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UNIVERSIDADE D
COIMBRA

Carlos Manuel Nunes Farinha

THE IMPACT OF DIFFERENT AQUATIC EXERCISE PROGRAMS ON CARDIOMETABOLIC MARKERS IN COMMUNITY DWELLING ELDERLY

Thesis for the degree of Doctor in Sport Science, in the field of Physical Activity and Health, supervised by Doctors José Pedro Leitão Ferreira, Ana Maria Miranda Botelho Teixeira and João Júlio de Matos Serrano, submitted to the Faculty of Sport Science and Physical Education in University of Coimbra.

October, 2022

Faculdade de Ciências do Desporto e Educação Física
da Universidade de Coimbra

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DEDICATORY

To God. That helped me to complete this stage of my life and allowed me to do it in good health. That brought me “angels” who supported me and lifted me whenever I needed them. Grateful.

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ABSTRACT

The elderly population is constantly growing worldwide. A large part of this population is affected by cardiometabolic diseases which make individuals more fragile and, consequently, with less autonomy. Scientific evidence has shown that the regular practice of physical exercise is an effective tool for the prevention and treatment of various pathologies associated with the aging process. However, physical exercise in water has been gaining popularity in this age group. Due to the specific properties of water, exercising in this environment becomes safer and more pleasurable. However, there are still few studies that have tested the impact of physical exercise programs in an aquatic environment in elderly. Thus, the main objective of this study was to test the impact of different physical exercise programs in an aquatic environment, on risk markers of cardiometabolic diseases, in community dwelling elderly.

The sample consisted of 102 participants who were randomly allocated into four groups: continuous aerobic exercise program (AerG) (n=25, 71.44±4.84 years); aerobic interval exercise program (IntG) (n=28, 72.64±5.22 years); combined exercise program (ComG) (n=29, 71.90±5.67 years); and control group (CG) (n=20, 73.60±5.25 years). Participants in AerG, IntG and ComG participated in three different physical exercise programs in aquatic environments for 28 weeks, while CG participants maintained their usual routines (without any systematic physical exercise) for the same period of time. All participants were evaluated on a broad set of cardiometabolic markers, pre and post-intervention. This thesis is organized into 5 studies:

The first study is the study protocol, which aimed to briefly describe the entire methodological process used to carry out this research.

The second study consisted of a cross-sectional analysis of baseline data and aimed to verify the association between aerobic capacity, handgrip strength and cognition with risk markers for cardiometabolic diseases and mental health in community dwelling elderly. The results showed that aerobic capacity was associated with markers of functional fitness (muscle strength of the lower and upper limbs, handgrip strength, flexibility of the lower and upper limbs, and agility and dynamic balance), cardiovascular (intima-media thickness of the carotid arteries, peak systolic velocity and peak diastolic velocity), biochemical (tumor necrosis factor, granulocytes and lymphocytes), cognitive function (Mini Mental State Examination) and mental health (Geriatric Depression Scale, Life

Satisfaction Scale and EuroQol). Handgrip strength was associated with anthropometric measures (height, weight, lean body mass and fat mass), functional fitness (aerobic capacity, muscle strength of the lower and upper limbs, agility and dynamic balance), biochemical markers (hematocrit, erythrocytes, hemoglobin concentration, glucose, cholesterol total and high-density lipoprotein), cognitive function (Mini Mental State Examination) and mental health (Rosenberg Self Esteem Scale, Geriatric Depression Scale, EuroQol and Perceived Stress Scale). And cognitive function was associated with anthropometric measures (legs circumference), physical fitness (muscle strength of the lower and upper limbs, handgrip strength, aerobic capacity, flexibility of the upper limbs, and agility and dynamic balance), cardiovascular (intima-media thickness of the carotid arteries) and biochemical markers (adenosine diphosphate), and mental health (Geriatric Depression Scale and EuroQol). These results suggest that aerobic capacity, handgrip strength and cognition may be associated with risk markers for cardiometabolic diseases.

The third study aimed to verify the effects of different physical exercise programs in an aquatic environment on body composition, functional fitness and cognitive function in community dwelling elderly. The results showed statistically significant differences in body composition after intervention for the percentage of fat mass, percentage of lean body mass and waist circumference in AerG, for body mass index, percentage of fat and lean body mass, and lower limb perimeter in IntG, and for body mas, percentage of fat and lean body mass, and perimeters of the lower limbs in ComG. In functional fitness, statistically significant differences were found for aerobic capacity and muscle strength in AerG, for aerobic capacity, flexibility and muscle strength in IntG, and muscle strength in ComG. In cognitive function, significant differences were observed for the Mini-Mental States Examination test in ComG. These results show the beneficial effects of physical exercise in an aquatic environment on body composition, functional fitness and cognition in community dwelling elderly. The combined program was potentially shown to be more beneficial on body composition and cognition, while the aerobic interval program was shown to be more effective on functional fitness.

The fourth study investigated the effects of different physical exercise programs in the aquatic environment on the immune profile in community-dwelling elderly. The results showed that after the intervention there were statistically significant differences for several hematological variables in AerG, IntG and ComG. Regarding immunological variables, statistically significant differences were found for IL-10 in AerG, for TNF-α and

TNF-α/IL-10 ratio in IntG, and for TNF-α and IL-1ß/IL-1ra ratio in ComG . The results of this study suggest that physical exercise in an aquatic environment can cause beneficial changes in the immune profile in elderly participants, by providing a lower pro-inflammatory profile. Aerobic interval exercise program and combined exercise program appear to be most effective in decreasing low-grade chronic inflammation by producing higher levels of anti-inflammatory cytokines. However, differences between exercise groups were small and may not be clinically significant.

In the fifth study, we aimed to verify the effects of different programs of physical exercise in the aquatic environment on the intima-media thickness of the carotid arteries, hemodynamic parameters and biomarkers of cardiovascular diseases. Regarding the carotid artery intima-media thickness, after the intervention, statistically significant differences were observed for diastolic diameter in AerG, for peak-systolic velocity in IntG and diastolic diameters and endo-diastolic velocity in ComG. Regarding the blood pressure, significant differences were found for systolic and diastolic blood pressure in AerG, for diastolic blood pressure in IntG, systolic and diastolic blood pressure, and heart rate in ComG. In the metabolic profile, significant differences were observed for glycemia in AerG and IntG. Regarding the chemokines, significant differences were found for MCP-1 and MIP-1α in AerG, and MCP-1 in ComG, after the intervention. These data suggest that physical exercise in an aquatic environment can improve cardiovascular health, regardless of the type of program. The programs of aerobic characteristics (combined program and continuous aerobic program) seem to have a more beneficial effect in the reduction of cardiovascular risk markers.

The results of this thesis showed that exercise in an aquatic environment can provide beneficial changes in most of the variables analyzed, evidencing that the practice of exercise in this environment can be considered an effective tool in the prevention and treatment of cardiometabolic diseases, in community dwelling elderly, and that it can be used as an alternative to the practice of physical exercise on land.

KEYWORDS

Aquatic exercise; hydrogymnastic; cardiometabolic rick factors; functional capacity; cognition; cardiovascular diseases; metabolic syndrome; immune system; aging.

RESUMO

A população idosa está em constante crescimento a nível mundial. Grande parte dessa população é afetada por doenças cardiometabólicas, que torna os indivíduos mais fragilizados e consequentemente com menos autonomia. Evidências científicas têm mostrado que a prática regular de exercício físico é uma ferramenta eficaz para a prevenção e tratamento para várias patologias associadas ao processo do envelhecimento. Entretanto, o exercício físico em meio aquático tem vindo a ganhar popularidade nesta faixa etária. Devido às propriedades específicas da água, a prática de exercício neste meio torna-se mais segura e prazerosa. No entanto ainda existem poucos estudos que testaram o impacto de programas de exercício físico em meio aquático na população idosa. Assim o objetivo principal deste estudo foi testar o impacto de diferentes programas de exercício físico em meio aquático, em marcadores de risco de doenças cardiometabólicas, em idosos residentes na comunidade.

A amostra foi constituída por 102 participantes que foram alocados aleatoriamente em quatro grupos: programa de exercício aeróbio contínuo (AerG) (n=25, 71.44±4.84 anos); programa de exercício intervalado aeróbio (IntG) (n=28, 72.64±5.22 anos); programa de exercício combinado (ComG) (n=29, 71.90±5,67 anos); e grupo de controlo (CG) (n=20, 73.60±5.25 anos). Os participantes do AerG, IntG e ComG participaram em três programas diferentes de exercício físico em meio aquático durante 28 semanas, enquanto os participantes do CG mantiveram as suas rotinas habituais (sem qualquer prática de exercício físico sistemático) durante o mesmo período de tempo. Todos os participantes foram avaliados num conjunto alargado de marcadores de risco cardiometabólico, pré e pós intervenção. Esta tese esta organizada em 5 estudos:

O primeiro estudo é o protocolo de estudo, que teve como finalidade descrever sucintamente todo o processo metodológico utilizado para a realização desta investigação.

O segundo estudo consistiu na análise transversal dos dados de linha base e teve como objetivo verificar a associação entre a capacidade aeróbia, força de preensão manual e cognição com marcadores de risco de doenças cardiometabólicas e saúde mental, em idosos residentes na comunidade. Os resultados mostraram que a capacidade aeróbia foi associada a marcadores de aptidão funcional (i.e., força muscular dos membros inferiores e superiores, força de preensão manual, flexibilidade dos membros inferiores e superiores, e agilidade e equilíbrio dinâmico), cardiovasculares (i.e., espessura íntima e média das

artérias carótidas, velocidade pico sistólica e velocidade pico diastólica), bioquímicos (i.e., Factor de necrose tumoral, granulócitos e linfócitos), função cognitiva (i.e., Mini Mental State Examination) e saúde mental (i.e., Escala de Depressão Geriátrica, Escala de Satisfação com a Vida e EuroQol). A força de preensão manual foi associada a medidas antropométricas (altura, peso, massa corporal magra e massa gorda), aptidão funcional (capacidade aeróbia, força muscular dos membros inferiores e superiores, e agilidade e equilíbrio dinâmico), marcadores bioquímicos (hematócrito, eritrócitos, concentração de hemoglobina, glicose, colesterol total e lipoproteína de alta densidade), função cognitiva (Mini Mental State Examination) e saúde mental (Escala de Auto Estima de Rosenberg, Escala de Depresão Geriátrica, EuroQol e Escala de Strees Percebido). E a função cognitiva foi associada a medidas antropométricas (perímetro dos membros inferiores), aptidão física (força muscular dos membros inferiores e superiores, força de preensão manual, capacidade aeróbia, flexibilidade dos membros superiores, e agilidade equilíbrio dinâmico), marcadores cardiovasculares (espessura íntima e média das artérias carótidas) e bioquímicos (difosfato de adenosina) e saúde mental (Escala de Depressão Geriátrica e EuroQol). Estes resultados sugerem que a capacidade aeróbia, força de preensão manual e cognição podem estar associadas a marcadores de risco de doenças cardiometabólicas.

O terceiro estudo teve como objetivo verificar os efeitos de diferentes programas de exercício físico em meio aquático na composição corporal, aptidão funcional e função cognitiva, em idosos residentes na comunidade. Os resultados mostraram diferenças estatisticamente significativas na composição corporal após intervenção para a percentagem de massa gorda, percentagem de massa muscular e perímetro da cintura no AerG, para índice de massa corporal, percentagem de massa gorda e muscular, e perímetro do membro inferior no IntG, e para peso, percentagem de massa gorda e muscular, e perímetros dos membros inferiores no ComG. Na aptidão funcional foram verificadas diferenças estatisticamente significativas para a capacidade aeróbia e força muscular no AerG, para capacidade aeróbia, flexibilidade e força muscular no IntG, e para a força muscular ComG. Na função cognitiva foram verificadas diferenças significativas para o teste Mini Mental States Examination no ComG. Estes resultados evidenciam os efeitos benéficos do exercício físico em meio aquático na composição corporal, aptidão funcional e na cognição, em idosos residentes na comunidade. O programa combinado mostrou ser potencialmente mais benéfico na composição corporal e cognição, enquanto o programa intervalado aeróbio mostrou ser mais eficaz na aptidão funcional.

O quarto estudo investigou os efeitos de diferentes programas de exercício físico em meio aquático no perfil imunológico, em idosos residentes na comunidade. Os resultados mostraram, que após a intervenção foram verificadas diferenças estatisticamente significativas para várias variáveis hematológicas no AerG, IntG e ComG. Em relação às variáveis imunológicas, foram encontradas diferenças estatisticamente significativas para IL-10 no AerG, para TNF-α e rácio TNF-α/IL-10 no IntG, e para TNF-α e rácio IL-1ß/IL-1ra no ComG. Os resultados deste estudo sugerem que o exercício físico em meio aquático pode provocar alterações benéficas no perfil imunológico em participantes idosos, ao proporcionar um menor perfil pró-inflamatório. O programa de exercício intervalado aeróbio e o programa de exercício combinado, parecem ser mais eficazes, diminuindo a inflamação crônica de baixo grau através da produção de níveis mais elevados de citocinas anti-inflamatórias. No entanto as diferenças entre os grupos de exercício foram pequenas e podem não ter significado clínico.

No quinto estudo objetivámos verificar os efeitos de diferentes programas de exercício físico em meio aquático na espessura intima-media das artérias carótidas, parâmetros hemodinâmicos e em biomarcadores de doenças cardiovasculares. Em relação à espessura intima-média das artérias carótidas, após intervenção, foram verificadas diferenças estatisticamente significativas para diâmetro diastólico no AerG, para velocidade pico-sistólico no IntG e para diâmetros diastólico e velocidade endo-diastólica no ComG. Na pressão arterial, foram encontradas diferenças significativas para a pressão arterial sistólica e diastólica no AerG, para a pressão arterial diastólica no IntG, e para a pressão arterial sistólica e diastólica, e frequência cardíaca no ComG. No perfil metabólico foram verificadas diferenças significativas para a glicémia no AerG e IntG. Relativamente às quimiocinas, diferenças significativas foram verificadas para MCP-1 e MIP-1α no AerG, e para MCP-1 no ComG, após intervenção. Estes dados sugerem que o exercício físico em meio aquático pode melhorar a saúde cardiovascular, independentemente do tipo de programa. Os programas de caraterísticas aeróbias (programa combinado e programa aeróbio contínuo) parecem ter um efeito mais benéfico na redução de marcadores de risco cardiovascular.

Os resultados desta tese mostraram que o exercício em meio aquático proporcionou alterações benéficas na maioria das variáveis analisadas, evidenciando que a prática de exercício neste meio pode ser considerada uma ferramenta eficaz na prevenção e tratamento de doenças cardiometabólicas, em idosos residentes na comunidade, e que pode ser utilizada em alternativa à prática de exercício físico em meio terrestre.

PALAVRAS-CHAVE

Exercício em meio aquático; hidroginástica; fatores de risco cardiometabólicos; aptidão funcional; aptidão cognitiva; doenças cardiovasculares; síndrome metabólica; sistema imunológico; envelhecimento.

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LIST OF ABBREVIATIONS

AerG	Continuous aerobic group
IntG	Aerobic interval exercise group
ComG	Combined exercise group (aerobic and muscle strength
CG	Control group
Hgt	Height
Wgt	Weight
BMI	Body mass index
VF	Visceral fat
FM	Fat mass
LBM	Lean body mass
WCir	Waist circumference
ACir	Arms circumference
LCir	Legs circumference
30s-CS	Chair stand test
30s-AC	Arm curl test
2min-ST	Two-minutes step test
CSR	Chair sit and reach
BS	Back scratch test
TUG	Timed up and go test
HG	Hand grip test
MMSE	Mini Mental State Examination
RSES	Rosenberg Self-Esteem Scale
CPSPP	Physical Self-Perception Profile For Clinical Populations
WHO5	World Health Organization Well-Being Index
SWLS	Satisfaction With Life Scale
EQ5D	EuroQol
GDS	Depression Scale
PSS	Perceived Stress Scale
IMT	Intima-media thickness
SD	Systolic diameter
DD	Diastolic diameter
PSV	Peak systolic velocity
EDV	End-diastolic velocity
HR	Heart rate

SBP	Systolic blood pressure
DBP	Diastolic blood pressure
RRmin	Intervalo R-R mínimo
RRmax	Intervalo R-R máximo
RRmean	Intervalo R-R média
RMSSD	Root mean square of the successive normal sinus R-R- interval difference
HDL	High-Density Lipoprotein
LDL	Low-Density Lipoprotein
CLT	Cholesterol total
AI	Atherogenic index
GLU	Glucose
TG	Triglycerides
LEU	Leukocytes
LI	Lymphocytes
Mo	Monocytes
GR	Granulocytes
ERI	Erythrocytes
Hb	Hemoglobin concentration
Hct	Hematocrit
MCV	Mean corpuscular volume
MCH	Mean globular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
RDW	Erythrocytes distribution width
PL	Platelets
MPV	Mean platelet volume
Pct	Procalcitonin
ADP	Adenosine diphosphate
IL-10	Interleukin 10
IL-1ra	Interleukin 1
IL-1β	Interleukin 1 beta
TNF-α	Tumor necrosis factor
MIP-1α	Macrophage inflammatory protein-1 Alpha
MCP-1	Monocyte chemotactic protein-1
med	Medication
all	Allergies
vdy	Visits to the doctor per year

sq Sleep quality

1. GENERAL INTRODUCION

1.1. Background

Ageing is considered to be a progressive and inevitable phenomenon, which leads to a reduction of several physical and metabolic functions (Filho et al., 2010). With this process, the human being is more susceptible to the appearance of certain pathologies, namely cardiometabolic diseases. These diseases are considered to be one of the main causes of mortality and morbidity in the world, thus arousing the scientific interest of researchers from different areas. According to the World Health Organization (WHO), cardiometabolic diseases are responsible for 63% of the annual 57 million deaths worldwide, with physical inactivity being strongly associated with these percentages: between 6 and 10% (Roque et al., 2013).

About five million annual deaths could be avoided if the world population were more active (OMS, 2020b). In 2014, only 23% of the adult population of Portugal complied with the physical activity recommendations, while the estimated percentage of mortality associated with physical inactivity was of 14% (SNS, 2017). Nowadays, more than 60% of the elderly who live in the community are sedentary (Collard et al., 2012), a fact that greatly contributes to their frailty. This frailty is associated with ageing and is characterized by a process of loss of some physiological reserves and functional capabilities, which in the future may bring aggravating health factors, namely loss of autonomy, institutionalization and mortality (Jang et al., 2018).

To counteract a sedentary lifestyle, strategies have been developed that implement more active and healthier lifestyles in society. One of these strategies is related to the dissemination of global guidelines about physical activity (namely, the frequency, duration, intensity, type and amount of physical activity practiced in the different age groups), with the main focus that countries develop evidence-based national health policies and support the implementation of the WHO Global Action Plan on physical activity 2018-2030 (OMS, 2020a). According to the same organization, the regular practice of physical activity can contribute to prevent and help control several non-transmissible chronic diseases, such as heart disease, type 2 diabetes, some types of cancer. It could also contribute to the reduction of symptoms of depression and anxiety (OMS, 2020a). At the same time, there has been a development of scientific interest in variables that are related to this type of pathologies, namely in terms of body composition (Arazi et al., 2018), functional fitness (Lopez et al., 2021), cardiovascular risk markers (Pereira et al., 2015), cognition (Chupel et al., 2018; Furtado et al., 2018) and immunity (Teixeira et al., 2016).

Physical exercise has been considered an effective method in attenuating the ageing process because it helps to stabilize the loss of physical and metabolic capacities, and especially allowing greater autonomy. Several studies developed by Chupel et al. (2017); Furtado et al. (2016); Novaes et al. (2014); Teixeira et al. (2016) point out that the practice of regular physical exercise is a strong ally against cardiometabolic diseases, helping in their prevention but also in their treatment.

In an aquatic environment, physical exercise has also been gaining popularity, particularly among the elderly population, insofar as some disadvantages, present in exercise on land, are minimized due to the specific properties of the aquatic environment. The buoyancy and the viscosity of water can help to reduce joint load, providing a safer and more protective environment for physical exercise. Programs carried out in an aquatic environment seem to be as effective as - or even more effective than - exercise practiced on land (Moreira et al., 2020).

Regarding body composition, functional fitness and cognition, Naylor et al. (2020), tested the impact of two walking programs (land-based and aquatic) and concluded that the aquatic program can be considered a safer and preferred exercise modality for the elderly, providing benefits in body composition similar or superior to those achieved in a land-based environment. The meta-analysis performed by Waller et al. (2016), which investigated the effects of exercise in an aquatic environment on the functional fitness of healthy elderly people, compared to the physical exercise practiced on land and a control group with no intervention, concluded that physical exercise in the aquatic environment appears to be effective in maintaining and improving functional fitness in healthy elderly and appears to be as effective, at least, and can be used as an alternative modality when land-based exercise is not feasible or desired. Kang et al. (2020) tested the effectiveness of a physical exercise program in an aquatic environment in the cognition of a group of elderly women aged between 60-80 years and concluded that this practice can contribute to the maintenance and improvement of their cognitive function.

On the other hand, regular physical exercise is also associated with the improvement of the immune system and the reduction of the occurrence of different types of chronic diseases, associated with ageing, through changes in immune function, namely the decrease in immune senescence, the increase of the innate immune function and decreased chronic inflammation (Abd El-Kader & Al-Shreef, 2018). In the same way, physical exercise, by causing the reduction of visceral fat, will stimulate a decrease in the production and release of adipokines and will contributing to the development of an anti-inflammatory environment at each exercise session (Gleeson et al., 2013). In addition to reducing visceral fat, physical exercise, through skeletal muscle contraction, directly stimulates the production of IL-6, which will stimulate the production of anti-inflammatory cytokines, such as IL-10 and IL-1ra, as well as the secretion of cortisol (Pedersen, 2017). In the study by Chupel et al. (2017), it is concluded that a 28-week intervention of a chair-supported muscle strength exercise program, was able to increase the anti-inflammatory balance in a group of elderly women. Similar results were observed in the study by Furtado et al. (2020), after the intervention with two different physical exercise program (i.e., muscle strength with elastic bands and combined exercise with chair support). In the study by Werner et al. (2019), the results showed an increase in leukocytes, granulocytes and lymphocytes, after a 26-week intervention of aerobic and interval exercise.

Physical exercise has also shown evidence of being an effective method in improving cardiovascular markers (Santos, 2020). The intima-media thickness of the carotid arteries (IMT) is considered an early marker of coronary artery disease and a risk factor for other types of cardiovascular diseases and stroke (Kablak-Ziembicka et al., 2008). According to the existing literature, this process can be attenuated with the recurrent practice of physical exercise, namely exercise with aerobic characteristics, which may contribute to the reduction of the luminal diameter of the arteries, as a localized effect, and/or to the increase of blood flow (Park & Park, 2017). Combined exercise programs have also been shown to be effective in improving cardiovascular parameters. The study by Son et al.(2017) of a 12-week combined exercise program (aerobic exercise and muscle strength exercise) in elderly women (75.0 ± 2.0 years) provided significant changes in arterial stiffness and in the participants' systolic and diastolic blood pressure.

A meta-analysis performed by Montero et al. (2015), aimed to quantify and compare the effect of two types of physical exercise program on arterial stiffness: combined exercise program (aerobic exercise and muscle strength exercise) and aerobic exercise programs. The results showed that no statistically significant differences were found between the two types of programs ($p = 0.12$). In the aerobic programs, a statistically significant reduction in arterial stiffness was found compared to the control groups (10 studies; std. mean difference = -0.52; effect $z = 4.15$; $p < 0.0001$). The same could not be said about the combined programs (11 studies; std. mean difference = -0.23; effect $z = 1.66$; $p = 0.10$). These results suggest that combined exercise programs may not be as effective as aerobic exercise programs in reducing arterial stiffness.

In recent times, the effectiveness of various physical exercise programs (aerobic, muscle strength and combined exercise) has been tested in variables related to cardiometabolic diseases, cardiovascular, functional and immunological levels (Timmons et al., 2018). However, most of these physical exercise programs were developed on land, so the effects of physical exercise in aquatic environments are not yet sufficiently explored in terms of research. Therefore, the development of scientific studies in an aquatic environment is essential, in order to understand the impact that these programs have on cardiometabolic markers, which may contribute to new lines of investigation related to prevention and treatment.

1.2. Research question

The elderly population is increasing all over the world, and it is estimated that by 2050 their number will double. A large part of this population lives with pathologies that are common in the aging process and that somehow make individuals frailer and less autonomous. Helping this population to live better and longer is the most important factor, but we can also, take into account, the impact that the loss of quality of life and independence have at an economic level in a society. If, on the one hand, healthier elderly people, with more active lifestyles, are more economically dynamic (with the practice of more leisure and cultural activities, etc.), on the other hand, more dependent and frailer elderly persons require an increase in public funds spending, both in terms of health and in terms of support for caregiving institutions, such as nursing homes and other private institutions of social solidarity.

