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CLINICAL TRIAL REPORT

The Effect of Two Somatic-Based Practices Dance and Martial Arts on Irisin, BDNF Levels and Cognitive and Physical Fitness in Older Adults: A Randomized Control Trial

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Background: Maintaining healthy brain function during ageing is of great importance, especially for the self-sufficiency of older adults. The main aim of this study was to determine the effects of dance and martial arts on exerkines Brain Derived Neurotrophic Factor (BDNF) and irisin blood serum levels.

Methods: This randomized controlled trial examined the effects of dance and martial arts on serum Brain-Derived Neurotrophic Factor (BDNF) and irisin levels, as well as cognitive function, mood, and physical measures in older adults. Seventy-seven independently living older adults (mean age 70.3±3.8 years) were randomized into three groups: dance (DG), martial arts (MaG), and control (CG), followed over 12 weeks. Generalized linear models were used to assess the interventions' effects.

Results: There was a significant increase in BDNF levels in both the DG (1.8 ± 4.9 , p < 0.05) and MaG (3.5 ± 6.3 , p < 0.05), while CG experienced a decrease (-4.9 ± 8.2 , p < 0.05). Between-group effects were significant for BDNF, with DG and MaG showing higher levels than CG (p < 0.05). No significant changes in irisin levels were found. Cognitive performance, particularly attention and mental flexibility (measured by the Trail Making Test A and B), significantly improved in the DG compared to CG (p < 0.05). Additionally, participants in DG showed improved mood based on the Geriatric Depression Scale (p < 0.05) compared to CG. Anthropometric T-scores were significantly associated with changes in irisin levels (p < 0.05) after intervention.

Conclusion: The study found that dance and martial arts upregulated BDNF levels, with dance showing notable improvements in cognitive function and mood in older adults. Changes in anthropometric measures were linked to increased irisin levels. These findings suggest that both dance and martial arts may promote healthy brain function in aging populations.

Trial Registration: NCT05363228.

Keywords: cognitive domains, ageing, cognitive performance, cognitive function, serum, biomarkers

Introduction

Physical activity (PA) has a positive effect on health and can ease some neurological conditions such as depression, epilepsy, stroke, Alzheimer's (AD) and Parkinson's disease (PD).^{1–4} The healthy older adult population has also demonstrated improvements in cognitive functions after PA,⁵ which is believed to be mediated by exerkines (cytokines secreted by organs in response to exercise).⁶ BDNF is one of the most prominent neurotrophic and neuroprotective exerkines in the central nervous system (CNS).⁷ BDNF is believed to be mediated by irisin, a myokine cleaved from the fibronectin type III domain-containing 5 (FNDC5) protein secreted by muscles in response to exercise.^{8–11}

In the last decade, a holistic point of view has defined the need to link the body with psycho-emotional experience; ie the need for PA that improves physical function but also considers social relationships, emotional experience, and body awareness.¹² These somatic approaches are appropriate for enhancing cognitive domains because they are designed to improve sensitivity, alter habitual movement patterns through sensory perception¹³ and include multi-faceted activities such as cognition, emotion, coordination, visual-spatial orientation, social interaction, and kinesthetic empathy.^{14,15} An essential feature of dance is the synchronization of movement and the connection to time through musical rhythms,¹⁶ promoting the imagery of spontaneous movement patterns, memories, and recollections.¹⁷ On the other hand, martial arts are mostly moderate-intensity group activities that allow older adults to exercise in a social environment and communicate with other participants in a similar way to dancing.¹⁵

In fact, both dance and martial arts are universal human behaviors associated with group rituals,¹⁸ and both have been shown to improve cognitive function in older adults.^{19–22} Moreover, dance could be superior to other physical activities²³ because of its complexity of coordination.²⁴ On the other hand, there is evidence that long-term dancing (more than 10 years) helped older individuals maintain healthy aging to the same extent as an adapted PA program.²⁵ Therefore, it is important to distinguish between different types of dances and approaches. For example, a recent randomized controlled trial (RCT) found decreased BDNF and increased irisin after folk dance and balance training, accompanied by improved physical performance, glucose homeostasis and blood pressure.²⁶ In this study, we focused on somatic practices, acknowledging their distinct nature and approaches to bodily movement.

To our knowledge, this is the first RCT to investigate the effect of two somatic-based practices, dance and martial arts on the secretion of chosen exerkines and cognitive performance. The main aim of our study was to determine the magnitude of the effect of the dance and martial arts on serum BDNF and irisin levels and to estimate whether changes in serum BDNF and irisin levels, anthropometrics, and physical performance were associated with changes in cognitive performance in older adults.

