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Neuromuscular Aging: A Case for the Neuroprotective Effects of Dancing

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Keywords
C-terminal agrin fragment · Neuromuscular junction · Physical activity · Muscle power · Dance

Abstract
Aim: We planned a cross-sectional investigation (study 1) and a longitudinal training intervention (study 2) to investigate whether recreational dancing affords greater neuroprotective effects against age-related neuromuscular junction (NMJ) degeneration compared to general fitness exercise training. ***Methods:*** In study 1, we recruited 19 older volunteers regularly practising dancing (older dancers [OD]) and 15 recreationally physically active older individuals (OA) and physical performance, muscle morphology, muscle function, and NMJ stability (from serum C-terminal agrin fragment [CAF] concentration) were assessed. In study 2, employing a longitudinal study design in a different cohort (composed of 37 older adults), we aimed to study whether a 6-month dancing intervention decreased CAF concentration compared to general fitness exercise training in older adults. ***Results:*** Our findings show that OD had a lower CAF concentration (suggesting an increased NMJ stability) compared to OA. This result was accompanied by superior functional performance despite no differences in muscle size. In study 2, we observed a reduction in CAF concentration only in the dancing group. ***Conclusion:*** Overall, these findings suggest that dancing is an effective training modality to promote neuroprotection and increase muscle function in healthy older individuals.

Introduction

It is now common knowledge that physical activity promotes multiple health benefits and represents one of the most effective strategies to counteract the age-related physiological decline of different systems [1]. Particularly, physical activity plays a fundamental role in slowing down the natural loss of muscle mass and strength associated with ageing [2]. Physical activity exerts positive ef-

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fects on muscle health through different strategies, such as by increasing muscle protein synthesis [3], maintaining mitochondrial structure and function [4], and reducing systemic inflammation levels [5], with the activation of specific molecular pathways. A mechanism that received relatively little attention in human ageing studies is represented by the preservation of neuromuscular junction (NMJ) health induced by physical activity. The NMJ constitutes a fundamental crossroad for the functional dialogue between muscle and nerve [6], and its degeneration, along with muscle fibre denervation and loss of motor units, is considered as an important contributor to muscle wasting in ageing [7–10]. Evidence from animal studies shows that chronic physical activity has a protective effect on NMJ stability [11, 12], mediated by the activity of several neurotrophins [13]. The direct observation of NMJs morphology in humans is difficult: indeed, despite some new promising approaches, the presence of NMJ structures in human muscle biopsies collected with traditional sampling procedures is generally low [14]. For this reason, human NMJ health status is generally inferred by biomarkers of muscle denervation or NMJ stability [9]. Of note, the circulating serum concentration of C-terminal agrin fragment (CAF) has been proposed as a biomarker of NMJ instability and applied in different contexts and populations [15–18]. CAF is released in the circulation upon agrin cleavage by neurotrypsin [19]. Since agrin is a proteoglycan that stabilizes the synaptic structures, higher CAF serum levels would be then indicative of a greater NMJ instability [15, 16]. The concentration of this biomarker has been shown to decrease in some longitudinal training studies involving different exercise modalities (resistance and power training) [15, 20, 21], suggesting that PA may play a role in the maintenance of human NMJ stability. In contrast, other studies reported opposite findings with unchanged [22, 23] or increased [24] CAF concentration after training interventions. Our laboratory has previously speculated that, in older adults, activities that require high levels of motor coordination and challenge the trainee with sensory and cognitive stimuli could be particularly effective in the preservation of the NMJ health [25]. To this extent, dancing is a form of physical activity that promotes neuroprotection and requires the integration of sensory information from multiple channels and whole-body fine-grained motor control [26–28]. We have recently shown that older active dancers exhibited lower CAF levels, accompanied by superior gait and dynamic balance performance, compared to sedentary peers [25]. Despite these promising findings, in the same previous study, we could not test whether dancing represents a superior form of physical activity to promote NMJ stability compared to other modalities.

Therefore, with this two-experiment study, we aimed to investigate whether recreational dancing practised on a regular basis, affords protections against NMJ degeneration in older individuals. In study 1, with a cross-sectional research design, we investigated the differences in CAF concentration, muscle morphology, and neuromuscular properties between older dancers (OD) and physically active age-matched peers. In study 2, employing a longitudinal study design on a different cohort, we aimed to study whether a 6-month dancing intervention could have neuroprotective effects (as inferred from CAF concentration) when compared to conventional fitness exercise training programmes in older healthy adults.