Scientific evidence has shown that the practice of physical exercise is considered an effective, non-pharmacological strategy to prevent and treat several very common pathologies in aging, namely related to cardiovascular and metabolic health. However, a large percentage of the elderly continue to have a sedentary life. In this way, it is pertinent to develop strategies that encourage the elderly to practice physical exercise, thus contributing to an increase in the quality of life of this population, through the reduction of limitations and prevention/attenuation of pathologies very common in this age group, such as cardiometabolic diseases.

The practice of physical exercise in an aquatic environment has been gaining popularity in the elderly population and can be considered an alternative strategy to exercise in a land environment when this is not desired or possible due to health problems (eg. orthopedic, rheumatological limitations or functional, vertigo, etc.). Due to its characteristics, aquatic environment make the practice of physical exercise more pleasant and safer for the elderly population (Moreira et al., 2020). The buoyancy of the water allows a reduction of the gravitational forces that act on the musculoskeletal system, allowing a better muscular relaxation and consequently a reduction of the biomechanical stress in the muscles and joints. Additionally, the hydrostatic pressure and the force of water viscosity provide a different proprioceptive and sensory feedback when compared to the land-based environment, allowing for better balance and postural control (Saleh et al., 2019).

A preliminary systematic research carried out within the scope of the present study revealed its innovative characteristics, since, according to our knowledge, few studies have been carried out that have evaluated the impact of physical exercise in an aquatic environment on cardiometabolic markers in the elderly. More specifically, no studies were found in which the impact of different types of aquatic physical exercise programs in cardiometabolic markers in the elderly population was researched.

Based on the above and to clarify the effects of different physical exercise programs in aquatic environments on cardiometabolic markers, the following research question was defined:

What is the impact of different physical exercise programs in an aquatic environment on risk markers of cardiometabolic diseases in community-dwelling elderly?

1.3. General and specific objectives

This thesis is organized in 5 independent studies, carried out with the aim of answering the question of the study. The studies were carried out in the form of manuscripts, which were later submitted to international peer-reviewed journals. The different studies were developed, taking into account the general and specific objectives defined for this research work. Those are presented below:

1. To verify the association between aerobic capacity, handgrip strength and cognition with risk markers for cardiometabolic diseases and mental health in community dwelling elderly:

   1.1. To verify the association between aerobic capacity with risk markers for cardiometabolic diseases and mental health in community dwelling elderly;

   1.2. To verify the association between handgrip strength with risk markers for cardiometabolic diseases and mental health in community dwelling elderly;

   1.3. To verify the association between cognition with risk markers for cardiometabolic diseases and mental health in community dwelling elderly.

2. To test the impact of different types of physical exercise programs in the aquatic environment on body composition, functional fitness and cognition in community dwelling elderly:

   2.1. To test the impact of different physical exercise programs in the aquatic environment on body composition in community dwelling elderly;

   2.2. To test the impact of different physical exercise programs in the aquatic environment on functional fitness in community dwelling elderly;

   2.3. To test the impact of different exercise programs in aquatic environments on cognition in community dwelling elderly.

3. To test the impact of different types of physical exercise programs in the aquatic environment on the immune profile in community dwelling elderly:

3.1. To test the impact of different exercise programs in the aquatic environment on the hematological profile in community dwelling elderly;

3.2. To test the impact of different aquatic exercise programs on the immune profile in community dwelling elderly.

4. To test the impact of different types of physical exercise programs in aquatic environments on cardiovascular risk markers in community dwelling elderly.

4.1. To test the impact of different water-based exercise programs on the intima and media thickness of the carotid arteries in community dwelling elderly;

4.2. To test the impact of different aquatic exercise programs on blood pressure and heart rate in community dwelling elderly;

4.3. To test the impact of different aquatic exercise programs on the metabolic profile in community dwelling elderly;

4.4. To test the impact of different exercise programs in the aquatic environment on chemokines (MCP-1 and MIP-1α) in community dwelling elderly.

1.4. Organization of PhD thesis document

This research consists of a sequence of experimental studies that seek to respond to the defined objectives and that served as a guideline for the organization of this document. This study is built in a systematic and orderly manner, using the type of model called the "Scandinavian model" of a doctoral thesis, supported by a set of scientific articles published or in the process of being published.

The work is structured in different parts and begins with an introduction, where we contextualize the problem and summarize our objectives, followed by a chapter regarding the state of the art, in which we intend to detail a little more about the theoretical aspects and investigate the underlying conceptual models for the performance of this work. Then we present the methodology chapter, where we describe in detail the entire methodological process, which includes the characterization of the sample, instruments and methods used for data collection, description of the different physical exercise programs and statistical analysis. We present a chapter with the results, where the studies done and published are presented and, finally, we present a chapter discussing the global results of the work, in which we seek to present a framework and an adequate justification for the sequence of the studies carried out, as well as a sequential organization of the results obtained in each of the studies.

To respond to the aforementioned objectives, we developed 1+4 studies that we now present:

- Study 1: Impact of Aquatic-Based Physical Exercise Programs on Risk Markers of Cardiometabolic Diseases in Older People: A Study Protocol for Randomized-Controlled Trials.

- Study 2: Association between aerobic capacity, grip strength and cognition function with cardiometabolic diseases risk markers and mental health in the non-institutionalized old adults: a cross-sectional analysis.

- Study 3: Impact of different aquatic exercise programs on body composition, functional fitness and cognitive function of non-institutionalized elderly adults: A randomized controlled trial.

- Study 4: The Impact of aquatic exercise programs on the immunologic profile of community dwelling older persons: A randomized controlled trial.

- Study 5: Impact of different aquatic exercise programs on the intima-media thickness of the carotid arteries, hemodynamic parameters, lipid profile and chemokines of community-dwelling old persons: A randomized controlled trial.

2. STATE OF THE ART

The fact that this thesis is published in a similar format to the Scandinavian model, with the inclusion of a set of publications, and the nature of the thesis in terms of the reduced space for the presentation and development of more detailed theoretical content - resulting from the limitations in the number of words imposed by the editors -, makes us choose to introduce a chapter on the state of the art, with the objective of contextualizing, from a theoretical point of view, the different themes and sub-themes related to the present work, giving it greater theoretical sustainability, which is always important when it comes to a doctoral thesis.

2.1. Ageing

According to the ACSM, the term "elderly" defines individuals aged 65 years or older and individuals aged between 50 and 64 years with clinically significant conditions or physical limitations that affect movement, physical fitness or physical activity (ACSM, 2018).

The world population is ageing in all countries, with an increase of around 3% per year in the number of persons aged 60 and over. In 2017, this population represented 13% of the world population (962 million), and it is estimated that this number will double in 2050 (2.1 billion) and triple in 2100 (3.1 billion). The population aged 80 and over has also been increasing, with this age group expected to triple by 2050, from 137 million in 2017 to 425 million (United Nations, 2021). This increase is mainly due to three factors: fertility, mortality and migration. In all regions of the world, since 1950, there has been a substantial increase in life expectancy (Tian et al., 2019). Although population ageing is strongly driven by declining fertility and increasing longevity, international migration also contributes to changing population age structures in some countries and regions (United Nations, 2021).

These additional years are often accompanied by the onset of comorbidities, disability and compromised quality of life (Awick et al., 2017). Ageing is one of the risk factors for the development of most chronic diseases, namely cardiovascular, metabolic, autoimmune and cancer (Wahl et al., 2019). It is considered a process in which several complex changes arise: at the biological level, ageing is associated with the accumulation of a wide variety of molecular and cellular damages, which over time correspond to a gradual loss in physiological reserves, an increase in the probability of contracting various diseases, a general decline in intrinsic capacity, and ultimately resulting in death (WHO, 2015); physiologically, ageing is characterized by a reduction in maximum heart rate, maximum cardiac output, maximum reserve of absolute and relative oxygen consumption, vital capacity, reaction time, muscle strength, flexibility, bone mass, fat-free body mass, tolerance to glucose, as well as increases in resting and active blood pressure, residual volume, body fat percentage and reaction time (ACSM, 2018). However, these changes are not linear or consistent and although they are vaguely associated with advancing age, they are also influenced by habits and lifestyles (WHO, 2015).

In addition to pharmacological therapies, lifestyle modifications - such as a balanced diet and physical exercise - have called attention to the correction of metabolic abnormalities, for the benefit of cardiovascular health and in delaying premature ageing (Tian et al., 2019). Physical activity in the elderly population has been strongly associated with several health benefits (Awick et al., 2017). Physical activity and/or physical exercises practiced on a regular basis were considered the best predictor of mortality, being able to attenuate the main characteristics of ageing, providing benefits in musculoskeletal health and helping to prevent various pathologies such as metabolic syndrome, cardiovascular diseases, type 2 diabetes, liver disease, some cancers, obesity, and also general mortality that is often independent of weight loss (Davies et al., 2019).

The ACSM guidelines recommend the practice of between 30 and 60 minutes/day (equivalent to at least 150 minutes per week) of moderate-intensity physical exercise, or between 20 and 60 minutes/day (equivalent to at least 75 minutes per week) of vigorous intensity physical exercise. People can also combine both intensities in their practice. These amounts of time and intensities can be accumulated in a single session or at intervals longer than 10 minutes, several times a day (ACSM, 2018).

2.2. Physical exercise and the elderly

Regular physical activity is essential for healthy ageing and is considered an excellent preventive and treatment strategy to counteract the harmful changes that occur during the ageing process (Galloza et al., 2017). Physical activity provides a variety of benefits: it reduces all-cause mortality; reduces the development of chronic diseases that are highly costly and challenging for the elderly population; reduces premature death; improves mental health (reduces anxiety and depression); helps slow cognitive decline; improves sleep quality; reduces the incidence of mortality from cardiovascular diseases; the incidence of hypertension; the incidence of some types of cancers; the incidence of developing type 2 diabetes; the risk of moderate or severe functional limitations; the risk of falling; the decline in bone health; and the development of body adiposity (Mora & Valencia, 2018; OMS, 2020a)(Mora & Valencia, 2018)(Mora & Valencia, 2018).

In the study by Watson et al. (2016), the results showed that the prevalence of physical inactivity is higher in adults with at least 1 of 7 chronic diseases (31.9%), while in adults without chronic pathologies the percentage of physical inactivity is 19.2%.

An exercise program for the elderly population should include a combination of aerobic, muscle strengthening, flexibility and balance exercises (Galloza et al., 2017). According to the World Health Organization, the practice of physical activity between 150-300 minutes per week, of moderate-intensity aerobic physical activity, or 75-150 minutes per week, of high-intensity aerobic physical activity is recommended for the elderly population. However, it is also recommended the practice of muscle strengthening exercise (2x per week), and multicomponent physical exercise that encompass functional balance and muscle strengthening (3x week) (OMS, 2020a).

Aerobic capacity levels decrease rapidly with advancing age, and this decline is exacerbated by many common comorbidities among the elderly (Fleg, 2012). Moderate to high-intensity aerobic physical exercise has a positive impact on cardiometabolic health, namely in some aspects that are harmed by ageing, such as improvements in cardiopulmonary capacity, blood pressure, control of glycaemic levels, in the control of cholesterol levels and vascular endothelial function (Ashton et al., 2020).

However, muscle strengthening exercise plays an important role in attenuating age-related physiological changes, and in addition to causing changes in skeletal muscle size and strength, there is also evidence that it may have a positive role in metabolic profile, body composition, systolic blood pressure, cardiopulmonary capacity and circulating inflammatory markers, and may also generate more prolonged improvements in body fat, metabolic profile and systolic blood pressure, when compared to exercise which is more aerobic-based (Ashton et al., 2020).

High-intensity interval exercise is a modality that can also bring benefits to the elderly population. When compared to moderate-intensity exercise, high-intensity interval training may have beneficially equal or superior results in terms of body composition, cardiorespiratory fitness, and cardiovascular health (Vélez et al., 2020). Another available option is combined physical exercise programs (aerobic and muscle strength exercise), which are considered one of the most appropriate strategies to simultaneously improve physical fitness (particularly muscle strength), cardiometabolic capacity, and to prevent the development of diseases associated with age. It also contributes to the improvement of blood pressure and glycated haemoglobin levels (Schneider et al., 2021), a very important marker in the identification and monitoring of diabetes.

Seniors must be physically active. As such, doing some form of physical activity is better than doing nothing. Thus, even if the WHO and ACSM recommendations cannot be fully complied with, doing some type of physical activity will certainly provide health benefits, and the level of effort to exercise should be related to the level of functional fitness and ability of the participants (Mora & Valencia, 2018), so its prescription should always be individualized and adapted to the abilities, limitations and goals of each individual (Galloza et al., 2017).

2.3. Physical exercise in water and the elderly

Exercise in water has been gaining popularity among the elderly population - with several disadvantages found in the land-based exercise being minimized (Moreira et al., 2020), offering specific mechanical advantages due to the principles of buoyancy, viscosity and drag (Cugusi et al., 2019). The aquatic environment is considered excellent for reaching maximum levels of training in individuals with and without pathologies (Saleh et al., 2019), while also being a great tool for rehabilitation due to the physical and mechanical properties of water (Saleh et al., 2019).

Buoyancy provides relief from weight bearing, and when combined with warm water temperature, it has been linked to reduced pain (Cugusi et al., 2019) and may have a therapeutic effect on painful joints (Avers, 2020). Exercise in an aquatic environment causes a reduction in the impact on the joints, being considered a viable environment for individuals who present pain, physical deconditioning or significant joint pathologies (Avers, 2020). The buoyancy caused by the aquatic environment decreases the compressive forces within the joints by 36% to 55%, while providing hydrostatic support for the upright position (Avers, 2020). The buoyancy and inertia of the aquatic environment facilitate the practice of physical exercise, causing a reduction in the mechanical action of friction with the ground on the joints (Junior et al., 2018). When comparing to land-based exercise, individuals that practise physical exercise in water are less exposed to the risk of traumatic fractures and their joints suffer less stress and impact (Lee et al., 2020).

Viscosity is considered an exceptional source of natural resistance, allowing the development of viscous drag, which provides a comfortable resistance that allows for muscle strengthening (Cugusi et al., 2019). Hydrostatic pressure and viscosity force provide individuals with proprioceptive and sensory feedback different from what is perceived on land, positively influencing the postural control system and balance (Saleh et al., 2019). The turbulence created by the water during the exercise sessions offers periods of instability that must be overcome and can result in positive changes in balance and postural stability, the same effect does not occur in land environments due to its more static nature (Avers, 2020)

The characteristics of exercise in an aquatic environment allow everyone to practice physical exercise, even when it can be considered unsafe when performed on land, such as in individuals with postural instability, high risk of falling, muscle weakness in the limbs. lower limbs and gait disorders (Cugusi et al., 2019). Exercise in an aquatic environment is considered a great tool to improve cardiorespiratory fitness, muscle strength, muscle power, agility, bone density, flexibility, balance and functional capacity (Moreira et al., 2020), and may provide beneficial effects on physical functioning in healthy elderly when compared to sedentary elderly, and can be at least as effective when compared to land-based exercise (Avers, 2020).

Studies carried out indicated the positive impact of exercise programs in the aquatic environment on quality of life and functional capacity in the elderly. In the study by Kargarfard et al. (2018), the results showed that physical exercise in an aquatic environment caused improvements in functional capacity, balance and perception of fatigue, after application of an 8-week exercise program, in a group of women with multiple sclerosis. Identical results were found in the study by Silva et al. (2018), where two 12-week aquatic exercise programs (aerobic and combined) provided benefit in functional capacity and perceived quality of life in a group of elderly women.

2.4. Cardiometabolic diseases

Cardiometabolic diseases are very present among the population. They are considered one of the main causes of death worldwide (Ferreira et al., 2018). Cardiometabolic complications are multifactorial diseases, and some aspects seem to be associated with their development, such as lifestyle, changes in the environment, as well as genetic and epigenetic factors (Ndisang & Rastogi, 2013).

Metabolic syndrome is characterized by a set of risk factors of a metabolic nature, which consequently translates into an increased risk of developing cardiovascular diseases. Metabolic syndrome is detected in individuals when they meet at least three of the following criteria: abdominal obesity, high levels of cholesterol, high levels of triglycerides, type 2 diabetes and high blood pressure (Boulangé et al., 2016).

Diabetes is a chronic metabolic disease characterized by high blood glucose levels that, over time, lead to serious damage to the heart, blood vessels, eyes, kidneys and nervous system (WHO, 2021c). The number of individuals with diabetes has steadily increased in recent decades, affecting about 422 million people worldwide and 1.5 million deaths annually are directly attributable to diabetes. This pathology can be divided into two types: type 1, when the pancreas produces little or no insulin, and type 2, when the body becomes insulin resistant or does not produce enough insulin (WHO, 2021c).

Cardiovascular diseases are a group of diseases of the heart and blood vessels that include coronary heart disease, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, pulmonary embolism, as well as other conditions such as heart attacks and strokes. which are responsible for more than 4 out of 5 deaths from cardiovascular diseases, with a third of these deaths occurring prematurely, in individuals under 70 years of age. Cardiovascular diseases are considered the leading cause of death worldwide, accounting for about 17.9 million deaths annually (32% of all global deaths). The main risk factors for cardiovascular diseases are associated with physical inactivity, unbalanced diets, consumption of alcohol, tobacco and obesity (WHO, 2021a).

Ageing is considered the greatest risk factor for the development of most cardiometabolic diseases (Wahl et al., 2019), due to the susceptibility of the elderly to various metabolic risk factors and premature cardiovascular ageing, which develops more frequently in individuals with metabolic diseases (Tian et al., 2019). Although average life expectancy has increased in recent decades, a recent outbreak of metabolic diseases such as obesity, dyslipidaemia, type 2 diabetes and arterial hypertension has drastically promoted premature ageing and reduced life expectancy (Tian et al., 2019).

Regular practice of physical exercise or physical activity contributes to preventing and controlling the occurrence of cardiovascular and metabolic diseases, with the risk of death being 20% to 30% higher in individuals who are insufficiently active compared to individuals who are sufficiently active (WHO, 2020b). Physical exercise of aerobic characteristics, regardless of whether there is a dietary intervention, improves cardiovascular function, reducing the risk of developing associated diseases, as well as all risk factors associated with the development of the metabolic syndrome, such as increased resistance to insulin and reduced glucose tolerance, which can progress to the development of type 2 diabetes, which may lead to the development of cardiovascular diseases in the future (Cugusi et al., 2015; Nagle et al., 2017).

Regular physical exercise is widely accepted as one of the main interventions that provide benefits in terms of blood pressure, functional fitness and quality of life among the elderly population (Ruangthai et al., 2020). Even though in the last decade several studies have pointed out the potential benefits of maintaining an active lifestyle, both in healthy individuals and in individuals with cardiovascular risk factors (Cugusi et al., 2015), one in four adults does not achieve global levels of recommended physical activity, which means that about 5 million deaths per year could be avoided if the world population were more active (WHO, 2020b). The main barrier that has been pointed out to this fact, in adults, is the lack of time, so the suggestion that effective and time-saving exercise regimens may be more likely to be adopted by habitually sedentary individuals seems pertinente (Nagle et al., 2017). For the elderly, the great barrier identified was the fear of falling, so exercise programs that allow the elderly to participate safely on a regular basis, improve strength and balance levels and reduce the risk of falls should be adopted in order to combat the sedentary lifestyle, in this age group, and to prevent the occurrence of cardiovascular and metabolic diseases (Ruangthai et al., 2020).

2.5. Body composition

Body composition refers to the compartmentalization and quantification of various components, namely fat mass, lean body mass and bone mass (Mundi et al., 2019). It can be addressed at five levels: atomic, molecular, cellular, tissue, and whole body. At the atomic level, there are 106 elements in nature, of which about 50 can be found in the human body (oxygen, carbon, hydrogen, nitrogen, sodium, potassium, phosphorus, chloride, calcium, magnesium and sulphur…). Due to the technological advancement, all these elements can be measured and analysed “live” in the human body. At the molecular level, there are five components (water, lipids, proteins, minerals and carbohydrates) but, in body composition, only the first four are considered. Carbohydrates are found in small amounts in the form of glycogen, mostly in the liver and skeletal muscle), and are not considered in estimates of body composition (Duren et al., 2008).

The assessment of human body composition allows providing insights into the nutritional status and functional capacity of the human body (Kuriyan, 2018). Currently, there are two types of methods to assess body composition: field methods and laboratory methods. Field methods include anthropometry, body mass index, waist circumference, waist-hip ratio, skinfold measurements, and bioimpedance analysis. Laboratory methods include hydrodensitometry, air displacement plethysmography, isotopic dilution method (hydrometry), dual energy X-ray absorptiometry (DEXA), computerized topography, magnetic resonance imaging, and whole-body potassium counter (Kuriyan, 2018).

Body composition can be measured at several levels. The monitoring of body changes in a given sample, resulting from the application of a certain physical exercise program, constitutes an additional element of information that helps in the prescription and development of more effective exercise programs (Gonçalves & Mourão, 2004). It also plays an important role in helping with dietary recommendations and body weight determination, monitoring children's growth and maturation, as well as accompanying changes in the ageing process in the elderly (Roche, Heymsfield, & Lohman, 1996; Heymsfield et al., 2005).

After searching on the Pubmed search engine, we found 8 intervention studies with physical exercise in aquatic environments, among the elderly population, carried out in the last 10 years, where body composition was evaluated. In total, 471 participants were evaluated with a mean age of 64.5 ± 5.54 years, with the following characteristics: mild osteoarthritis in the knee; peripheral arterial disease; rheumatoid; postmenopausal women; and active and healthy participants. Interventions ranged from 14 to 24 weeks and the following exercise programs were applied: intensive water resistance training; water walk; water multi-jump training; aquatic aerobic exercise, multimodality exercise; water resistance interval training. The methods used to assess body composition were dual energy x-ray absorptiometry (DXA) and the bioimpedance method. The main results revealed that exercise in an aquatic environment provided a decrease in fat mass and an increase in lean body mass (Martínez-Rodríguez et al., 2021; Waller et al., 2017), a slight increase in BMI as a possible derivation of the increase in lean body mass (Park et al., 2019), however, with no statistically significant differences regarding body composition as a result of the intervention (Ramírez-Villada et al., 2016; Siqueira et al., 2017). When comparing exercise programs in aquatic and land-based environments, the results revealed no statistically significant differences between the two groups; however, in the intervention groups in aquatic environments, a reduction in the percentage of fat mass, in the percentage of fat in the dominant upper limb, and an increase in lean body mass in the lower limbs (Bergamin et al., 2013; Naylor et al., 2020).

2.6. Functional fitness

Functional fitness is one of the dimensions that undergoes the most critical changes during ageing (Daneres & Voser, 2013), being directly related to functional independence (Garatachea & Lucia, 2013) and to indicators of physical and the cognitive frailty syndrome (Furtado et al., 2018). Loss of muscle strength and loss of balance are also observed during the ageing process, both of which are associated with a reduction in functionality, an increased risk of falls and also a decrease in cognitive capacity, leading individuals to a loss of autonomy (Sculthorpe et al., 2017; Furtado et al., 2017).

Rikli e Jones (1999), consider functional fitness to be very important for individuals with physical limitations and the elderly, and define it as "the physiological capacity for the development of normal activities of daily living in a safe and independent way, without excess of fatigue". The components of functional fitness that involve health are related to aspects of prevention and reduction of the risk of the onset of diseases and with greater availability to perform daily activities (Copeland et al., 2018; Roberta Rikli & Jones, 1999). Spirduso et al. (2005), mention that the assessment of functional fitness, from a health perspective, is the basis for developing an adequate exercise prescription.

One of the most used methods in scientific studies to assess functional fitness is the Functional Fitness Test (FFT) Battery, developed by Rikli e Jones (1999). It is a battery consisting of 7 tests that allow assessing cardiovascular capacity, muscle strength of the upper and lower limbs, flexibility, agility, balance and speed. All tests are easy and quick to apply and do not require very sophisticated or expensive equipment.

