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ABSTRACT

Introduction: Aquatic Nordic walking (ANW) is a novel whole-body low-impact exercise that can be practiced by a variety of older adults with chronic conditions. However, its efficacy on several aspects of health is largely unknown. **Purpose**: To determine the effects of regular ANW on glycemic control and vascular function in older adults with type 2 diabetes and mild cognitive impairment. **Method**s: Thirty-three older adults with type 2 diabetes aged 60-75 years were randomly allocated to non-exercising control (n=17) or aquatic Nordic walking (ANW; n=16) groups. Nordic walking was performed in a pool at water temperature of 34-36°C 3 times per week for 12 weeks. **Results**: Measures of functional physical fitness including chair stand, timed up and go, chair sit and reach, reach and back scratch, and 6-minute walk test scores were all improved after ANW (all $p<0.05$). Plasma glucose, glycosylated hemoglobin (HbA_{1c}), and homeostasis model assessment of insulin resistance (HOMA-IR) decreased (all $p<0.05$) in ANW. Vascular reactivity as assessed by brachial flow-mediated dilation (FMD) increased, and arterial stiffness as assessed by brachial-ankle pulse wave velocity decreased in ANW (all $p<0.05$). No significant changes were observed in the control group. Middle cerebral artery pulsatility index decreased with ANW under normocapnia condition ($p<0.05$). Cerebrovascular conductance increased with ANW under hypercapnia condition. Montreal Cognitive Assessment (MoCA) score increased in the ANW group ($P<0.001$). Changes in MoCA scores were positively associated with corresponding changes in brain-derived neurotrophic factor (BDNF) ($r=0.540$, $P=0.031$). **Conclusions**: Nordic walking in water was a safe and effective innovative exercise modality to improve glycemic control, vascular function, physical fitness, cerebrovascular reactivity and cognitive function in older adults with type 2 diabetes.

Key Words: GLYCEMIC CONTROL, VASCULAR FUNCTION, HEALTH-RELATED PHYSICAL FITNESS

INTRODUCTION

Type 2 diabetes mellitus is a complex metabolic disease that can lead to a variety of complications including cardiovascular disease and dementia (1). Both glycemic control and vascular dysfunction are implicated in the pathogenesis of both cognitive dysfunction and cardiovascular disease in diabetes (2). Most of the key measures of macrovascular and microvascular functions, including endothelium-dependent vasodilation is depressed, and central artery stiffness is elevated in older adults with diabetes (3). A hypothesized physiological connection is that diabetes-induced vascular stiffening can lead to increased transmission of pulsatile stress into the microcirculation and impair oxygen delivery to the brain (4). Due to the lack of energy reserve, the brain relies heavily on the perfusion through the vascular system to support its energy demand. Additionally, excessive blood flow pulsatility is thought to cause cerebral microvascular damage as high flow and low resistance organs like the brain would be highly susceptible to the damages induced by pulsatile stress (5). The impairment in vascular function can elicit the disturbance in the stringently regulated cerebral homeostasis and triggers a pathological cascade leading to cognitive decline. A strong correlation between aortic stiffness and middle cerebral pulsatility has been reported in patients with cerebrovascular disease (6). In adults with type 2 diabetes, the stiffening of cerebral vessels is associated with reduced cerebrovascular responsiveness to cognitive tasks and poorer cognitive performance (7).

Regular physical activity has been recommended for older patients with type 2 diabetes (8). However, the magnitude of benefits induced by regular exercise tends to be less than ideal on peripheral vascular and cerebrovascular functions (9). An innovative mode of exercise that exerts potentially large impacts on both functions is Nordic walking exercise conducted in water. Nordic walking on land is a simple and practical form of whole-body exercise that combines cardiovascular exercise with strength exercise for upper body muscles. It activates almost 90

percent of whole body muscles (10) and is an effective mode of exercise as it improves physical fitness (11) and muscular strength (12). However, the effects of Nordic walking on cognitive and cerebrovascular function are largely unknown in elderly patients with type 2 diabetes. In this context, the somatotropic map of primary motor cortex (13) indicates that the involvement of shoulders, arms, hands, and fingers involved in Nordic walking could activate greater parts of motor cortex and augment cerebral perfusion.

Aquatic exercise can be an excellent modality of whole-body exercise for people of all ages as it can be performed in the natural properties of water: buoyancy, hydrostatic pressure, and warming or cooling properties of the water surrounding exercisers (14). Water-based exercise training produces a variety of beneficial impacts on glycemic control, physical fitness measures, and both macro and microvascular functions in older patients with type 2 diabetes and that these effects in many key measures tend to be greater in water-based exercises than in land-based exercises (15). For instance, water-based exercise can induce a greater increase in cerebral blood velocity than land-based exercise of matched intensity (16). Aquatic-based treadmill exercise augments cerebral blood velocity across a range of intensities as middle cerebral artery blood velocity can be maintained while exercising at lower intensities with greater depths of water immersion (17). Due to the hydrostatic pressure, water immersion translocates blood volume to upper body and more specifically to the brain as measured by increased blood flow velocity in the middle and posterior cerebral arteries (18). A recent study in healthy older adults reported that water immersion enhanced episodic memory (19). Taken together, these observations indicate that a combination of Nordic walking and water immersion would be a very promising and innovative exercise mode for older patient population in general and older diabetics in particular.

Accordingly, the primary aim of the present study was to investigate the effects of aquatic Nordic walking on cerebrovascular and cognitive function in elderly patients with type 2 diabetes. Because the glycemic control and vascular function are implicated in the development of cerebral dysfunction in diabetes, these measures were included as secondary variables. Because this specific mode of exercise has not gone through detailed research examination, measures of physical fitness and blood chemistry were also included. We hypothesized that Nordic walking in water would exert a variety of beneficial impacts on physical fitness, glycemic control, vascular function, cerebrovascular reactivity, and cognitive impairment in elderly patients with type 2 diabetes.

METHODS

Participants

A total of 36 older adults with type 2 diabetes aged 60-74 years were recruited from hospitals in Bangkok, Thailand by invitations using face-to-face inquiries. The inclusion criteria included hemoglobin A_{1c} (HbA_{1c}) of 7-9% (20); mild cognitive impairment (Montreal Cognitive Assessment scores of 18-24) (21); no diabetic complication (retinopathy, nephropathy, and neuropathy); no musculoskeletal disorders limiting walking; not treated with insulin injection; no previous exercise training within the past 6 months; and screening and passing by physical activity readiness questionnaire. All subjects gave written informed consent prior to participation in the study. The study was approved by the institutional review board at Chulalongkorn University.

Exercise intervention

The participants were first stratified in sex, age, and Montreal Cognitive Assessment scores, and then were randomly allocated into either non-exercise control (n=18) or aquatic Nordic walking (n=18) groups using a simple random sampling technique. One participant in the control group and 1 participant in the exercise group dropped out due to medical reasons that were unrelated to the present study (cataract operation and COVID-19 infection).

