Physiology & Behavior 227 (2020) 113135

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Effect of exercise and grape juice on epigenetic modulation and functional outcomes in PD: A randomized clinical trial

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ARTICLE INFO

Keywords:
Parkinson disease
Aquatic exercise
BDNF
Epigenetic
Grape juice

ABSTRACT

Objective: This study aimed to investigate the impact of an aquatic physical training program associated with grape juice (*Vitis labrusca*) consumption on functional outcomes, Brain-Derived Neurotrophic Factor (BDNF) and global histone H4 acetylation levels in peripheral blood from individuals with Parkinson's disease.
Methods: Nineteen participants were randomized to Aquatic Exercise (AQ, $n = 9$) and Aquatic Exercise + Grape Juice (AQ + GJ, $n = 10$) groups and performed to 4 weeks of an aquatic intervention (twice a week, approximately 60 min/session). The AQ + GJ groups also consumed 400 mL of grape juice per day during this period. Functional capacity (six-min walk test, 6MWT), mobility (The Timed Up and Go, TUG) and the risk of falls (Berg Balance Scale, BBS) were evaluated before and after intervention. In addition, blood collections were carried out for biomarker analysis (e.g. BDNF and global histone H4).
Results: The aquatic exercise program induced functional improvement in individuals with Parkinson's disease, specifically ameliorating their mobility and functional capacity. In addition, enhanced levels of BDNF and histone H4 acetylation were found after the intervention. Grape juice consumption did not potentiate these effects, since any significant differences between the AQ and AQ + GJ groups were not found in all analysed variables.
Conclusions: The present study provides important insights about aquatic exercise-modulated BDNF levels in individuals with Parkinson's disease in combination with functional improvements, suggesting that histone acetylation status may interact to dictate the molecular mechanisms involved in this response. Parkinson disease, aquatic exercise, BDNF, epigenetic, grape juice.

1. Introduction

Parkinson's disease (PD), the second most common chronic neurodegenerative disease, affects approximately 1%~2% of the elderly population [1]. It is characterized by four cardinal motor symptoms: resting tremor, rigidity, bradykinesia and postural instability, resulting from degeneration of dopaminergic nigrostriatal pathways [1,2]. As a consequence of these symptoms, mobility impairments and a lack of balance are usually observed in individuals with PD contributing to the risk of falls [3].

It is largely accepted that reduced levels of brain-derived neurotrophic factor (BDNF) in the substantia nigra might contribute to the death of dopaminergic neurons, thus worsening neurodegeneration and mediating, at least in part, the motor impairment observed in these individuals [4,5].

BDNF is a neurotrophin known to promote neuronal protection, survival and remodelling; axonal and dendritic growth and synaptogenesis [6,7]. It is assumed that peripheral and central BDNF levels are strictly correlated [8]; therefore, plasmatic/serum levels of BDNF seem to show a strong correlation with cerebrospinal fluid levels [9]. In this sense, clinical trials have reported reduced levels of BDNF in peripheral blood from PD patients [10,5].

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https://doi.org/10.1016/j.physbeh.2020.113135
Received 13 June 2020; Received in revised form 10 August 2020; Accepted 11 August 2020
Available online 14 August 2020

In view of these considerations, strategies that enhance BDNF levels might be considered for this population. In this context, a growing body of research has highlighted the role of different exercise programs as an essential part of managing PD capable of increasing BDNF levels accompanied by significant improvements in motor behavior at any stage [11,12]. However, the molecular mechanisms engaging this response has not yet been elucidated.

Experimental studies have highlighted that exercise promotes increased BDNF via epigenetic modulation, specifically by inducing histone hyperacetylation levels [13,14]. Histone acetylation is widely associated with enhanced transcriptional activity and increased gene expression, whereas deacetylation is typically associated with transcriptional repression, a process catalysed respectively by histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes [15]. Interestingly, it is well established that exercise might be considered a powerful epigenetic modulator in different populations such as schizophrenic[16], obese [17], diabetic [18] and chronic obstructive pulmonary disease [19] patients; however, to our knowledge, there are no studies demonstrating this response in PD individuals.