There are several studies that point out that the practice of physical exercise in an aquatic environment provides benefits for the elderly population, in terms of functional fitness, identical to those acquired through the practice of exercise on land. In a study carried out by Novaes et al. (2014), two different physical exercise programs (muscle strength training and water aerobics) were applied to 38 postmenopausal women. The results showed that both programs provided significant changes in some markers of functional and cardiorespiratory skills, with no statistically significant differences between the two programs. A meta-analysis performed by Waller et al. (2016), analysed the effect of exercise in an aquatic environment on functional fitness in healthy elderly people, compared to physical exercise practised on land. Their conclusion was that physical exercise in an aquatic environment seems to be effective in maintaining and improving functional fitness in healthy elderly people and, when compared with exercise in a land environment, exercise in an aquatic environment seems to be at least as effective and can even be used as an alternative modality when exercise on land is not feasible or desired (Waller et al., 2016), i.e. when there are impediments or limitations to its performance, under usual conditions and on land, resulting from acute or chronic pathological situations. In the same line of results, a systematic review carried out recently by Lopez et al. (2021), who verified the impact of exercise programs in an aquatic environment, on functional fitness, in adults were between 50 and 80 years old, demonstrated that the use of exercise in an aquatic environment, when performed at least 2 to 5 times per week, lasting from 30 to 60 minutes per day and with an intensity of 50 to 95% of maximum heart rate, can lead to significant improvements in functional fitness.

2.7. Cognitive function

As the average life expectancy in societies increases, cognitive decline among the elderly has been considered an increasing problem, as the probability of memory loss and decline in the performance of cognitive tasks also increases, leading to mild cognitive impairment (Lasierra et al., 2021).

There are several risk factors of different types that are associated with cognitive decline, such as age, cardiovascular diseases, low levels of education, depression, constant anxiety, vitamin B12 and vitamin D deficiency, and certain lifestyles that include smoking and physical inactivity (Lasierra et al., 2021).

Another associated risk factor is the ageing of the vascular system, which causes a cascade of changes that negatively affect the cerebrovascular system, leading to a decrease in cerebral perfusion, that is related to cognitive decline, since continuous blood flow is necessary to maintain structural integrity and neuronal activity. In addition to brain perfusion, ageing also causes structural and functional changes in the brain that are related to cognitive decline. Cognitive decline tends to be seen more predominantly in executive functions (which may affect working memory, divided attention, memory and processing speed), probably due to the fact that the frontal regions of the brain are very susceptible to low cerebral perfusion and are affected from the onset of ageing (Intzandt et al., 2021).

Mild cognitive impairment is a strong predictor of functional capacity, particularly among the elderly population, being considered the typical stage of transition between the normal ageing process and the onset of dementia (Yong et al., 2021). Mild cognitive impairment is present in about 16% of the elderly, more predominantly in men than in women, with an annual conversion rate for diseases such as non-specific dementia and Alzheimer's disease of 34% and 28%, respectively, in individuals older than 60 (WHO, 2017).

Dementia is considered a syndrome in which there is a deterioration of cognitive function beyond what is expected, and the usual consequences of biological ageing, currently affecting about 55 million individuals worldwide, with an increase of 10 million new cases every year. Alzheimer's disease is the most prevalent form of dementia, accounting for about 60-70% of cases (WHO, 2021b).

Cognitive impairment is accompanied by reduced balance, decreased vision, gait difficulties and other factors that can lead to the occurrence of falls in the elderly population, with elderly people with cognitive problems usually falling two to three times more often than elderly people cognitively healthy, being that 60-80% of them fall every year (Racey et al., 2021).

Reducing risk factors and participating in physical activity and/or exercise programs have been considered effective non-pharmacological interventions to attenuate cognitive decline (Yong et al., 2021).

Studies carried out in the area of physical exercise and health reveal that the fact that individuals with mild cognitive impairment and moderate dementia exercise can significantly contribute to an improvement in their quality of life and to a reduction in the percentage of depression. A study carried out by Venturelli, Scarsini e Schena (2011), showed that the application of a specific walking program with the duration of 30 minutes, 4 times a week, provided a 13% reduction in cognitive dysfunctions compared to the 47% reduction detected in elderly who did not participate in the walking program.

Muscle strength training also seems to have a positive effect on cognitive performance, as shown in the study by Chupel et al. (2017), in which a muscle strength physical exercise program was applied to 16 women with cognitive impairment (exercise group) for 28 weeks, compared to 17 other women who did not participate in any physical exercise program (control group). The results obtained showed that the muscular strength exercise provided an increase in the cognitive profile of the participants of the exercise group.

In an aquatic environment, the study by Kang et al. (2020), tested the effectiveness of a physical exercise program in a group of elderly women aged 60-80, and concluded that the practice of physical exercise in an aquatic environment can contribute to the maintenance and improvement of cognitive function.

2.8. Immune profile

The immune system is made up of several complex layers that enhance the body's defense, having the function of protecting, recognizing, attacking and destroying foreign elements, such as viruses, bacteria and parasites (to which our body is constantly exposed), contributing to body homeostasis (Gleeson et al., 2013).

«All blood cells originate in the bone marrow from common stem cells and can differentiate as follows: Erythrocytes, Megalocytes and Leukocytes. Erythrocytes are called red blood cells, and their main function is to transport oxygen. Megakaryocytes are precursors of platelets, and their function is to clot the blood. Leukocytes are called white blood cells, and are divided into three large groups: Granulocytes (60-70%), whose function is to help the body fight bacterial infections; Monocytes (5-15%), whose function is phagocytosis, intracellular death, presentation of antigens to lymphocytes, and production of cytokines (IL-1β, TNF-α, IL-10, IL-1ra, etc.); and Lymphocytes (15-25%), which are subdivided into NK cells, whose main role is to defend the body against tumours and virus-infected cells, and T and B cells whose function is to recognize unknown antigens, which, once identified, cause cells to generate specific responses adapted to elimination, and to effective action against specific pathogens" (Gleeson et al., 2013).

Ageing is considered a complex process where there are fundamental changes in gene expression and in the functions of biological pathways. At the cellular level, the organism is totally affected by ageing, senescent cells accumulate in aged tissues and organs, leading to the development of diseases (Aalaei-Andabili & Rezaei, 2016). With ageing, there is a deregulation/decrease in immune system functions, a process called immunosenescence (Dorneles et al., 2016; Hall-López et al., 2015), which strongly contributes to the increase in morbidity and mortality associated with infectious diseases in the elderly population (Crooke et al., 2019). Immunosenescence is characterized by impaired maintenance, function and responsiveness of the immune system (Aalaei-Andabili & Rezaei, 2016), causing a reduction in the ability to maintain effective humoral and cellular immune responses against pathogens, leading to a systemic low-grade inflammatory state that contributes to the dysregulation of various components of the innate and adaptive immune systems (Crooke et al., 2019).

With immunosenescence humoral and cellular immunities are impaired (Aalaei-Andabili & Rezaei, 2016), contributing to a decrease in the function of NK cells, an increase in inflammation, a decrease in the number of naive T cells capable of responding to new antigen challenges and an increase in the number of senescent cells. The listed changes strongly contribute to the development of inflammatory diseases (Bigley et al., 2013), increased risk of autoimmune diseases, infectious diseases and neurocognitive disorders (Teixeira et al., 2016).

The regular practice of physical exercise has been considered as an important, alternative, safe and inexpensive tool to reduce immunosenescence, both by acute effects and at baseline levels. Acute effects are changes that occur during and immediately after a bout of physical exercise, whereas baseline effects are changes in resting levels when the acute effects induced by exercise have already been eliminated (Dinh et al., 2017).

Physical exercise causes anti-inflammatory effects through two mechanisms: increased production and release of anti-inflammatory cytokines from skeletal muscle contraction; and reduction of tall-like receptors on monocytes and macrophages, with subsequent inhibition of responses, such as production of pro-inflammatory cytokines. Exercise directly stimulates the production of IL-6 from muscle contractions, which when released into the muscle promotes the production of IL-10, IL-1ra and cortisol and acts in a harmonium-like manner to stimulate liver glucose production and lipolysis in adipose tissue (Gleeson et al., 2013).

The practice of physical exercise provides the elderly with a healthy maintenance of physical condition and improvements in the immune system, which are very beneficial effects that can prevent the development of tumour pathologies (Ko et al., 2014). Studies carried out on the subject point in the direction that physical exercise positively benefits the immune system (Dorneles et al., 2016; Hall-López et al., 2015; Teixeira et al., 2016). The conclusion of the meta-analysis performed by Dinh et al. (2017), was that physical exercise has relevant effects on the expression of surface markers of immune cells, and that a single session of physical exercise causes the mobilization of NK and cytotoxic memory T lymphocytes to the peripheral blood compartment and promotes apoptosis of senescent lymphocytes, while long-term exercise tends to promote the activity of NK cells and T lymphocytes that express CD28.

2.9. Cardiovascular markers

Cardiovascular diseases are a group of diseases of the heart and blood vessels that cause about 31% of deaths worldwide, 85% of which are caused by heart attacks and strokes. It is estimated that 80% of these deaths could be avoided by controlling the main risk factors: smoking, unbalanced diet and physical inactivity. About 150 minutes of moderate physical activity per week can contribute to a reduction in the risk of ischemic heart disease, risk of stroke and hypertension by approximately 30% (WHO, 2020a). Cardiovascular diseases can be understood as a group of disorders that occur in the heart and blood vessels. They manifest themselves through sudden death, myocardial infarction, angina pectoris, stroke or peripheral vascular disease (Martins et al., 2012).

Cardiovascular diseases can be predicted through increased carotid artery intima-media thickness (IMT), and its increase is considered a manifestation of subclinical atherosclerosis (Costanzo et al., 2010). IMT is also considered a surrogate marker in clinical trials for cardiovascular disease (Nezu et al., 2016; Polak e O'Leary, 2016).

Although serum biomarkers have been associated with the risk of developing atherosclerosis, IMT has the great advantage of being able to directly visualize the blood vessel wall (Centurión, 2016). Among the various existing subclinical markers, IMT, pulse wave velocity and stiffness are well-established structural measures associated with the risk of cardiovascular diseases (Magalhães et al., 2019). Additionally, IMT is also correlated with some coronary risk factors, such as smoking, sex, diabetes, cholesterol, and hypertension (Liu et al., 2020).

IMT is a marker used worldwide because it is a simple, non-invasive and reproducible measure (Nezu et al., 2016). According to the American Heart Association/American College of Cardiology guidelines, IMT was recommended for cardiovascular risk assessment in asymptomatic adults with intermediate risk of cardiovascular disease (Naqvi e Lee, 2014). The meta-analysis by Liu and colleagues (Liu et al., 2020), aimed to verify whether IMT was considered an accurate diagnostic method for coronary artery disease. After analysing the results, it was concluded that IMT can be considered as an accurate diagnostic tool, with the addition that a cut-off value of 1 mm can provide much more accurate diagnostic results for coronary artery disease (Liu et al., 2020).

IMT is defined as the distance between the intima and the adventitia layer of the carotid artery and is measured using ultrasound (Momeni et al., 2018; Santos, 2020). In the ultrasound image, it is represented as a double line density and the space between these two lines corresponds to the IMT (Naqvi e Lee, 2014; Nezu et al., 2016).

Carotid artery ultrasound must be performed using a linear array transducer, operating at a frequency of at least 7MHz, at a depth that must vary between 30 to 40 mm, although in some cases (larger necks or deeper vessels), this depth may be greater. In the B-mode ultrasound procedure, the common carotid artery must be observed from its origin to the carotid bifurcation, internal carotid artery and external carotid artery, in cross-sectional and longitudinal sections (Nezu et al., 2016).

The increase in IMT was positively and strongly associated with age, being one of the consequences of ageing (Munckhof, Jones, Hopman, de Graaf, et al., 2018). In the meta-analysis by Munckhof et al. (2018),), there was a strong and positive association between age and IMT in a healthy population, demonstrating a gradual and linear increase in IMT that did not differ between decades of age ($r = 0.91$; $p < 0.001$). This linear relationship between age and IMT was also observed in a population with cardiovascular diseases ($r = 0.36$; $p < 0.001$). The conclusion is that IMT is strongly related to age, regardless of the presence or absence of cardiovascular diseases or risk factors. In the healthy population, the mean IMT was 0.64 ± 0.16 mm, with an increase of 0.006 mm per year, while in the population with cardiovascular diseases, the mean IMT was 0.74 ± 0.13 mm, with an increase of 0.008 mm per year.

This increase may be due to hypertrophy of the intima-media or both layers (Momeni et al., 2018). According to Lorenz et al. (2006) e Touboul et al. (2012), IMT is considered normal when it measures between 0.40 mm and 0.89 mm, thickening when it measures between 0.90 mm and 1.40 mm and, when larger than 1.40 mm, there is the possibility that there are plaques. In middle-aged patients, a mean IMT between 0.6 and 0.7 mm is considered normal, and a mean greater than 1 cm is considered a marker of atherosclerosis and probably ischemic heart disease (Momeni et al., 2018).

Reduction in IMT was associated with a reduced risk of developing cardiovascular disease (Willeit et al., 2020). Thus, it becomes important to study interventions that promote this reduction, namely non-pharmacological interventions.

The study by Magalhães et al. (2019), aimed to verify the impact of one year high-intensity interval exercise program and muscle strength, versus a continuous exercise program of moderate intensity and muscle strength, on arterial indices (structural and functional), in participants with type 2 diabetes. After the intervention, both programs caused a reduction in IMT, but only the high-intensity interval program improved peripheral arterial stiffness indices. Suggesting that high-intensity interval exercise programs can be considered a significant tool for improving long-term vascular complications in participants with type 2 diabetes.

Diabetes is associated the development of atherosclerosis and coronary artery disease, being that two of the main complications of individuals with diabetes are the development of microvascular diseases (e.g., neuropathies and nephropathy), and the development of macrovascular diseases (e.g., ischemic heart disease, myocardial infarction, cardiac dysfunction, sudden cardiac death, etc.) (Momeni et al., 2018). In a study by the same authors, an association was found between IMT and the result of the exercise tolerance test in patients with type 2 diabetes, suggesting the possible use of IMT as an inexpensive and non-invasive method for evaluating ischemic heart disease in diabetic patients.

Another intervention (Kim et al., 2021) aimed to verify the impact of a 12-week combined (aerobic and muscle strength) program on IMT in middle-aged women with abdominal obesity. After the intervention, there was a significant reduction in IMT (0.61 ± 0.13 to 0.58 ± 0.12 mm; $p < 0.05$) in the exercise group but not in the control group. Additionally, the reduction in IMT was associated with reduced waist circumference ($r = 0.41$; $p < 0.01$) and peak oxygen consumption ($r = - 0.53$, $p < 0.01$).

However, in the study of young women by Karabinus et al. (2021), higher handgrip strength, measured using a handgrip dynamometer, was associated with lower IMT ($r = -0.23$); $p < 0.05$). Suggesting that increased muscle strength may reduce the risk of cardiovascular disease through favourable effects on subclinical carotid atherosclerosis.

In short, physical exercise can be considered a good way to reduce IMT, namely high-intensity exercise programs and programs that provide for the development of muscle strength, such as combined programs.

With ageing, arteries lose elasticity, thus increasing systolic pressure (which will consequently cause an increase in systolic diameter) and blood flow velocity, which can lead to turbulent blood flow and respective atheromatous plaques, which consequently can lead to obstruction or occlusion of the vessel (Santos, 2020). According to the same author, the systolic diameter (SD) corresponds to the highest point of each pulse in which the vessel is subjected to greater pressure and the diastolic diameter (DD) corresponds to the point of lowest arterial pressure at which the vessel is exposed. During the ageing process, there are also changes in the values of maximum heart rate, blood vessels, diameter of the carotid arteries, structural changes and a decrease in hormone production (Regufe & Maia, 2011).

Other early markers for cardiovascular disease are the chemokines Monocyte Chemoattractant Protein-1 (MCP-1) and Macrophage Inflammatory Protein-1 Alpha (MIP-

1α). They play a significant role in the development of coronary artery disease (Amin et al., 2020). Several cardiovascular diseases are associated with high levels of pro-inflammatory cytokines that promote atherosclerotic plaque formation through an accumulation of macrophages, lipid-laden cells, T cells, and other degenerative matter in the inner layer of blood vessel walls. Subsequently, several inflammatory molecules, cytokines and chemokines are released by the action of macrophages and lead to tissue damage and consequently to inflammation (Amin et al., 2020). MCP-1 is produced by different cell types (monocytes, macrophages, smooth muscle cells, endothelial cells, etc.) and MIP-1α is induced by various pro-inflammatory agents (TNFα, IL-1, etc.) and is negatively affected by anti-inflammatory agents (IL-4, IL-10, etc.) (Bianconi et al., 2018). Both are correlated with the risk of developing inflammation, cardiovascular disease (namely atherosclerosis), increased risk of myocardial infarction and death (Maurer & Von Stebut, 2004).

According to the existing literature, physical exercise is seen as a powerful tool for reducing the risk of cardiovascular diseases, namely the exercise of aerobic characteristics, which may contribute to reduce the luminal diameter of the arteries, as a localized effect and/or reflecting on the increase blood flow (Park & Park, 2017). Combined programs have also been shown to be effective in improving cardiovascular parameters. In a study carried out by Son et al. (2017), a 12-week combined (aerobic and muscle strength) intervention program in elderly women (75 ± 2 years) provided significant changes in arterial stiffness and systolic and diastolic blood pressure. However, a meta-analysis performed by Montero et al. (2015), concluded that the benefits gained from combined physical exercise (aerobic and muscle strength) do not seem to differ significantly when compared to aerobic exercise alone. Regarding the MCP-1 and MIP-1α markers, and according to Gleeson et al. (2013), physical exercise can act as a method to inhibit the release of chemokines in human adipose tissue, contributing to the reduction of the pathogenesis of several cardiovascular diseases. Aerobic exercise programs seem to be more effective in reducing MCP-1 and MIP-1α chemokines (Barry et al., 2017). However, further studies are needed to clarify the effects of physical exercise on these two variables, namely the effects of physical exercise in an aquatic environment.

3. METODOLOGIA

The methodological process underlying the realization of this research work was described in detail in a study protocol article, published in International Journal of Environmental Research and Public Health [IF:3,390] and which will then be presented under the designation of study 1.

Study 1: Impact of aquatic-based physical exercise programs on risk markers of cardiometabolic diseases in older people: a study protocol for randomized-controlled trials

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1. Abstract

Cardiometabolic diseases are one of the primary causes of mortality and morbidity worldwide and sedentary lifestyles are contributing factors to these pathologies. Physical exercise has been recognized as an important tool in the prevention and treatment of these diseases. However, there are still some doubts about the efficacy of certain type of physical exercise programs for older participants. The main goal of this study is to assess the impact of different aquatic-based physical exercise programs on risk markers of cardiometabolic diseases in older people. The study group will consist of non-institutionalized individuals, within the age group of 65 or older. The sample will be randomly divided into four groups, three experimental groups (EG) and one control group (CG). Participants from the EGs will be exposed to three physical aquatic-based exercise programs for a period of 28 weeks (continuous aerobic, interval aerobic and combined). The evaluated parameters include anthropometry, physical functions, mental health, cognitive function, carotid arteries intima-media thickness, heart rate variability and biochemical markers. The results will allow an interpretation of the impact of different aquatic-based physical exercise programs on cardiometabolic diseases markers and can also be used as a tool for professionals to prescribe adequate and more efficient physical exercise programs.

Keywords: exercise; aquatic-based; hydro gymnastics; elderly; cardiometabolic diseases

2. Introduction

Ageing is considered a progressive and inevitable phenomenon, where the reduction in various physical and metabolic functions occurs [1]. As they age, humans become increasingly sensitive to certain pathologies such as cardiometabolic diseases. These diseases are considered to be the main cause of mortality and morbidity worldwide and have attracted great interest in various fields of scientific investigation. The World Health Organization (WHO) reports that cardiometabolic diseases are responsible for 63% of 57 million annual deaths, with a significant correlation of 6 to 10% of these deaths due to physical inactivity [2].

Nowadays, more than 60% of the elderly population live a sedentary lifestyle [3]. This contributes to physical and mental frailty. Physical frailty is characterized by a reduction in physical activity levels, unintended weight loss, fatigue, reduction in handgrip strength, and a decrease in walking speed [4]. The same authors characterize cognitive frailty as the decline in all functional mental abilities that regulate the lifestyle of an individual. These mental activities range from simple to complex. Both types of frailty are associated to the process of physical and mental decline, with a deterioration in physical and mental capabilities that may lead to a serious decrease in health conditions, loss of autonomy, institutionalization and mortality [5]. To counteract sedentary lifestyles, WHO has developed strategies for a more active and healthy society. These strategies include the creation of guidelines that state the frequency, duration, intensity, type and amount of physical activity that are recommended for different age groups. Such guidelines target the prevention of non-transmissible chronic diseases [6]. Simultaneously, there has been an increase in research using a set of variables that are related to these types of pathologies, such as body composition [7], functional fitness [8], and variables related to the cardiovascular [9], cognitive [4] and immune systems [10].

Physical exercise is considered an effective tool during the process of ageing, as it helps to stabilize the loss of physical and metabolic capacities, mitigates the overall progress of ageing and enables more autonomy. Various studies indicate that regular physical exercise plays an important role in the prevention and treatment of cardiometabolic and cognitive diseases [10–14].

Different studies have been conducted recently to verify the efficacy of various physical exercise programs (aerobic, muscular strength and combined) on variables related to these pathologies. Such studies have analyzed the impact of exercise on cognition, cardiovascular, metabolic, immunological, functional and mental levels [15–18]. However, most of these studies have focused on land-based exercise and have not involved specific aquatic exercise programs, which are popular and successful among older participants.

The present investigation and its specific experimental design using different types of aquatic-based exercise programs aims to assess the impact of different aquatic-based physical exercise programs on risk markers of cardiometabolic diseases in older people.

3. Materials and Methods

3.1. Design

This investigation will be based on a randomized intervention, using three different aquatic-based exercise programs conducted in parallel for 28 weeks. The three programs include a continuous aerobic program, an interval aerobic program and a combined (aerobic and muscular strength) program. Variables related to cardiometabolic diseases will be evaluated, i.e., cardiovascular, mental health, and cognitive variables and biochemical markers.

All exercise programs will be conducted at the Piscina Muncipal da Sertã, two times a week (non-consecutive days), with 45-min sessions. All variables related to cardiometabolic diseases will be evaluated at two specific assessment moments: M1, before implementation of the physical exercise programs (baseline) and M2, after the conclusion of the 28 weeks aquatic-based physical exercise programs (post-intervention).

Each evaluation will be divided into three phases: Phase 1—Anthropometry evaluation, physical functions, cognitive and mental health levels; Phase 2—Evaluation of carotid arteries' intima-media thickness and heart rate variability; and Phase 3—Evaluation of biochemical markers.

The blood collection for biochemical analysis will be conducted by a specialized and certified lab. The measurement of the carotid arteries intima-media thickness will be conducted by a specialist in the area of cardiology. All the remaining data will be collected and organized by the research team members.

3.2. Participants

The participants were recruited in the central area of Portugal, more specifically in the region of Sertã. The sample was recruited by the non-probabilistic method for convenience. One hundred and fifty individuals from the community were personally invited, but only 102 agreed to participate in the study (mean age of 72.32 ± 5.2 and BMI 29.47 ± 4.85). Participants will be selected according to the following inclusion criteria: individual of both genders; age equal to or above 65 years of age; non-institutionalized individuals; they give permission to be part of the study and if the participant presents with a clinical condition or comorbidity, it must be stable and enable participation in aquatic-based exercises classes as approved by local medical staff. The exclusion criteria are: individuals less than 65 years of age; individuals with medical diagnosed pathologies that jeopardize their health while preforming aquatic based exercises, participants that attend less than 50% of all the sessions and participants who cannot complete all of the proposed tests.

The participants will be distributed randomly into different exercise groups based on their registration for one of the schedules offered for the hydro-gymnastics sessions. There will be three distinct schedules (9.00, 9.50 and 10.40), with the constraint being that the participants must attend the same schedule for the whole year. The 9.00 session includes the continuous aerobic program, the 9.50 session includes the interval aerobic program, and the 10.40 session includes the combined exercise program. This information will not be provided to participants before they register for the sessions so it will not be possible to establish any association between the schedule and the different exercises programs. Such information will be communicated later in the study. The control group will be randomly recruited from the community and will include those individuals that have not been involved in any kind of physical exercise during the last year.

Before each assessment moment, participants will be taken to a testing room, where the assessment tests will be performed. The room will be large and isolated, and the temperature will be controlled, and each assessment stages should be organized to provide maximum comfort and privacy to the participants during the tests. The research team will give the participants information about the tests they will perform for data collection, explain the purpose of each test, and explain the order and duration of the tests. Participants will be able to question researchers about any doubts that they may have regarding the tests and any possible consequences. During the assessment, participants may pause the evaluation and continue on another day if they feel very fatigued or if they are not able to complete all the tests at that time. In this situation, a new date will be scheduled to continue the tests.

3.3. Protocols

The percentage of adherence to the physical exercise programs, for each program, will be calculated considering the total attended sessions: (S × 100)/T, where "S" indicates the number of sessions that have been attended by the participant during the study and "T" indicates the total number (56) of physical exercise sessions. The participants' attendance will be recorded in a database. If a participant has two consecutive absences, they will be contacted and given motivational reinforcement to incentivize them to resume their physical exercise sessions.