The participants in the exercise group practiced Nordic walking on land twice to become familiar with the exercise movement using poles. Additionally, they practiced once in water to set exercise intensity by using a heart rate monitor (Polar H10, Kempele, Finland). The Nordic poles were properly adjusted such that poles were in vertical position with the tip on the ground, with elbow bent at 90°. The Nordic walking technique described by Svensson (22) was followed including heel strike, upper body lean forward, opposite between arm and leg, and straight arm swing. Exercises were performed in a swimming pool at water temperature of 34-36°C immersed to the chest level 3 times per week for 12 weeks. Each exercise bout was 40 minutes in duration at 40-50% of heart rate reserve for the first 6 weeks and 50-60% heart rate reserve for the last 6 weeks. The total exercise session consisted of a 10-minute warm-up and a 10-minute cool-down, giving a total session time of 60 minutes per day. The control group were instructed not to change their lifestyle and physical activity for the intervention duration and continued standard medical treatments.

Measurements

Participants were requested to avoid alcohol, caffeine, physical exercise, and supplements for at least 24 hours prior to testing.

Body composition was evaluated with a dual-energy x-ray absorptiometry (GE Healthcare, Madison, WI). A set of physical function measures for the senior fitness test developed by Purath et al. (23) were conducted. The tests included reach and back scratch tests and chair sit and reach tests for upper extremity and lower extremity flexibility, arm curl and chair stand tests to assess upper and lower extremity strength, timed up and go tests for agility and dynamic balance, and 2-minute step testing for cardiovascular endurance. In addition, 6-minute walk test was added to the testing battery (24). The participants were instructed to walk as fast as possible along markers which were 20-m apart for 6 minutes to cover the maximum distance.

Brachial-ankle pulse wave velocity (baPWV) was assessed using a noninvasive vascular testing device (VP-1000plus; Omron Healthcare, Kyoto, Japan) as previously described. Carotid artery intima-media thickness (IMT) was determined from an ultrasound machine (EPIQ 5 Ultrasound System; Philips Healthcare) equipped with B-mode ultrasonography with a high frequency linear-array transducer. The longitudinal ultrasound images were obtained over the common carotid artery between 1 to 2 cm proximal to the bifurcation. Arterial compliance was measured in the right common carotid artery using the combination of ultrasound imaging of a common carotid artery diameter and a noninvasive blood pressure (VP-1000plus; Omron Healthcare, Kyoto, Japan) (25). The ultrasound images were analyzed with the use of image analysis software (Carotid Analyzer; Medical Imaging Applications).

Flow-mediated dilation (FMD) was measured as an index of endothelium-dependent vasodilation by using the ultrasound machine (EPIQ 5 Ultrasound System; Philips Healthcare) equipped with a high-resolution linear-array transducer. The brachial artery of a dominant arm was imaged 5 cm above the antecubital fossa. The cuff set placed on the forearm was inflated

rapidly to 50 mmHg above systolic blood pressure for 5 minutes and deflated during recovery as previously described (26). Artery diameters were analyzed by ultrasound images via digital viewing software (Brachial Analyzer; Medical Imaging Applications). FMD was calculated using the formula: (peak post-occlusion diameter − baseline diameter)/(baseline diameter) × 100.

Blood velocity in the middle cerebral artery (MCA) was measured noninvasively using transcranial Doppler ultrasonography (TCD) (EPIQ 5 Ultrasound System; Philips Healthcare). A 1.8 MHz transcranial Doppler probe was attached to the patient's head at the right temporal window (27) in a supine position with the head of bed slightly elevated (30-45%). After identifying the middle cerebral artery, pulsed-wave Doppler is used to measure the velocity of blood flow within the vessel continuously. The Doppler sample gate is centered over the MCA's red color flow signal to obtain a spectral Doppler waveform. The difference between the long axis of the MCA itself and the angle of insonation was minimized (to <10-15) for all participants. After the signals were identified at a depth of 45-60 mm, the probe was fixed with a probe folder so as to not change the insonating angle (28).

MCA blood velocity was measured separately in three different conditions; normocapnia, hypocapnia, and hypercapnia conditions as previously described (29). The initial partial pressure of carbon dioxide (PCO_2) was monitored as hypocarbic/hyperventilated (PCO_2 <35mm Hg), normocarbic (PCO_2 35–45mm Hg), and hypercarbic/hypoventilated (PCO_2 >45mm Hg). Hypocapnic cerebral vasoconstriction immediately before hypercapnic stimuli may influence ensuing vasodilation. Ventilatory gases were continuously measured and acquired via breath-by-breath using a flow sensor.

After the participant had rested in the supine position for at least 10 minutes, a nose clip was placed and the participant breathed through a mouthpiece with a Y-valve, with one end open

to room air and the other end connected to a 5L rebreathing bag. Baseline MCA blood velocity, heart rate, blood pressure, and $EtCO_2$ were recorded simultaneously for 3 minutes. During these measurements, participants were instructed to breathe normally and to avoid body movement or Valsalva-like maneuvers. After baseline data collection, participants were instructed to perform voluntary hyperventilation for 20 seconds (1 breath/s) to induce a brief period of hypocapnia. Heart rate and blood pressure (CARESCAPE V100, GE Dinamap, USA) were obtained during the first 10 s of hyperventilation while cerebral blood flow in MCA was recorded during the last 20 s of hyperventilation. Following hyperventilation, a 5-minute recovery period was provided to allow cerebral hemodynamics to return to the baseline. After end-tidal CO_2 and MCA blood velocity returned to the baseline following hyperventilation, a respiratory valve was switched to an air reservoir containing 5% CO_2 and 21% O_2, and the participants were asked to breathe spontaneously for 3 min. The air reservoir was continuously filled from a cylinder whose air pressure was manually adjusted to subjects respiratory volume.

Then participants breathed air containing a gas mixture of 5% CO_2 and 21% O_2 balanced with nitrogen spontaneously for 3 minutes to induce hypercapnia. Briefly, at the end of a deep inspiration, the Y-valve of the mouthpiece was switched to the rebreathing bag for 3 minutes to induce a progressive increase in arterial CO_2 and then followed by a recovery period for 4 minutes. During rebreathing, a small amount of oxygen was added to the rebreathing bag based upon each subject's basal metabolic rate to maintain constant arterial blood oxygen saturation. The data were recorded during the last minute of hypercapnia.