Methods

Study 1: Cross-Sectional Investigation

Participants

A total of 34 older adults practising light aerobic physical activities were enrolled in the study. Inclusion criteria were the absence of musculoskeletal injuries in the last 2 years as well as neurological and cardiovascular pathologies. Among the participants, we recruited 19 older volunteers regularly practising dancing (OD: 11 males and 8 females; age: 76.9 ± 6.3 yrs; height: 1.60 ± 0.11 m; and mass: 71.4 ± 13.4 kg) and 15 recreationally physically active older individuals (OA; 7 males and 8 females; age: 78.6 ± 6.6 yrs; height: 1.56 ± 0.06 m; and mass: 76.7 ± 15.4). The OD practised one or different forms of dancing (i.e., sequence dancing, Morris dancing, and rapper sword dancing) on a regular basis (at least twice a week, about 90 min per session, for a minimum of 2 consecutive years). The OA participated in recreational physical activities (e.g., brisk walking, swimming, golf, fitness) at least twice/week (60–90 min per session). The OD were recruited by contacting local dancing clubs in the area of the city of Derby (UK), while the OA were recruited mainly through the Derby Bridge Club. All volunteers provided their written consent to participate in the study, after being informed of the purpose of the study and all the procedures and potential risks involved.

Protocol

Data collection was carried out between October 2016 and March 2017 in the MRC-ARUK Centre for Musculoskeletal Ageing, University of Nottingham, Derby, UK. Participants were asked to visit the laboratory three times, separated by 5/7 days. On the first visit, all volunteers (a) underwent a health questionnaire and screening and (b) a blood sampling; (c) performed the short physical performance battery (SPPB) and (d) handgrip strength test (HG). During the second visit, (e) muscle morphology was assessed by ultrasonography and (f) muscle power using the Nottingham power rig. Finally, on the last visit, (g) muscle strength was assessed, and (h) a muscle fatigue test was performed. Participants were asked to refrain from intense physical activities in the

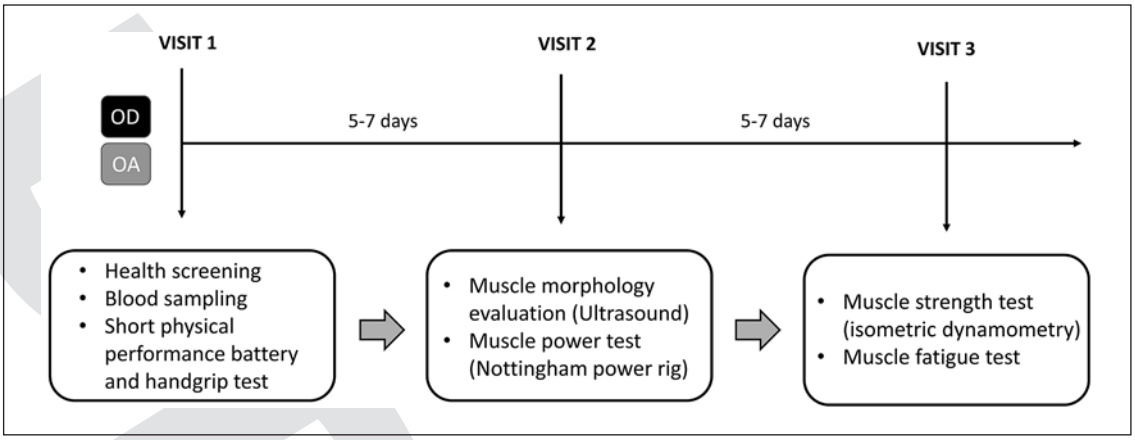

Fig. 1. Graphical representation of study 1 design.

24 h preceding laboratory visits. In addition, tests were carried out in the morning or at least 3 h after lunch to avoid possible influence of the postprandial state. The study was approved by the University of Nottingham Ethics Committee and conformed to the ethical standards of the Declaration of Helsinki. A graphical representation of the study design is presented in Figure 1. Members of the study team that performed the analyses were blinded to the groups.