During the study period, all adverse effects or health problems attributed to the physical exercise sessions or evaluation tests will be reported. Parameters such as muscle pain, excessive fatigue and general pain will also be reported and inserted in a database. Exercise technicians and researchers will be responsible for data collection as well as for gathering and communicating all relevant data.

Three physical exercise programs will be conducted for a time period of 28 weeks, two times per week (non-consecutive days) and have the following common characteristics: all sessions will have a duration of 45 min, taking into account previous studies [10] that suggest sessions of this duration seem to be sufficient to provide changes in several parameters in the elderly population; they will be aquatic-based (the water level will be between 0.80 and 1.20 m with a temperature of approximately 32 ◦C); and they will be conducted to the rhythm of music (bpm) that can be adjusted to achieve the target HR. Sessions are divided into three parts: the initial part, main part and final part, with common exercises in the initial and final part of the three programs. The initial part or warm-up has a duration of 10 min and the purpose is to assist participants to adapt to the water environment, more specifically, for participants' to acclimatize and prepare for muscular and metabolic stimulation. Simple aquatic-based exercises will be conducted, e.g., displacement and isolated movements. The exercises increase in complexity and intensity during this initial phase. The final part has a duration of 5 min. This part will be divided into two phases: return to calm (relaxation) where relaxing exercises are conducted with the purpose of returning the participants' heart rate (HR) value to a resting level. The second phase is composed of stretching routines that stretch the most exercised muscle groups stimulated in the main part of the session and reduce the level of lactic acid and the occurrence of post-exercise pain. The main part is different in the three physical exercise programs and their characteristics are described in Table 1. All physical exercise programs will be planned and implemented according to the recommendations of the American College of Sports Medicine [19] and conducted by specialized physical exercise technicians (with a degree in sports science) with specialization in hydro-gymnastics (instructor course—level 1).

Table 1 - Characteristics of the three physical exercise programs applied for 28 weeks (continuous aerobic, interval aerobic and combined).

Program	Description	Intensity (week 1–13)	Intensity (week 14–28)	Exercises
Continuous Aerobic	30 min exercise aerobic (moderate intensity)	60–65% maximum HR	65–70% maximum HR	Basic hydro-gymnastics exercise, with some variations: running, bounce, kicking, pendulum jumping, skiing, twister and horse.
Interval Aerobic	10 min exercise aerobic (moderate intensity)	60–65% maximum HR	65–70% maximum HR	Basic hydro-gymnastics exercise, with some variations: running, bounce, kicking, pendulum jumping, skiing, twister and horse.
	5 min exercise aerobic (high intensity)	70–75% maximum HR	75–80% maximum HR	
	10 min exercise aerobic (moderate intensity)	60–65% maximum HR	65–70% maximum HR	
	5 min exercise aerobic (high intensity)	70–75% maximum HR	75–80% maximum HR	
Combined	15 min exercise aerobic (moderate intensity)	60–65% maximum HR	65–70% maximum HR	Basic hydro-gymnastics exercise, with some variations: running, bounce, kicking, pendulum jumping, skiing, twister and horse.
	15 min muscular strengthening exercises	2 steps 12 repetitions	3 steps 16 repetitions	Exercises with auxiliary equipment (dumbbells, pool noodles, etc.): elbow extension/flexion; shoulder extension/flexion; shoulder abduction/adduction; hip abduction/adduction; hip flexion/extension; knee flexion/extension; dorsal and plantar flexion of the ankle.

HR monitors (Polar V800) will be used on the participants during all sessions of the different physical exercise program. The intensity of each exercise program will be monitored using the data provided by the Polar V800 and adjusted accordingly. As a precaution and safety measure, the intensity will be indirectly calculated using the following equation [20]:

$$\text{Target HR} = ((\text{Maximum HR} - \text{Resting HR})\ \%\ \text{intensity}) + \text{Resting HR} \quad (1)$$

Maximum HR is calculated with the following equation for senior populations [21]:

$$\text{Maximum HR} = 207 - (0.7 \times \text{age}) \quad (2)$$

The control group consists of non-institutionalized individual participants who have not partaken in any physical exercise during the preceding year. These participants will be encouraged to conduct their daily activities as usual, except for the data collection (M1 and M2) organized by the researchers.

3.4. Instruments

3.4.1. Individual Characterization

In the first assessment moment (M1), all participants will fill in a clinical survey to help with the clinical characterization of each participant. This document includes the following information: civil status, regular medication, infections, allergies, diseases, annual doctor consultations, average hours of daily sleep, supplement use, latest blood panel, information on dietary habits, smoking habits and drug use.

3.4.2. Environmental Characteristics

The physical exercise programs are aquatic-based, and will take place in water at temperatures that follow regional health guidelines (30–32 degrees Celsius) in the swimming pool complex of Piscina Municipal da Sertã (indoor pool), Portugal. During the study, daily monitoring of parameters such as the pool water temperature, free chlorine, combined chlorine, pH, relative humidity and pier external temperature will be conducted and the results will be inserted in a database by two facility staff members. An external certified company will conduct a bi-weekly analysis of the following parameters: pH, conductivity, free chlorine, total chlorine, temperature and, bacteriological tests (total germs, total coliforms, Escherichia coli, fecal enterococci, total staphylococci, coagulase-producing staphylococci, and pseudomonas aeruginosa).

3.4.3. Anthropometry

Anthropometric measurements will be conducted by a certified investigator by FCDEF-UC. The following parameters will be evaluated: (i) stature, using a portable stadiometer, Seca Bodymeter® (model 208, Hamburg, Germany) with a precision of 0.1 cm; (ii) weight, body mass index (BMI), visceral fat, percentage of fat and lean body mass using a portable scale from Seca® (model 770, Hamburg, Germany) with 0.1 kg accuracy; and (iii) waist circumference, arms and legs using a retractable fiberglass tape (model Hoechst mass-Rollfix®, Sulzbach, Germany) with an accuracy of 0.1 cm.

3.4.4. Physical Function

Physical function will be assessed using the Senior Fitness Test, developed and reviewed by Rikli and Jones [22] and validated for the Portuguese population [23]. It is composed of the following test items:

- Chair stand, assesses lower body strength and consists of the maximum number of full stands that can be concluded in 30 s. Necessary equipment: chair and stopwatch.
- Arm curl, assesses upper body strength and consists of the maximum number of bicep curls that can be completed in 30 s while holding a hand weight. Necessary equipment: 2.27 kg hand weight for women and 3.63 kg for men, chair and stopwatch.
- 2-min step, assesses aerobic endurance and consists of maximum number of full steps completed in 2 min, a full step is recorded when each knee reaches the point midway between the patella (kneecap) and iliac crest (top hip bone). Necessary equipment: stopwatch, sticky-tape and ruler.
- Chair sit and reach, assesses lower body flexibility and is conducted from a sitting position where one of the participant's legs is extended while the other is flexed and where hands are reaching towards the toes. This test is assessed in cm and is positive (+) if the extended fingers pass the tip of the toes or negative (−) if the extended fingers do not pass the tip of the toes. Necessary equipment: chair and ruler.
- Back scratch, assesses upper body flexibility and is conducted with one hand reaching over the shoulder in the direction of the floor and the other hand up the middle of the back in the direction of the head. This test is assessed in cm and is positive (+) if both hands overlap and is negative (−) if overlapping does not occur. Necessary equipment: ruler.
- Timed up and go, assesses agility and dynamic balance and is conducted from a starting sitting position where the participant stands up and walks, as fast as possible, to and from a distance 2.44 m (marked by a cone). Necessary equipment: chair, cone and stopwatch.
- Hand grip, assesses hand grip strength and consists of asking the participant to grip a dynamometer with maximum achievable force, the output value of the device is then registered. Necessary equipment: Lafayette hydraulic manual dynamometer (model J00105).

3.4.5. Cognitive Function

Cognitive function will be assessed with the Portuguese version of the Mini Mental State Examination (MMSE) [24]. The MMSE, evaluates the following cognitive areas: orientation, short term memory, attention and calculation capacities, long term memory and language capabilities. The final score has a maximum of 30 points, and scores below 24 can be used as an aid in the assessment of dementia. The test will be used as an instrument to create a cognitive profile with the following criterium [25]: severe cognitive impairment (scores between 1 and 9 points); moderate cognitive impairment (scores between 10 and 18 points); mild impairment (score between 19 and 24 points), normal cognitive profile (scores between 25 and 30 points).

3.4.6. Mental Health

Mental health will be assessed using the following scales and questionnaires validated for the Portuguese population: the Rosenberg Self-Esteem Scale (RSES) [26]; Physical Self-Perception Profile for Clinical Populations (CPSPP) [27]; World Health Organization Well-Being Index (WHO-5) [28]; Satisfaction With Life Scale (SWLS) [29]; EuroQol (EQ-5D) [30]; Geriatric Depression Scale (GDS) [31] and; Perceived Stress Scale (PSS) [32].

- RSES, assesses global self-esteem and is composed of 10 items that are answered using a 4-point Likert scale, the answers vary from “I totally agree” to “I totally disagree”. In items 1, 2, 4, 6 and 7 the score is reversed. Global self-esteem is represented by the summation of all individual scores, providing a final score ranging from 10 to 40 points, where higher scores indicate higher self-esteem.
- CPSPP, is an instrument designed to provide a self-assessment summary of the physical characteristics of elderly groups in clinical and rehabilitation settings. A scale is defined by six subscales of three items that evaluate the following subdomains: functionality, physical health, sports competence, physical attractiveness, physical strength and physical self-worth. Answers to the items are displayed in an alternative structured format that is designed to eliminate social desirability bias. The score can vary between 3 to 12, with higher scores representing better performance.

- WHO-5, is an instrument that assesses psychological well-being. It is a self-administrated short questionnaire composed of 5 items with positive words, these words are related to a positive mood (good mood, relaxation), vitality (being active and waking up fresh and rested) and general interests. Each item is classified on a 5-point Likert scale, ranging from 0 (not present) to 5 (constantly present). The scores are summed, with the final score ranging from 0 to 25 points. The final score is then converted to a scale of 0 to 100 (by multiplying by 4), where higher scores represent a higher level of well-being and better quality of life. A final score equal to or below 50 points represents poor well-being but does not necessarily mean depression. A final score equal to or below 28 possibly indicates clinical depression.
- SWLS, assesses global cognitive parameters of life satisfaction. It is composed of 5 items with a 7-point Likert scale. The answers indicate the level of agreement the participant feels with each item. The final score ranges from 1 to 35 points, where higher final scores indicate higher satisfaction with life.
- EQ-5D, is an instrument that assesses general health status. It consists of two parts: the EQ-5D health descriptive system and the EQ visual analogue scale. The descriptive system consists of five dimensions (mobility, personal care, usual activities, pain/discomfort and anxiety/depression). The participant is asked to indicate their health status by selecting the most appropriate options in the five dimensions. The visual analogue is self-assessed and is conducted in a scale from the lowest rate (0) "the worst health you can imagine" to the highest rate (100) "the best health you can imagine".
- GDS, assesses life satisfaction, interruptions in activities, annoyances, isolation, energy, joy and memory problems. It consists of fifteen easy to understand questions and has a binary answer system (0 or 1 point) for answers of "no" and "yes", respectively. A participant who obtains a final score between 0 and 5 points is considered healthy; scores between 6 and 10 points indicate signs of mild to moderate depression; scores between 11 and 15 points indicate signs of severe depression.
- PSS, is an instrument to measure perceptions of stress. It is composed of 14 items, where 7 items are considered as positive aspects while the rest are considered as negative aspects. The questions are about feelings and thoughts during the last month. A point reversal is conducted on items 4, 5, 6, 7, 9, 10 and 13. The final score may vary between 14 and 70 points and a higher score indicates higher stress levels.

3.4.7. Assessment of Carotid Arteries Intima-Media Thickness

The carotid arteries intima-media thickness assessment takes place with the participant lying down in a dorsal position. Then, the following parameters are evaluated using a sphygmomanometer from Riester (Model RI-championN®, Jungingen, Germany): heart rate (HR), systolic blood pressure (SP) and diastolic blood pressure (DP). The intima-media thickness of the right and left carotid arteries are measured with a Doppler two-dimensional ultrasound and are assessed with the AIRC study protocol [33]. The following values are then recorded through a portable ultrasound from General Electric® (VIDe, Vancouver, Canada) with probe linear 11 L: Intima-media thickness (IMT); systolic diameter (SD), diastolic diameter (DD), peak systolic velocity (PSV) and end-diastolic velocity (EDV).

3.4.8. Heart Rate Variability (HRV) Measurement

HRV will be assessed according to the procedures of Abad et al. [34] using Polar V800 heart rate monitors. Participants will place the sensor, which is synchronized with the V800 clock, on their chest beneath their pectoral muscles. Then, the participants will be asked to lie down in a dorsal position, in silence, with open eyes and with a calm respiration. The test will have a duration of 10 min in a calm, silent and low-light environment. After the conclusion of the test, HRV measurement data are downloaded from the Polar Flow Web Service.

3.4.9. Biochemical Markers

Blood samples will be drawn via venipuncture from fasting participants. For each participant a total of 18.5 mL of blood will be drawn. Of the 18.5 mL, 3.5 mL will be used by the clinic to assess lipid panel values (HDL, LDL, glucose, triglycerides). The remaining 15 mL, processed by the college laboratory, will be divided into three tubes: two serum separator tubes and one ethylenediaminetetraacetic acid (EDTA) tube. Once the test tubes arrive at the university laboratory a complete blood count (CBC) will be conducted using an automatic hematology analyzer Coulter Act Diff, Beckman Coulter, USA. Next, the test tubes are centrifuged for10 min at 3500× g rotations per minute and stored in cryogenic test tubes. Levels of HbA1C, IL-1, IL-1ra, IL-6, IL-10, TNF-α, Adiponectin, Leptin, MIP-

1α, MCP-1, SOD, MMP-9, are subsequently analyzed with ELISA Invitrogen® CA kits (Bender MedSystems GmbH, Vienna, Austria).

3.5. Ethical Aspects

The researcher will be responsible for the data integrity and validity during the entire study. All data collected will be confidential and used exclusively for scientific purposes. Anonymization procedures will be conducted under the Data Protection Regulation of 25 May 2018. Data anonymization will be implemented by attributing a code to each participant. Each participant will receive a unique identification code that will correspond to their process; this code will be visually accessible with the use of “code cards”. These “code cards” will be used by the participants during data collection. Once the data collection is finalized the participants will be asked to destroy their “code cards”, thus finalizing the anonymization process.

Data will be stored in an Excel Microsoft Office 2016 database. Access to this database will be protected with a password and will be restricted to the main researcher responsible for the data collection. Data backups will be carried out regularly by the main researcher.

Participation in this study is purely voluntary, with no reprisals for non-participation. All subjects will be asked for their informed consent before they start participating in the study. The study will be conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee for Health of the Faculty of Sport Sciences and Physical Education of the University of Coimbra (reference: CE/FCDEF-UC/00462019).

The results from this study will be disseminated through publication in various scientific journals. The participants, if desired, will receive a copy of the main results and publications.

3.6. Statistical Analysis

The size and statistical power of the sample will be calculated using the G*Power software application [35]. The following parameters will be considered: F test (ANOVA);

effect size: 0.25; α-level: 0.05; statistical power: 0.95; number of groups: 4; number of measures: 2 (pré and post intervention); a 30% margin for possible losses and refusals. Therefore, the initial size of the total sample was estimated at 76 participants.

The collected data will be subjected to descriptive statistical analysis where values such as maximum, minimum, mean and standard deviation will be calculated for each variable in each assessment moment. Afterwards, data normality will be tested by considering the response to three conditions: z-values from Skewness and Kurtosis tests; p-value from Shapiro-Wilk test; and visual inspection of generated histograms. Parametric data will be analyzed using the T-Student test for independent samples to compare the groups and two paired samples to compare the different moments (M1 and M2). Nonparametric data will be analyzed using the Mann–Whitney U test to compare the groups and the Wilcoxon test to compare the different moments (M1 and M2). Statistical analysis will be performed using the Statistical Package for the Social Sciences (SPSS) statistical software, version 25.0. The level of significance used will be $p \leq 0.05$.

4. Expected Results/Discussion

The main aim of this study is to assess the impact of different aquatic exercise programs (a continuous aerobic program, aerobic interval program and combined program) on risk markers of cardiometabolic diseases in older people. The study is guided by scientific-based evidence on the practice of physical exercise in the elderly population [11,36].

The practice of regular physical exercise is considered as the optimum tool in the prevention and treatment of various types of cardiometabolic diseases. Thus, it is important that research on this topic continues; by doing so, new intervention methods can be identified and their efficacy validated. The offer of aquatic physical exercise programs is highly successful among the elderly because they transform physical exercise into something more pleasant and suitable for this particular population. However, the high costs of maintaining aquatic environments and the additional difficulties associated with assessment methodologies and specific equipment needed to monitor exercise in aquatic environments, means this topic not yet been sufficiently explored in the literature.

After completion of the data collection and analysis, the participants in the experimental groups are expected to show positive developments with regard to the anthropometric level, physical function, the intima-media thickness of the carotid arteries, heart rate variability, cognitive function, mental health and a number of biochemical markers. It is also expected that statistically significant differences will be found between the exercise groups for some of the variables. In the control group, no changes are expected in the analyzed variables. It is believed that the expected results can be attributed to the physical and physiological effects of the aquatic environment associated with the different proposed exercise protocols. The results will be published after the study is completed.

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4- RESULTS

In this chapter, 4 studies that were developed sequentially in order to answer the research question and objectives of this thesis, will be presented. The wide range of variables analysed, justify its organization into 4 independent studies (1 cross-sectional study and 3 intervention studies), which were submitted to international peer-reviewed journals.

The first study is a cross-sectional study (study 2), entitled "Association between aerobic capacity, grip strength and cognition function with cardiometabolic diseases risk markers and mental health in the non-institutionalized old adults: a cross-sectional analysis". This study was carried out through the analysis of the data collected in the first evaluation moment (pre-intervention). This study aimed to analyze possible relationships between the different variables under study, to detect within each of the groups of variables, those that could play a more relevant role in intervention studies. The variables, aerobic capacity (2min-ST) handgrip strength (HG) and cognitive function (MMSE), were the ones that showed the highest statistically significant correlation values. These variables were immediately identified as relevant in the following study, the first intervention study.

Study 3 "Impact of different aquatic exercise programs on body composition, functional fitness and cognitive function of community dwelling elderly adults: A randomized controlled trial". It aimed to test the impact of different types of physical exercise programs performed in aquatic environments on body composition, functional fitness and cognition in community dwelling elderly. We tested the impact of different programs on body composition, in order to characterize the samples at an anthropometric level, and because several indicators of body composition were also correlated with HG and MMSE, in study 2. Body composition could also be correlated with several cardiometabolic markers. The results of this study showed that the different exercise programs in the aquatic environment caused statistically significant changes in body composition, functional fitness and cognition. Some of these variables (eg. 2min-ST, HG, MMSE) were correlated with immunological markers (eg TNF-α, LI, GR, ERI, Hb, Hct, GLU, CLT, HDL, ADP), according to the results of study 2. These data led us to believe that different aquatic physical exercise programs could also cause significant changes in the immune profile, that are investigated in the second intervention study:

Study 4 "The Impact of aquatic exercise programs on the immunologic profile of community dwelling older persons: A randomized controlled trial", aimed to test the impact of different types of aquatic physical exercise programs on the immune profile of community dwelling elderly people. The results of this study showed that the different exercise programs in the aquatic environment can contribute to the development of a positive response in the immune profile, reflected in a less pro-inflammatory environment, contributing to the reduction of the development of cardiovascular diseases. According to Kumari et al. (2018), the TNF-α/IL-10 ratio was associated with the risk of coronary artery disease, suggesting that this marker may play a vital role in the development of cardiovascular diseases. These data suggest that different aquatic exercise programs could also provide significant changes in cardiovascular markers. Thus, it became relevant to study the impact of aquatic exercise on other cardiovascular risk markers, namely the carotid artery intima-media thickness (IMT), and other variables related to the development of cardiovascular diseases (metabolic profile, blood pressure and cytokines MPC-1 and MIP-1α), giving rise to the third intervention study:

Study 5 "Impact of different aquatic exercise programs on the intima-media thickness of the carotid arteries, hemodynamic parameters, lipid profile and chemokines of community-dwelling old persons: A randomized controlled trial", which aimed to test the impact of different types of aquatic physical exercise programs on cardiovascular risk markers in community dwelling elderly. The results of this study showed that the different exercise programs can beneficially contribute to the reduction of the development of cardiovascular diseases, through the improvement of markers related to IMT, metabolic profile, blood pressure and MPC-1 and MIP-1α cytokines.

Study 2: Association between aerobic capacity, grip strength and cognition function with cardiometabolic diseases risk markers and mental health in the non-institutionalized old adults: a cross-sectional analysis

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Farinha, Carlos, Teixeira, A. M., Serrano, J., Santos, H., Silva, F. M., Cascante-Rusenhack, M., Luís, P., & Ferreira, J. P. Association between aerobic capacity, grip strength and cognition function with cardiometabolic diseases risk markers and mental health in community dwelling elderly: a cross-sectional analysis. In Diversity & Inclusion Issues - Effects of physical fitness and cognition in old people´s cardiometabolic disease risk markers, 2nd ed.; Ferreira, J.P., Campos, M.J.; Publisher: Coimbra, Portugal, 2022; volume 1, pp. 7–28).

1. Abstract

The elderly population is constantly growing worldwide. One of the characteristics of aging is the decrease in functional fitness and cognitive function, leading to the appearance of cardiometabolic disorders. Methodology: The aim of this study is to verify the association between aerobic capacity, handgrip strength and cognition with risk markers for cardiometabolic diseases and mental health in community dwelling elderly. The study consists of a cross-sectional analysis of baseline data from a 28-week randomized controlled trial, with a sample of 102 participants (mean age 72.32 ± 5.25 years). The sample was evaluated for anthropometry, functional fitness, heart rate variability, carotid artery intima and mean thickness (IMT), cognitive function, mental health and biochemical markers. Correlations were evaluated using Pearson's statistical analysis and interpreted according to Cohen's (1988). Results: Statistically significant correlations were found between aerobic capacity (2min-ST) and markers of functional, cardiovascular, biochemical, cognitive function and mental health fitness. Handgrip strength (HG) was statistically significantly correlated with anthropometric measurements, various indicators of functional fitness, biochemical markers, cognitive function, and mental health variables.

Finally, cognitive function (MMSE) was correlated with anthropometric measures, functional fitness, cardiovascular and biochemical markers, and mental health. These data suggest that aerobic capacity, handgrip strength and cognitive function may be hypothetically associated with cardiovascular disease risk markers.

Keywords: Aerobic capacity; hand strength; cognition; cardiometabolic risk factors; Elderly.

2. Introduction

The elderly population is in constant growth. Estimates indicate that the percentage of world population with more than 60 years of age will nearly double between 2015 and 2050, from 12% to 22% (OMS, 2018). This ageing pattern is advancing at a large rate compared to what has been previously verified. Countries now face an enormous challenge in the preparation of social and health systems to meet the needs of this demographic change (OMS, 2018).

During aging, individuals are more prone to molecular and cellular damage, which leads to the gradual advance of physical and psychological disorders. Aging also increases the occurrence of various types of diseases, such as cardiometabolic diseases, and finally to death (Lira et al., 2020; Morgan et al., 2016). A decrease in cognition levels is also present during aging, specifically in executive functions (Kirk-Sanchez & McGough, 2013). Physical function is likewise influenced by aging, more specifically with a relevant decrease in aerobic capacity (Garatachea et al., 2015), muscular strength (Dugan et al., 2018) and balance (Borges et al., 2014; Olchowik et al., 2015).

Aerobic capacity is one of the key indicators of cardiorespiratory fitness and is directly influenced by aging (Garatachea et al., 2015). The average rate of decline in the VO2 max, in the elderly population per decade, is equal to or greater than 4-5 ml/kg/min. Progressive muscular aging contributes to a reduction in the capacity of oxygen usage, which in turn is caused by: reduction of lean body mass (LBM), reduction in muscle capillary density, endothelial dysfunction, changes in skeletal muscle microcirculation, and reduced muscle oxidative capacity (Garatachea et al., 2015).

In elderly, LBM is also affected, a progressive decline in this parameter is observed after the age of 25 to 30 (Nieuwpoort et al., 2018), causing problems of functional health, independence, and reduced well-being (Stojanović et al., 2021). A progressive reduction in muscular strength can be caused by the quantitative loss of the cross-sectional area of the muscles (Garatachea et al., 2015), that is, the skeletal muscles atrophy and become progressively weaker (McPhee et al., 2016). Hand grip strength has often been applied as a general metric for whole body strength assessment (Hershkovitz et al., 2019). Lower values of hand grip strength are indicators of cardiometabolic diseases, morbidity and early mortality (Alonso et al., 2018). Reduced hand grip strength is also associated with the reduced ability to perform daily activities (Hershkovitz et al., 2019), this reduced ability leads to an increased risk of falling and to mental and depressive health problems (Fukumori et al., 2015).