Pulsatility index (PI) was calculated as the difference between systolic and diastolic velocity divided by mean velocity (27, 30). Cerebrovascular reactivity (CVR) was defined as a percent change in the middle cerebral artery velocity (MCAv) over an absolute change in end-

tidal CO_2 (27). The change in CVR was estimated from the three different conditions of end-tidal CO_2 levels including normocapnia to hypocapnia, normocapnia to hypercapnia, and hypocapnia to hypercapnia. Moreover, cerebrovascular conductance index (CVCi) was computed to account for the effects of changes in mean arterial pressure on MCA blood velocity during 3 conditions (27). CVR were averages of >5 recorded values in each participant. The formula used to determine CVR and CVCi are shown below:

$$\text{CVR (\%/mmHg)} = \%\Delta \text{ MCAv } / \Delta\text{End-tidal } CO_2$$

$$\text{CVCi (cm/sec*mmHg}^{-1}\text{)} = \text{MCAv /MAP}$$

The Montreal Cognitive Assessment (MoCA) evaluates multiple cognitive domains including visuospatial/executive function, naming, memory, attention, language, abstraction, delayed recall, and orientation. The MoCA was used also for cognitive screening to detect mild cognitive impairment (MCI) between 18 – 24 scores from 30 scores (21) as MoCA is highly sensitive and effective in detecting MCI in patients with type 2 diabetes (31). Concurrent sensitivity and specificity of MoCA for identifying MCI patients in Thailand are 70% and 95% with high internal consistency (Cronbach α = 0.744) (21). The Mini-Mental State Examination (MMSE) is consisted of seven domains including orientation to time, orientation to place, registration, attention and calculation, word recall, language, and visual construction. The Trail making test part B was used to measure the executive function. Stroop color and word test was used to assess the ability to inhibit cognitive interference (32).

After 12-hour overnight fasting, a venous blood sample was collected from the antecubital vein. Complete blood count (via automated cell counter), concentrations of fasting

plasma glucose (enzymatic assay using hexokinase reaction), insulin (chemiluminescent microparticle immunoassay), high sensitivity C-reactive protein (immunoturbidimetric assay), lipids and lipoproteins (enzymatic assays), and glycosylated hemoglobin or HbA_{1c} (enzymatic assay) were measured in the certified clinical laboratory within the Faculty of Allied Health Science at Chulalongkorn University. Malondialdehyde (MDA) concentration was estimated by thiobarbituric Acid (TBA) Assay (Cayman, Ann arbor, MI, USA). Nitric oxide concentration was measured in a standard Griess reagents (Promega, Madison, MI, USA). Tumor necrosis factor-α (TNF-α), adiponectin, interleukin 1β (IL-1β), interleukin-6 (IL-6), and brain-derived neurotrophic factor (BDNF) were analyzed with the commercially available ELISA assay kits (Sigma-Merck, Saint Louis, MO, USA). Homeostasis Model Assessment of insulin resistance (HOMA-IR) was calculated using the following formula (33): [Fasting serum insulin (uU/mL) x Fasting plasma glucose (mg/dL)]/405.

Statistical Analyses

Sample size calculation was conducted using G* power version 3.1.9.2 data analysis software (Franz Faul, Institute of Psychology, Kiel University, Germany) with the methods of ANOVA with repeated measures, within-between interaction, with power = 0.8, α-level = 0.05, and effect size = 0.267. This effect size was from previous study that examined the effect of aerobic training on cerebral blood flow in elderly with chronic kidney disease (34).

Descriptive data are expressed as means ± SD. All data were analyzed using SPSS (version 23; IBM, Armonk, NY). Grubb's outlier test (35) was used to detect outliers in the data sets using GraphPad statistical software (San Diego, CA). As a result, 1-3 outlier points were eliminated from some variables before the statistical analyses. Inclusion/elimination of these data

points did not alter the primary findings. Prior to the parametric analyses, the normal distribution was confirmed using a Shapiro Wilk test. A 2 × 2 (group × time) repeated measure ANOVA, followed by a post hoc test with LSD multiple comparison, were used to evaluate changes in all variables. Pearson's correlation coefficient was utilized to evaluate correlations between the percent pre-and post- test changes in BDNF, CVR normocapnia to hypocapnia condition, CVR hypocapnia to hypercapnia condition, CVCi hypercapnia condition and changes in Montreal Cognitive Assessment scores.

RESULTS

All the physical characteristics and blood pressure at baseline were similar between the groups (Table 1). The participants in ANW completed 100% of the training sessions (all 36 exercise sessions). Body weight, BMI, fat mass, and lean mass did not change in both groups. Body fat percentage (P=0.009) and heart rate at rest (P=0.010) decreased significantly in ANW. Brachial blood pressure did not change significantly in both groups.

As shown in Table 2, flexibility as assessed by chair sit & reach and reach & back scratch improved with ANW (P=0.001, P=0.027) while no such improvements were seen in the control group. Muscle strength as estimated by chair stand and arm curl increased in ANW (P=0.001, P=0.001). Timed up and go test and 6-minute walking performance improved in ANW (P=0.001) while decreased in the control group (P=0.001). Two-minute step test improved only in ANW.

As showed in Table 3, carotid IMT and baPWV decreased in ANW (P=0.001, P=0.015). In addition, brachial FMD and arterial compliance increased in ANW (P=0.012, P=0.010) and higher than control (P=0.005, P=0.001). No such changes were observed in the control group.

As illustrated in Table 4, MCA blood velocity was not different between the groups at baseline and did not change with either intervention. Pulsatility index decreased in ANW (Pre 1.25±0.15 vs. Post 1.16±0.10, P=0.003) under normocapnia conditions but did not change significantly under hypocapnia or hypercapnia conditions. CVCi increased with ANW under hypercapnia conditions (Pre 0.42±0.90 cm/sec*mmHg^{-1} vs. Post 0.47±0.13 cm/sec*mmHg^{-1}, P=0.006). There were no significant changes in cerebrovascular reactivity (CVR) in both groups under normocapnia to hypercapnia and hypocapnia to hypercapnia conditions. $\Delta CVCi/\Delta EtCO_2$ did not change significantly in either group.

There were no significant changes in hematological variables in any of the groups (Table 5). Glycemic control indicators including plasma glucose concentration, HbA1c, and HOMA-IR decreased in ANW (P=0.033, P=0.001, P=0.044) and lower than control (P=0.005, P=0.020, P=0.015). Plasma lipid and lipoprotein concentrations and inflammatory markers did not change in either group. The concentration of nitric oxide and BDNF increased in ANW (P=0.002, P=0.028) and higher than control (P=0.030, P=0.023).

As illustrated in Table 6, Montreal Cognitive Assessment score increased in the ANW group (P=0.001). Notable changes were observed in domains of executive, abstraction, delayed recall, and orientation. MMSE score did not change significantly in both groups. There were no significant changes in Stroop color test, trail making test part B in both groups.

The associations between cerebrovascular function and cognitive function measures of pre-post percent differences in the ANW group are shown in Figure 1. Changes in Montreal cognitive assessment scores were positively associated with corresponding changes in BDNF (r=0.540, P=0.031), CVR normocapnia to hypocapnia condition (r=0.741, P=0.001), CVR hypocapnia to hypercapnia condition (r=509, P=0.044), and CVCi hypercapnia condition

(r=0.550, P=0.027). There were no significant associations between HbA1c and cerebral vascular measures.