Methods

A randomized, three-arm, single-blind, controlled trial was conducted in two centers: 1) University Hospital Kralovske Vinohrady (UHKV), Charles University, Third Faculty of Medicine, Prague and 2) Charles University, Faculty of Physical Education and Sport, Prague, from May 2021 to December 2022. The study was approved by the Faculty of Physical Education and Sport ethics committees and the University Hospital Kralovske Vinohrady, and all the participants signed an informed consent. The trial was registered on clinicaltrials.gov with registration ID NCT05363228 03/05/2022.

Participants

Seventy-seven independently living older adults participated in the study. Inclusion criteria were age 65–80 years, no mobility limitations, less active according to the Rapid Assessment of Physical Activity (RAPA),²⁷ complete independence in activities, normal score according to the Eight-item Informant Interview to Differentiate Aging and Dementia (AD8-CZ)²⁸ and good vision and hearing. Exclusion criteria were as follows: neurological diseases (epilepsy, significant head injury, stroke, brain surgery, brain tumor, etc)., psychiatric diseases or treatments (schizophrenia, bipolar disorder, drug addiction, alcoholism, etc)., organ failure (heart, kidney, etc)., oncological diseases in the last five years or patients after chemotherapy or radiotherapy, surgery under general anesthesia in the last three months, use of cognitive enhancers. Participants who reported a much higher than average level of PA according to the RAPA were excluded. Depression was not an exclusion criterion. We set our sample size at a minimum total number of participants of N = 68 based on an a priori power analysis using G*Power 3.1.²⁹ Based on prior findings,^{15,30} we sought to detect a medium effect size (f² = 0.15) with 80% power and a probability of error of 0.05.

Trial Design

Participants were randomly assigned to a dance group (DG) and control group (CG) in the first year of the study and to a martial arts group (MaG) and CG in the second year. 12-week interventions lasted 90 minutes per session, twice a week. This amount was based on the WHO recommendation for older adults of at least 150–300 minutes of moderate-

to-vigorous intensity activity throughout the week for substantial health benefits.³¹ Simple randomization based on a single sequence of random assignments using a shuffled deck of cards (even-numbered control, odd-numbered intervention) was used each year. AB generated the random allocation sequence. AB enrolled and allocated participants to interventions. Each participant was examined at the University Hospital Kralovske Vinohrady for cognitive assessment and at the Faculty of Physical Education and Sport for physical fitness testing. Blood samples were taken during the visit to the University Hospital Kralovske Vinohrady. Participants completed cognitive tests and physical fitness batteries, self-reported depression levels, functional status and years of education. The evaluators responsible for administering the tests were blinded to the participants' assigned interventions, and the instructors (dance or martial arts) were not involved in administering the pre- and post-assessments. After all measurements, participants followed the intervention/maintenance of daily activities for 12 weeks, beginning in August (pre-test) and ending in December (post-test).

Exercise Interventions

Dance

The interventions took place twice a week, lasted 90 minutes per session, and lasted 12 weeks from September to November 2021. The location and structure of each session were the same and adapted to the group of older adults.

Characteristics of the dance sessions: energy expenditure is mostly aerobic and intermittent, with varying intensity and global muscle involvement and dynamic muscle activity.

Each session consisted of a 15-minute welcome in a circle (attention, breathing, body awareness, landing, grounding, relationships with others and with the environment through observation and rhythmic patterns) followed by a 30-minute warm-up technique (concerning the body on an anatomical level, including developmental pattern exercises, bones, muscles, fasciae, skin structures awareness through touch, stretching or resistance). Peek of the class included 30 minutes of controlled improvisation based on different choreographic scores, addressing different areas (spatial trajectories, shaping, walking, expressive qualities of movement, props, phrasing, attention, active participation, and relationships). The session ended with 15 minutes of cool-down (breathing, focusing on oneself) and verbal reflection. The motivation to move in a particular but individual way is carefully prepared and facilitated through verbal scores, suggestions and invitations based on imagination, stories, symbolism, or choreographic scores. Initially, the sessions were based on mirroring, imitation and clear identification of movement or spatial pathways, gradually giving way to creativity, spontaneity, and improvisation. A qualified professional dance teacher prepared, facilitated, and supervised each session.