Measurements

Serum CAF

Blood samples for CAF measurement were obtained in the morning by venepuncture of the median cubital vein after overnight fasting, using commercial collection tubes (Vacuette® Z Serum Sep Clot Activator; Greiner Bio-One, Kremsmünster, Austria). Samples were kept on ice until they were centrifuged (IEC Centra CL3R; Thermo electron Corporation, Waltham, MA, USA) at +4°C, 20 min at 3,200 rpm. The supernatant, corresponding to the serum fraction, was aliquoted and stored at −80°C until analyses. CAF levels were determined in the serum using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (NTCAF ELISA; Neurotune AG, Schlieren, CH, USA) following the manufacturer's instructions. All samples were assessed in duplicate. The obtained CAF concentration was read through a microplate ELISA reader. To calculate CAF serum concentrations, a standard curve was prepared and read at 450 nm. Serum CAF concentration was interpolated from the CAF linear standard curve and corrected for sample dilution.

Physical Performance

Physical performance was assessed using the SPPB that includes three subtests that evaluate the standing balance, the usual gait speed over a 4-m walk, and the ability to rise repetitively 5 times from a chair [29]. Each SPPB component test (balance, gait, and chair stand) was scored from 0 (worst performance; inability to perform the test) to 4 (best performance) [29]. For the balance task, the participants were asked to stand with their feet side by side, followed by the semi-tandem (heel of one foot alongside the big toe of the other foot) and tandem (heel of one foot directly in front of and touching the other foot) positions for 10 s each. The gait speed test consisted of a 4-m walk at the participant's usual pace, where time was recorded. Finally, participants were asked to stand up and sit down 5 times from a chair as quickly as possible with their arms folded across their chests.

Handgrip Test

HG performance was measured using a hand-held dynamometer (JAMAR hydraulic hand dynamometer). Participants were required to stand with their legs slightly apart and their upper limbs relaxed and were asked to tighten the dynamometer as strongly as possible for 3 s. The test was performed with their dominant hand (defined as the hand that the participants were more prone to use during motor tasks like writing, brushing their teeth, or catching a ball) and the force was measured in pounds per square inch. The participants performed three trials and the highest force value was chosen for data analysis. Relative HG strength (rHG) was computed by dividing this value by the body mass.

Muscle Architecture

Longitudinal images of the right vastus lateralis (VL) muscle were collected using B-mode ultrasonography (Mylab70; Esaote, Genoa, Italy), with the participants resting in a supine position. A 100-mm linear array transducer (10–15 MHz) was employed to assess VL fascicle length (Lf), pennation angle (PA), and muscle thickness (MT), as previously described in detail [30, 31]. The ultrasound images were obtained with the transducer positioned at 50% of femur length (mid-distance between great trochanter and midpatella point) in the mid of the muscle belly (detected as the midpoint between the medial and lateral borders). Care was taken in adjusting the transducer to the fascicle plane, applying minimal pressure. A sufficient amount of transmission gel was applied to the transducer for all scans. Lf, PA, and MT were analysed using ImageJ software (1.52v; National Institutes of Health, Bethesda, MD, USA). Lf was determined by digitalizing the visible portion of the fascicle and then extrapolating it with a straight line until the extension of the superficial aponeurosis. PA was measured as the insertion angle between the fascicle and the deep aponeurosis, while MT was defined as the linear distance between the two aponeuroses. The ultrasound sarcopenia index (USI) (i.e., the ratio between Lf and MT) was also computed [32] (Fig. 2).

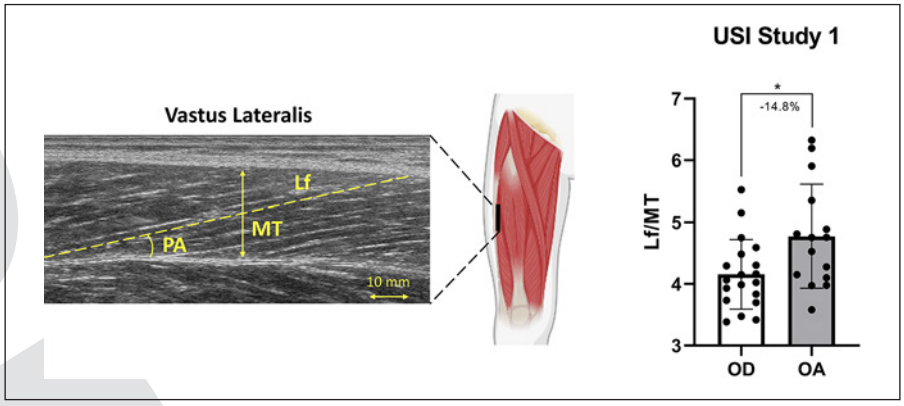

Fig. 2. Differences between recreationally active OD and OA in all the USI in study 1. Lf, fascicle length; PA, pennation angle; MT, muscle thickness.