DISCUSSION

Three months of moderate-intensity aquatic Nordic walking produced favorable changes in multitudes of key measures in elderly patients with type 2 diabetes and mild cognitive impairment. Markers of glycemic control, indices of health-related physical fitness, key subclinical measures of vascular dysfunction were all improved significantly with aquatic Nordic walking. Additionally, selected measures of cerebrovascular reactivity improved with aquatic Nordic walking and these improvements were associated with corresponding improvements in cognitive function as well as BDNF. Taken together, these results indicate that Nordic walking performed in water is a highly effective innovative exercise in improving glycemic control, physical fitness, vascular function, cerebrovascular reactivity and cognitive function in older adults with type 2 diabetes and mild cognitive impairment.

Twelve weeks of Nordic walking exercise on land produced significant reductions in body weight, BMI, HbA_{1c} (36), muscular flexibility, and aerobic fitness (37) in patients with type 2 diabetes. We have previously demonstrated that an addition of water immersion augmented the exercise training benefits of moderate cycling training in patients with type 2 diabetes (15). Because exercise is performed in water, viscosity of water results in a drag resistance during movements of arms and legs resulting in greater exertion under water (38) leading to increased metabolic rate and hence higher caloric expenditure during exercise (10). Accordingly, we hypothesized that Nordic walking performed in water could induce multitudes of benefits with great magnitudes. In addition to a decrease in body fat percentage, aquatic

Nordic walking examined in the present study produced significant reductions in fasting plasma glucose, HbA_{1C}, and insulin resistance as estimated by the HOMA-IR. We also observed significant increases in flexibility, both upper and lower muscular strength, and cardiovascular fitness in elderly patients with type 2 diabetes.

Aquatic Nordic walking improved endothelium-dependent vasodilation as assessed by brachial flow-mediated dilation, indices of arterial stiffness as assessed by baPWV and carotid arterial compliance and reduced a marker of subclinical atherosclerosis, carotid artery IMT. These functional and structural changes in the vasculature are supported by circulating humoral markers as a significant increase in plasma nitric oxide concentration was observed in the present study. These collective results indicate that water-based Nordic walking exercise is effective in improving a variety of macrovascular function in older patients with type 2 diabetes. Physiological mechanisms underlying these beneficial effects are not clear but it is plausible to hypothesize that Nordic walking performed in water may have increased blood flow and shear stress to a greater extent and, in turn, improved endothelium-dependent vasodilation and NO bioavailability (26).

Type 2 diabetes is related to an elevated risk of cerebrovascular disease and vascular dementia (39). Arterial stiffness and subsequent loss of the intrinsic buffering capacity increases hemodynamic pulsatility precipitating cerebrovascular damage and thus cognitive decline (30). A significant negative correlation between HbA_{1c} and cerebral blood flow in all cortical regions might reflect a risk of preclinical oligemia due to altered glycemic control, leading to potential neuronal injury (40). The participants in the present study were elderly patients with type 2 diabetes who exhibited mild cognitive impairment as assessed by Montreal Cognitive Assessment score below 25. Following Nordic walking in water training, average Montreal

Cognitive Assessment score was increased approximately 15%. The improvement in the cognitive function was associated with the corresponding changes in cerebrovascular function as assessed by transcranial Doppler technique. Nordic walking in water improved cognitive performance in type 2 diabetes. This was associated with increased MCA blood velocity possibly medicated by $PETCO_2$ effects since the cerebrovasculature is very reactive to changes in $PETCO_2$ and contribute to changes in cerebral perfusion (41). Additionally, cerebrovascular resistance was reduced as reflected by higher CVCi during hypercapnia and lower pulsatility index in middle cerebral artery in all conditions. There are a number of possible explanations for the improved cerebrovascular function and the associated improvement in cognitive function induced by Nordic walking in water. As represented in the somatotropic map of primary motor cortex, use of fingers, arms, and shoulders involved in the Nordic walking activates greater areas of the motor cortex in the brain (42). Moreover, hydrostatic pressure surrounding exercisers translocates blood volume to upper body and increase blood flow and perfusion to the brain (16). The augmented blood flow by a combination of water immersion and aerobic exercise has the potential to enhance shear stress-mediated cerebrovascular adaptation linked with improved brain health (17).

Cerebral circulatory responses to hypocapnia are mediated, at least in part, by endothelial NO (43). We observed increases in functional measures of peripheral endothelial function as well as in biochemical markers of endothelial function in the present study. Arterial stiffness has been implicated in the development of dementia (44) as it is associated with age-related differences in cerebrovascular conductance (27). In addition, patients with diabetes also demonstrate increased pulsatility index in the common carotid artery (45). Arterial stiffening, central pulse pressure, and forward wave energy are major contributors to the transmission of

pulsatility into cerebral vessels (46) and could be damaging to the cerebral microvasculature. In the present study, arterial stiffness as measured by baPWV and cerebrovascular stiffness as measured by MCA pulsatility index (PI) were improved by aquatic Nordic walking. Previous findings demonstrated cerebral reactivity assessed during a cognitive challenge had positive associations with the cognitive domains of response speed, memory, attention and executive function (47). The improvement in cognitive function was also associated with the corresponding increase in BDNF in the aquatic Nordic walking group. BDNF is a neurotropic factor that plays an important role in protection of neurons from eventual damage, neurogenesis, and neuroplasticity (48). Indeed, the microcirculation in area of the brain associated with long-term memory is especially vulnerable to pulsatile stress (49). Taken together, aquatic Nordic walking may exert cognitive enhancing effects possibly through a variety of underlying mechanisms in patient with type 2 diabetes.

Aquatic Nordic walking is a whole-body exercise in which both arms and legs are rhythmically moving forwards and backwards to overcome drag resistance exerted by water. Accordingly, this can be considered one of the multisensory stimulation trainings to emphasize motor coordination between arms and legs. Because exercise is conducted in water, effects of buoyancy minimize excessive joint stress during exercise (50). High thermal conductivity of water lessens heat-related problems that older adults are more prone to (14). As demonstrated in the present study, multitudes of favorable changes carrying clinical and functional significance can be elicited by moderate intensity of exercise that older patient populations can enjoy. There were no participants in the Nordic walking in water group who suffered from injury. Anecdotal evidence during the exercise supervision suggests that all the exercisers enjoyed this mode of exercise. In fact, the exercise adherence/compliance was extremely high at 100%. This

innovative mode of whole-body exercise is a promising exercise that should be evaluated in larger exercise intervention studies involving different patient populations.

There were several limitations in the current study that must be considered. First, the number of participants studied was relatively small although this sample size is larger than most other exercise training intervention studies involving high risk patient populations. Second, we did not include control/comparison groups that performed Nordic walking on land as well as walking only or pole swing only groups. Third, because Nordic walking applied to water-based condition is new, there are a number of issues that are unknown. For example, the level of water immersion during Nordic walking needs further investigation. Fourth, because transcranial Doppler flowmeter was used to determine cerebrovascular parameters, a lack of arterial diameter measurement resulting in an inability to derive volumetric blood flow is another limitation of the present study.