According to the literature, polyphenols sources, such as the purple grape juice, could be an important choice to prevent neurodegenerative damage in experimental models without [20,21] or with exercise [22]. Also, some studies were proposing that the with purple grape juice consumption could an important ergogenic, which might potentiate exercise effects [23,24]. Furthermore, a link between histone acetylation status and the neuroprotective effects induced by grape juice and the consumption of other polyphenol-rich foods has been suggested by several authors [25, 26]. However, this response has not been investigated in PD patients.

Thus, the aim of our study was to evaluate the impact of an aquatic physical training program associated with grape juice consumption on motor/functional outcomes and on BDNF and global histone H4 acetylation levels in peripheral blood from PD individuals. Finally, in order to better elucidate the knowledge base of exercise and PD, the relationship between motor ability, functional outcomes and biomarkers were also assessed.

2. Methods

2.1. Participants and ethical considerations

This study was approved by the Ethics Research Committee of Centro Universitário Metodista-IPA, Brazil (no. 2.533.844) and the Brazilian Registry of Clinical Trials (ReBEC, number: RBR-2c4xxb). Written informed consent was provided before the protocol began, and the investigation was in full compliance with the Helsinki Declaration.

Sample size was calculated with G. Power Software, considering the H4 histone acetylation levels in a previous study from our group [16]. To calculate the sample size, the effect size (1.3), two tails, alfa error probability (0.05) and power (0.8) were considered, resulting in a sample number of 10 per group. However, considering the non-adherence at the protocol, we decided to recruit 20% more than this number, 12 per group.

Individuals who met the following criteria were included: diagnosis of idiopathic PD, score of 1 to 3 on the Hoehn &Yahr (H&Y) scale and who informed that hadn't have done physical activity lasting at least 1 month. They need to be received the regular drug treatment and have the ability to understand verbal instructions to perform tests and the intervention. All of them were taking the L-DOPA class medication.

The exclusion criteria were cognitive impairment (Mini-Mental State Examination, MMSE ≤ 24), use of Histone deacetylases (HDAC) inhibitor drugs, deep brain stimulation surgery (DBS), history of vertigo, surgeries of the lower limbs during the last year, use of prostheses of the lower limbs, severe heart diseases or other associated neurological diseases.

Participants of both genders and aged over 48 years were randomly (www.randomizer.org) distributed in two groups: Aquatic Exercise (AQ, $n = 12$) and Aquatic Exercise + Grape Juice (AQ + GJ, $n = 12$). The randomization was blinded to the assay's performance students. All participants continued to receive their usual medication at the (disease stage-adjusted) doses normally prescribed by their usual neurologist.

2.2. Study design

Firstly, PD motor symptom severity was assessed (by means of the motor subsection of the Unified Parkinson's disease Rating Scale (UPDRS-III) and the disease severity (by the Hoehn and Yahr scale) of each participant. All the clinical functional outcome measures and the blood collections for biomarker analysis were assessed before and after the intervention. The patients were not familiar with tests that were selected in order to control for learning effects, and the same evaluator performed each test at all times. The intervention and subsequent blood collections were always performed at the same time (10 a.m.–11 a.m.), and an observer blinded to the study carried out all analyses.

The AQ + GJ and AQ groups underwent an aquatic physical training program, carried out in the indoor swimming pool (depth 1.95 m, length 25 m, mean water temperature 31 °C) of Universidade Federal do Rio Grande do Sul (UFRGS). In accordance with other similar programs [27,28], the intervention lasted 4 weeks (twice a week, approximately 60 min each session) in the morning. The training intensity was controlled using Borg's subjective feeling of effort scale [29,30]. All assessments as well the intervention were conducted in the "on" state of medication (after 1–2 h of medication intake). During the intervention period, AQ + GJ also drank the juice, consuming 400 mL/daily.