Muscle Cross-Sectional Area

In addition to VL architecture, muscle cross-sectional area (CSA) of the right quadriceps femoris (QF) muscles were measured using the same ultrasound device. A 47-mm, 7.5-MHz linear array transducer was utilized to obtain transversal ultrasound images using the extended-field-of-view technique. The operator placed the transducer on the medial portion of the leg at 50% of femur length and moved it along the transverse plane until the lateral borders of the VL. Care was taken in keeping the transducer pressure on the skin as constant as possible. CSA values were calculated by tracing the contours of QF using ImageJ software (1.52v; National Institutes of Health).

Muscle Power Test

The Nottingham power rig device (Medical Engineering Unit, University of Nottingham Medical School, Nottingham, UK) was used to assess leg extension muscle power (LEP) during a hip and knee extension movement [33]. Participants were seated in an upright position and were instructed to press as hard and fast as possible a pedal forward until full leg extension. This pedal accelerates a flywheel, and its resultant final angular velocity was used to calculate the mean LEP. Volunteers were required to perform a minimum of 5 repetitions [31, 34]. If within the 5 repetitions, the participant did not improve his/her performance from the 4th to the 5th repetition, the highest LEP value among the 5 trials was employed for data analysis. Conversely, if a progressive increase in power values was observed from the 4th repetition, unlimited additional trials were allowed until a maximal power value was obtained. The protocol was repeated three times and the higher power value (W) was selected. The relative LEP (rLEP) was finally calculated as LEP normalized to body mass (W/kg).

Muscle Isometric Strength Test

Isometric maximum voluntary torque (MVT) of the knee extensors was measured using an isokinetic dynamometer (Cybex Norm; Cybex International Inc., NY, USA) at a fixed joint angle of 70° (full knee extension = 0°). After a brief warm-up, consisting of 10 short submaximal contractions, subjects performed two maximum voluntary contractions lasting ~4 s, with a rest period of 30 s between contractions [34, 35]. Real-time visual feedback on torque production and strong verbal encouragement were provided during the two trials. Participants underwent a familiarization with this test procedure during their second visit to the laboratory. The trial with the highest MVT value was considered for data analysis. Knee extensor MVT was then normalized to QF CSA to obtain the specific force (MVT/CSA; $N*m/cm^2$).

Muscle Fatigue Test

Muscle fatigue was assessed on the same isokinetic dynamometer at a joint angle of 70° by asking the participants to perform an isometric knee extension contraction at 60% MVT until a torque drops to <50% for at least 3 s (task failure) whilst measuring VL surface EMG. Participants underwent a familiarization with this test procedure during their second visit to the laboratory. Time-to-task failure(s) were used to quantify muscle fatigue. Torque steadiness was also computed as the coefficient of variation (CV) in torque during the submaximal contraction.

Study 2: Longitudinal Study Design

Participants and Protocol

This second study was carried out as a part of a larger investigation performed in older adults, aiming to study the effects of dancing versus conventional strength/endurance training on brain structure and function, neurogenesis, cognitive performances, and motor skills [26–28]. While most of these data have been already published in previous studies [26–28], the present investigation focused exclusively on CAF changes (such data are not published). The participants' recruitment process and the experimental protocol have been described in detail previously [26–28]. Briefly, 57 healthy older individuals (>65 years old) were recruited and randomly assigned either to a dance group (DG) or to a fitness group (FG). Participants in both groups were novices and did not have previous dance/exercise experience. CAF data were available in a subgroup of the total sample: DG included 9 females and 10 males (age: 72.3 ± 3.9 yrs; height: 1.70 ± 0.08 m; mass: 79.5 ± 11.0 kg), while the FG 9 females and 9 males (age: 70.6 ± 2.8 yrs; height: 1.74 ± 0.09 m; mass: 82.0 ± 11.5 kg). Both groups performed training sessions twice a week for 6 months. Each dancing or fitness class lasted 90 min and was supervised by experienced instructors. The two training programmes were comparable in terms of exercise intensity based on heart rate assessment, as explained in detail previously [26, 27]. The dance intervention consisted of choreographies of five different genres (Line Dance, Jazz Dance, Rock "n" Roll, Latin-American Dance, and Square Dance) with increasing coordinative demands and time pressure. Participants in the FG completed a conventional strength/endurance training pro-