CONCLUSIONS

In conclusion, the results of the present study demonstrated that Nordic walking in water exerted beneficial effects on glycemic control, health-related physical fitness, peripheral vascular function, cerebrovascular reactivity, and cognitive impairment in the elderly patients with type 2 diabetes.

Acknowledgements

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REFERENCES

1. Messier C, Awad N, Gagnon M. The relationships between atherosclerosis, heart disease, type 2 diabetes and dementia. *Neurol Res*. 2004;26(5):567-72.
2. Chen S, Shen Y, Liu YH, et al. Impact of glycemic control on the association of endothelial dysfunction and coronary artery disease in patients with type 2 diabetes mellitus. *Cardiovasc Diabetol*. 2021;20(1):64.
3. Sena CM, Pereira AM, Seiça R. Endothelial dysfunction - a major mediator of diabetic vascular disease. *Biochim Biophys Acta*. 2013;1832(12):2216-31.
4. Chirinos JA, Segers P, Hughes T, Townsend R. Large-artery stiffness in health and disease: JACC state-of-the-art review. *J Am Coll Cardiol*. 2019;74(9):1237-63.
5. Skow RJ, Brothers RM, Claassen J, et al. On the use and misuse of cerebral hemodynamics terminology using transcranial Doppler ultrasound: a call for standardization. *Am J Physiol Heart Circ Physiol*. 2022;323(2):H350-7.
6. Webb AJ, Simoni M, Mazzucco S, Kuker W, Schulz U, Rothwell PM. Increased cerebral arterial pulsatility in patients with leukoaraiosis: arterial stiffness enhances transmission of aortic pulsatility. *Stroke*. 2012;43(10):2631-6.
7. Nealon RS, Howe PR, Jansen L, Garg M, Wong RH. Impaired cerebrovascular responsiveness and cognitive performance in adults with type 2 diabetes. *J Diabetes Complications*. 2017;31(2):462-7.
8. Mazzeo RS, Tanaka H. Exercise prescription for the elderly: current recommendations. *Sports Med*. 2001;31(11):809-18.
9. Qiu S, Cai X, Yin H, et al. Exercise training and endothelial function in patients with type 2 diabetes: a meta-analysis. *Cardiovasc Diabetol*. 2018;17(1):64.
10. Sharma P, Agarwal M, Tiwari S. Nordic walking: a novel exercise regimen for cardio-metabolic health. A brief review. *J Physiol*. 2017;16:25-30.
11. Piotrowicz E, Zieliński T, Bodalski R, et al. Home-based telemonitored Nordic walking training is well accepted, safe, effective and has high adherence among heart failure patients,

including those with cardiovascular implantable electronic devices: a randomised controlled study. *Eur J Prev Cardiol*. 2015;22(11):1368-77.

12. Takeshima N, Islam MM, Rogers ME, et al. Effects of Nordic walking compared to conventional walking and band-based resistance exercise on fitness in older adults. *J Sports Sci Med*. 2013;12(3):422-30.
13. Rao SM, Binder JR, Hammeke TA, et al. Somatotopic mapping of the human primary motor cortex with functional magnetic resonance imaging. *Neurology*. 1995;45(5):919-24.
14. Tanaka H. Swimming exercise: impact of aquatic exercise on cardiovascular health. *Sports Med*. 2009;39(5):377-87.
15. Suntraluck S, Tanaka H, Suksom D. The relative efficacy of land-based and water-based exercise training on macro- and microvascular functions in older patients with type 2 diabetes. *J Aging Phys Act*. 2017;25(3):446-52.
16. Pugh CJ, Sprung VS, Ono K, et al. The effect of water immersion during exercise on cerebral blood flow. *Med Sci Sports Exerc*. 2015;47(2):299-306.
17. Parfitt R, Hensman MY, Lucas SJE. Cerebral blood flow responses to aquatic treadmill exercise. *Med Sci Sports Exerc*. 2017;49(7):1305-12.
18. Carter HH, Spence AL, Pugh CJ, Ainslie P, Naylor LH, Green DJ. Cardiovascular responses to water immersion in humans: impact on cerebral perfusion. *Am J Physiol Regul Integr Comp Physiol*. 2014;306(9):R636-40.
19. Bressel E, Louder TJ, Raikes AC, Alphonsa S, Kyvelidou A. Water immersion affects episodic memory and postural control in healthy older adults. *J Geriatr Phys Ther*. 2019;42(4):E1-6.
20. Rosenzweig JL, Weinger K, Poirier-Solomon L, Rushton M. Use of a disease severity index for evaluation of healthcare costs and management of comorbidities of patients with diabetes mellitus. *Am J Manag Care*. 2002;8(11):950-8.
21. Tangwongchai S, Phanasathit M, Charernboon T, et al. The validity of Thai version of the Montreal cognitive assessment (MoCA-T). *Dement Neuropsychol*. 2009;3(2):172.
22. Svensson M. Nordic walking. United States of America: *Human Kinetics*; 2009. 219 p.

23. Purath J, Buchholz SW, Kark DL. Physical fitness assessment of older adults in the primary care setting. *J Am Acad Nurse Pract*. 2009;21(2):101-7.

24. Rikli RE, Jones CJ. The reliability and validity of a 6-minute walk test as a measure of physical endurance in older adults. *J Aging Phys Act*. 1998;6(4):363-75.

25. Costa EC, Kent DE, Boreskie KF, et al. Acute effect of high-intensity interval versus moderate-intensity continuous exercise on blood pressure and arterial compliance in middle-aged and older hypertensive women with increased arterial stiffness. *J Strength Cond Res*. 2020;34(5):1307-16.

26. Mitranun W, Deerochanawong C, Tanaka H, Suksom D. Continuous vs interval training on glycemic control and macro- and microvascular reactivity in type 2 diabetic patients. *Scand J Med Sci Sports*. 2014;24(2):e69-76.

27. Jaruchart T, Suwanwela NC, Tanaka H, Suksom D. Arterial stiffness is associated with age-related differences in cerebrovascular conductance. *Exp Gerontol*. 2016;73:59-64.

28. Lau VI, Jaidka A, Wiskar K, et al. Better with ultrasound: transcranial doppler. *Chest*. 2020;157(1):142-50.

29. Tarumi T, Gonzales MM, Fallow B, et al. Cerebral/peripheral vascular reactivity and neurocognition in middle-age athletes. *Med Sci Sports Exerc*. 2015;47(12):2595-603.

30. Heffernan KS, Augustine JA, Lefferts WK, et al. Arterial stiffness and cerebral hemodynamic pulsatility during cognitive engagement in younger and older adults. *Exp Gerontol*. 2018;101:54-62.

31. Alagiakrishnan K, Zhao N, Mereu L, Senior P, Senthilselvan A. Montreal cognitive assessment is superior to standardized mini-mental status exam in detecting mild cognitive impairment in the middle-aged and elderly patients with type 2 diabetes mellitus. *Biomed Res Int*. 2013;2013:186106.