Martial Arts

Like the dance intervention, the martial arts intervention took place twice a week, lasted 90 minutes per session and ran for 12 weeks; however, it took place from September to November 2022. Each session was designed specifically for the older adults, considering their needs and abilities and creating a supportive environment. Each session consisted of a 15-minute welcome and evaluation of the previous session while walking. This was followed by 30 minutes of muscle activation through warm-up. The warm-up always started from head to tail and then moved to a warm-up on the floor. The session included games that verbally motivated people to move through imagination. After the game, the main 30-minute session began with punching training, moving from shadow work on the palms to pad work, then to bags and then back to pair work with pad work. As soon as the palms were tired enough, the situation was relieved with a game or activity that released the whole arm. After a few lessons, the exercise also focused on the lower limbs and kicks. This was followed by more work in pairs or the entire group. A model situation was usually played out - how to solve it with a punch, kick or other maneuver that was the session's content. This part was followed by exercise in the form of Tai Chi, or more specifically, chakra activation or relaxation of the part of the muscle most stressed during the lesson. This part aimed to improve flexibility, balance, and muscle strength, reduce stress and anxiety, and support cognitive function. As with the dance intervention, the session ended with 15 minutes of cool-down.

Control Group

In the CG, participants were either placed on a waiting list. The benefits of cooperation in the study were joining the exercise group of their choice, dancing or martial arts after the trial, or receiving a financial reward. In the CG,

participants were asked to maintain their usual lifestyle as fewer active individuals according to RAPA were selected for the study, no specific recommendations for physical activity were required.

Serum Sample Collection and Preparation

During the first and third visit 9 mL of venous blood was drawn within the week, no earlier than 48 hours pre and postintervention, from the antecubital vein of the arm and collected into Vacutainer tubes between 7.30 a.m. and 9 a.m. Participants were advised to refrain from unusual or intense PA for at least 24 hours prior to collection.³²

The samples were then stored at room temperature for a maximum of 60 minutes to facilitate clotting and obtain highquality serum.³³ Sample collection, clotting time and storage was performed by an experienced nurse and laboratory technician according to University Hospital standards.

After, the blood samples were separated by centrifugation at $1500 \times g$ for 10 minutes at $20^{\circ}C$ and stored frozen at $-80^{\circ}C$ until assayed. To estimate BDNF and irisin serum levels, we used the enzyme-linked immunosorbent assay (ELISA) kits in the Microplate Photometer HiPo MPP-96 (BioSan) reader. For the BDNF analysis, we used an ELISA kit from Thermofisher SCIENTIFIC (USA). The samples were diluted 5 times before analysis to fit the concentration range declared by the producer. The measured absorbance was recalculated according to the calibration curve and converted to the concentration in the sample. Irisin was analyzed using a kit from Sigma-Aldrich, Germany. The sample was not diluted; therefore, the measured absorbance corresponded directly to the concentration of irisin in the sample according to the calibration curve.

Cognitive Measures

Following a small snack immediately after the blood collection, participants completed a battery of neuropsychological tests in a quiet room through trained research team members who administered it under the supervision of a psychologist. The Rey Auditory Verbal Learning Test (RAVLT) assessed verbal memory and learning abilities. From RAVLT, we used the tests of immediate short-term memory (A1), as well as the ability and strategies to learn new information (ΣA).^{34–36} We used two fluency verbal tests initially developed by Thurstone³⁷ 1) the phonemic fluency test of words with the initial letter "P" and 2) the animal fluency test that also evaluates semantic memory in addition to language, both under time constraints of 60 seconds. The subject is asked to say as many words as possible under a given condition and in a specified time frame. More words demonstrate better performance.³⁸ The Digit Symbol (DS) subtest of the Wechsler Adult Intelligence Scale (WAIS-III) was used to test processing speed. The DS subtest consists of a key consisting of the numbers 1-9, each paired with a unique, easily drawn symbol such as a "-", "=" or "". The numbers 1-9 series are presented below the key in random order and repeated several times. The subject then has 120 seconds to fill in the corresponding symbol for each number.^{39,40} The Trail Making Test (TMT) in both versions A and B was used to assess attention and mental flexibility. In TMT A, the subject uses a pencil to connect a series of 25 encircled numbers in numerical order. In part B, the subject connects 25 encircled numbers and letters in numerical and alphabetical order, alternating between numbers and letters. For example, the first number, "1", is followed by the first letter, "A", then the second number, "2", then the second letter, "B", etc. Speed was measured, and participants were asked to complete the test as quickly as possible, with less time, which meant better performance on both tests. 41-43

Self-Report Subjective Questionnaires

The battery included the Geriatric Depression Scale (GDS) questionnaire to assess their mood over the previous three months and after the trials. Scores on the GDS range from 0 to 15. The higher the score, the more depressed the responses on the GDS.⁴⁴ The Questionnaire of Functional State (FAQ-CZ)⁴⁵ was used to assess to what extent they were able to carry out everyday activities. The FAQ-CZ consists of 10 items and is scored on a 0–3-point scale. The raw score of the FAQ-CZ was used to calculate the percentage of independence.

