points (p = 0.023) and from 6.2 ± 2.0 to 7.0 ± 2.1 (p = 0.011) points, respectively after CEFP session (middle and right panel, Figure 1 (A)). Forward word span scores did not increase in MCI group (from 4.1 ± 1.2 to 4.6 ± 1.6 point, p = 0.327) but increased from 3.5 ± 1.7 to 4.6 ± 1.8 points (p = 0.029) in non-MCI group (middle and right panel, Figure 1B. Similarly, serum BDNF levels did not increase in MCI group (from 787.0 ± 258.5 to 923.4 ± 292.4 pg/ml, p = 0.074) but increased from 610.8 ± 221.1 to 757.9 ± 267.9 pg/ml (p = 0.017) in non-MCI group (middle and right panel, Figure 1C. Changes in forward digit span scores, forward ward span scores, and serum BDNF levels between the pre- and post-CEFP showed no intergroup differences (p = 0.702, p = 0.683, and p = 0.474, respectively, data not shown). Forward digit span scores, forward ward span scores, and serum BDNF levels after CEFP session were found to be negatively correlated with age (r = -0.490, p = 0.006; r = -0.518, p = 0.003; and r = -0.481, p = 0.007, respectively), however, changes in these variables between pre- and post-CEFP were not associated.

Discussion

During ageing, memory, cognition, language abilities, body structure and function gradually deteriorate, and many studies have been conducted to improve or maintain cognitive function. Physical activity has been shown to have a positive effect on information processing of the central nerve system, to increase brain blood flow and neurotransmitter secretion, to delay brain ageing by continual stimulation of the brain, and improves physical fitness (Bherer et al., 2013; Gates and Valenzuela, 2010; Gomez-Pinilla and Hillman, 2013; Hillman et al., 2008). Most previous studies have reported that exercise habits and dance are promising non-pharmaceutical interventions that might delay cognitive deterioration or improve cognitive function in old age (Bherer et al., 2013; Gomez-Pinilla and Hillman, 2013; Kim et al., 2011; Wayne et al., 2014). In the present study, we investigated the effects of single session of a cognitive function improvement-focused exercise program, CEFP, on cognitive function, especially on short-term memory and serum BDNF levels in middle aged women (40-59 years old). A single session of CEFP intervention significantly improved short-term memory function and increased BDNF concentrations in healthy middle-aged women, especially those without MCI.

To the best of our knowledge, this is the first study to utilize dance combined with thera-band exercise to improve cognitive function. We designed a combination of dance-based aerobic exercises together with resistance band exercises (CEFP) based on the principles of exercise associated with improvements in cognitive performance (Chang et al, 2012; Gates and Valenzuela, 2010; Kaufman et al., 2007). In addition, we asked participants to do these exercises after memorizing numbers (from 1 to 8) which were associated with each dance or resistance exercise respectively, as a type of cognitive-related 'physical' training. Average energy expenditure per kg of body weight and average exercise intensity during the CEFP session were 2.56 kcal/kg and 3.8 METs, respectively, which indicates the exercise intensity exercise level used was equivalent to quick walking or light jogging.

Memory span is a commonly used measure of short-term memory and was used in the present study (Richardson, 2007). Comparisons were performed pre- versus post-CEFP for all subjects and for MCI and non-MCI groups separately. As expected, results showed the participants had a significantly higher median forward digit span score (119.2% of baseline) compared with baseline. In addition, both groups had higher median forward digit span scores compared with baseline (120.6%, P=0.023 for the MCI group, 117.9%, P=0.011 for the non-MCI group). Forward word span scores also increased significantly from baseline for all subjects and in the non-MCI group. These findings are compatible with previous studies that investigated the association between exercise and cognition in older adults. In the present study, analyses of gain scores were conducted to compare between pre- and post-CEFP memory span scores, because word and digit learning may contribute more heavily towards memory and attention, respectively. Most, but not all, intervention studies have shown aerobic exercise training improves cognitive performance in older people. However, the reported effects of fitness training on cognition in randomized intervention trials tend to vary in an elderly population (Hillman et al., 2008). In addition, a Cochrane systematic review of twelve randomized controlled trials in 754 people aged over 55 years without known cognitive impairment demonstrated no effect of aerobic exercise on cognitive function (Young et al., 2015). Also some studies reported that one session of moderate aerobic exercise was not enough to improve digit span scores in a depressed elderly population (Vasques et al., 2011) or middle-aged adults (Netz et al., 2007). On the other hand, Chu and colleagues (Chu et al., 2015) reported an improvement in cognitive function after a 30 minutes of acute exercise in older adults, especially for those with initially higher fitness levels. In addition, Loprinzi and Kane (2015) reported that concentration-related cognition scores were significantly higher after a 30-minute acute bout of moderate-intensity aerobic exercise in young adults.