Table 1. Differences between recreationally active OD and OA in all the neuromuscular parameters investigated in study 1

	OD mean (SD)	OA mean (SD)	Δ% mean	*p* value
CAF levels, pM	209.7 (116.6)	369.7 (182.3)	−76.3	0.0067*
SPPB (score)	11.95 (0.23)	11.33 (1.17)	+5.19	0.0451**
rHG, psi/kg	0.44 (0.1)	0.36 (0.10)	+17.83	0.0292**
VL Lf, cm	7.99 (1.06)	7.95 (1.25)	+0.4	0.9357
VL PA, deg	14.34 (2.35)	13.30 (2.31)	+7.25	0.2054
VL MT, cm	1.94 (0.29)	1.72 (0.42)	+11.43	0.0785
USI (Lf/MT)	4.16 (0.56)	4.77 (0.84)	−14.79	0.0158**
QF CSA, cm^2	49.20 (9.11)	49.16 (14.72)	+0.08	0.7959
rLEP, W/kg	3.22 (1.38)	2.31 (0.81)	+28.4	0.0299**
MVT, *N**m	142.1 (46.69)	140.7 (43.80)	+0.98	0.9314
MVT/CSA, $N*m/cm^2$	2.91 (0.85)	2.9 (0.60)	+1.2	0.899
Time to failure, s	88.39 (38.97)	68.76 (31.98)	+22.21	0.1016
Torque steadiness, %	7.8 (2.11)	6.82 (3.2)	+12.53	0.301

CAF, C-terminal agrin fragment; SPPB, short physical performance battery test; rHG, relative HG strength; VL, vastus lateralis; Lf, fascicle length; PA, pennation angle; MT, muscle thickness; USI, ultrasound sarcopenia index; QF CSA, quadriceps cross-sectional area; rLEP, relative leg extension power; MVT, maximum voluntary torque. * $p < 0.01$. ** $p < 0.05$.

gramme composed mainly of repetitive exercises and low demands in terms of whole-body coordination and memory. Each session comprised units of endurance, strength endurance, and flexibility training. The endurance training was performed on cycle ergometers, while strength training with equipment such as barbells, rubber bands, and a fitness ball. The flexibility exercises mainly consisted of stretching exercises. Approval for the study was obtained from the Ethics Committee of the Otto-von-Guericke University, Magdeburg (Germany). All subjects gave written informed consent to the investigation and obtained reimbursement for their travel expenses.

Measurements Study 2

Serum CAF

For the procedures regarding the assessment of CAF concentration, we refer the reader to the Methods section of study 1.

Statistical Analysis

Study 1

The normality of the datasets was evaluated through qualitative visual inspection of Q-Q plot, skewness and kurtosis calculation, and Shapiro-Wilk normality test. The QF CSA, time to failure during the fatigue test, torque steadiness, $RMS_{fatigue}$, and $MDF_{f/l}$ failed to pass the normality tests, while all the other parameters did. When normality tests were not passed, a correction using the natural logarithm (Ln) or inverse function was applied and the normality of the distribution was tested again. After correction, all parameters resulted normally distributed, and thus, a parametric statistic was applied where appropriated. Unpaired *t* tests were employed to compare the performance between OD and OA in all parameters, except for the SPPB (representing a categorical variable), where the Mann-Whitney test was performed. The magnitudes of changes were evaluated as standardized mean difference Hedges' g. Correlations were obtained using Pearson's product-moment correlation coefficient (*r*). The level of significance was set at $p < 0.05$. Statistical analysis was performed using GraphPad Prism software (version 8.00; GraphPad Software, San Diego CA, USA).