Anthropometric and Strength Measures

During the second and fourth visits, the anthropometric and strength measurements were conducted in the morning within the week pre and post-intervention. Height was measured using a SECA 213 portable stadiometer, and weight and body composition were measured using bioelectrical impedance (InBody 720, Biospace Co., Ltd. Korea). Skeletal

muscle mass (SMM) and fat estimations were obtained from the InBody device. Skeletal muscle index (SMI) was calculated as SMM (kg)/height² (m²).⁴⁶ Handgrip strength was measured using a TKK 5401 digital dynamometer (Takei, Japan). The best value from the three trials was used. The isometric knee extensor strength of the dominant limb was tested using a Humac Norm dynamometer (Cybex CSMI, Stoughton, MA). Prior to testing, participants completed a supervised general warm-up. They were tested according to standardized guidelines. Gait speed was assessed using the 4-metre walk test,⁴⁷ and the chair stand was measured as time for 5 repetitions.⁴⁸

Data Analysis

Means and standard deviations (SDs) were calculated for each continuous variable, and proportions for each categorical variable. We then used the Kolmogorov–Smirnov test to test the normality of the distribution of each continuous variable. As the normality assumption was violated in most of the variables, we used non-parametric methods in our analyses. Kruskal–Wallis test and chi-squared tests were used to test for similarity between groups on baseline measures. We then calculated T-scores from each cognitive, anthropometric and muscle strength variable as T-score = z*10 + 50, where z-score was calculated as z-score = $(X - \overline{X})/SD$, where x is the raw score, \overline{X} is the mean of the study sample and SD is the standard deviation of the study sample. From the individual T-scores, we calculated the average T-scores for each domain - cognitive, anthropometric, and physical. The Wilcoxon signed-ranks test was used to test for within-group differences at pre-post. Generalized linear models for gamma with log link distribution adjusted for age and pre-intervention levels of tested dependent variables were used as the main tool to test the effect of the intervention. In addition, generalized linear models for gamma with log link distribution were used to examine the association between changes in BDNF and irisin levels and changes in mean z-scores of cognitive domains, anthropometric and strength measures, controlling for the effect of the intervention groups. All analyses and graphs were performed using IBM SPSS Statistics 24.

Results

174 people completed the web form, and 150 met the study criteria. We then selected 77 participants whose written PA was 2 out of 5 (highest PA) according to the RAPA. 77 older adults were randomly assigned to three groups - DG, MaG and CG. During the interventions, 34.4% of participants dropped out (Figure 1). The mean age of participants was 70.3 (3.8) years, and they were deemed non-obese, with a mean BMI of 26.7 (4.9) and higher education levels. The majority



Figure I Flow chart of study design.

	DG n = 25	MaG n = 25	CG n = 27	Р
Age (years)	69.6 (3.5)	71.1 (4.3)	70.1 (3.7)	0.349
Females (%)	80.0	88.0	81.5	0.723
Education (years)	15.3 (3.0)	16.2 (3.0)	16.3 (2.4)	0.414
Height (cm)	166.5 (8.2)	165.2 (10.2)	166.2 (9.0)	0.777
Weight (kg)	74.2 (11.6)	69.8 (12.9)	74.1 (17.0)	0.440
BMI (kg.m ⁻²)	26.9 (4.1)	25.5 (6.3)	26.7 (4.9)	0.489
FAQ-CZ (%)	95.6 (7.9)	98.8 (2.5)	96.4 (6.8)	0.436
Total adherence (%)	80.0	64.0	85.2	0.175

 Table I Descriptive Characteristics of the Study Participants

Abbreviations: DG, dance group; MaG, martial arts group; CG, control group; p, significance value calculated using the Kruskal–Wallis test for continuous data and the Chi-square test for categorical data; BMI, Body Mass Index; FAQ-CZ, Questionnaire of Functional Czech Republic State.

of the participants, 83.1%, were women. Overall adherence to the intervention was 76.6%. The highest dropout rate was 36.0% in MaG (Table 1).