In terms of resistance exercise, previous studies have reported that 6-12 months of moderate or high intensity resistance exercise improved memory performance in men aged 65 to 75 years (Cassilhas et al., 2007; Liu-Ambrose et al., 2010). This mixed pattern of results may be explained by the different types of cognitive function measured, the exercise (duration, intensity and type), or the age of subjects. In the present study, we used dance as a component of aerobic exercise, because dance has been shown to have a positive effect on both motor and cognitive function. Kattenstroth et al. (2013) reported that 60-min dance once a week for 6 months improved posture, cognitive, tactile, and motor performance in the neurologically healthy elderly. In contrast, a 60-min Cha-Cha dance twice a week for 6 months improved verbal fluency, word list delayed recall, and word list recognition, but not immediate recall in older adults with metabolic syndrome (Kim et al., 2011). These inconsistent results can be partly explained by the different types of dance and different subject populations. It is essential to develop a well-structured fitness program to achieve a specialized purpose. In the present study, we also included a combination of various dance types in order to focus cognitive function on brain cell activation in the CEFP. Interestingly, our results suggest that a combination of dance and thera-band (resistance) exercise might improve immediate memory function. The results of previous studies suggest that aerobic exercise and resistance exercise included in a combined exercise program are beneficial for cognition. The reason for these effects is that it helps to improve cognitive ability through neuroplastic activity, cranial nerve formation and increased secretion of active neutrophins including BDNF, and hormones such as insulin growth factor-1 (Cassilhas et al., 2007; Gates et al., 2010; Hogan et al., 2013; Huang et al., 2014; Kim et al., 2011; Laske et al., 2010).

BDNF concentration was increased by median 118.7% of baseline in all subjects. In addition, although there was no significant difference in changes of forward digit or word span scores or BDNF levels between the MCI and non-MCI groups, pre-post differences in BDNF concentrations were observed in the non-MCI group, but not in MCI after CEFP, which is consistent with previous studies. Considering the findings of the present and previous studies, changes in BDNF concentrations caused by CEFP seems to be larger in individuals without MCI compared with those with MCI. Previous studies have also reported that the physical fitness-cognitive performance association is mediated by BDNF and is enhanced after a single session of exercise or regular exercise (Langdon and Corbett, 2012; Piepmeier and Etnier, 2015; Szuhany et al., 2015). BDNF is released in the prefrontal cortex, amygdala, and hippocampus, which are closely related to learning, memory, and cognitive function (Del Arco et al., 2011).

The present study has some limitations. First, of the several cognitive domains, such as, memory, learning, execution, processing, literacy, and numeracy, only immediate memory was measured. Second, our study was neither randomized nor blinded, and participant expectancy may have been a strong motivating factor. In addition, no control group was included. Third, the generalizability of the study is uncertain, because of the small sample size, especially in MCI and non-MCI. Fourth, the change in immediate memory was small, even if statistically significant. Therefore, few data may fall within the overlapping area. However, there was a large change in BDNF levels, especially for non-MCI. The changes with digits or words will not be a factor related to familiarity of the testing situation and better understanding of the test demands, because no practice trials for memory span test were included. We still do not know how long improved memory lasts after exercise and how long plasma BDNF levels remain elevated. Despite these limitations, the present study demonstrated that a brief cognitive functional improvement-focused combined exercise program (CEFP) significantly improved short-term memory and increased serum BDNF levels in middle-aged women.

Conclusions

In conclusion, the present study shows that a single, 50-minute brain fitness program improved short-term memory and increased serum BDNF levels in healthy middle-aged women, especially those without MCI. Long-term follow-up of randomized controlled trials in different age groups based on the present study is required to confirm these preliminary findings. It remains to be determined whether the rise in BDNF plays a causative role in the improvement in cognitive test scores with a single session of CEFP intervention. Other biomarkers such as insulin growth factor-1, neuron restrictive silencer factor may need to be measured together with BDNF.

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Key points

- A single 50-minute CEFP comprised 5 minutes of general warm up and mat stretching, 20 minutes of low intensity activities with 8 dance exercises (RPE 11-12), another 20 minutes of moderate intensity activities including 8 resistance exercises using elastic bands ((RPE 13-15), and finally a 5-minute general cool down with yoga.
- Both MCI and non-MCI groups produced higher forward digit span test scores and had higher serum BDNF levels after CEFP but forward word span scores were increased only in non-MCI.
- A single 50-minute brain fitness program improved short-term memory and increased serum BDNF levels in healthy middle-aged women, especially those without MCI.

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