Cognitive functions decline with senescence, this decline is notable in executive functions (difficulties with daily activities, slower response times, reduced information comprehension speed, decline in the realization of tasks involving exchanges of attention and decreased inhibitory control capacity) (Kirk-Sanchez & McGough, 2013). Mild cognitive impairment is considered to be the transition between a normal cognitive profile and dementia and is characterized by a greater than expected cognitive decline, without significantly affecting daily activities. In its turn dementia is characterized by a progressive and severe decline which causes loss of ability to perform activities of daily living (Karssemeijer et al., 2017).

Studies have identified correlations between aerobic capacity, muscular strength, cognition and cardiometabolic risk markers. Chong et al. (2020) verified the existence of correlations between hand grip strength, systolic and diastolic blood pressure values, BMI, protein C and diabetes levels. Other authors (Mainous et al., 2015), correlated hand grip strength with diabetic and hypertension values. Another study (Rebollo-Ramos et al., 2020), tested the correlation between aerobic fitness with different levels of adiposity, blood pressure, lipid profile, inflammatory profile (IL-6 and TNF-α) and lifestyles, in a sample with ages between 18 and 40 years. Regarding cognition Yeh et al. (2015) correlated cognitive performance with cardiometabolic variables and hyper white matter intensities, in individuals aged between 50 and 85 years. Furtado et al. (2020), tested the association between frailty and several geriatric characteristics in a group of institutionalized women aged between 75 and 85 years. In the study (Chupel et al., 2017), significant correlations were found between the Mini Mental State Examination (MMSE) test score and the 8-foot up and go test (8-foot) and chair stand test (30s-CS) in a group of old women (82.7 ± 5.7 years) with moderate cognitive impairment. In the same direction, the study (Furtado et al., 2017), correlations were found between indicators of physical fragility and the 8-foot (positive strong), the 30s-CS and arm curl test (30s-AC) (negative moderate) and no correlation was found between frailty indicators and body mass índex (BMI) in a sample of 119 women (81.96 ± 7.89 years).

Despite being a topic that is frequently addressed in current literature, a lack of studies of the aforementioned associations in the community dwelling elderly is notable (Aparicio et al., 2017; Hernandez-Martinez et al., 2019; Lee et al., 2016; Tay et al., 2019). Therefore, the necessity of conducting more studies that use as a basis community dwelling elderly is of importance. These studies aid in discovering evidence that support the efficiency of using aerobic capacity, grip strength and cognition in preventing cardiometabolic diseases.

The purpose of this study is to investigate the association between the aerobic capacity, hand grip strength and cognition with a wide range of cardiometabolic markers (anthropometry, physical function, immunological markers and cardiovascular markers) and mental health, in community dwelling elderly.

3. Methodology

3.1. Study design

Participants in this study are part of the sample of the study protocol published by Ferreira et al. (2020). The present study consists of a cross-sectional analysis of baseline data collected from 28 weeks randomized and controlled intervention study with physical exercise in aquatic environment with elderly aged equal to or above 65 years old, living in Sertã region, Centre of Portugal. All participants attended a community aquatic physical exercise program ran by the Municipality of Sertã in the local swimming pool. This was an exploratory case-control, local, population-based survey (Pearce, 2012) that collected information on the effects of different aquatic exercise programs on anthropometrics, physical function, cognitive function, mental health, heart rate variability, carotid arteries intima-media thickness, and biochemical markers in community dwelling elderly. In addition, this study was designed to provide information on the trends and results expected when using a representative probabilistic sample of this population in future studies.

3.2. Sample selection criteria

The participants were recruited with a non-probability convenience sampling method in the central area of Portugal (Sertã). The sample size was calculated using G-Power software (Effect size: 0.25; α-level: 0.05; power: 0.95; n=76). The following eligibility criteria was used to form the sample: a) both female and male individuals; b) age equal to or above 65 years; c) community dwelling elderly; d) ability of participants to autonomously travel from there residence to Sertã municipal pool. Furthermore, ineligible criteria were also defined: a) individuals with medically diagnosed pathologies that jeopardize their health while preforming aquatic based exercises; b) severe cognitive impairment, that is, a score below 9 in the Mini Mental States Examination or a clinically diagnosed mental illness. After applying the previously mentioned eligibility and ineligible criteria, the final sample was composed of 102 individuals (24 male and 78 female) with a average age of 72,32 ± 5,25 years.

3.3. Ethical aspects

All participants or their legal representatives signed consent forms. The study protocol was approved by the Ethical Committee of the Faculty of Sport Science and Physical Education at University of Coimbra (Reference code CE/FCDEF-UC/00462019) and previously published (Ferreira et al., 2020). The study protocol respected the Portuguese Resolution (Art. 4th; Law n° 12/2005, 1st series) and complied with research guidelines in humans of the Helsinki Declaration (Petrini, 2014).

Data collected is confidential and used exclusively for scientific investigation purposes, obeying all the anonymization procedures defined by the Data Projection Regulation, applied since May 25, 2018.

3.4. Assessment instruments

3.4.1. Result measurements

Blood samples were drawn at a professional clinic using certified methods (Clinic-Affidea Sertã). The carotid arteries intima-media thickness assessment was conducted by a cardiology specialist. The rest of the data was collected and organized by members of the research team. Data quality was assessed using internal consistency reliability (ICR) measure.

3.4.2. Anthropometry measurements

Anthropometry measurements were conducted by a certified investigators by FCDEF-UC. The following parameters were assessed: height (Hgt), evaluated with a portable stadiometer, Seca Bodymeter® (model 208, Germany), with a precision of 0.1 cm; weight (Wgt), body mass index (BMI), visceral fat (VF), percentage of fat mass (FM) and muscle body mass (LBM) using a portable scale TANITA BC-601 with a precision of 0.1 cm with 0.1 kg accuracy; and waist circumference (WCir), arms (ACir) and legs (LCir) were measured using a retractable fiberglass tape (model Hoechstmass-Rollfix®, Germany) with an accuracy of 0.1 cm.

3.4.3. Physical Function

Physical Function was assessed with the Senior Fitness Test Battery (Rikli & Jones, 1999), validated for the Portuguese population by Baptista and Sardinha (2005). Lower body muscular strength was evaluated with the chair stand test (30s-CS) (repetitions/30s); Upper body strength was evaluated with the arm curl test (30s-AC) (repetitions/30s); aerobic capacity was assessed with the two-minutes step test (2min-ST) (repetitions /2min); Lower body flexibility was measured with the chair sit and reach (CSR) (centimetres) and upper body flexibility was assessed with the back scratch test (BS) (centimetres); agility and dynamic balance was assessed via the timed up and go test (TUG) (seconds); and hand grip strength was evaluated with the hand grip test (HG) (kg)..

3.4.4. Cognitive function and mental health

Cognitive function was calculated with the Portuguese version of the Mini Mental State Examination - MMSE (Morgado et al., 2009), which evaluates the following areas of cognition: orientation, short term memory, attention and calculation capacities, and long term memory and language aptitudes. The final score has a maximum of 30 points, and scores below 24 can be used as an aid in the assessment of dementia. The test will be used as an instrument to create a cognitive profile with the following criterium (Mungas, 1991): severe cognitive impairment (scores between 1 and 9 points); moderate cognitive impairment (scores between 10 and 18 points); mild impairment (score between 19 and 24 points), normal cognitive profile (scores between 25 and 30 points).

Mental health was assessed with following questionnaires and scales validated for the Portuguese population: Rosenberg Self-Esteem Scale – RSES (Neto, 1996), which assess global self-esteem; Physical Self-Perception Profile For Clinical Populations – CPSPP (Ferreira et al., 2017), which is a self-assessment of physical characteristics in elderly groups and is composed of the following dimensions: functionality, physical health, sports competence, body attraction, physical strength and physical self-worth; World Health Organization Well-Being Index - WHO5 (Canavarro et al., 2009), which gauges psychological well-being; Satisfaction With Life Scale – SWLS (Neto & Oliveira, 2004), which evaluates global cognitive parameters of life satisfaction; EuroQol – EQ5D (Ferreira et al., 2013), which assess the general health of the participants; Geriatric Depression Scale - GDS (Apóstolo et al., 2014), assess life satisfaction, interruptions in activities, annoyances, isolation, energy, joy and memory problems; Perceived Stress Scale - PSS (Trujillo & Cabrera, 2007), used to measure perception of stress..

3.4.5. The carotid arteries intima-media thickness (IMT)

The (right and left) IMT was measured with a portable Doppler ultrasound of make and model General Electric VIDe® with an 11L probe, using the AIRC Study protocol (Stein et al., 2008). The measurements consisted of the following parameters: Intima-media thickness (IMT); systolic diameter (SD); diastolic diameter (DD); peak systolic velocity (PSV); end-diastolic velocity (EDV). Heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were also evaluated with a RI-Champion N® sphygmomanometer.

3.4.6. Heart rate variability (HRV)

HRV was assessed by following the procedures devised by Abad et al. (2017). Polar V800 (Polar Electro Oy, Finland) heart rate monitors were used, for 10 minutes, with the participants in a calm, silent and low light environment. The following values will be recorded: minimum R-R interval (RRmin); maximum R-R interval (RRmax); mean R-R range (RRmean); Root mean square of the successive normal sinus R-R- interval difference (RMSSD).

3.4.7. Biochemical markers

Blood samples were drawn via venepuncture, with 12 hour fasting participants. The following parameters were evaluated: HDL cholesterol (HDL), LDL cholesterol (LDL), cholesterol total (CLT), glucose (GLU), triglycerides (TG), leukocytes (LEU), lymphocytes (LI), monocytes (Mo), granulocytes (GR), erythrocytes (ERI), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean globular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocytes distribution width (RDW), platelets (PL), mean platelet volume (MPV), procalcitonin (Pct), adenosine diphosphate (ADP), using an automatic hematologic analyser Coulter Act Diff, Beckman Coulter, USA; Levels of interleukin 10 (IL-10), interleukin 1 (IL-1ra), interleukin 1 beta (IL-1β) and tumor necrosis factor (TNF-α) were also analysed with ELISA Invitrogen® CA kits.

3.5. Data analysis

The collected data was subject to descriptive statistical analysis, were values such as maximum, minimum, mean and standard deviation were calculated for each variable. Afterward, data normality was tested considering the response to 3 conditions: z-values from Skewness and Kutosis tests; p value from Shapiro-Wilk test; and histogram redundancy. Statistical tests, Pearson's r-moment and Spearman's r-product correlation tests were applied to the data to establish the relationship between the different variables under study. Relationship between the different variables were interpreted according to Cohen’s (1988) cutoff values which consider that r = -/+.10 to -/+ .29 means weak correlation, r = -

/+.30 to -/+ .49 means moderate correlation and r = -/+.50 to -/+ 1.0 means strong correlation.

The whole statistical analysis was performed using version 26.0 of the statistical software, Statistical Package for the Social Sciences (SPSS), with a level of significance of $p \leq 0.05$.

4. Results

The sample was composed of 102 community dwelling elderly, with a mean age of 72.32 ± 5.25 years, 24 males (mean age 74.00 ± 4.39) and 78 females (mean age 71.81 ± 5.40). The short query answers revealed that 95 % of the participants used medication (MED) in a regular basis, 61% mentioned not to have any allergies (ALL), 41% responded that they attended a doctor (VDY) in average 3 or more times a year, 49% classified their sleep (SQ) as being good. The participant´s anthropometric characteristics are presented in Table 2.

Table 2 - Participant´s anthropometric profile characterization analyzed by gender

	All sample (N=102)							
	Female (N=78)				Male (N=24)			
	Minimum	Maximum	Mean	Std. Dev.	Minimum	Maximum	Mean	Std. Dev.
Age	65	86	71.81	5.40	65	82	74.00	4.39
Hgt (cm)	1.47	1.66	1.54	0.04	1.59	1.76	1.67	0.05
Wgt (kg)	49.4	104.3	71.23	11.96	63.9	97.7	78.82	9.97
BMI (kg/m²)	20.7	42.9	29.92	5.07	20.5	38.0	27.98	3.76
VF (%)	6	18	11.59	2.90	14	28	14.58	5.20
FM (%)	27.4	57.1	42.73	6.26	11.5	33.8	27.22	4.73
LBM (%)	18.4	41.0	24.32	3.33	26.8	37.5	30.97	2.15

Note: Height (HG); Weight (WG); Body mass index (BMI); Visceral fat (VF); Fat mass (FM); lean body mass (LBM).

In terms of aerobic capacity, the final mean result for the study sample was 77,22. The relationship between aerobic capacity and the different variables assessed in the present study (anthropometry, physical function, cognitive function, mental health, immunological markers and cardiovascular markers) showed a strong and positive correlation between the 2min-ST variable and the 30s-CS (r=.562; n=102; p<0.001); 30s-

AC (r=.513; n=102; p<0.001); and a strong and negative correlation with the TUG variable (r=-.591; n=102; p<0.001). There was a moderate and positive correlation between the 2min-ST variable and the HG (r=.426; n=102; p<0.001), EDV–R (r=.326; n=102; p=.001), SWLS (r=.314; n=102; p=.001), and EQ5D – scale (r=.394; n=102; p<0.001). Finally, there was a weak and positive correlation between the aerobic capacity variable and CSR–L (r=.198; n=102; p=.046); BS–R (r=.212; n=102; p=.032); BS–L (r=.230; n=102; p=.020); EDV–L (r=.232; n=102; p=.019); PSV–R (r=.248; n=102; p=.012); TNF-α (r=.210; n=102; p=.034); LI (r=.286; n=102; p=.004); and MMSE (r=.275; n=102; p=.005). There was a weak and negative correlation between the 2min-ST variable and the IMT–R (r=-.271; n=102; p=.006), GR (r=-.287; n=102; p=.003) and GDS (r=-.291; n=102; p=.003). These results are presented in Figure 1 and 2.

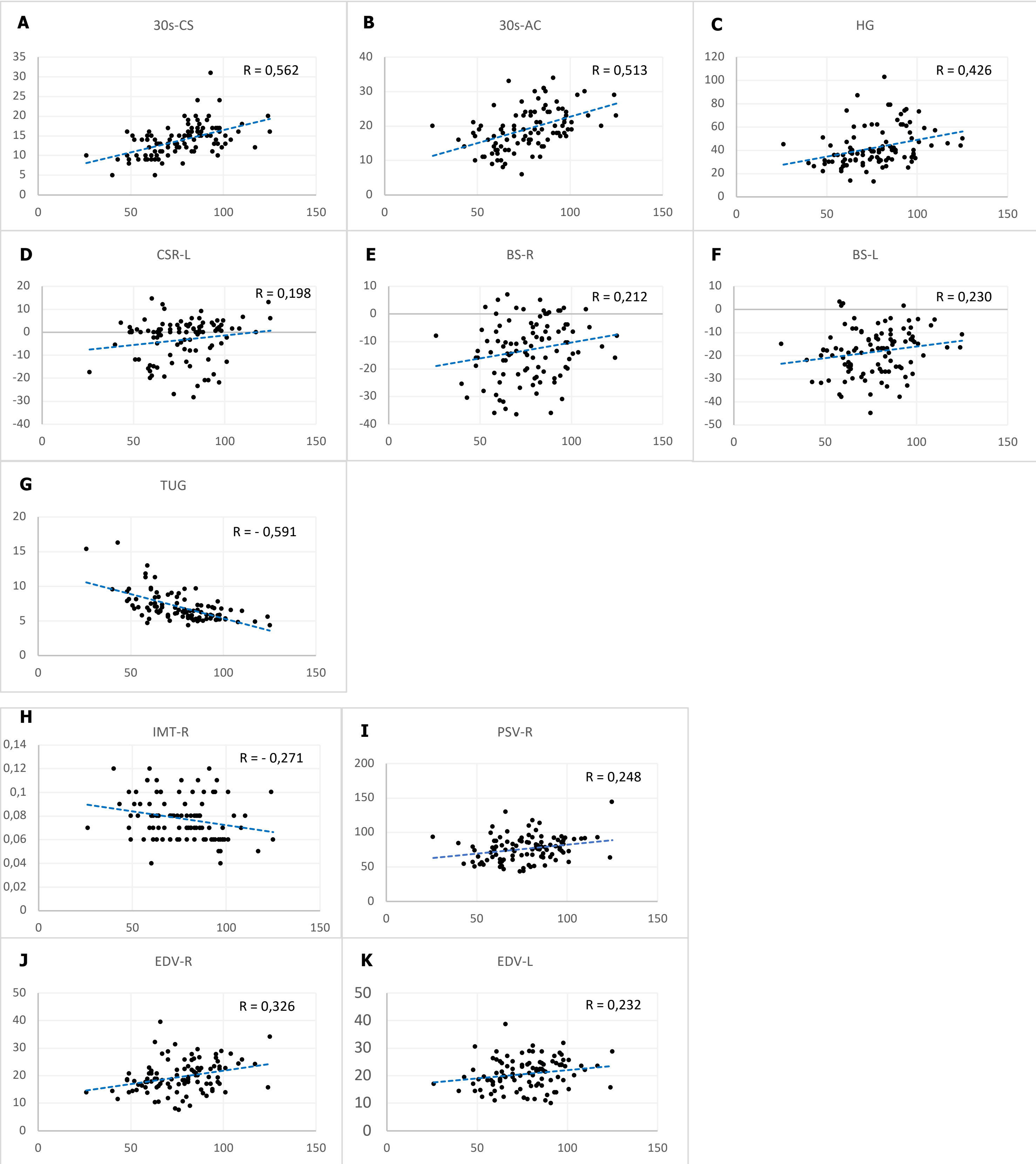

***Figure 1** - Relationship between aerobic capacity variable assessed through the 2min-ST and chair stand test (30s-CS) (A), arm curl test (30s-AC) (B), hand grip test (HG) (C), chair sit and reach test (CSR) (D), back scratch test - right (BS-R) (E), back scratch test – left (BS-L) (F), timed up and go test (TUG) (G), intima-media thickness (IMT) (H),peak systolic velocity (PSV) (I), end-diastolic velocity – right (EDV-R) (J) and end-diastolic velocity – left (EDV-L) (K).*

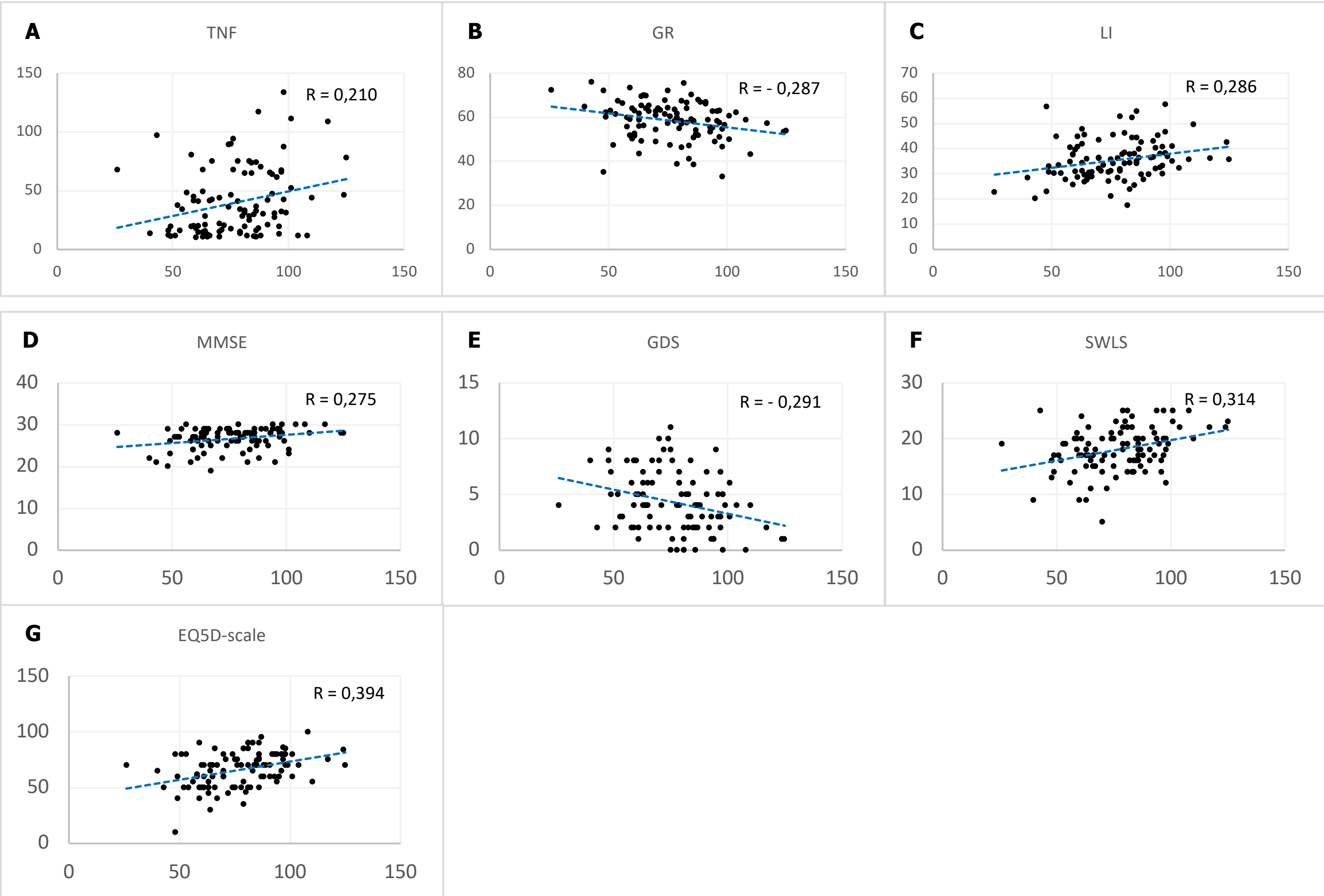

Figure 2 *- Relationship between aerobic capacity variable assessed through the 2min-ST and tumor necrosis factor (TNF-α) (A), granulocytes (GR) (B), lymphocytes (LI) (C), mini mental state examination (MMSE) (D), geriatric depression scale (GDS) (E), satisfaction with life scale (SWLS) (F) and euroqol (EQ5D) (G).*

In relation to handgrip strength, the final mean result for the study sample was 42kg. Value was calculated according to the sum of the two best results from the HG from both hands (ACSM, 2018). The relationship between hand grip test (HG) and the different variables assessed in the present study (anthropometry, physical function, cognitive function, mental health, immunological markers and cardiovascular markers) showed a strong and positive correlation between the HG variable and the Hgt ($r=.522$; $n=102$; $p<0.001$). There was a moderate and positive correlation between the HG variable and LBM ($r=.381$; $n=102$; $p<0.001$), 2min-ST ($r=.426$; $n=102$; $p<0.001$), 30s-AC ($r=.365$; $n=102$; $p<0.001$), and MMSE ($r=.342$; $n=102$; $p<0.001$). There was a moderate and negative correlation between the HG variable and FM ($r=-.348$; $n=102$; $p<0.001$) and TUG ($r=-.403$; $n=102$; $p<0.001$). Finally, there was a weak and positive correlation between the

HG variable and Wgt (r=.253; n=102; p=.010); 30s-CS (r=.289; n=102; p=.003); ERI (r=.233; n=102; p=.018); Hb (r=.270; n=102; p=.006); Hct (r=.275; n=102; p=.005); RSES (r=.233; n=102; p=.019); and EQ5D–scale (r=.217; n=102; p=.028). There was a weak and negative correlation between GLU (r=-.197; n=102; p=.047); CLT (r=-.255; n=102; p=.010); HDL (r=-.285; n=102; p=.004); GDS (r=-.290; n=102; p=.003); and PSS (r=-.239; n=102; p=.015). These results are presented in Figure 3 and 4.