2.3. Aquatic physical training program

The intervention was based on the Deep Water Running technique. In addition, each session included the application of specific exercises for balance, strength, agility, coordination and body control. Participants performed all stages of the class with the use of floating belts, allowing them to remain upright in fluctuation. The structure of the classes is shown in Table 1.

The training was controlled by different intensities (speeds) and volumes (distance covered). The training volume was determined by the total session time and by calculating the percentage of the distance covered in the six-minute walk test adapted to the aquatic environment (6MWT), which was determined individually for each patient. The training intensity was determined on the basis of the subjective gait intensity (comfortable, intermediate and maximum), confirmed by Borg's subjective feeling of effort.

Importantly, the intervention was conducted by physiotherapists specialized in the area of aquatic rehabilitation and trained physical therapy students. Also, the participants received constant verbal motivation during the training sessions.

2.4. Grape juice consumption

The grape juice (Vitis labrusca L. variety Bordon) was kindly provided by Garibaldi Winery (RS, Brazil) and was from the harvest of 2017 and all from the same lot. The juice was packaged in 200 mL tetra

Table 1
Session´s structure.

Part	Duration	Exercises
1	5–10 min	Joint warm-up, coordination and attention activities
2	30 − 40 min	Deep Water Running with distance and speed determined in periodization.
3	10–15 min	Exercises of balance, strength, agility, coordination, double-task and body control.
4	5–10 min	Stretches and final relaxation.

packs®. The total phenolic compounds, evaluated by Folin-Ciocalteau method [31], observed in grape juice was 2.08 ± 0.019 g EAG/L, the flavonoid content, evaluated according to [32], was 0.258 ± 0.049 g EAG/L. The predominant polyphenols evaluated by HPLC, were gallic acid (4.92 ± 0.11 μg/mL), epigallocatechin (0.33 ± 0.005 μg/mL), epicatechin (3.08 ± 0.06 μg/mL), catechin (15.41 ± 0.59 μg/mL) and epigallocatechin gallate (4.13 ± 0.05 μg/mL).

2.5. Functional and motor outcome evaluation

2.5.1. Functional capacity

Functional capacity was evaluated by the six-min walk test (6MWT) carried out according to the recommendations of the American Thoracic Society [33]. This test evaluates the individual's aerobic capacity when walking for six minutes and covering the greatest distance possible. The test is performed in a 30 m corridor, with markings every 3 m. The participant walks alone along the corridor, without the examiner on the side so as not to alter the speed of the individual's gait. The participant receives information in advance about how the test will take place. Before and after the walk, the participant's vital signs are monitored, and every min the therapist informs the participant of the time remaining to complete the 6 min [34,35].

2.5.2. Mobility

The Timed Up and Go (TUG) test was used to evaluate mobility. This test is considered simple operational tool and features associated with a higher gait speed, balance, functional index, overall health decline, inability to activities of daily living and falls [36].Participants were required to start from a sitting position, get up from a standard chair, walk 3 m, turn around, return to the chair, and sit again. If the task is performed for a period longer than 20 s, this is an indication of increased risk for falls and functional dependence [36]. The test was performed three times, and each participant's shortest time was recorded [37,38,39].

2.5.3. Risk of falls

The risk of falls was assessed using the Berg Balance Scale (BBS), which consists of 14 tests that evaluate an individual's ability to sit, stand, spin around, look over their shoulders, stand over a one-way support and transport steps. Its maximum score is 56 points, and the higher the value, the greater the functional balance. Lower scores represent lower balance performance, and values below 46 indicate a risk of 40% for at least one fall episode in the next year [40].