32. Bajaj JS, Thacker LR, Heuman DM, et al. The stroop smartphone application is a short and valid method to screen for minimal hepatic encephalopathy. *Hepatology*. 2013;58(3):1122-32.

33. Dianatinasab A, Koroni R, Bahramian M, et al. The effects of aerobic, resistance, and combined exercises on the plasma irisin levels, HOMA-IR, and lipid profiles in women with metabolic syndrome: a randomized controlled trial. *J Exerc Sci Fit*. 2020;18(3):168-76.

34. Stringuetta Belik F, Oliveira ESVR, Braga GP, et al. Influence of intradialytic aerobic training in cerebral blood flow and cognitive function in patients with chronic kidney disease: a pilot randomized controlled trial. *Nephron*. 2018;140(1):9-17.

35. Grubbs FE. Procedures for detecting outlying observations in samples. *Technometrics*. 1969;11(1):1-21.

36. Sentinelli F, La Cava V, Serpe R, et al. Positive effects of Nordic walking on anthropometric and metabolic variables in women with type 2 diabetes mellitus. *Sci Sports*. 2015;30(1):25-32.

37. Pippi R, Di Blasio A, Aiello C, et al. Effects of a supervised Nordic walking program on obese adults with and without type 2 diabetes: The C.U.R.I.A.Mo. centre experience. *J Funct Morphol Kinesiol*. 2020;5(3):62.

38. Haupenthal A, Ruschel C, Hubert M, de Brito Fontana H, Roesler H. Loading forces in shallow water running in two levels of immersion. *J Rehabil Med*. 2010;42(7):664-9.

39. Luchsinger JA, Reitz C, Patel B, Tang MX, Manly JJ, Mayeux R. Relation of diabetes to mild cognitive impairment. *Arch Neurol*. 2007;64(4):570-5.

40. Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci*. 2011;12(12):723-38.

41. Shoemaker LN, Wilson LC, Lucas SJE, Machado L, Thomas KN, Cotter JD. Swimming-related effects on cerebrovascular and cognitive function. *Physiol Rep*. 2019;7(20):e14247.

42. Roux FE, Niare M, Charni S, Giussani C, Durand JB. Functional architecture of the motor homunculus detected by electrostimulation. *J Physiol*. 2020;598(23):5487-504.

43. Iadecola C. Does nitric oxide mediate the increases in cerebral blood flow elicited by hypercapnia? *Proc Natl Acad Sci U S A*. 1992;89(9):3913-6.

44. Bailey TG, Klein T, Meneses AL, et al. Cerebrovascular function and its association with systemic artery function and stiffness in older adults with and without mild cognitive impairment. *Eur J Appl Physiol*. 2022;122(8):1843-56.

45. Bill O, Mazya MV, Michel P, et al. Intima-media thickness and pulsatility index of common carotid arteries in acute ischaemic stroke patients with diabetes mellitus. *J Clin Med*. 2022;12(1):246.

46. Lefferts WK, DeBlois JP, Augustine JA, Keller AP, Heffernan KS. Age, sex, and the vascular contributors to cerebral pulsatility and pulsatile damping. *J Appl Physiol (1985)*. 2020;129(5):1092-101.

47. Cilhoroz BT, DeBlois JP, Lefferts WK, et al. Exploration of cerebral hemodynamic pathways through which large artery function affects neurovascular coupling in young women. *Front Cardiovasc Med*. 2022;9:914439.

48. Budni J, Bellettini-Santos T, Mina F, Garcez ML, Zugno AI. The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. *Aging Dis*. 2015;6(5):331-41.

49. Conley AC, Karayanidis F, Jolly TAD, Yang MH, Hsieh S. Cerebral arterial pulsatility and global white matter microstructure impact spatial working memory in older adults with and without cardiovascular risk factors. *Front Aging Neurosci*. 2020;12:245.

50. Alkatan M, Baker JR, Machin DR, et al. Improved function and reduced pain after swimming and cycling training in patients with osteoarthritis. *J Rheumatol*. 2016;43(3):666-72.

FIGURE LEGEND

Figure 1: Associations of percent changes (Pre and Post-test) in Montreal Cognitive Assessment (MoCA) scores with corresponding changes in brain-derived neurotrophic factor (BDNF) (A), cerebrovascular reactivity (CVR) normocapnia to hypocapnia (B), CVR hypocapnia to hypercapnia (C), and cerebrovascular conductance index (CVCi) during hypercapnia (D).

Figure 1

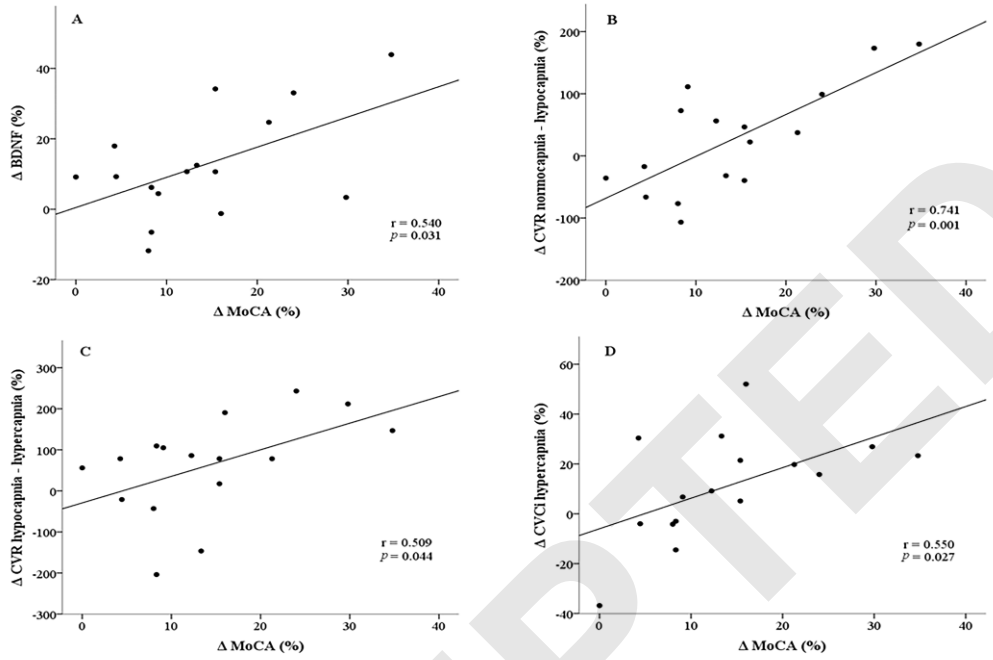

TABLE 1. Selected characteristics before and after 12 weeks of non-exercising control and aquatic Nordic walking in elderly participants with type 2 diabetes.