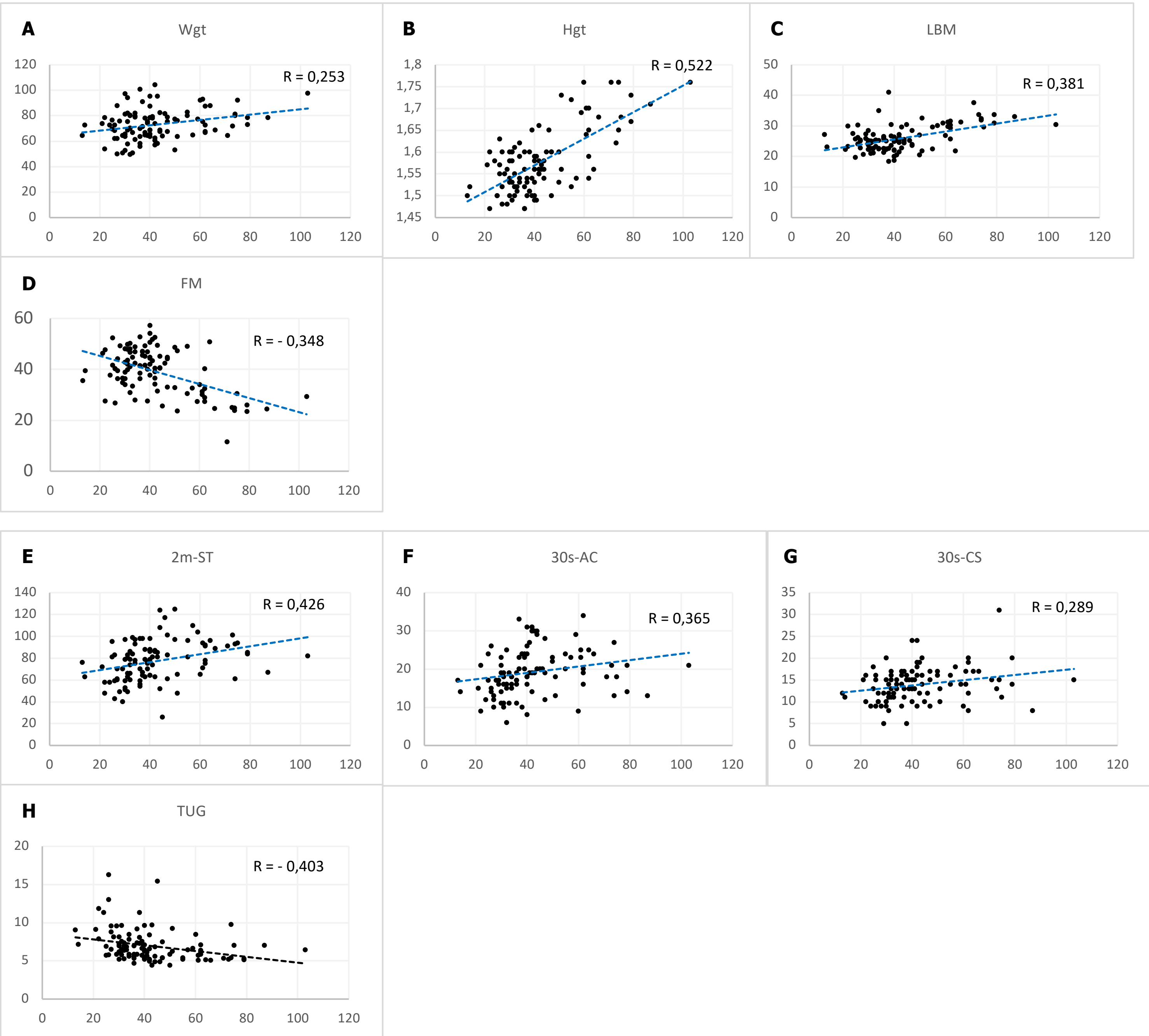

Figure 3 *- Relationship between hand grip strength assessed through the HG and weight (Wgt) (A), height (Hgt) (B), lean body mass (LBM) (C), fat mass (FM) (D), two-minutes step test (2min-ST) (E), arm curl test (30s-AC) (F), chair stand test (30s-CS) (G) and timed up and go test (TUG) (H).*

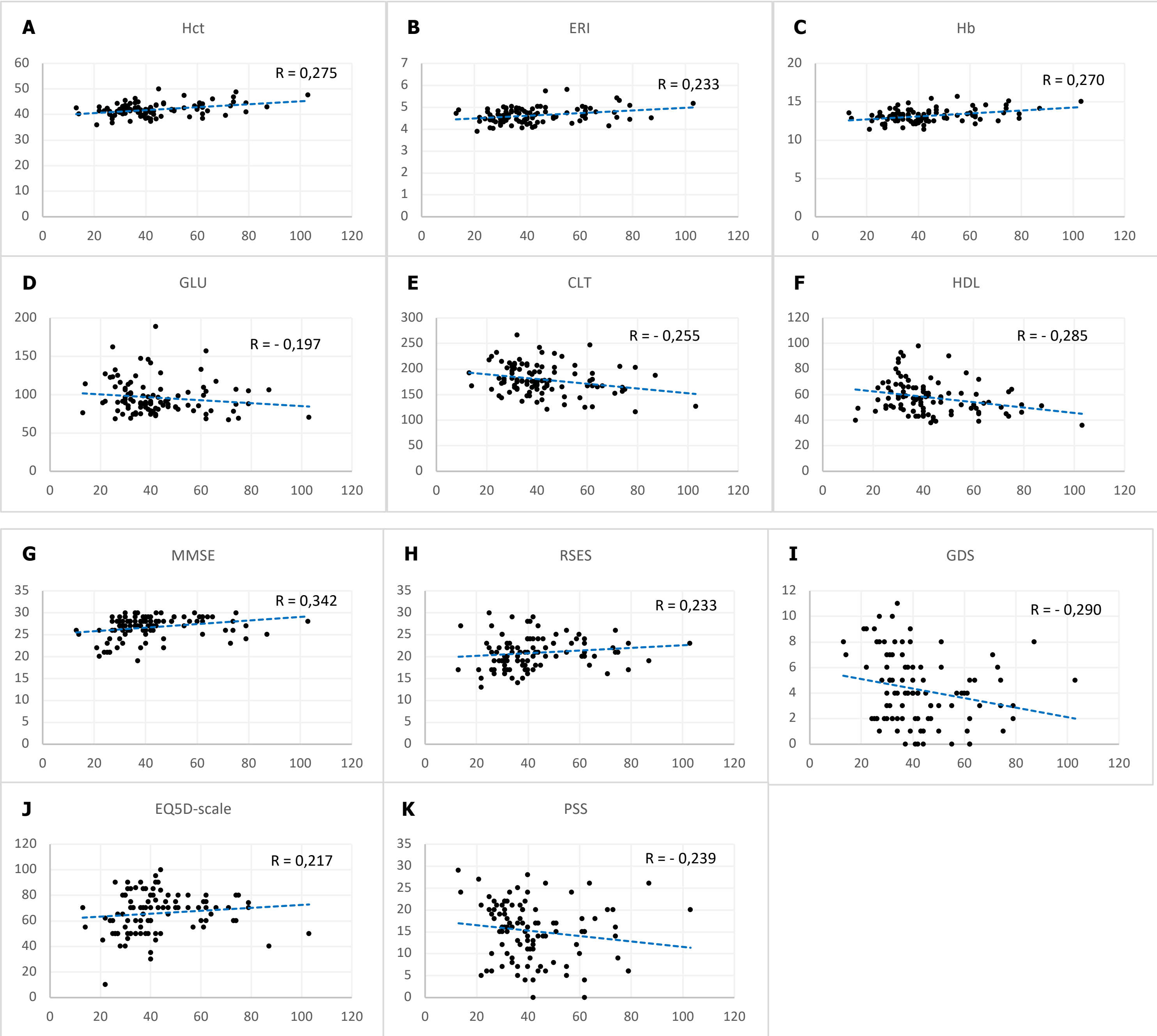

Figure 4 *- Relationship between hand grip strength assessed through the HG and hematocrit (Hct) (A), erythrocytes (ERI) (B), hemoglobin concentration (Hb) (C), glucose (GLU) (D), cholesterol total (CLT) (E), high-density lipoprotein (HDL) (F), mini mental state examination (MMSE) (G), rosenberg self-esteem scale (RSES) (H), geriatric depression scale (GDS) (I), euroqol (EQ5D) (J) and perceived stress scale (PSS) (K).*

Finally, cognitive function was assessed using the MMSE, with the total sample achieving an average score equivalent of 26.71 points, being considered as a normal cognitive profile (Mungas, 1991). The relationship between cognitive function assessed using the MMSE score and the different variables assessed in the present study (anthropometry, physical function, cognitive function, mental health, immunological markers and cardiovascular markers) revealed a moderate and positive correlation between HG (r=.342; n=102; p<0.001), EQ5D–scale (r=.307; n=102; p=.002), and a moderate and negative between TUG (r=-.300; n=102; p=.002). Finally, there was a weak and positive correlation between LCir–R (r=.247; n=102; p=.012); LCir–L (r=.222; n=102; p=.025); 2min-ST (r=.275; n=102; p=.005); BS–R (r=.210; n=102; p=.035); 30s-CS (r=.194; n=102; p=.050); and 30s-AC (r=.253; n=102; p=.010). There was also a weak and negative correlation between IMT–L (r=-.213; n=102; p=.031), ADP (r=-.208; n=102; p=.036) and GDS (r=-.201; n=102; p=.043). These results are presented in Figure 5.

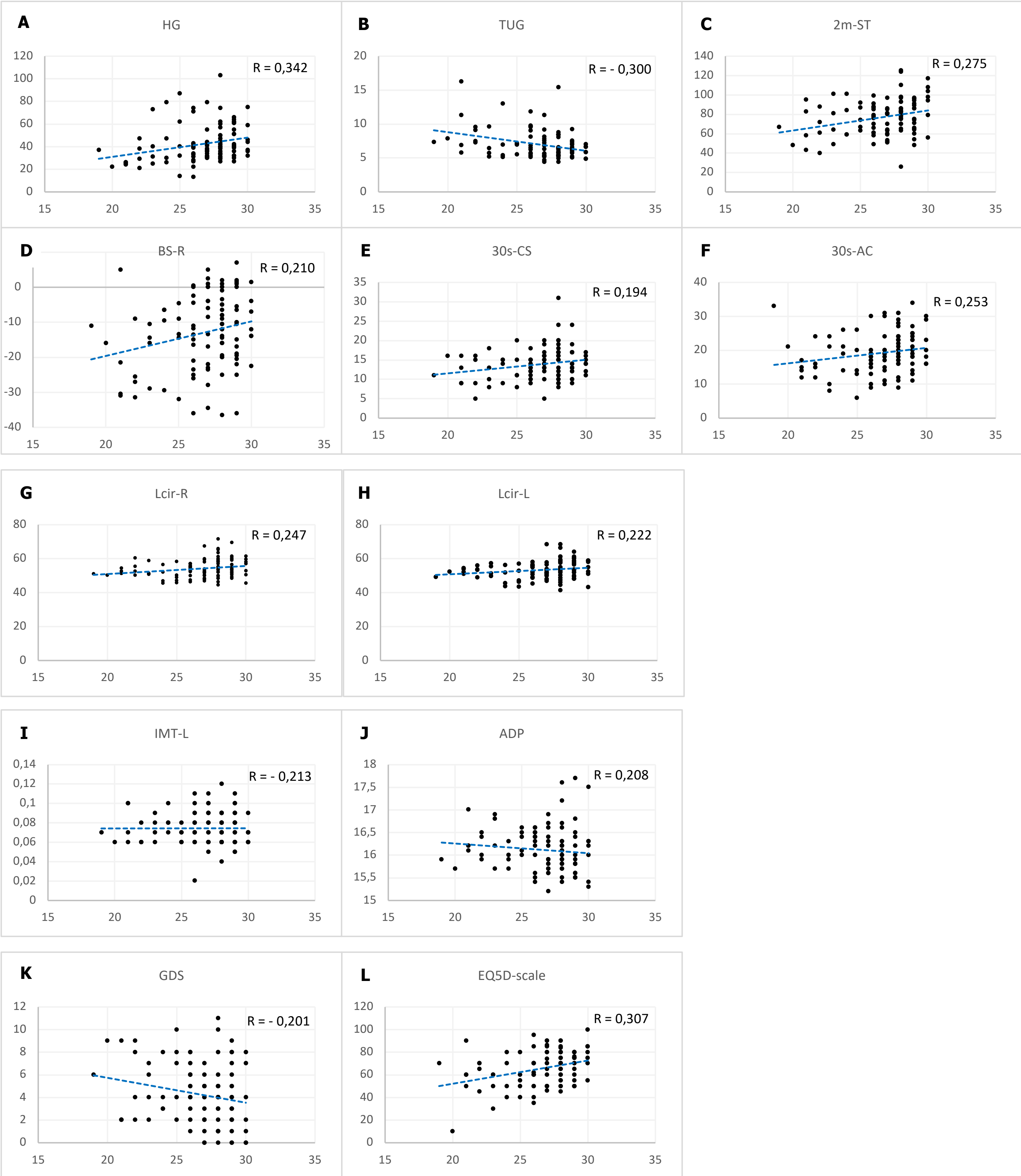

Figure 5 *- Relationship between cognitive function assessed through the MMSE and hand grip test (HG) (A), timed up and go test (TUG) (B), two-minutes steo test (2min-ST) (C), back scratch test – right (BS-R) (D), chair stand test (30s-CS) (E), arm curl test (30s-AC) (F), legs circumference – right (LCir-R) (G), legs circumference – left (LCir-L) (H), intima-media thickness (IMT) (I), adenosine diphosphate (ADP) (J), geriatric depression scale (GDS) (K) and euroqol (EQ5D) (L).*

5. Discussion

The purpose of this study was investigate the association between the aerobic capacity, hand grip strength and cognition with a wide range of cardiometabolic markers (anthropometry, physical function, immunological markers and cardiovascular markers) and mental health, in community dwelling elderly.

The results of this study suggesting a strong positive correlation between aerobic fitness (assessed by 2min-ST) and 30s-CS and 30s-AC, and a strong negative correlation between TG. A moderate positive correlation between 2min-ST and HG, EVD-R, SWLS and EQ5D-scale. A weak positive correlation between 2min-ST and CSR-L, BS-R, BS-L, EDV-L, PSV-R, TNF-α, LI and MMSE, and a weak negative correlation between IMT-R, GR and GDS.

Our results are in agreement with those found in other studies. Regarding physical function, Teodorczyk et al. (2016) also demonstrated a strong and positive correlation between aerobic capacity and lower limb muscle strength, assessed using an isometric dynamometer armchair. Likewise, in the study of Kaymaz et al. (2018), positive and moderate correlations were also found between aerobic capacity (i.e., assessed through the incremental back-and-forth walk test and through the resistance walk test) and upper limb muscle strength (i.e., assessed through the one-repetition maximal test for the upper limb strength). Pedrosa and Holanda (2009) demonstrated a strong and negative correlation between aerobic capacity and agility/dynamic balance. Similarly, Taylor-Piliae et al. (2012) also found a moderate and positive correlation between aerobic capacity with lower limb strength, and with balance (i.e., assessed using the unipedal stance test). Katsimpris et al. (2021) demonstrated a strong and positive correlation between aerobic capacity and handgrip strength. Regarding flexibility, in the study of Chang et al. (2019) a moderate and positive correlation was verified between aerobic capacity and flexibility (back-scratch test). However, in studies of Santos et al. (2018) e Huang et al. (2018), no statistically significant correlations were found between these two variables.

Other studies have also verified the association between aerobic capacity and the intima and media thickness of the carotid arteries. In particular, the study of Lee et al. (2009) which demonstrated a strong and negative association between aerobic capacity and the risk of developing carotid atherosclerosis, and the study of J. Lee et al. (2019), which also demonstrated an inverse association between aerobic capacity and the development of carotid artery disease and with the intima and medium thickness of the carotid arteries.

As for biochemical markers, in the study of Lacedonia et al. (2016) there was also a moderate and negative correlation between aerobic capacity (6-minute walking test) and granulocyte levels. Contrary to the results found in the present study, Pérez & Núñez (2018), found a weak and negative correlation between aerobic capacity and the TNF-α marker. In the study of Neves et al. (2021), there was also a moderate and negative correlation between these two variables. Likewise, the results of Okan (2020) showed a negative correlation between aerobic capacity (6-minute walking test) and the neutrophil/lymphocyte ratio.

Regarding psychological health, studies of Marques et al. (2017) e Kouwijzer et al. (2020), also showed a moderate and positive relationship between aerobic capacity and life satisfaction. In the study of Kim et al. (2019) a moderate and positive correlation was found between aerobic capacity and quality of life. And in the study of Baldasseroni et al. (2014), a moderate and negative correlation was found between aerobic capacity and the geriatric depression scale. As for the cognitive level, the study of Yazar et al. (2018), showed a moderate and positive correlation between aerobic capacity (6-minute walking test) and the MMSE.

The 2m ST is considered an adequate tool to assess aerobic fitness, as it has minimal requirements that make it easy to apply to different study scenarios (Ricci et al., 2019). Our results reinforce the importance of improving aerobic capacity, which is an important component that can significantly improve the predictive ability of cardiovascular disease risk in both the short and long term (Lin et al., 2015). According to the same authors, aerobic capacity is an indicator used in clinical practice, both in the diagnosis and prognosis of cardiovascular diseases.

Regarding muscle strength, we verified the strong positive correlation between HG and Hgt. A moderate positive correlation between HG and LBM, 2min-ST, 30s-AC and MMSE, and a moderate negative correlation between HG and FM and TUG. A weak positive correlation between HG and Wgt, 30s-CS, ERI, Hb, Hct, RSES and EQ5D, and weak negative correlation between GLU, CLT, HDL, GDS and PSS.

The obtained results are aligned with those found in the literature. Regarding body composition, Torralvo et al. (2018) also demonstrated a strong and positive correlation between handgrip strength and height. However, in the study of Pizzigalli et al. (2017) this correlation was moderate and positive. Buehring et al. (2018) also demonstrated a moderate and positive correlation between handgrip strength and lean body mass, assessed by different methods (i.e., dual-energy X-ray asborptiometry, bioelectric impedance and creatine dilution DB-CR), e Torralvo et al. (2018) demonstrated a strong and positive correlation between handgrip strength and lean body mass (i.e., bioelectric impedance). Torralvo et al. (2018) also demonstrated a strong and negative correlation between handgrip strength and fat mass. However, in the study of Bentes et al. (2017), there were no statistically significant correlations between handgrip strength and weight.

Regarding functional fitness, Katsimpris et al. (2021) also demonstrated a strong and positive correlation between handgrip strength and aerobic capacity. Alonso et al. (2018) demonstrated a moderate and positive correlation between handgrip strength and lower limb muscle strength. C. Liu et al. (2017), also demonstrated a moderate and positive relationship between HG and upper limb muscle strength. (30s-AC). Lam et al. (2016) e Alonso et al. (2018) demonstrated a weak and negative correlation between handgrip strength and agility and dynamic balance (i.e., TUG).

At the immunological level, in the study of Belury et al. (2021), ERI values were also positively associated with handgrip strength. Hu et al. (2018) demonstrated a positive correlation between handgrip strength and Hb levels. in the same sense, Sayre et al. (2017) demonstrated a positive association between HG and Hb and Hct levels. In the lipid profile, blood glucose values were negatively associated with HG, that is, participants with higher blood glucose levels had a lower result in HG (Åström et al., 2021; Liang et al., 2020) and Pedrero-Chamizo et al. (2020) found a weak and negative correlation between HG and total cholesterol. In HDL levels, the same authors found a weak and positive correlation with HG, contradicting the results verified in the present study.

In terms of cognitive function and mental health, our study found a statistically significant correlation between HG and cognitive function, quality of life, level of depression and perceived stress. In the study of Y. Liu et al. (2021), the results also showed a weak and positive correlation between handgrip and cognitive level (i.e., MMSE). However, such association was not found in previous studies (Chupel et al., 2017) assessing the same variables in elderly participants. in the study of Hu et al. (2018), the results also showed a positive association between handgrip strength and quality of life. Ozer et al. (2021) showed a weak and negative correlation between the HG and the Geriatric Depression Scale, in participants aged over 60 years. in the study of Poornima et al. (2014), the results also showed a weak and negative correlation between handgrip strength and perceived stress (assessed using the PSS). Additionally, in our study, a statistically significant correlation was found between HG and self-storage (assessed using the Rosenberg scale). No other studies were found where this correlation was verified.

These results are relevant since reduced muscular strength, in the elderly, impacts everyday tasks. A significant increase in the risk of falling and loss of functional independence is also associated with muscular strength reduction (Alonso et al., 2018). Hand grip strength is fast and simple to assess. It has been considered as an important clinical test to be used to identify increased risk of morbidity and mortality (Hershkovitz et al., 2019; Wu et al., 2019). Hand grip strength is often used in the detection of deteriorations related to aging and is observed in the LBM and strength (McGrath et al., 2018) parameters.

Regarding cognition function, we verified the moderate positive correlation between MMSE and HG and EQ5D-scale, and a moderate negative correlation between MMSE and TUG. A weak positive correlation between MMSE and LCir-R, LCir-L, 2min-ST, BS-R, 30s-CS and 30s-AC, and weak negative correlation between IMT-L, ADP and GDS.

The results obtained are in line with those found in other studies. With regard to functional fitness, Y. Liu et al. (2021) and Ramnath et al. (2018) also demonstrated a moderate and positive correlation between cognitive fitness and handgrip strength. However, Emerenziani et al. (2020) had already demonstrated a strong and positive correlation between these two variables. n the study of Chupel et al. (2017), moderate and negative correlations were also demonstrated between cognitive function and agility/dynamic balance. In the same vein, in the study of Borges et al. (2018) a weak and negative correlation was shown between cognition and agility/dynamic balance while, in the study of Ramnath et al. (2018), there were no significant correlations between the two variables. Still in functional fitness, Chupel et al. (2017), also found moderate positive associations between MMSE and upper and lower strength (30s-CS and 30s-AC), and cardiorespiratory fitness (2min-ST), in institutionalized elderly. According to the authors, these associations can be explained by ascertaining that physical fitness tests involve, in addition to physical requirements, some attention, concentration and understanding from the perspective of the participants. Similarly, Yang et al. (2018) presented the correlation that lower and upper limb strength, lower limb flexibility, dynamic agility/balance and aerobic capacity are independently associated with cognitive function. According to the authors, little is known about the mechanisms between cognitive and physical functions. However, physical fitness tests require refined brain control mechanisms to start tasks, in addition to motor unit recruitment and motor coordination. Similarly, Yang et al. (2018) also showed that participants with cognitive impairment scored lower on several tests of functional fitness (30s-CS, 30s-AC, 2min-ST, CSR and BS).

In relation to the intima and mean thickness of the carotid arteries, our results are in agreement with those found in the study by Yue et al. (2016), where they demonstrated that participants with cognitive impairment had a higher IMT value, compared to participants with a normal cognitive level. This result reinforces the results of the study by Gorgone et al. (2009), that found a moderate and negative correlation between the MMSE and the IMT.

Regarding the relationship between cognitive functions and mental health, our results are supported by the study by Chae et al. (2020), where a lower score on the Quality of Life questionnaire was associated with a cognitive decline (MMSE), suggesting that quality of life metrics should be used as a tool to detect cognitive changes in the elderly, and thus prevent or delay cognitive decline. Similarly, Samuel et al. (2016) concluded that compromised cognitive function, in the elderly, has a negative effect on health-related quality of life metrics, regardless of gender, education or the existence of chronic illnesses. On the other hand, in the study by Voros et al. (2020), no significant associations were found between elderly cognitive function (assessed using the MMSE) and quality of life (Older People Quality of Life questionnaire). As in our study, Ozer et al. (2021) also demonstrated a weak and negative correlation between the MMSE and the GDS.

Additionally, in our study, a statistically significant correlation was found between MMSE and the variables ADP, LCir-R and LCir-L. No other studies were found where these correlations were verified. These data suggest that cognitive function can also influence the immune profile and body composition, more precisely the perimeters of the lower limbs.

Cognitive function assessment has considerable scientific value. With aging, a decline in cognition is observed. This decline originates in the expansion of cerebral spinal fluid, in the progressive deterioration of the microstructure of white matter and other subcortical nuclei (hippocampus, cerebellum and striatum) (Cherup et al., 2018).

The results found in the present study showed that aerobic capacity (2min-ST), handgrip strength (HG) and cognitive function (MMSE) can be correlated with a wide range of cardiometabolic markers. More studies are needed to corroborate the evidence found. It is also relevant to develop more studies that test interventions that can improve these markers, such as exercise in an aquatic environment.

6. Conclusion

Our results suggest that aerobic capacity (assessed using 2min-ST) may be related to functional fitness, cardiovascular, biochemical, cognition and mental health markers. They also suggest that handgrip strength (assessed using the GH) may be correlated with anthropometric measurements, functional and biochemical fitness markers, with cognitive function and mental health. Regarding cognitive function (MMSE), the results suggested that it may be associated with anthropometric measures, functional fitness, cardiovascular and biochemical markers, and also with mental health. These data show that aerobic capacity, handgrip strength and cognitive function may hypothetically be related to risk markers for cardiometabolic diseases.