2.6. Biochemical analysis

2.6.1. Sample preparation

A sample of venous blood (15 mL) was collected and separated in tubes with Ethylenediamine tetraacetic acid(EDTA) for epigenetic and BDNF level measurements. Blood samples collected in EDTA tubes were diluted in a proportion of 4:3 in phosphate-buffered saline (PBS, 136 mM, NaCl, 2.7 mMKCl, 7.8 mM Na2HPO4, 1.7 mM KH2PO4; pH 7.2–7.4) and centrifuged (1500 rpm, 20 min, at room temperature) on Ficoll-Histopaque 1077 (Sigma, MO, USA), as described by Bicalho et al. [41]. After that, peripheral blood mononuclear cells (PBMCs) were collected from the interface between plasma and Histopaque, washed 2 x in PBS (2000 rpm, 5 min, at room temperature) and frozen for the epigenetic analysis. Plasma was also collected after the first centrifugation, aliquoted in microtubes and frozen for BDNF analysis. The determination of biomarkers was carried out by an observer blinded to the interventions of the study.

2.6.2. Global H4 histone acetylation level analysis

The global histone acetylation levels of H4 in PBMCs were determined using the commercial kit (Colorimetric Detection, catalogue number P-4009, respectively, EpiGentek USA) according to the manufacturer's instructions. The values were expressed as ng/mg protein, and the protein concentration of each sample was measured by the Coomassie Blue method using bovine serum albumin as the standard [42].

2.6.3. BDNF level analysis

Plasma BDNF levels were determined with the ELISA method, from Boster Biological Technology (#EK0307) according to the manufacturer's instructions. Briefly, the sample and BDNF-specific standards were added to a microplate and incubated for 2 h at room temperature. Subsequently, the solutions were discarded, and the Biotinylated Anti-Human BDNF antibody was added and incubated for 90 min at room temperature. The plate was washed three times with wash buffer, and Avidin-Biotin-Peroxidase Complex was added to each well. The plate was incubated at room temperature for 40 min. The solution was discarded, and the plate went through the washing process. Colour Developing Reagent was added, and the solution was incubated for 30 min at room temperature in the absence of light. The stop solution was added, and the plate was read in a spectrophotometer at a wavelength of 450 nm. BDNF plasma levels were expressed in ng/mL.

2.7. Statistical analysis

All collected data were inserted in a spreadsheet (Microsoft Excel®) and analysed in SPSS® software version 22.0 for Windows®. Normality was verified using the Shapiro-Wilk test. The outliers were detected by the Outlier calculator from GraphPad Prism, and the values were removed. Data on sample characterization and body composition were described as mean ± standard deviation (SD). To evaluate the main outcomes of the study, a GEE (generalized estimation equation) with gamma distribution was performed. Results were expressed as mean ± standard error (SE). Also, the mean percentage (%) of the difference between the pre and post times was presented. The% differences were analysis by *t*-test or Mann-Whitney test. Correlations amongst biomarkers, functional outcomes, Hoehn and Yahr scale and UDPRS were analysed by Pearson test. Differences were considered significant at $p < 0.05$.

3. Results

Twenty-four PD patients were recruited; however, five of them dropped out during the training period due to a decline in general health status; therefore, 19 patients successfully completed the 4-week intervention, attending a minimum of 70% of the sessions. The flow diagram, according to the Consolidated Standards of Reporting Trials (CONSORT), is depicted in Fig. 1.

The sample characterization is illustrated in Table 2. It is important to note that the AQ and AQ + GJ groups proved to be homogenous in all basal characteristics, including gender, age, motor symptoms and disease severity.

Regarding functional outcomes, as described in Table 3, a better performance in functional capacity ($p = 0.001$) and balance ($p = 0.007$) was found after the intervention in both the AQ and AQ + GJ groups. However, BBS scores remain unchanged in both groups ($p > 0.05$). Importantly, no differences between groups were observed in any evaluated parameter ($p > 0.05$; Table 3).

A significant increase in BDNF levels was observed in the AQ and AQ + GJ groups after the intervention ($p = 0.003$), while no differences between groups were found, as highlighted in Fig. 2A. Similarly, the intervention was able to enhance global histone H4 acetylation levels in both groups ($p = 0.031$, Fig. 2B).