Variables	Non-exercising Control		Aquatic Nordic Walking		ANOVA Statistics			
	Pre	Post	Pre	Post	Time	Group	Interaction	**Effect size**
Male/Female (n)	7/10	-	5/11	-				
Age (years)	69.2±5.3	-	68.9±3.7	-				
Height (cm)	161±8	-	159±6	-				
Body weight (kg)	62.9±9.3	62.5±10.2	61.4±10.1	60.5±9.1	0.033	0.621	0.427	0.021
BMI (kg/m²)	24.8±2.7	24.6±3.1	24.2±3.6	23.9±3.3	0.041	0.561	0.330	0.031
Fat mass (kg)	20.9±5.9	20.6±6.0	21.8±7.0	20.9±6.2	0.014	0.768	0.176	0.058
Body fat (%)	34.0±8.2	34.0±7.8	35.8±7.8	34.6±7.4*	0.009	0.655	0.040	0.129
Lean mass (kg)	40.8±8.2	40.9±8.2	37.6±6.2	37.6±5.9	0.003	0.612	0.593	0.009
Bone mass (kg)	1.10±0.19	1.09±0.18	1.07±0.11	1.08±0.12	0.527	0.744	0.057	0.133
Waist circumference (cm)	86.7±7.2	87.6±8.1	86.7±8.8	86.7±9.3	0.351	0.856	0.351	0.028
Heart rate at rest (bpm)	72±9	73±8	75±14	70±10*	0.010	0.957	0.023	0.172
Systolic BP (mmHg)	130±16	125±18	128±11	122±11	0.003	0.612	0.593	0.009
Diastolic BP (mmHg)	72±8	69±9	73±8	69±8	0.002	0.893	0.694	0.005
Mean BP (mmHg)	92±10	87±11	92±8	86±9	0.001	0.845	0.643	0.007

Values are means ± SD. *P<0.05 vs Pre. BMI = Body mass index, BP = Blood pressure.

TABLE 2. Measures of physical fitness before and after 12 weeks of non-exercising control and aquatic Nordic walking in elderly participants with type 2 diabetes.

Physical fitness	**Non-exercising Control**		**Aquatic Nordic Walking**		**ANOVA Statistics**			
	Pre	Post	Pre	Post	Time	Group	Interaction	**Effect size**
Chair sit & reach (cm)	5.2±7.8	4.5±8.9	1.9±8.8	6.4±9.5*	0.001	0.811	0.004	0.236
Reach & back scratch (cm)	-6.3±15.8	-7.7±14.7	-9.7±10.9	-6.3±10.6*	0.027	0.815	0.025	0.152
Chair stand 30 sec (times)	15.5±3.7	15.7±4.3	15.3±2.6	19.6±3.2*†	0.001	0.006	0.001	0.370
Arm curl 30 sec (times)	15.9±3.1	16.6±4.0	17.7±2.2	20.6±2.5*†	0.001	0.002	0.049	0.124
2-min step test (times)	168±25	174±29	171±26	193±23	0.009	0.171	0.109	0.083
Timed up and go test (sec)	7.25±1.37	7.95±1.46*	7.39±0.9	6.80±0.82*†	0.001	0.009	0.001	0.573
6-min walking (m)	496±96	471±88*	452±61	492±73*	0.001	0.677	0.001	0.428

Values are means ± SD. *P<0.05 vs Pre, † P<0.05 vs Control

TABLE 3. Vascular function and structure before and after 12 weeks of non-exercising control and aquatic Nordic walking in elderly participants with type 2 diabetes.

Vascular measures	Non-exercising Control		Aquatic Nordic Walking		ANOVA Statistics			
	Pre	Post	Pre	Post	Time	Group	Interaction	**Effect size**
Carotid IMT (mm)	0.68±0.13	0.67±0.13	0.68±0.16	0.62±0.13*	0.001	0.606	0.025	0.151
baPWV (cm/sec)	1721±302	1747±205	1802±225	1656±176*	0.015	0.950	0.039	0.143
Arterial compliance (units)	0.24±0.16	0.26±0.11	0.30±0.12	0.48±0.17*†	0.010	0.001	0.029	0.164
Brachial FMD (%)	4.8±2.9	4.5±2.7	4.9±2.2	7.3±2.7*†	0.012	0.005	0.037	0.133

Values are means ± SD. *P<0.05 vs Pre, † P<0.05 vs Control.

IMT = intima-media thickness, baPWV = brachial-ankle pulse wave velocity, FMD = flow-mediated dilation.

TABLE 4. Cerebrovascular function before and after 12 weeks of non-exercising control and aquatic Nordic walking in elderly participants with type 2 diabetes.

Cerebrovascular function	Non-exercising Control		Aquatic Nordic Walking		ANOVA Statistics			
	Pre	Post	Pre	Post	Time	Group	Interaction	**Effect size**
Mean CBFV (cm/s)								
Normocapnia	36.3±8.7	34.3±7.5	39.6±12.8	40.2±6.8	0.724	0.180	0.534	0.013
Hypocapnia	28.4±7.2	27.6±6.9	33.4±13.0	30.6±8.1	0.368	0.136	0.599	0.010
Hypercapnia	37.9±7.9	37.3±6.8	40.7±11.5	43.0±10.8	0.602	0.162	0.374	0.027
$EtCO_2$ (mmHg)								
Normocapnia	40.5±11.5	33.7±9.8*	38.8±8.2	39.9±5.5	0.008	0.404	0.028	0.146
Hypocapnia	31.0±10.7	25.2±8.2*	29.5±6.5	30.1±5.1	0.006	0.478	0.028	0.146
Hypercapnia	56.4±9.5	46.9±8.9*	51.0±7.9	49.9±4.6	0.001	0.629	0.002	0.263
MCA PI								
Normocapnia	1.33±0.20	1.38±0.22	1.25±0.15	1.16±0.10*†	0.009	0.001	0.003	0.255
Hypocapnia	2.06±0.51	2.08±0.44	1.91±0.30	1.78±0.17	0.604	0.022	0.482	0.017
Hypercapnia	1.29±0.20	1.27±0.21	1.18±0.19	1.09±0.15	0.105	0.016	0.286	0.037
CVCi (cm/sec*$mmHg^{-1}$)								
Normocapnia	0.39±0.08	0.38±.080	0.40±0.10	0.43±0.08	0.542	0.297	0.193	0.058
Hypocapnia	0.32±0.10	0.30±0.08	0.35±0.11	0.35±0.08	0.621	0.204	0.597	0.009
Hypercapnia	0.38±0.80	0.35±0.09	0.42±0.09	0.47±0.13*†	0.007	0.005	0.006	0.223
CVR (%/mmHg)								
Normocapnia - Hypocapnia	2.7±2.07	2.11±1.56	3.33±2.24	3.66±1.48	0.788	0.032	0.350	0.031
Normocapnia - Hypercapnia	0.36±1.03	0.65±1.04	1.09±1.27	1.25±1.91	0.549	0.056	0.863	0.001
Hypocapnia - Hypercapnia	1.7±1.19	1.85±1.39	1.62±1.42	2.56±1.74	0.099	0.436	0.224	0.049
$\Delta CVCi/\Delta EtCO_2$ (%/mmHg)								
Normocapnia - Hypocapnia	1.82±2.34	1.76±3.34	1.82±2.06	3.41±1.32	0.223	0.155	0.188	0.055

Normocapnia - Hypercapnia	0.64±1.64	0.76±1.38	1.13±1.97	1.15±1.77	0.886	0.217	0.919	0.001
Hypocapnia - Hypercapnia	1.7±1.19	1.85±1.39	1.62±1.42	2.56±1.74	0.099	0.436	0.224	0.049

Values are means ± SD. **P*<0.05 vs Pre, † *P*<0.05 vs Control

CBFV = cerebral blood flow velocity, $EtCO_2$ = end-tidal CO_2, MCA = middle cerebral artery, CVCi = cerebrovascular conductance index, CVRi = cerebrovascular reactivity index.