Study 2

The normality of the dataset was evaluated through qualitative visual inspection of a Q-Q plot, skewness and kurtosis calculation, and Shapiro-Wilk normality test. Sphericity was tested with Mauchly's test. The serum CAF concentration values passed the normality tests. The CAF concentration changes were evaluated using a two-way repeated-measures ANOVA (group [i.e., DG or FG]; time [i.e., PRE or POST intervention]). Sidak's post hoc test was employed for multiple comparisons analysis. The magnitudes of changes were evaluated as standardized mean difference Hedges' g. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using GraphPad Prism software (version 8.00; GraphPad Software, San Diego CA, USA).

Results

Study 1

A priori power analysis was performed based on the changes in CAF concentration to determine the convenient sample size. For an effect size calculated based on our previous work [25], a required power ($1 - \beta$) of 0.8 and an error $\alpha = 0.05$, the total sample size resulted in 14 subjects for OA and 18 for OD. Therefore, the partici-

pants recruited in this work represented an appropriate sample. All the participants completed the experimental procedures. However, due to technical problems four subjects (two OD and two OA) were not included in the CAF analysis (i.e., non-detectable CAF concentration), one OD in the MVT test (i.e., knee pain complaint – did not perform the task) and OA in the muscle fatigue test (i.e., muscle soreness – did not perform the task). No differences were observed in age, height, and body mass between the two groups. All the differences between the two groups are presented in Table 1.

Serum CAF

OD exhibit significant lower serum CAF concentration compared to OA ($p = 0.0067$; $g = 1.049$, 95% CI: 0.29–1.84). CV for CAF concentration was 5.7% ± 4.8%.

SPPB and Handgrip Test

The SPPB score ($p = 0.0451$; $g = 0.76$, 95% CI: 0.07–1.48) and rHG strength ($p = 0.0292$; $g = 0.77$, 95% CI: 0.08–1.49) were significantly higher in the OD with respect to OA.

Muscle Architecture and CSA

Conversely, no differences were observed between the two groups in all the muscle morphology parameters: Lf ($p = 0.9357$; $g = 0.03$, 95% CI: −0.65–0.7), PA ($p = 0.2054$; $g = 0.43$, 95% CI: −0.43–1.13), MT ($p = 0.0785$; $g = 0.61$, 95% CI: −0.07–1.31) and QF CSA ($p = 0.7959$; $g = 0.09$, 95% CI: −0.59–0.76). However, the USI significantly differed ($p = 0.0158$; $g = 0.86$, 95% CI: 0.16–1.58), with higher values observed in OA (Fig. 2).

Maximum Muscle Power and Maximum Isometric Strength

The rLEP was significantly greater (+29.4%) in OD than OA ($p = 0.0299$; $g = 0.77$, 95% CI: 0.07–1.48), while no significant differences in MVT were found ($p = 0.9314$; $g = 0.03$, 95% CI: −0.66–0.72) and MVT/CSA ($p = 0.899$; $g = 0.045$, 95% CI: −0.64–0.74).

Muscle Fatigue Test

The time to failure ($p = 0.1016$; $g = 0.57$, 95% CI: −0.01–1.28) during the muscle fatigue test was greater in OD (+22.2%) than in OA, while no differences between the two groups in torque steadiness ($p = 0.301$; $g = 0.358$, 95% CI: −0.34–1.06) were found.

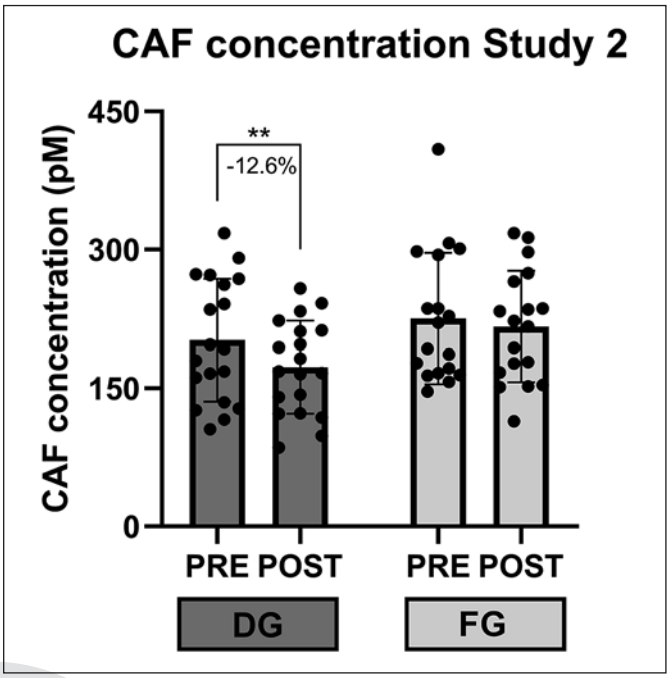

Fig. 3. CAF concentrations changes after a 6-month training intervention based on dancing (DG) or conventional fitness (FG) in study 2. $^{**}p < 0.01$.