No significant correlation amongst biomarkers, UDPRS, Hoehn and Yahr scale and functional outcomes were observed. However, significant correlations between Hoehn and Yahr scale and UDPRS ($r = 0.610$; $p = 0.006$), TUG ($r = 0.585$; $p = 0.011$) and BERG ($r = -0.592$; $p = 0.010$) were observed. In addition, UDPRS significant

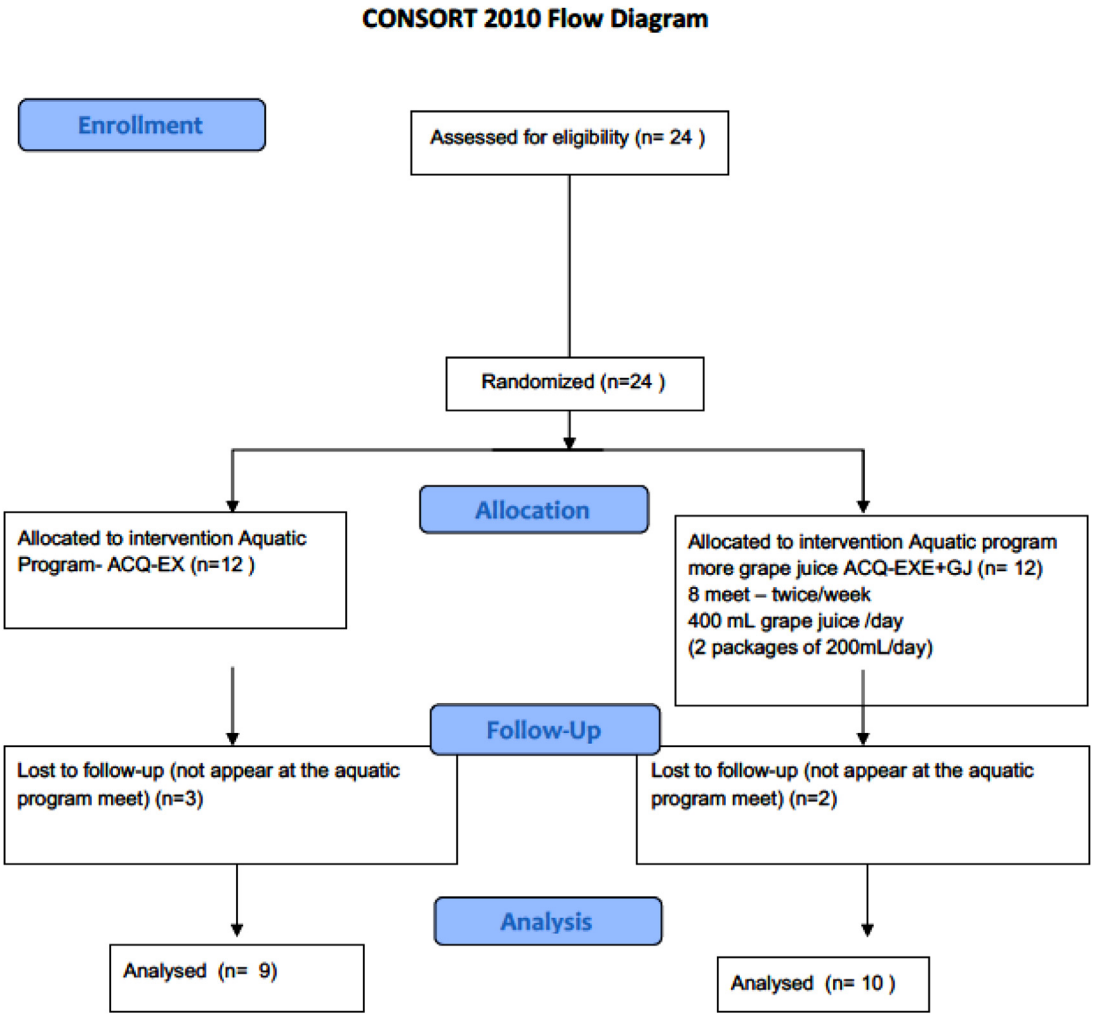

Fig. 1. Consort flow diagram.