There were no significant changes in serum irisin levels in any of the groups, although a greater reduction in irisin was observed in the CG. The 12-week interventions resulted in a significant increase in serum BDNF levels in MaG (p < 0.05), whereas there was a significant decrease in CG. The between-group effect was significant for BDNF levels in both DG and MaG compared to CG (p < 0.05). In the cognitive domain, represented by T-scores, there was a significant improvement after the dance intervention (p < 0.05), and the between-group effect was significant in DG compared to CG (p < 0.05). Participants' performance on the RAVLT A1 deteriorated significantly (p < 0.05) in CG after 12 weeks, but there was no significant between-group effect. The between-group effect was significant in DG compared to CG (p < 0.05) in TMT A. There was a significant improvement in TMT-B in the DG (p < 0.05), and the between-group effect was also significantly compared to CG (p < 0.05). Participants increased their gait speed ability after the dance intervention but not significantly compared to CG. Finally, there was an improvement in GDS in the DG (p < 0.05), and the between-group effect was significant compared to CG. Finally, there was an improvement in GDS in the DG (p < 0.05), and the between-group effect was significant compared to the CG (Table 2). With the exception of TNT B, DS, physical T-scores and handgrip, all pre-treatment measures were significantly associated with post-intervention change, meaning that with few exceptions, the lower the serum level or test performance, the greater the change predicted. Changes in the levels of selected variables for individual participants are shown in Figure 2, with the solid line indicating improvement and the dashed line indicating deterioration.

According to the generalized linear models of the association between changes in BDNF and irisin levels and changes in mean cognitive domain T-scores, only anthropometric T-scores were significantly associated with changes in irisin levels (p = 0.026). There was no association with any other score changes after the intervention.

Discussion

This is the first RCT to investigate exerkines (irisin and BDNF) blood level changes concerning cognitive and physical fitness in healthy older adults after a 12-week dance and martial arts intervention (MaG) program. We found a significant increase in serum BDNF levels in MaG group and a significant decrease in serum BDNF in the control group (CG). Moreover, we found a significant improvement in cognitive T-scores, TMT – A and TMT – B after the dance intervention. On the other hand, performance on the RAVLT A1 in CG deteriorated significantly. Our results are largely in consensus with numerous studies which suggested that regular exercise is associated with improved memory, enhanced cognitive flexibility, physical performance, and mood due to elevated levels of some prominent exerkines.

Exerkines Levels

Exerkines are cytokines that are released in response to acute bouts of exercise, but chronic exercise also leads to changes in circulating humoral factors, even during periods of rest. These changes suggest that exerkine levels may serve as