Correlations

Interestingly, serum CAF levels significantly negatively correlated with rHG strength ($p = 0.012$; $r = -0.4529$ [95% CI: −0.6991 to −0.1107]) and rLEP ($p = 0.0348$; $r = -0.3866$ [95% CI: −0.6556 to −0.0306]). A trend toward significance was also observed between CAF levels and SPPB score ($p = 0.0981$; $r = -0.3077$ [95% CI: −0.6013–0.05913]).

Study 2

Significant time ($p = 0.0027$; $\eta_p^2 = 0.484$) effect was observed for serum CAF concentration, with a trend toward significance also for group ($p = 0.0962$; $\eta_p^2 = 0.139$) effect and group-by-time interaction was found ($p = 0.0919$; $\eta_p^2 = 0.112$). Post hoc analysis showed a reduced CAF concentration in the DG ($p = 0.0022$; $g = 0.48$ [95% CI: −0.15–1.14]) but not FG ($p = 0.5166$; $g = 0.13$ [95% CI: −0.52–0.79]) group after the intervention (Fig. 3). No differences in CAF concentration between the two groups were observed at PRE ($p = 0.4482$; $g = 0.33$ [95% CI: −0.31–0.99]), while a trend toward significance was detected at POST ($p = 0.0714$; $g = 0.77$ [95% CI, −0.12–1.46]). CV for CAF concentration was 2.5% ± 2.2%.

Discussion

The present work, consisting of two separate studies, aimed to investigate whether dancing afforded neuroprotective effects against age-related NMJ degeneration and whether these were greater than those induced by general fitness training or recreational physical activities. In study 1, we showed, with a cross-sectional observation, that OD presented greater NMJ stability (i.e., lower CAF concentration), lower USI values, better general physical performance (SPPB score), greater HG strength, and greater lower limb muscle power compared to age-matched recreationally active individuals. In study 2, we demonstrated that CAF concentration decreased significantly in response to a 6-month dancing intervention but not in response to general fitness training. Overall, these findings suggest that dancing, in healthy older individuals, affords a protective effect on NMJ stability. While this concept is well established in animal studies [13], there is still a paucity of evidence in human populations [8]. For instance, studies employing CAF as a biomarker of NMJ stability reported decreases [15, 20, 21], no changes [22, 23], or increases [24] in CAF concentration after a variety of different training interventions. In addition, we previously reported that older active individuals exhibited lower CAF concentration compared to age-matched controls with low physical activity levels [25]. Possible explanations for these inconsistencies observed between training studies may be related to several factors, such as exercise modality and dosage, age, and level of physical fitness of the participants. Among these, the modality of physical activity, in particular, seems to play a key role. As dancing is a type of activity that requires high motor coordination and proprioception, it may be ideal for promoting neuroplasticity. Our study 2 revealed that a dancing intervention not only prevented the expected age-related increase in CAF concentration, but that also could reduce the concentration of such biomarkers in healthy older adults. Previous studies conducted on our same cohort (study 2) showed that dancing can improve cognitive function, increase plasma brain-derived neurotrophic factor, and induce neurogenesis of specific brain areas to a greater extent compared to conventional fitness training [26–28]. In particular, a brain-derived neurotrophic factor may be a possible mediating factor of the superior NMJ stability of the OD observed in this work, since this neurotrophin is known to be involved in the regulation of the synaptic transmission efficiency of the NMJs [13, 36]. Therefore, dancing may induce a higher production of neurotrophins compared to other modalities of light physical activity. It is also tempting to speculate that activities requiring high motor coordination and cognitive demands, such as dancing, could selectively stimulate the action of other specific neurotrophins involved in NMJ regulation [13]. The aerobic component of dancing may have also contributed to such neuroprotective effects through the release of PGC-1α (known to promote NMJ remodelling) [37] and by reducing mitochondrial oxidative damage [38]. Future studies should investigate deeper the mechanisms by which dancing can afford protection of the NMJ in advanced age.