Table 2
Characteristics and Motor symptom severity (UPDRS-III) and disease severity (Hoehn and Yahr scale) of PD participants.

	AQ(n = 09)	AQ +GJ (n = 10)	p
Gender (female/male)	1(11.1%)/8(88.9%)	1 (10%)/9 (90%)	0.937
Age (years)	65.5 ± 2.16	68.33 ± 0.413	0.551
Hoehn & Yahr Scale			
Stage 1	4 (44.4%)	3 (30%)	0.445
Stage 1.5	2 (22.2%)	1 (10%)	
Stage 2	0 (0%)	3 (30%)	
Stage 2.5	2 (22.2%)	1 (10%)	
Stage 3	1 (11.1%)	1 (10%)	
Stage 4	0 (0%)	1 (10%)	
UPDRS score (mean ± SD)	14.88 ± 5.46	16.5 ± 7.10	0.590

The data are presented as mean ± standard deviation (numeric data) or relative frequency (categorical data).The comparison between groups was performed using the Student t-test for independent data or chi-square test ($p<0.05$). UPDRS: Unified Parkinson's Disease Rating Scale.

correlated with TUG (r = 0.588, p = 0.001) and BBS ($r=-0.626$; p = 0.005).

4. Discussion

Adequate levels of exercise and healthy dietary practices have the advantage of being non-invasive and highly efficacious. Although the impact of physical exercise practice and grape juice consumption *per se* on clinical parameter and biomarker modulation have been investigated in different populations, the combination of both strategies has been poorly studied.

To the best of our knowledge, we report here the first evidence demonstrating the effect of exercise, regardless of grape juice consumption, on functional outcomes and levels of BDNF and global H4 acetylation modulation in PD patients. The main finding of the study was that an aquatic exercise program induced functional improvement and the BDNF enhancement could be contributed to this result, probably by engaging histone hyperacetylation status. However, this effect was not potentiated by grape juice consumption since no significant differences between the AQ and AQ+GJ groups were found in any of the analysed variables.

As expected, we demonstrated that the intervention was able to ameliorate functional capacity and mobility in PD patients from the AQ and AQ+GJ groups. Our data are in agreement with several authors, demonstrating that this exercise modality has the potential to improve motor symptoms in these individuals [43,44]. The aquatic environment offers specific advantages for the prescription of physical exercises for this population due to the hydrostatic and hydrodynamic principles of buoyancy, viscosity and drag [45]. The decrease in joint compression forces generated by the buoyant force also contributes to the facilitation of movement and thus to the performance of muscle strengthening exercises and gait training [46,47]. Aquatic exercise, through the properties of water, such as hydrostatic pressure, turbulence and buoyancy, creates instability that increases sensory stimulation and, consequently, causes balance reactions that can contribute to improve

Table 3
Effect of aquatic physical training program associated or not with grape juice consumption on functional outcomes in PD patients.

Variable	**Before**Mean ± SD	**After**Mean ± SD	**Effect** Condition	(p-value) Moment	Condition XMoment
6MWT (Distance)					
AQ +GJ	398.57 ± 17.66	440.00 ± 28.96	0.58	**0.001***	0.11
AQ	340.00 ± 35.27	465.37 ± 46.20			
TUG (Seconds)					
AQ +GJ	11.56 ± 1.11	8.03 ± 0.93	0.40	**0.007***	0.28
AQ	12.79 ± 1.83	11.03 ± 3.49			
BERG (Score)					
AQ +GJ	51.55 ± 1.54	52.44 ± 1.53	0.3	0.35	0.70
AQ	53.77 ± 1.34	54.12 ± 1.26			

Data presented as mean ± standart derivation (numeric data).