	DG (n = 20)		MaG (n = 16)		CG (n = 23)		DG (n = 20)	MaG (n = 16)	CG (n = 23)
	Pre	Post	Pre	Post	Pre	Post	Δ	Δ	Δ
IRISIN (ng.m L^{-1})	495.0 (74.8)	496.2 (56.5)	446.5 (75.0)	451.0 (69.9)	478.8 (73.7)	469.0 (67.1)	1.3 (27.7)	4.5 (58.2)	-9.8 (34.3)
BDNF (ng.mL ⁻¹)	50.2 (7.7)	52.0 (7.0)	49.7 (7.5)	53.2 (7.3)*	52.5 (7.9)	47.6 (6.1)*	I.8 (4.9) [†]	3.5 (6.3) [†]	-4.9 (8.2)
Cognitive T-scores	49.3 (6.7)	51.6 (6.5)*	49.0 (6.0)	48.3 (7.2)	51.4 (4.8)	49.8 (4.4)	2.3 (3.1) [†]	-0.7 (3.2)	-1.5 (4.1)
RAVLT A1 (points)	6.7 (2.0)	6.8 (1.9)	6.1 (1.3)	5.9 (1.7)	7.2 (1.7)	6.6 (1.9)*	0.1 (1.9)	-0.2 (1.6)	-0.6 (1.8)
RAVLT ∑A1-5 (points)	49.9 (11.4)	51.4 (8.8)	52.2 (7.9)	49.1 (7.9)	54.0 (7.4)	51.2 (6.6)	1.5 (7.5)	-3.1 (7.6)	-2.9 (5.9)
Words	17.9 (4.4)	18.1 (4.2)	18.6 (7.3)	18.0 (4.1)	19.8 (5.3)	18.4 (5.4)	0.3 (3.2)	-0.6 (4.7)	-1.4 (4.5)
Animals	24.7 (6.5)	24.7 (6.1)	24.4 (7.8)	23.9 (6.4)	25.6 (4.8)	24.9 (6.4)	-0.1 (4.3)	-0.6 (4.3)	-0.7 (4.8)
TMT – A (s)	39.5 (10.4)	35.0 (10.0)	40.9 (10.0)	38.6 (12.0)	39.3 (10.4)	42.0 (11.1)	-4.5 (10.4) [†]	-2.3 (10.1)	2.7 (12.4)
TMT – B (s)	96.3 (24.7)	78.0 (17.2)*	88.6 (16.3)	98.4 (49.8)	88.7 (20.5)	92.2 (19.9)	-18.4 (19.6) [†]	9.8 (45.0)	3.4 (13.3)
DS (points)	61.6 (14.7)	64.0 (20.8)	56.8 (11.5)	58.5 (10.1)	60.1 (10.5)	62.4 (8.2)	2.5 (8.2)	1.7 (9.4)	2.4 (7.0)
Anthropometric T-scores	50.0 (6.8)	49.6 (6.7)	48.3 (5.8)	48.3 (5.2)	51.2 (7.3)	51.5 (7.1)	-0.4 (2.2)	0.00 (2.0)	0.3 (2.2)
SMI (kg.m ⁻¹)	9.6 (1.0)	9.6 (0.9)	9.1 (0.9)	9.0 (1.0)	9.6 (1.1)	9.6 (0.9)	-0.0 (0.3)	-0.1 (0.2) [†]	0.1 (0.3)
Fat (kg)	22.6 (7.0)	23.2 (6.8)	22.9 (7.0)	22.4 (6.6)	21.4 (8.7)	21.5 (8.4)	0.6 (1.8)	-0.5 (2.0)	0.1 (1.7)
VFA (cm ²)	111.0 (38.7)	113.2 (38.2)	112.0 (39.1)	111.2 (36.3)	102.9 (47.1)	103.1 (45.5)	3.0 (10.8)	-0.8 (12.1)	0.2 (10.0)
Physical T-scores	49.7 6.2)	50.7 (7.1)	47.2 (4.9)	47.2 (4.6)	52.2 (6.4)	51.4 (7.0)	1.0 (3.8)	-0.1 (2.8)	-0.8 (4.4)
Handgrip (kg)	28.8 (8.3)	29.9 (8.1)	26.4 (6.7)	26.6 (6.1)	30.2 (7.4)	30.7 (7.5)	1.2 (2.5)	0.2 (2.0)	0.6 (2.4)
Knee extension (Nm)	138.2 (36.1)	141.2 (35.6)	117.1 (30.7)	121.9 (31.5)	139.1 (43.4)	139.0 (44.0)	3.1 (28.1)	4.8 (15.8)	-0.1 (22.1)
Gait speed (m.s ⁻¹)	1.2 (0.1)	1.3 (0.2)*	1.2 (0.2)	1.2 (0.2)	1.3 (0.2)	1.3 (0.2)	0.1 (0.2)	0.0 (0.2)	0.0 (0.3)
Chair stand (s)	6.7 (1.3)	6.7 (1.9)	7.3 (0.7)	7.2 (1.1)	6.6 (1.6)	6.7 (1.5)	0.0 (1.6)	-0.2 (1.3)	0.0 (1.5)
GDS (points)	2.3 (2.2)	1.2 (1.3)*	1.1 (1.0)	1.0 (1.4)	2.1 (2.0)	2.4 (2.3)	-1.1 (1.8) [†]	-0.1 (1.4)	0.3 (1.4)

Table 2 Pre and Post Measures and Changes in All Tested Variables

Notes: data are presented as mean (SD); *Wilcoxon Signed Ranks Test p < 0.05; [†]effect differs from CG according to generalized linear models for gamma with log link distribution adjusted for age and pre-intervention level at p < 0.05. Abbreviations: DG, dance group; MaG, martial arts group; CG, control group; Δ, post-pre changes; BDNF, Brain-Derived Neurotrophic Factor; RAVLT, Rey Auditory Verbal Learning Test; RAVLT A1, first learning trial; RAVLT $\sum A1-5$, learning curve; RAVLT A7, delayed recall; TMT, Trail Making Test; DS, Digit Symbol subtest from the Wechsler Adult Intelligence Scale III.; SMI, Skeletal Muscle Index; VFA, visceral fatty acids; GDS, Geriatric Depression Scale.