In study 1, we found small, yet significant, correlations between CAF concentration and some physical function parameters (i.e., rHG, rLEP, and SPPB), similarly to previous studies [17, 22, 25]. Since these same parameters differed between OD and OA, we can speculate that the greater NMJ stability of the OD may have contributed to the better functional performance observed in such motor tasks. Most of all, OD had significantly greater (+28.4%) muscle power than the OA population. This finding is particularly noteworthy as the preservation of muscle power in old age is functionally more important than preserving strength alone since any daily action requires the production of work in a given timeframe (thus power). Indeed, it has been recommended that exercise strategies in old age should focus on improving muscle power [34, 39]. In this respect, our findings suggest that dancing may be a useful intervention also for increasing muscle power. This is because muscle power is the product of an optimum combination of force and velocity and thus even modest changes in force may become relevant if through greater recruitment of motor units, higher contraction velocities and thus power, are attained. While it is known that OD have an improved physical function and postural balance control compared to sedentary individuals [25, 40], it is also noteworthy that our results highlighted differences with respect to recreationally physically active peers.

The OD participants not only presented lower CAF values, indicative of better preserved NMJ stability, and a superior physical function but also lower USI values (−14.8%), suggesting that dancing may protect against age-related alterations in muscle architecture proportions associated with sarcopenia more than other physical activities with similar duration and frequency [32]. Whether this mitigation of sarcopenia is the result of the mechanical stimuli involved in dancing or the influence of better preserved NMJ stability, or both, should be further investigated.

Some limitations must be acknowledged. In study 1, the two groups performed a similar physical activity volume (frequency and duration of the activities), but we could not match the two groups for training intensity. The groups were not specifically matched for length of activity exposure in years. While OD recruitment was based on eligibility criteria of dancing for at least 2 years or more, the OA recruitment was not conditioned on years of participation in physical activity (although some OA volunteers, during the initial screening, have specifically declared to have been practising their light physical activities for more than 2 years). However, the total exposure time to dancing could have been higher than the exposure time to physical activity of the OA group. Therefore, it is possible that the long adaptation to dancing might have explained the superiority of dance over other types of physical activities.

In study 2, we did not perform any measures on muscle morphology. However, as explained in the manuscript, this second experiment was part of a complex larger investigation, in which, for organizational reasons, the collection of muscle morphological data was not possible. Moreover, we acknowledge that a control group was not included. Nevertheless, a previous report showed no differences in CAF concentration after 1 year without intervention in older adults [22]; thus, we expect that changes in CAF are unlikely to occur in 6 months in a control group. Finally, we acknowledge that in both studies sample size was relatively small.

In conclusion, in this two-study paper, we showed that (a) OD had better preserved NMJ stability, physical function, and muscle geometric proportions compared to OA, and (b) a 6-month dancing but not general fitness intervention can decrease serum CAF concentration. Overall these findings suggest that dancing may represent a superior exercise modality to promote neuroprotection and neuromuscular function in healthy older individuals.

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Statement of Ethics

Studies 1 and 2 were approved by the D of the University of Nottingham, UK (B13032014 SoMSGEM) and Otto-von-Guericke University, Magdeburg, Germany (Nr:22/12), respectively. Study 2 was also registered as DRKS clinical trial (Nr: DRKS00012605). Written informed consent was obtained from all the participants and all the experimental procedures were performed in accordance with the Declaration of Helsinki.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Marco Narici, Martino V. Franchi, Anita Hökelmann, Notger G. Müller, and Jon Lund designed the study. Jon Lund supervised clinical procedures. Francesca Badiali, Martino V. Franchi, Elena Monti, Stefano Longo, Patrick Müller, and Kathrin Rehfeld performed data collection. Francesca Badiali, Martino V. Franchi, Elena Monti, Fabio Sarto, and Debbie Rankin carried out data analysis. Martino V. Franchi and Fabio Sarto wrote the manuscript with input from all authors. All the authors contributed to the interpretation of the results and approved the final version of the manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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