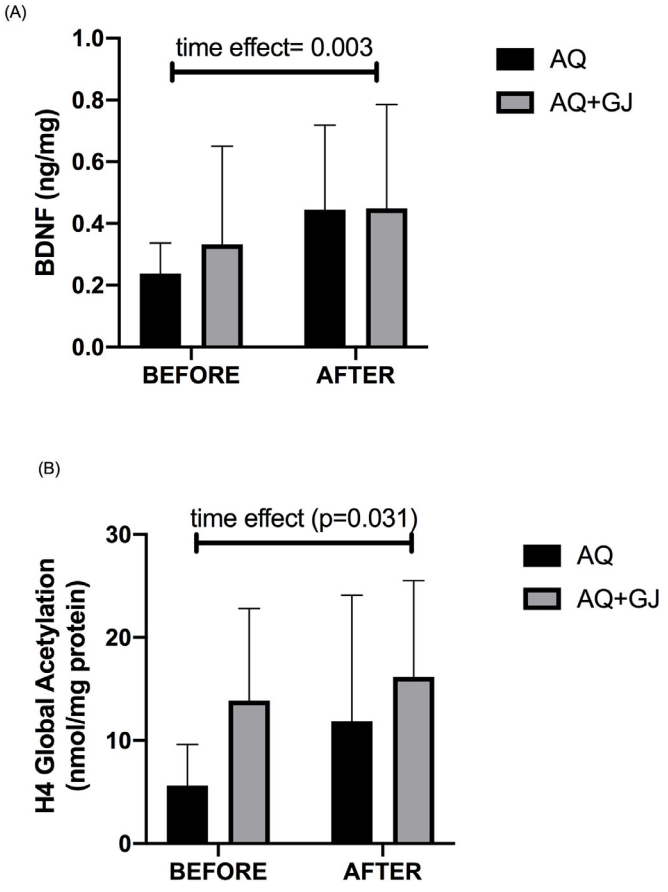

Fig. 2. Effect of aquatic physical training program associated or not with grape juice consumption on BDNF (A)and global histone H4 acetylation(B) levels in PD patients.

postural control and mobility in patients with PD [45].

It is important to note that the symptomatic progression of the PD is defined through the stages of the Hoehn Scale and Yahr, a tool that uses numbers from 1 to 5 to classify the severity of PD pathology. It is demarcated by worsening gait conditions and postural instability and the patients that are classified in stages 1, 2 and 3 have mild to moderate disability, while those in stages 4 and 5 have more severe disability [48]. Considering that the sample of our study included patients of stages 1–4 of the disease according to this scale and that we observed significant improvements in their clinical-functional status after the exercise protocol, we can suggest that the proposed intervention might be effective to improve the motor function of patients with PD, regardless the degree of disability.

On the other hand, no changes in BBS scores were observed. In contrast, Volpe and colleagues (2014) showed that hydrotherapy rehabilitation treatment for 2 months (5 days a week, 60 min/session) significantly improved this variable [43]. A possible reason for this divergent result is that participants of the current study were submitted to a protocol with sessions for 1 month only twice a week. Taken together, these findings led us to hypothesize that more prolonged and higher frequencies of aquatic-based exercise programs are required to be effective in preventing falls in PD patients.

Although the literature addressing the effects of grape juice on motor abilities in patients with PD is lacking, several experimental studies show evidence of neuroprotective effects in different brain areas strictly linked to PD pathophysiology, including the striatum and substantia nigra [20,21]. Furthermore, mobility-related activity improvements in response to grape juice intake have also been reported in rodents [49,50]. Despite these findings, we found that grape juice consumption did not seem to influence exercise effects on motor outcomes from PD patients, since no differences between the AQ and AQ +GJ groups were observed.

Interestingly, our research group recently analysed the impact of grape juice consumption during 4 weeks alone or combined with a therapeutic exercise protocol (concurrent exercise program, three times a weekly, 60 min each session) on several functional variables in healthy elderly women. Increased functional capacity was observed in both groups after the intervention, while a significant improvement in handgrip strength and BBS was observed only in the group that consumed grape juice without exercising [39]. Altogether, these findings led us to infer that grape juice consumption effects might be influenced by factors such as the population (healthy or patient) and the type of exercise protocol (land-based versus aquatic environment interventions).