Figure 2 Pre and post means with 95% CIs and changes in levels of selected variables for individual participants by study group. (A) Irisin (ng.mL⁻¹), (B) BDNF (ng.mL⁻¹), (C) Cognitive T-scores, (D) Anthropometric T-scores, (E) Physical T-scores, (F) GDS (points); *Wilcoxon Signed Ranks Test; Δ Generalized linear models for gamma with log link distribution adjusted for age and pre-intervention level.

a marker of long-term adaptations to regular physical activity, highlighting the lasting effects of chronic training on the body's physiological systems.⁴⁹

The FNDC5/irisin effect on brain function was investigated only recently,⁵⁰ and its beneficial effect has been proven particularly in AD patients.⁵¹ Our recent meta-analysis showed that long-term PA and exercise might increase irisin blood levels primarily in specific populations, including healthy and obese older adults; however, the results of individual studies exhibited high heterogeneity, which must be taken into account when interpreting irisin data.⁵² Our results did not confirm a significant change in serum irisin levels in any of our intervention groups, although a significant reduction in irisin was observed in the CG. In humans, some studies have shown increases in irisin levels in men following cardiovascular exercise,^{8,53} while others indicated no significant effect.^{54–56} The variability in findings may be attributed to the timing of sample collection, as irisin concentrations have been shown to exhibit temporal fluctuations following

acute exercise. Specifically, irisin levels are modulated by acute physical activity for up to 48 hours post-exercise, which may account for inconsistencies in measurement outcomes across studies.⁵⁷

Additionally, a recent meta-analysis reporting the effect of resistance exercise on irisin levels described that irisin levels significantly increased in PA lasting less than 12 weeks and decreased in PE lasting longer than 16 weeks.⁵⁸ Other meta-analyses confirm that resistance training is crucial for increasing circulating irisin, especially in older adults.^{59,60} Rodziewicz-Flis et al²⁶ also reported a significant increase in irisin serum levels after 12 weeks of folk dance. We assume that the nature of our dance intervention, based on perception and body connection rather than resistance training principles, could explain this difference between the results of both studies. In fact, folk dance can be regarded as a type of resistance training that focuses on leg strength, which was demonstrated in folk dancers when compared to healthy peers.⁶¹

It is suggested that peripheral PA-induced irisin can pass through the blood-brain barrier and influence BDNF levels.⁶² However, it remains unknown which neuronal receptor induces this pathway after being activated by irisin.⁶³ However, it is postulated that the faciliatory effect of irisin on brain activity may be indirect by the induction of a rise in BDNF levels.⁶⁴ In fact, our 12-week interventions resulted in a significant increase in serum BDNF levels in MaG and a significant decrease in CG. The Δ post-pre changes were positive in both intervention groups and negative in CG, which was significant between each intervention group compared to CG. This agrees with recent studies, where the peripheral concentration of BDNF significantly increased following the judo training compared with the control group in older people⁶⁵ and where regular Taekwondo training improved cognitive function and increased BDNF levels in older women.⁶⁶ Marinus et al⁶⁷ demonstrated that resistance training is an essential component of the PA program in older adults to increase baseline BDNF levels; however, Dinoff et al⁶⁸ did not find an effect of chronic resistance training on BDNF after balance training and folk dance. Possible reasons for this inconsistency include population heterogeneity, differences in type, intensity, and duration of the exercise intervention, and BDNF measurement from different blood components such as serum or plasma.⁶⁹ We hypothesize that both of our somatic-based interventions exhibit greater similarities to aerobic training characteristics than resistance training.

More importantly, it was shown that serum BDNF concentrations systematically vary over the year, increasing in the spring-summer period and decreasing in the autumn-winter period. This finding is important as subtle effects of season on depressive behaviors have been described and long-known.⁷⁰ Research studies have demonstrated that BDNF level in peripheral blood is correlated negatively with the severity of depression in rodents and humans.^{71,72} Our results did not show significant increases in serum BDNF after the dance intervention, but a significant decrease in depressive signs in DG assessed by GDS was observed, confirming that dance can indeed ameliorate mood in older adults and reduce depressive signs.^{73–75} Teixeira-Machado et al⁷⁶ claim that dance practice improves neuroplasticity, and scientists link endurance exercise with BDNF expression in the brain concretely showing that neuroplasticity could well be induced by acute or chronic exposure to PA.^{7,77–79}