7. References

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Study 3: Impact of different aquatic exercise programs on body composition, functional fitness and cognitive function of non-institutionalized elderly adults: a randomized controlled trial

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1. Abstract

Aquatic physical exercise programs have become progressively more popular among elderly people. Some of the major physical exercise program disadvantages on land are minimized due to the specific properties of the aquatic environment. The purpose of the present randomized controlled study is to verify the effects of different aquatic physical exercise programs on body composition, functional fitness and cognitive function in non-institutionalized elderly people. For this study, 102 elderly individuals were randomly allocated into four different groups: AerG (n = 25, 71.44 ± 4.84 years); IntG (n = 28, 72.64 ± 5.22 years); ComG (n = 29, 71.90 ± 5.67 years) and CG (n = 20, 73.60 ± 5.25 years). Individuals from the groups AerG, IntG and ComG participated in three different aquatic physical exercise programs for a period of 28 weeks. The CG participants kept to their usual routines. All participants were evaluated for body composition, functional fitness and cognitive function at two time moments, i.e., pre- (M1) and post-intervention (M2). Significant differences for body composition were found between M1 and M2 for FM ($p < 0.001$), LBM ($p < 0.001$) and WCir ($p < 0.01$) in the AerG, for BMI ($p < 0.05$), FM ($p < 0.05$), LBM ($p < 0.001$) and Lcir-R ($p < 0.05$) in the IntG, and for WGT ($p < 0.01$), FM (p

< 0.05), LBM ($p < 0.01$), Lcir-R ($p < 0.05$) and Lcir-L ($p < 0.01$) in the ComG groups. For functional fitness, differences were found between M1 and M2 for 2min-ST ($p < 0.001$), 30s-CS ($p < 0.001$), 30s-AC ($p < 0.05$), HG-R ($p < 0.001$) and HG-L ($p < 0.001$) in the AerG, for 2min-ST ($p < 0.05$), BS-R ($p < 0.05$), 30s-CS ($p < 0.001$), 30s-AC($p < 0.01$), HG-R ($p < 0.001$) and HG-L ($p < 0.001$) in the IntG, and for 30s-CS ($p < 0.001$), HG-R ($p < 0.001$) and HG-L ($p < 0.001$) in the ComG groups. The present study evidenced the beneficial effects of physical exercise in an aquatic environment on body composition, functional fitness and cognitive function in non-institutionalized elderly adults. The ComG water-based exercise program showed more beneficial effects in the improvement of body composition and cognitive function variables, while the IntG and AerG programs were more effective in the improvement of functional fitness.

Keywords: physical exercise; water-based exercise; aging; elderly

2. Introduction

Regular physical activity is a recognized cost-effective intervention for public health and is associated with an ever-widening constellation of health, economic, and other benefits, playing an important role in the prevention and management of many major chronic conditions. Thus, the implementation of programs, practices, and policies to facilitate more physical activity and limit sedentary behavior could result in significant health improvements and other benefits, as well as reducing the burden and cost of chronic disease to healthcare systems [1]. In this respect, sedentary behavior is associated with negative changes in the neuromuscular systems of healthy older adults, resulting in a decrease in physical functioning [2]. Body composition, functional fitness and cognition are variables that undergo negative changes during aging, and these changes can lead to the development of cardiometabolic diseases [3].

Evidence reveals that physical exercise on land has been identified as an effective method for improving body composition [4] and functional capacity [5], and reducing the cognitive impairment associated with aging [6]. However, exercise in an aquatic environment has progressively gained popularity, particularly in the elderly population, as it minimizes or overcomes some existing disadvantages from land-based exercise programs due to specific water properties, such as buoyancy and water viscosity. Such properties help to reduce strain on the joints and offer additional support for balance and other problems associated with a lack of strength, as is very frequent in frailty, providing a safer and more protective environment for physical exercise. In addition, aquatic exercise programs seem to be at least as or even more effective as land-based exercise programs [7] for improving elderly people´s health and well-being.

A recent intervention study [8] that tested the impact of two walking programs (land-walking versus water-walking), concluded that the water program can be considered safer and is a preferred activity for elderly people, providing benefits in body composition that are similar or higher than those achieved in land-based exercise programs. Results showed that older participants benefit more from the lower impact forces and decreased risk of falls associated with water-walking, without compromising improvements in their cardiorespiratory fitness.

A meta-analysis performed by Waller et al. [3] analyzed the effects of water-based exercise programs on functional fitness in healthy older adults compared to physical exercise programs performed on land. The authors concluded that physical exercise in aquatic environments seems to be effective in the maintenance and improvement of functional fitness in healthy older people. Moreover, when compared to exercise on land, exercise in an aquatic environment seems to be at least as effective and can be used as an alternative type of exercise, when exercise on land is not feasible or desirable [3].

Regarding cognitive function, another intervention study [8] tested the effectiveness of an aquatic exercise program in a group of older women, aged between 60 and 80 years old, and reported that aquatic exercises can contribute to the maintenance and improvement of cognitive function.

For all these reasons, aquatic exercise programs appear to be a viable alternative to exercise on land, and it is crucial to continue exploring the potential of this environment in order to clarify its benefits and the effects of different water-based exercise programs. Thus, the aim of this randomized controlled trial is twofold. Firstly, to perceive the impact of different water-based exercise programs (continuous aerobic, aerobic interval and combined) on body composition, functional fitness and cognitive function in non-institutionalized older people. Secondly, to perceive which of the three water-based exercise programs will be more effective in improving function in non-institutionalized older people. According to the existing literature [3,5,8–11] we predicted that differences would be found in the experimental groups, as opposed to the CG, and that the ComG program would be more effective than the AerG and IntG programs.

3. Materials and Methods

3.1. Study Design

A randomized controlled trial was conducted in the central region of Portugal, between October 2018 and July 2020. The study included non-institutionalized older adults participating in three different aquatic physical exercise programs for a period of 28 weeks. A complete study protocol was previously published in [12]. Participants were recruited in order to analyze the impact of three aquatic exercise programs (over 28 weeks), namely: continuous aerobic exercise, aerobic interval exercise and combined exercise. Data regarding body composition, functional fitness and cognition were collected at two specific moments, pre-intervention (M1) and post-intervention (M2).

This study conformed to the Declaration of Helsinki guidelines and the protocol was approved by the Faculty of Sports Science and Physical Education, University of Coimbra Ethics Committee (reference: CE/FCDEF-UC/00462019). All participants provided written informed consent prior to participation.

3.2. Participants and Sample Size

The size and statistical power of the sample were calculated using the G*Power software application [13]. The following parameters were considered: F test (ANOVA); effect size: 0.25; α-level: 0.05; statistical power: 0.95; number of groups: 4; number of measures: 2 (pre- and post-intervention); margin for possible losses and refusals: 30%. Therefore, the initial size of the total sample was estimated at 76 participants.

Initially, 174 individuals from the community were personally invited to participate in the study. After applying the inclusion and exclusion criteria, 152 individuals were randomized into four different groups: the continuous aerobic exercise group (AerG, n = 36), aerobic interval exercise group (IntG, n = 41), combined exercise group (ComG, n =

48), and control group (CG, n = 27). According to the experience of the research team and according to previous studies [6,7,14], the rate of dropping out from exercise programs in the elderly population is high, so we gathered more participants. An external researcher used a computer-generated list of random numbers to allocate participants to each group in a ratio of 1:1:1:1. Researchers were blinded to ensure group randomization.

The inclusion criteria were the following: a) participants of both sexes; b) 65 years of age or older; c) non-institutionalized older people; d) having the autonomy to move from their residence to the municipal swimming pool; e) individuals who gave permission to take part in the study by signing the consent form; f) individuals with medical authorization to practice physical exercise in an aquatic environment, in cases where they have some type of clinical condition or comorbidity. The exclusion criteria were the following: a) individuals with clinically diagnosed pathologies that put their health and that of others at risk during the practice of physical exercise in an aquatic environment; b) having severe cognitive impairment, that is, a score lower than 9 points in the MMSE or mental illness that has been clinically diagnosed; c) participants who attended less than 50% of the physical exercise sessions; d) participants who failed to complete all of the proposed assessment tests.

All three experimental groups (AerG, IntG and ComG) performed different types of water-based exercise simultaneously for 28 weeks. Participants from the control group (CG) were asked to maintain their normal daily activities, including not performing any type of systematic physical exercise during the same time period.

Figure 6 shows the entire allocation process for the different groups. Fifty participants were excluded from the study due to the following reasons: personal reasons (n = 11); the participant attended less than 50% of the exercise program sessions (n = 14); the participant did not complete all assessment tests (n = 12); injury not related to the intervention program (n = 4); and disease (n = 9).

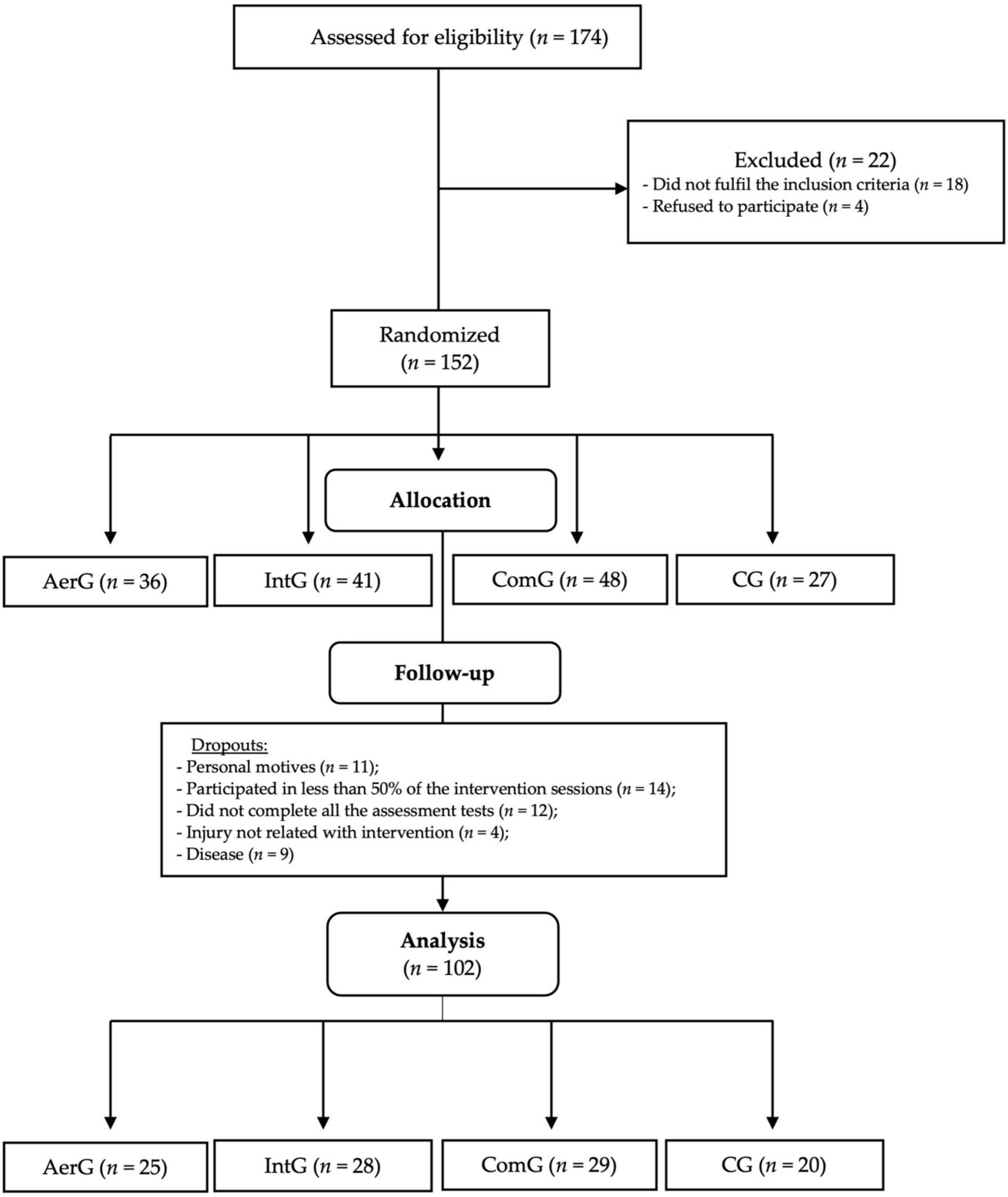

Figure 6 - *Allocation process for the different groups: continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG).*

3.3. Outcomes Measurements

3.3.1. Clinical and Demographic Characteristics

Data regarding age (AG), marital status (MS), regular medication (RMED), allergies (ALL), disease (DIS), annual medical appointments (AMA), sleep quality (SQ), alcohol, tobacco and drugs consumption (ATD) were assessed at baseline (M1) using a specific questionnaire developed for this purpose.

3.3.2. Anthropometry

Anthropometric measurements were conducted by two certified investigators by FCDEF-UC. The following parameters of anthropometry were evaluated: height (HGT), using a portable stadiometer, Seca Bodymeter® (model 208, Hamburg, Germany) with a precision of 0.1 cm; weight (WGT); body mass index (BMI); visceral fat (VF); percentage of fat mass (FM) and muscle body mass (LBM) using a portable scale TANITA BC-601 with a precision of 0.1 cm with 0.1 kg accuracy; and waist circumference (Wcir); arm circumference (Acir) and leg circumference (Lcir), using a retractable fiberglass tape (model Hoechst mass-Rollfix®, Sulzbach, Germany) with an accuracy of 0.1 cm.

3.3.3. Physical Function

Physical function was assessed using the senior fitness test, developed by Rikli and Jones [15] and validated for the Portuguese population [16]: the strength of the lower and upper limbs was assessed using the chair stand test (30 s-CS) and arm curl test (30 s-AC), respectively (repetitions/30 s); aerobic capacity was assessed using the two-minute step test (2min-ST) (number of steps); flexibility of lower and upper limbs was tested with the chair sit and reach test (CSR) and back scratch test (BS), respectively (centimeters); and agility and dynamic balance through the timed up and go test (TUG) (seconds). Hand strength was assessed through the handgrip test (HG) using the Jamar hand dynamometer (Lafayette Instrument Company, Lafayette, IN, USA) (kg).

3.3.4. Cognitive Function

Cognitive function was assessed with the Portuguese version of the mini-mental state examination (MMSE) [17]. The MMSE evaluates the following cognitive areas: orientation, short-term memory, attention and calculation capacities, long-term memory, and language capabilities. The final score has a maximum of 30 points, and scores below 24 can be used as an aid in the assessment of dementia. The test was used as an instrument to create a cognitive profile with the following criteria [18]: severe cognitive impairment (scores between 1 and 9 points); moderate cognitive impairment (scores between 10 and 18 points); mild impairment (score between 19 and 24 points), normal cognitive profile (scores between 25 and 30 points).

3.4. Intervention Protocol

The exercise programs were implemented by sport sciences and fitness experts with specific training in water aerobics and were developed according to the exercise prescription guidelines recommended by the American College of Sport Medicine (ACSM) for the elderly [19].

All exercise program sessions had a duration of 45 minutes, twice a week, for 28 weeks and were performed in a water environment (the water level was between 0.80 and 1.20 m, with a temperature of approximately 32 °C), using the rhythm of the music as a tool to control the intensity level of exercise. Each session had a maximum limit of 16 participants, with a ratio of 1 exercise coach for every 16 participants. Water exercise sessions were organized into three different sections: initial, main and final parts.

The initial part, or the warm-up, lasted between 10 and 15 minutes, at low intensity (30–40% max HR), and were the same in the three water exercise programs. During this initial part, it was intended that the participants would adapt to the aquatic environment, i.e., to the water temperature, and provide muscular and metabolic stimulations to prepare the body for the main part of the session. Thus, simple exercises in water were used, such as displacements and isolated movements, with a progressive increase in complexity and intensity throughout the initial part.

The main part for each one of the three water exercise program sessions had a duration of 20 to 30 minutes and all were characterized as follows:

- In the water-based continuous aerobic program, aerobic exercises were used continuously throughout the main part of the session (20 to 30 minutes), with a target intensity of 60 to 70% of the maximum heart rate, according to the recommendations of the ACSM for the elderly [19];
- In the water-based interval aerobic program, the main part of the session consisted of performing exercises with different intensities, such as: short duration exercises of

30 seconds, with an intensity of 70–80% of the maximal heart rate, followed by active recovery intervals of 1 minute, using exercises with an intensity of 60–70% of the maximal heart rate;

- In the water-based combined training program (continuous aerobic and muscular strength), the main part of the session was divided into two phases, with equal time periods. The first phase consisted of aerobic exercises on a continuous basis, with a target intensity of between 60 and 70% of the maximal heart rate. In the second phase, muscle-strengthening exercises were applied (water environment): 6 to 8 different exercises, with auxiliary equipment to create more resistance to movement (e.g., “spaghetti” and dumbbells), covering muscle strengthening from trunk (e.g., rowing, inverted crunches, etc.), upper limbs (e.g., elbow flexion and extension, shoulder rotation, etc.) and lower limbs (e.g., knee flexion and extension, leg abduction and adduction, etc.). Strengthening exercises were implemented using 2 to 3 sets of 12 to 16 repetitions at a moderate intensity (6–7) on the Borg scale.

The final part of the water exercise sessions lasted between 5 and 10 minutes and was the same for each of the three water exercise programs. This part consisted of two phases: return to calm, where relaxation exercises were applied to resume the participants’ heart rate to values close to the resting state, and stretching, where exercises using a greater range of motion were used to stretch the main muscle groups used throughout the sessions.

3.5. Monitoring the Intensity of the Physical Exercise

For safety and intensity target control reasons, all participants were randomly using heart rate monitors (Polar, RS800CX, Finland) during the exercise sessions, in all three water exercise programs. Depending on the heart rate values obtained, adjustments were performed to maintain the target intensity established for each water exercise program.

The intensity of the different water exercise programs was predicted indirectly using the Karvonen formula [20]:

Target heart rate = ((maximal heart rate – resting heart rate) × % intensity) + resting heart rate.

Additionally, and to calculate the maximal heart rate, the formula created by [21] for the elderly was used:

$$\text{Maximal HR} = 207 \text{ beats per minute} - (0.7 \times \text{chronological age})$$

3.6. Statistical Analysis

The collected data was subject to descriptive statistical analysis, where values such as maximum, minimum, mean and standard deviation were calculated for each variable in each assessment moment. Afterward, data normality was tested by considering the response to three conditions: z-values from the Skewness and Kurtosis tests; p-values from the Shapiro–Wilk test; and visual inspection of generated histograms. All longitudinal comparisons were performed using complete case analysis. Parametric data were analyzed using T-Student test for independent samples to compare the different moments (M1 and M2), and an ANOVA of one factor and post hoc Tukey's test to analyze the differences between groups. Nonparametric data was analyzed using the Wilcoxon test to compare the different moments (M1 and M2), and the Kruskal–Wallis and Bonferroni tests to analyze differences between groups. Statistical analysis was performed using the statistical package for the social sciences (SPSS) statistical software, version 27.0. The level of significance used was $p \leq 0.05$.

4. Results

The final 102 participants completed the entire selection process (AerG: n = 25, 71.44 ± 4.84 years, 80% female; IntG: n = 28, 72.64 ± 5.22 years, 89.3% female; ComG: n = 29, 71.90 ± 5.67 years, 75.9% female; CG: n = 20, 73.60 ± 5.25 years, 55% female). Despite there being no inclusion/exclusion criteria based on gender, the sample was unintentionally formed mostly of female participants, mainly as a result of the higher number of female elderly participants who usually attend community aquatic exercise programs, compared to males. No adverse event was reported during the intervention, showing that the practice of physical exercise in an aquatic environment is a safe modality for the elderly population. Individual characteristics for each group at baseline are presented in Table 3.

Table 3 – Major sample characteristics at baseline.

Characteristics	AerG (n = 25)	IntG (n = 28)	ComG (n = 29)	CG (n = 20)	Value p
AG (years)	71.44 ± 4.84	72.64 ± 5.22	71.90 ± 5.67	73.60 ± 5.25	0.504
MMSE	27.00 ± 2.00	27.00 ± 2.00	26.00 ± 3.00	26.00 ± 3.00	0.489
BMI (kg/m²)	28.20 ± 3.30	29.10 ± 4.80	30.80 ± 5.30	29.50 ± 5.80	0.272
RMED	**n (%)**	**n (%)**	**n (%)**	**n (%)**	
Yes (%)	23 (92%)	27 (96.4%)	26 (89.7)	19 (95%)	0.768
No (%)	2 (8%)	1 (3.6)	3 (10.3%)	1 (5%)	
MS	**n (%)**	**n (%)**	**n (%)**	**n (%)**	
Married (%)	23 (92%)	20 (71.4%)	21 (72.4%)	19 (95.0%)	0.116
Divorced (%)	0 (0.0%)	2 (7.1%)	0 (0.0%)	0 (0.0%)	
Widow (%)	2 (8.0%)	6 (21.4%)	6 (20.7%)	1 (5.0%)	
Single (%)	0 (0.0%)	0 (0.0%)	2 (6.9%)	0 (0.0%)	
ALL	***n*** **(%)**	***n*** **(%)**	***n*** **(%)**	***n*** **(%)**	
Yes (%)	10 (40.0%)	9 (32.8%)	14 (48.3%)	7 (35.0%)	0.638
No (%)	15 (60.0%)	19 (67.9%)	15 (51.7%)	13 (65.0%)	
DIS	**n (%)**	**n (%)**	**n (%)**	**n (%)**	
No disease (%)	6 (24.0%)	5 (17.9%)	6 (20.7%)	3 (15.0%)	0.794
Cardiovascular (%)	4 (16.0%)	7 (25.0%)	4 (13.8%)	5 (25.0%)	
Metabolic (%)	6 (24.0%)	5 (17.9%)	5 (17.2%)	7 (35.0%)	
Cardiovascular and metabolic (%)	9 (36.0%)	11 (39.3%)	14 (48.3%)	5 (25.0%)	
ATD	**n (%)**	**n (%)**	**n (%)**	**n (%)**	
None (%)	22 (88.0%)	25 (89.3%)	26 (89.7%)	19 (95.0%)	0.772
Alcohol (%)	0 (0.0%)	0 (0.0%)	1 (3.4%)	0 (0.0%)	
Tabaco (%)	3 (12.0%)	3 (10.7%)	1 (3.4%)	1 (5.0%)	
Both (%)	0 (0.0%)	0 (0.0%)	1 (3.4%)	0 (0.0%)	

Note: Age (AG); mini mental state examination (MMSE); body mass index (BMI); regular medication (RMED); marital status (MS); allergies (ALL); diseases (DIS); alcohol, tabacco and drugs consumption (ATD).

Group characteristics were very similar between groups at baseline. As no significant statistical differences were found for AG, MMSE, BMI, RMED, MS, ALL, DIS and ATD variables among participants from the AerG, IntG, ComG and CG groups. Most participants were taking medication intended to control their cholesterol, blood pressure and diabetes values. Body composition results were analyzed by type of aquatic exercise program before and after the intervention, and are presented in Table 4.

Table 4 – Body composition results variation analysed by type of aquatic exercise program before and after intervention.