Although we did not evaluate the impact of intervention on UPDRS and Hoehn and Yahr Scale, as expected, the baseline correlations between both tools and motor outcomes demonstrate that the disease

impairment as severity is associated with worse results in the clinical-functional outcomes in the current study, such as mobility impairment and higher risk of falls (assessed by TUG and BBS). On the other hand, functional capacity, evaluated by 6MWT seems to be an aspect that is not influenced by the severity and progression of the disease since it was not correlated with both instruments. These findings indicate that there is an important link between the deterioration caused by the advance of the disease and the aggravation in the conditions of balance and mobility in this population, reinforcing the importance that strategies that can improve these outcomes, such as physical exercise, should be indicated for patients with PD.

Another important point to discuss is the enhancement of BDNF after the intervention, which should be contributed in the exercise-induced functional outcome improvements in PD individuals of both groups on a molecular level. These data disagree with those recently obtained by da Silva Germanos and colleagues (2019) showing that 1 month of an aquatic exercise program (two sessions per week/1 hour per session) was not able to change BDNF levels in this population [51]. However, our findings are in accordance with other studies that used prolonged land-based interventions [52,53]. Specifically, 8 weeks of interval training (three 1-hour sessions weekly) on a stationary cycloergometer significantly increased BDNF levels, alleviating parkinsonian rigidity and decreasing muscle tone [52]. In addition, Zoladz and colleagues (2014) found that a moderate-intensity interval training (three 1-hour training sessions weekly) over 8 weeks resulted in elevated peripheral BDNF levels and improved motor symptoms in PD individuals [53]. Therefore, we may postulate that BDNF modulation in response to exercise in PD individuals should depend on the protocol duration, being more sensitive to long-lasting exposure, regardless of whether it is performed in an aquatic environment or not.

Finally, a novel finding that emerged from this study was that aquatic physical training evoked significant increases in BDNF levels in PD patients, at least in part by engaging histone hyperacetylation status [15]. To date, this is the first study demonstrating that this population is vulnerable to epigenetic changes induced by exercise, corroborating several other clinical trials with other populations showing that exercise is capable of modulating the epigenome [16,19].

Some lines of evidence strongly point towards the fact that the loss of acetylation homoeostasis appears to represent a critical and decisive mechanism commonly underlying neuronal dysfunction in neurodegeneration processes [54]. On the other hand, some insights have been obtained into the role of histone hyperacetylation in neuroprotection, memory improvement and amelioration of several neurodegenerative diseases [54,55].

Furthermore, Morrison and colleagues (2006) demonstrated that HDAC inhibitors and consequently increased histone acetylation levels can protect in both cell cultures and in vivo models of neurodegenerative diseases [56]. Furthermore, HDAC inhibitors reversed neuronal apoptosis induced by oxidative stress observed in several neurodegenerative diseases such as PD. Considering that increased levels of global histone H4 acetylation were observed after the intervention, our results revealed that, in addition to HDAC inhibitors, aquatic exercise could be an interesting strategy for PD patients, acting with therapeutic potential to induce neuroplasticity though BDNF modulation.

5. Conclusion

In summary, our data present a first attempt to bridge the gap between aquatic exercise-induced epigenetic changes in patients with PD, emphasizing and reinforcing the importance of a physically active lifestyle for this population. When combining our findings, it appears entirely possible that the improvement in functional outcomes after the intervention is related, at least in part, to BDNF enhancement engaged by histone H4 hyperacetylation status. In addition, our correlation findings support the premise of an important link between PD severity and progression and worse balance and mobility behaviors.

Acknowledgments

The authors acknowledge support from IBRAVIN, Garibaldi Winery, Centro Universitário Metodista-IPA; Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS)/Brazil and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq/Brazil.

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