Anthropometrics and Physical Fitness

Our study only found significant improvements in gait speed after the dance intervention. There were no other improvements in physical or anthropometric measures. However, regarding the association between selected variables, anthropometric T-scores Δ were significantly associated with irisin without group effect. Planella-Farrugia et al⁸⁰ suggest that irisin may be considered a marker for improved muscular performance in older adults. For example, irisin correlates with muscle quality in Charcot-Marie-Tooth patients as a biomarker of muscle mass and strength loss.⁸¹ However, Baek et al⁸² did not observe the association between irisin serum level and clinical muscle parameters in humans. On the other hand, BDNF's neuroprotective properties could indirectly impact muscle strength by promoting effective neuromuscular communication and thus preventing muscle weakness or atrophy.^{83,84} Higher BDNF levels have been associated with increased activation of fast-twitch muscle fibers, which generate force and power,⁸⁵ primarily through its role in promoting muscle adaptation, growth, and overall function.^{86–88}

Cognitive Fitness

Recently, cognition has been associated with frailty,⁸⁹ impaired steady balance and poor mental flexibility in older people.⁹⁰ Dance and other sensory-rich movement practices attract (neuro)science because they are complex activities that support adaptation to our environment during ageing.⁹¹ It includes self-managing based on soft skills such as connecting with intuition, promoting emotional and physical integration, self-esteem, empathy, mutual validation, and the ability to take risks. Our participants showed significant improvement in cognitive T-scores after the dance intervention and Δ post-pre change in cognitive T-scores when DG was compared to CG. Further, we found better participants' performance in short-term memory assessed by the RAVLT A1 in DG compared to MaG and significant deterioration in CG. A recent meta-analysis summarized that dance interventions benefit specific cognitive domains, such as global cognition, memory, and executive function.⁹² Ma et al⁹³ provide evidence that regular rhythmic movement, like dance, improves global cognitive function but not executive functions. In practices based on body awareness, the creative process leads to forgotten or new resources, stimulates new associations and releases energy for decision-making and action, thus creating relationships.¹⁸ Numerous studies suggest that a long-term dance intervention could be better than repeated PA for inducing neuroplasticity in the ageing human brain, which is related to the multimodal nature of dance.^{19,94} However, the duration of the intervention and the intensity of the dancing exercise might be the key factor for brain changes and cognitive improvements, ¹⁹ as documented earlier on PA.^{10,95} Moreover, dance was shown to be superior to aerobic exercise after 12 weeks in older adults.⁹⁶ Our results did not show a significant beneficial effect on cognition in more cognitive domains in both intervention groups compared to CG. The explanation could be the test's low sensitivity for revealing changes in cognitive processes, the ceiling effect in pre-tests, and the possibility that changes in the brain precede the changes in measurable behavior.

Limitations and Future Proposal

Although we recruited the study participants based on the lowest RAPA score, most participants led fairly active lifestyles (social and sports activities), which became apparent from talks during interventions. Moreover, according to the FAQ-CZ score and cognitive and physical measurements, most study participants reported higher education levels and demonstrated good mental and physical condition. Also, the majority of the study participants were females. Therefore, all these might have influenced the study results due to the ceiling effect in the pre-tests. Moreover, a significant (more than 20%) dropout of the participants during the intervention period should also be considered a study limitation. Future studies should focus on follow-up and investigate the motivations behind adherence, as it is well known that neural plasticity in older adults depends on individual behavior,^{97,98} and can be positively modified by PA.^{99,100} Also, more extended intervention periods would benefit from observing changes related explicitly to cognitive domains. The ultimate goal should be finding a physical exercise protocol that works best to improve physical and cognitive performance deficits in specific populations of older adults.

Conclusion

Exerkines represent excellent biomarkers in our quest to comprehend the complex relationship between exercise and brain health. Even though our study found no significant changes in serum irisin levels across the groups, changes in anthropometric T-scores (Δ) (ie increased muscle mass and decreased fat mass) were significantly correlated with irisin levels, independent of group effects. We also found a significant increase in brain-derived neurotrophic factor (BDNF) levels in the MaG group, which suggests that BDNF can be upregulated by such somatic-based practice. Our data suggest that dance and martial arts could be promising non-pharmacological programs for the ageing brain, and scientists should further focus on providing evidence of their beneficial effects at the molecular level.

Declarations

The study was approved by the Charles University Faculty of Physical Education and Sport ethics committees (245/2020) and the Charles University Hospital Kralovske Vinohrady (EK-VP/13/0/2020), and all the participants signed an informed consent. All methods were performed following the Declarations of Helsinki.

Data Sharing Statement

The data supporting this study's findings are available from the corresponding author, MS, upon reasonable request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

This manuscript has not been previously submitted or published and is not under consideration in any other peerreviewed media. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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