TABLE 5. Biochemical variables before and after 12 weeks of non-exercising control and aquatic Nordic walking in elderly participants with type 2 diabetes.

Biochemical variables	Non-exercising Control		Aquatic Nordic Walking		ANOVA Statistics			
	Pre	Post	Pre	Post	Time	Group	Interaction	**Effect size**
WBC (10^3 /ul)	7.1±1.6	7.1±1.8	6.8±1.6	6.2±1.3	0.128	0.288	0.140	0.069
RBC (10^6 /ul)	4.6±0.5	4.5±0.4	4.6±0.4	4.5±0.5	0.119	0.952	0.536	0.012
Hemoglobin (g/dl)	13.2±1.0	12.9±0.8	13.2±0.5	12.9±0.9	0.019	0.914	0.641	0.007
Hematocrit (%)	40.4±2.8	39.5±2.3	40.2±1.8	39.9±2.7	0.134	0.906	0.454	0.018
Glucose (mg/dl)	147±25	152±40	135±21	117±22*†	0.033	0.005	0.047	0.129
HbA1c (%)	7.9±0.7	7.6±0.6	7.8±0.7	7.0±0.7*†	0.001	0.020	0.023	0.156
Insulin (uU/mL)	7.4±2.6	6.8±2.7	6.5±1.9	7.7±2.9	0.484	0.966	0.087	0.101
HOMA-IR (units)	2.94±1.15	3.41±1.56	2.76±1.25	2.17±1.03*†	0.044	0.015	0.012	0.203
Cholesterol (mg/dl)	164±26	165±22	176±44	171±35	0.536	0.419	0.339	0.030
Triglyceride (mg/dl)	110±47	110±46	110±33	96±31	0.395	0.557	0.378	0.027
HDL (mg/dl)	50±11	52±9	51±11	53±12	0.033	0.735	0.858	0.001
LDL (mg/dl)	98±21	105±24	103±33	96±28	0.928	0.850	0.081	0.101
CRP (mg/L)	1.74±1.1	1.82±1.33	1.77±2.38	1.74±2.24	0.093	0.966	0.797	0.002
IL-1β (pg/ml)	0.52±0.02	0.50±0.02	0.53±0.04	0.53±0.02	0.101	0.012	0.150	0.070
IL-6 (pg/ml)	39.8±2.3	39.6±2.8	39.1±3.8	39.8±2.9	0.750	0.795	0.590	0.010
Nitric oxide (μM)	6.66±2.16	7.17±3.23	6.65±2.13	10.20±3.91*†	0.002	0.030	0.045	0.141
Adiponectin (pg/ml)	13.8±5.1	12.7±4.7	12.2±5.2	11.1±4.8	0.255	0.286	0.987	0.001
MDA (pg/ml)	1.83±0.57	1.82±0.48	1.91±0.53	1.72±0.38	0.184	0.943	0.273	0.039
TNF–α (pg/ml)	84.7±11.9	86.4±13.8	84.0±13.5	80.8±6.3	0.759	0.387	0.323	0.036
BDNF (pg/ml)	241±16	216±45	225±32	249±26*†	0.028	0.023	0.002	0.284

Values are means ± SD. *P<0.05 vs Pre, † P<0.05 vs Control

WBC = White blood cell, RBC = Red blood cell, HbA1c = Glycosylated hemoglobin, HOMA-IR = Homeostatic model assessment of insulin resistance, HDL = High density lipoprotein, LDL = Low density lipoprotein, CRP = High-sensitivity C-reactive protein, IL = Interleukin, MDA = Malondialdehyde, TNF-α = Tumor necrosis factor alpha, BDNF = Brain-derived neurotrophic factor

TABLE 6. Cognitive function before and after 12 weeks of non-exercising control and aquatic Nordic walking in elderly participants with type 2 diabetes.

Cognitive function	Non-exercising Control		Aquatic Nordic Walking		ANOVA Statistics			Effect size	Kruskal-Wallis test		
	Pre	Post	Pre	Post	Time	Group	Interaction		df	X^2	P
MoCA (score)	21.5±2.2	21.2±2.8	22.3±1.5	25.6±1.8*†	0.001	0.001	0.001	0.458	-	-	-
#Visuospatial/executive (score)	4 (3–4.5)	4 (2.5-4)	3.5 (3-4)	5 (4-5)†	-	-	-	-	1	16.4	0.001
#Naming (score)	3 (3-3)	3 (3-3)	3 (3-3)	3 (3-3)	-	-	-	-	1	2.0	0.163
#Attention (score)	5 (4-5)	5 (4-5.5)	5 (4-5)	5 (4-6)	-	-	-	-	1	1.0	0.311
#Language (score)	2 (1-2)	1 (1-2)	2 (1-2)	2 (1-2)	-	-	-	-	1	3.2	0.075
#Abstraction (score)	1 (0.5-1)	1 (0-1)	1 (1-1)	2 (1-2)†	-	-	-	-	1	9.5	0.002
#Delayed recall (score)	2 (0.5-3)	3 (2-4)	3 (2-3)	4 (3-4)†	-	-	-	-	1	4.6	0.033
#Orientation (score)	6 (5.5-6)	6 (5-6)	6 (6-6)	6 (6-6)†	-	-	-	-	1	6.7	0.010
MMSE (score)	26.8±2.6	26.8±1.9	27.7±1.4	28.5±1.0†	0.279	0.023	0.212	0.050	-	-	-
Stroop color test (sec)	217±45	228±50	213±38	205±28	0.774	0.350	0.066	0.105	-	-	-
Trail making test part-B (sec)	176±53	176±56	160±64	145±63	0.416	0.217	0.372	0.026	-	-	-

Values are means ± SD or median (interquartile range). *P<0.05 vs Pre, † P<0.05 vs Control

MoCA = Montreal Cognitive Assessment, MMSE = Mini-Mental State Examination.

Kruskal Wallis test was used to calculate p-values.