	AerG			IntG			ComG			CG					
	M1 Mean (SD)	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**Time x group (M1)**	**Time x group (M2)**
WGT (kg)	70.5 (8,1)	71.0 (7,6)	0.150	71.3 (14.3)	71.9 (14.5)	0.097	75.1 (11.0)	76.0 (11.5)	0.007*	75.5 (13.3)	75.2 (13.5)	0.589	0.334	0.350
BMI (kg/m²)	28.2 (3.3)	28.5 (3.0)	0.360	29.1 (4.8)	30.1 (6.2)	0.041**	30.8 (5.3)	31.0 (5.1)	0.677	29.5 (5.8)	29.4 (5.9)	0.489	0.272	0.476
VF (%)	11.0 (3.0)	11.0 (3.0)	0.388	12.0 (3.0)	12.0 (3.0)	0.096	13.0 (3.0)	13.0 (3.0)	0.839	13.0 (6.0)	13.0 (6.0)	1.000	0.128	0.267
FM (%)	38.9 (7.3)	37.1 (8.4)	0.009**	41.0 (6,7)	40.0 (6.7)	0.016*	40.3 (9.8)	39.4 (9.8)	0.031**	34.9 (10.9)	34.6 (10.7)	0.310	0.134	0.145
LBM (%)	26.5 (4.3)	27.2 (3.8)	0.007**	24.5 (3.0)	25.4 (3.0)	0.001**	25.5 (4.3)	26.3 (4.3)	0.002**	27.7 (4.7)	27.9 (4.7)	0.188	0.079	0.160
Wcir (cm)	99.7 (8.7)	96.5 (9.0)	0.018*	102.2 (11.4)	101.1 (11.8)	0.216	105.1 (11,5)	103.4 (10.1)	0.109	103.1 (15.8)	102.5 (17.0)	0.652	0.409	0.181
Acir-R (cm)	31.5 (2.2)	31.5 (2.2)	0.729	32.7 (4.1)	32.2 (4.5)	0.107	32.7 (3.2)	32.4 (3.2)	0.331	31.7 (3.5)	31.6 (3.2)	0.745	0.659	0.734
Acir-L (cm)	31.6 (2.5)	31.3 (2.0)	0.381	32.3 (4.2)	31.7 (4.3)	0.850	32.4 (3.3)	32.2 (3.5)	0.475	31.6 (3.2)	31.4 (3.3)	0.278	0.861	0.616
Lcir-R (cm)	54.0 (4.6)	53.5 (4.9)	0.443	54.9 (4.2)	53.8 (5.0)	0.019*	54.6 (6.2)	53.2 (6.2)	0.025*	52.5 (6.2)	51.8 (4.7)	0.247	0.093	0.375
Lcir-L (cm)	53.7 (4.0)	53.1 (4.5)	0.266	53.8 (3.8)	53.3 (4.7)	0.265	54.3 (6.4)	52.6 (6.7)	0.008*	51.0 (5.9)	50.6 (4.6)	0.466	0.153	0.312

Note: * T-Student test results; ** Wilcoxon test results; Correlation is significant at 0.05 level. Weight (WGT); body mass index (BMI); visceral fat (VF); fat mass (FM); muscular mass (LBM); waist circumference (Wcir); right upper limb perimeter (Acir-R); left upper limb perimeter (Acir-L); right lower limb perimeter (Lcir-R); left lower limb perimeter (LCIr-L).

Global body composition results revealed that no significant statistical differences were found between groups, before and after intervention (Table 2). As a result of intervention with aquatic exercise, an increase in WGT was found in all exercise groups, with significant statistical differences on ComG ($p = 0.007$; $\Delta = 1.2\%$), but not in the CG. A similar increase was found for BMI in all aquatic exercise groups, with significant statistical differences in IntG ($p = 0.041$; $\Delta = 3.4\%$), but not in the CG.

FM variable results revealed a significant statistical reduction in all aquatic exercise groups as a result of the intervention AerG ($p = 0.009$; $\Delta = -4.6\%$), IntG ($p = 0.016$; $\Delta = -2.4\%$) and ComG ($p = 0.031$; $\Delta = -2,2\%$), but not in the CG. LBM variable showed a significant increase in all aquatic exercise groups AerG ($p = 0.007$; $\Delta = 2.6\%$), IntG ($p = 0.001$; $\Delta = 3.7\%$) and ComG ($p = 0.002$; $\Delta = 3.1\%$), but not in the CG. Anthropometric results also showed a significant statistical reduction for Wcir in the AerG ($p = 0.018$; $\Delta = -3.2\%$), for Lcir-R in the IntG ($p = 0.019$; $\Delta = -2.0\%$) and in the ComG ($p = 0.025$; $\Delta = 2.6\%$), and for Lcir-L in the ComG ($p = 0.008$; $\Delta = -3.1\%$).

For functional fitness variables (Table 5), there were no significant statistical differences between groups before intervention ($p > 0.05$). However, we found significant statistical differences for 2 m-ST ($p = 0.05$) between participants from AerG and IntG, after intervention, as well as for CSR-R (p = 0.006), CSR-L (p = 0.004) and 30 s-CS (p = 0.018) variables, between participants from the AerG and CG, after intervention. In all identified cases, the results were higher in the AerG. Considering the time effects due to the intervention (M1 and M2), we found significant statistical differences for 2 m-ST in AerG (p = 0.000; Δ = 16.8%) and in the IntG (p = 0.015; Δ = 10.8%). For the BS-R variable, there was a global trend showing a reduction in the performance level in all groups but was just statistically significant for IntG (p = 0.026; Δ = 15.1%). For the 30 s-CS variable, we found a significant average increase in all three aquatic exercise groups: AerG (p = 0.000; Δ = 20.0%), IntG (p = 0.000; Δ = 15.4%) and ComG (p = 0.000; Δ = 23.1%), and an average reduction in the CG. For the 30 s-AC variable, we found an increase in average for all groups, and it was statistically significant in the AerG group (p = 0.010; Δ = 9.5%) and Ignt (p = 0.005; Δ = 11.8%). For the HG variable, we found a statistically significant increase in average in the three aquatic exercise groups, for both the right hand—AerG (p = 0.000; Δ = 27.3%), IntG (p = 0.000; Δ = 23.8%) and ComG (p = 0.000; Δ = 23.8%), and the left hand—AerG (p = 0.000; Δ = 19.0%), IntG (p = 0.000; Δ = 20.0%) and ComG (p = 0.000; Δ = 23.8%), but not for the CG.

Table 5 – Functional fitness results variation analysed by type of aquatic exercise program before and after intervention

	AerG			**IntG**			**ComG**			**CG**				
	M1 Mean (SD)	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**Time x group (M1)**	**Time x group (M2)**
2min-ST (no of steps)	80.9 (17.4)	94.5 (21.2)	0.000**	71.5 (16.5)	79.2 (20.5)	0.015*	81.6 (19.2)	85.4 (22.7)	0.250	74.3 (18.9)	79.4 (21.7)	0.147	0.069	0.048†
CSR-R (cm)	−0.5 (6.6)	1.2 (8.7)	0.182	−3.7 (10.6)	−2.5 (10.5)	0.174	−3.5 (7.8)	−3.3 (8.1)	0.819	−7.6 (9.7)	−7.6 (7.0)	0.954	0.099	0.013†
CSR-L (cm)	0.6 (7.2)	1.1 (8.8)	0.467	−3.9 (9.9)	−2.7 (11.6)	0.190	−5.8 (9.9)	−3.6 (8.6)	0.073	−3.5 (7.3)	−6.8 (8.2)	0.113	0.054	0.039††
BS-R (cm)	−9.9 (10.4)	−11.3 (11.4)	0.387	−11.9 (11.5)	−13.7 (12.8)	0.026**	−14.3 (9.7)	−15.6 (9.7)	0.245	−16.6 (9.9)	−17.1 (10.2)	0.195	0.157	0.361
BS-L (cm)	−14.4 (7.2)	−15.9 (8.8)	0.098	−17.4 (8.6)	−18.1 (12.1)	0.631	−21.0 (10.8)	−21.5 (9.4)	0.369	−20.6 (10.7)	−21.2 (10.1)	0.197	0.056	0.241
TUG (s)	6.1 (1.1)	5.9 (0.9)	0.201	7.4 (1.8)	7.4 (2.2)	0.546	7.4 (3.0)	7.4 (2.9)	0.702	6.8 (1.7)	6.6 (1.9)	0.443	0.110	0.065
30s-CS (reps/30s)	15.0 (3.0)	18.0 (5.0)	0.000*	13.0 (4.0)	15.0 (4.0)	0.000*	13.0 (3.0)	16.0 (4.0)	0.000**	15.0 (5.0)	14.0 (4.0)	0.229	0.185	0.016†
30s-AC (reps/30s)	21.0 (6.0)	23.0 (6.0)	0.010*	17.0 (7.0)	19.0 (6.0)	0.005**	20.0 (5.0)	22.0 (6.0)	0.053	19.0 (6.0)	20.0 (6.0)	0.192	0.119	0.109
HG-R (kg)	22.0 (6.0)	28.0 (6.0)	0.000**	21.0 (9.0)	26.0 (9.0)	0.000**	21.0 (9.0)	26.0 (9.0)	0.000**	24.0 (9.0)	25.0 (11.0)	0.259	0.411	0.317
HG-L (kg)	21.0 (6.0)	25.0 (6.0)	0.000*	20.0 (9.0)	24.0 (9.0)	0.000**	21.0 (9.0)	26.0 (8.0)	0.000**	21.0 (10.0)	22.0 (10.0)	0.175	0.578	0.271

Note: * T-Student test results; ** Wilcoxon test results; †ANOVA and Turkey test results; ††Kruskal Wallis and Bonferroni test results. Correlation is significant at the 0.05 level. Two-minute step test (2min-ST); chair sit and reach test – right (CSR-R); chair sit and reach test – left (CSR-L); back scratch test – right (BS-R); back scratch test – left (BS-L); timed up and go test (TUG); chair stand test (30s-CS); arm curl test (30s-AC); hand grip test – 95apá95 (HG-R); hand grip test – left (HG-L).

Table 6 shows the cognitive results variation, analyzed according to the type of aquatic exercise program, before and after intervention.

Table 6 – Cognitive function results variation analysed by type of aquatic exercise program before and after intervention

	AerG			IntG			ComG			CG				
	M1 Mean (SD)	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**Time x group (M1)**	**Time x group (M2)**
MMSE	27.0 (2.0)	28.0 (2.0)	0.294	27.0 (2.0)	28.0 (2.0)	0.138	26.0 (3.0)	27.0 (2.0)	0.008**	26.0 (3.0)	26.0 (3.0)	0.651	0.489	0.255

Note: * T-Student test results; **Wilcoxon test results. Correlation is significant at the 0.05 level. Mini mental state examination (MMSE).

Regarding the cognitive function variable, no significant statistical differences were found between groups before and after the intervention (see Table 6). However, after the intervention, we found an increase in the MMSE average scores in the three aquatic exercise groups (between M1 and M2), with significant statistical differences in the ComG group ($p = 0.008$; $\Delta = 3.8\%$), but not in the CG.

5. Discussion

The main purpose of the present study was to assess the impact of different aquatic exercise programs on the body composition, functional fitness and cognitive function of non-institutionalized elderly people. A preliminary systematic search revealed the innovative characteristics of the present study, as no other study was identified as assessing similar variables in an aquatic environment with elderly participants.

For body composition, global results revealed that significant statistical differences were found for most of the variables analyzed, as a result of the intervention with aquatic exercise (AerG, IntG and ComG), while no differences were found in the CG. A more detailed analysis of the results revealed significant statistical differences over time (from M1 to M2), i.e., between pre- and post-intervention time moments in the AerG for the FM, LBM and Wcir variables, in IntG for the BMI, FM, LBM and LCIr-R variables, and in the ComG group for the WGT, FM, LBM, Lcir-R and Lcir-L variables. The variance of the mean was higher for the FM variable in the AerG (Δ = −4,6%), for the LBM variable in the IntG (Δ = 3.7%), and for the Lcir-D variable in the ComG (Δ = −2.6%). The increase of the WGT in all aquatic exercise groups may be associated with the significant statistical increase of LBM found in all exercise groups.

A study conducted by [8] that tested the effectiveness of two walking programs at moderate intensity (both aquatic and land environment) on the body composition of elderly participants showed that both programs had beneficial effects on body composition. Like our results, [8] revealed that exercise in an aquatic environment provided a significant reduction in abdominal fat. In spite of there being no significant statistical differences for VF in our study, Wcir was reduced in all aquatic exercise groups, and that reduction was statistically significant in the AerG group. Additionally, in the same study, significant differences were found for LBM in the aquatic walking group. Similar results were found in the present study for all three aquatic exercise groups, with the IntG group obtaining the highest mean variation from all analyzed groups. The study by [8] also found significant differences for the LBM variable in the aquatic walking group. Similar results were found in the three aquatic exercise groups in our study, with the IntG group obtaining the highest mean variation when compared with the other two groups. However, in the study by [8], no significant statistical differences were found for BMI. In our study, the BMI increased in all aquatic exercise groups, and differences were statistically significant in the IntG group.

Another study, conducted by [3], tested the effectiveness of a high-intensity aquatic resistance program in a group of postmenopausal women, before and after 4 months of intervention. Significant statistical differences in FM were reported. The present study also found significant statistical differences for FM in all aquatic exercise groups, with the AerG group reporting the highest mean value.

Previous results show that physical aquatic exercise seems to have a beneficial impact on body composition. Regarding the hypothetical effects of different types of aquatic exercise programs on body composition, functional fitness and cognitive function, results show that they all seem to have a similar positive effect on the FM and BMI variables, but the ComG group also showed significant statistical differences in the Lcir variables, providing additional evidence to confirm that combined aquatic exercise programs may have a more positive effect on body composition.

Global results regarding functional fitness showed significant statistical differences in all aquatic exercise groups (AerG, IntG and ComG), but not in the CG. A more comprehensive analysis of the results showed that significant statistical differences were found between AerG and IntG for the 2 m-ST variable, and between AerG and CG for CSR-R, CSR-L and 30 s-CS variables, as a result of the intervention with aquatic exercise. Additionally, significant statistical differences were also found in the AerG group for 2 m-ST, 30 s-CS, 30 s-AC, HG-R and HG-L variables, in the IntG for 2 m-ST, BS-D, 30 s-CS, 30 s-AC, HG-R and HG-L variables and in ComG for 30 s-CS, HG-R and HG-L variables before and after intervention (M1 versus M2). Delta (Δ) variation of the mean was higher for 2 m-ST (Δ = 16.8%) and HG-D (Δ = 27.3%) variables in the AerG, for 30 s-AC (Δ = 11.8%) variable in the IntG, and for 30 s-CS (Δ = 23.1%) and HG-L (Δ = 23.8%) variables in the ComG group.

A study run by [22] assessed functional fitness in 13 individuals before and after a 12-week aquatic exercise intervention program. Sessions consisted of warm-up exercises on land, walking in the water, aquatic exercises with different materials, and swimming. Results showed significant statistical differences in agility, balance and the muscle strength of the lower limbs. A similar study conducted by [14] analyzed the impact of a 3-month aquatic exercise program with music and found significant statistical differences in upper- and lower-limb muscular strength, flexibility, aerobic capacity agility and dynamic balance. A third study conducted by [23], based on a 24-week high-intensity jumping exercise program, showed improvements in agility, dynamic balance, and lower limb muscular strength. Furthermore, [5] developed a 14-week land aerobic chair-based exercise intervention program and found significant statistical differences for upper-limb muscular strength, agility and dynamic balance. In the same study, a strength-training program using elastic bands showed significant statistical differences for lower-limb strength as a result of the intervention.

As evidenced in the studies above, the results of the present study also showed significant statistical differences in the muscle strength of upper and lower limbs and in hand-grip strength in all three aquatic exercise groups. Regarding aerobic capacity, significant differences were also reported between AerG and IntG, with AerG showing a higher mean variation. On the other hand, no significant statistical differences were found regarding flexibility, agility and dynamic balance. Thus, the present results support the idea that aquatic exercise programs seem to have positive effects on functional fitness, mainly in variables related to upper- and lower-limb strength. In spite of there being no evidence found in the present study, it seems that aquatic exercise programs also tend to have a positive impact on agility and dynamic balance [24,25] in older people. Such results are in agreement with those presented by [5] when comparing elderly institutionalized women participating in a chair-based aerobic exercise walking program, versus a chair-based elastic-band muscle strength program.

Finally, and regarding cognitive function, we found a global trend for positive improvements in cognitive function (i.e., through MMSE instrument) as a result of intervention with aquatic exercise in all exercise groups, but not in the CG. A previous study by [26] tested the efficacy of a water-based exercise program on physical fitness improvement and cognitive function stimulation in adult healthy women. Results showed significant improvements in participants' cognitive function and the mental health domain, regardless of the group in which they were initially included, providing evidence that water-based exercise is capable of enhancing cognitive function and quality of life through improvements in mental health in healthy adult women. Similar results were reported by [9] when testing the efficacy of a 16-week moderate-intensity aquatic exercise program on elderly women´s BDNF levels and cognitive function. Thus, most studies show that aquatic exercise programs are beneficial to cognitive function and that moderate-intensity aerobic exercise seems to have high benefits on elderly people´s cognition levels.

However, the present study also tested the efficacy of a combined aquatic exercise program (aerobic and muscle strength) on elderly non-institutionalized participants. The positive effects of strength-training exercise programs on elderly women´s cognition have previously been reported by [6]. The authors found significant statistical differences in older women´s levels of cognition after their participation in a 28-week elastic-band strength-training intervention program, supporting the idea that strength training is also an effective type of exercise program to be used by groups with cognitive impairment. In our study, combined exercise (aerobic and muscular strength) was responsible for a significant increase in MMSE results ($p = 0.008$) in older participants from M1 to M2, revealing that the combination between the two types of exercise programs may be even more beneficial for improving cognitive function in elderly people.

As regards the limitations of the study, we refer to the fact that it used a simple randomization method, instead of using a blocked randomization method, which would guarantee a balance in the number of participants in each group, reducing sequence unpredictability. Another limitation was in measurements: inter-observer reliability was not measured; however, the evaluators jointly trained the measurers to have the same methods and both evaluators were the same at the evaluation moments (M1 and M2).

Further research is needed, using different types of aquatic exercise programs, to confirm this hypothesis, as the limited number of studies published in the literature regarding aquatic exercise in older people was a limitation when trying to discuss the present results and for clarifying which type of exercise program is more effective in the elderly adult population.

6. Conclusions

Results from the present study provided evidence for the beneficial effects of physical exercise in an aquatic environment on body composition, functional fitness (namely in lower- and upper-limb muscle strength, handgrip strength and aerobic capacity), and cognitive function in non-institutionalized older adults. The present study also revealed that different types of aquatic exercise programs may have different impacts on body composition, functional fitness and cognitive function. ComG was more effective in the improvement of body composition and cognitive function variables, while IntG and AerG were more effective in the improvement of functional fitness.

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Study 4: The impact of aquatic exercise programs on the immunologic profile of community dwelling elderly: A Randomized Controlled Trial

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1. Abstract

Evidence shows that physical exercise is important in maintaining an efficient immune system during ageing. However, there are few studies that test the impact of aquatic exercise programs on the immune system. This study aims to analyze the impact of different physical exercise programs in aquatic environments on the immune profile in community dwelling elderly. One hundred and two elderly were randomly allocated into four groups: a continuous aerobic exercise group (AerG) (n=25, 71.44±4.84 years); an interval aerobic exercise group (IntG) (n=28, 72.64±5.22 years); a combined exercise group (ComG) (n=29, 71.90±5.67 years); a control group (CG) (n=20, 73.60±5.25 years). The AerG, IntG and ComG participants took part in three different aquatic exercise programs over a 28-weeks period. The CG participants maintained their usual routines during the same time period. Blood samples were collected from all participants in order to access hematologic indicators, by means of cell count, and the immune profile by ELISA. After 28 weeks, significant differences were found for several hematologic variables in the AerG, IntG and ComG with increases in mean corpuscular hemoglobulin (MCH), mean corpuscular hemoglobulin concentration (MCHC), and hemoglobulin (Hb). In terms of cytokines, statistically significant differences were found in the AerG with decrease for TNF-α, in the IntG with increase for IL-10 and decrease for TNF-α, TNF-α /IL-10 ratio, and in the ComG with decrease for TNF-α, IL-1ß/IL-1ra ratio. The present study suggests that aquatic exercise programs were able to improve the immune profile of the participants. Those in the exercise intervention groups showed a shift towards a lower pro-inflammatory profile while the non-exercising group showed the opposite behaviour. The IntG and ComG aquatic exercise programs appeared to be more effective than the AerG program in decreasing chronic low-grade inflammation by mediating the production of higher levels of anti-inflammatory cytokines. However, the differences found between the exercising groups were small and may not have clinical significance.

Keywords: physical exercise; aquatic environment; hydrogymnastics; ageing; interleukins; inflammageing.

2. Introduction

The immune system is characterized as a complex network of cells and molecules that operate to protect the body from disease, prevent the entry of invading microorganisms and facilitate wound healing (Simpson et al., 2015).

Ageing is associated with immunosenescence, i.e., a progressive decline in the immune function, which causes changes in innate and adaptive immunity. These changes are related to increased morbidity from infectious causes, leading to the appearance of several age-related diseases, including cardiovascular and metabolic diseases (Sellami et al., 2018). Immunosenescence is characterized by the following aspects: decreased response to new invading infectious agents; unsupported memory T cell response; a higher susceptibility to autoimmune diseases; low-grade chronic inflammation (inflammation) (Rodriguez et al., 2020).

The ageing process is characterized by a decrease in naive T cells and the consequent increase in memory T cells (Weltevrede et al., 2016). Its reduction is considered to be a crucial factor in decreasing the ability to recognize the pathogen, which increases the probability of infection (Spielmann et al., 2011). T cells are subject to continuous remodeling, that results from their interaction with different stressors from the internal and external environment. This interaction leads to the decrease of T cells, which is accompanied by a decline in the T cell receptor clonal diversity and consequent increase in the memory cell subpopulation, with an accumulation of terminally differentiated T cells that are dysfunctional or depleted (Rodriguez et al., 2020).

Regular physical exercise is associated with a reduction of different types of chronic diseases associated with ageing, by means of changes in immune function, specifically in the decreasing of immune senescence, the increasing of the innate immune function and the decreasing of chronic inflammation (Abd El-Kader & Al-Shreef, 2018). In contrast, a sedentary lifestyle leads to accumulation of visceral fat, which is infiltrated by macrophages and pro-inflammatory T cells. This causes pro-inflammatory macrophages to predominate and the inflamed adipose tissue to release adipokines and TNF-α, which leads to a state of systemic inflammation (causing insulin resistance, tumor growth, neurodegeneration and atherosclerosis). In comparison, because physical exercise reduces visceral fat, it will stimulate a decrease in the production and release of adipokines, causing an anti-inflammatory environment during each exercise session (Gleeson et al., 2013). In addition to the reduction of visceral fat, physical exercise, more specifically skeletal muscle contractions, directly stimulates the production of IL6, that will produce anti-inflammatory cytokines, such as IL-10, IL-1ra, and cortisol secretion (Pedersen & Fischer, 2007). A study by Chupel et al. (2017) concluded that a 28-weeks exercise intervention with chair muscular strength exercise program supported the increase of anti-inflammatory balance in a sample of elderly women. Similar results were observed by Furtado et al. (2020), after an intervention with two different physical exercise programs (a combined exercise program, with muscle strength exercise using elastic bands and a chair-based combined exercise). In another study by Werner et al. (2019), the results showed an increase in leukocytes, granulocytes and lymphocytes after a 26-weeks intervention with aerobic exercise and interval aerobic exercise. Contrastingly, the muscle strength exercise did not produce the same increase. However, Kapasi et al. (2003) concluded that an 8-month intervention with combined exercise (aerobic exercise and muscle strength exercise) did not induce changes in lymphocyte subpopulations. Likewise, Shimizu´s et al. (2011) study showed that the number of leukocytes, lymphocytes and monocytes did not change after a 12-weeks muscle strength intervention program.

The studies mentioned above have shown that physical exercise can be a powerful tool to improve the immune profile of elderly participants. However, it is still not clear which type of exercise is the most effective. Additionally, few studies have analyzed the impact of physical exercise programs within these indicators, in an aquatic environment (Bansi et al., 2013; Santos, 2020). Considering the limitations previously mentioned, the purpose of this study is to compare the impact of different physical exercise aquatic programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) on the immunologic profiles of community dwelling elderly people, by determining which of the three aquatic exercise programs used was the most effective.

3. Materials and methods

3.1. Study design

A randomized controlled trial was conducted in the Beira Interior region, Portugal. A sample of community dwelling elderly participants were submitted to 28-weeks aquatic exercise intervention (October-May). The entire study protocol was previously published (Ferreira et al., 2020). To analyzed the impact of different aquatic exercise programs on the immunologic profile of elderly participants, three different physical exercise programs were used: continuous aerobic (AerG), interval aerobic exercise (IntG) and combined exercise (ComG). Blood samples were collected at two specific time moments, namely: pre-intervention (baseline, M1) and post-intervention (after 28 weeks, M2). This study was carried out according to the recommendations of the Declaration of Helsinki for Human Studies. The protocol was approved by the Ethics Committee for Health of the Faculty of Sport Sciences and Physical Education, University of Coimbra (reference: CE/FCDEF-UC/00462019). Written informed consent was obtained from all participants prior to any protocol-specific procedures.

3.2. Participants and sample size

The size and statistical power of the sample were calculated using the G*Power software application (Franz et al., 2007). The following parameters were considered: F test (ANOVA); effect size: 0.25; α-level: 0.05; statistical power: 0.95; number of groups: 4; number of measures: 2 (pre and post intervention); margin of possible losses and refusals: 30%. Therefore, the initial size of the total sample was estimated at 76 participants.

Initially, 174 individuals from the community were invited to participate in the study. After the application of the inclusion and exclusion criteria, 152 individuals were randomized into four groups: continuous aerobic exercise group (AerG, n=36); interval aerobic exercise group (IntG, n=41); combined exercise group (ComG, n=48); control group (CG, n=27). According to the experience of the research team and previous studies, the dropout rate from exercise programs among elderly population is high, so we gathered more participants to fulfil the sample size and compensate potential dropouts (Chupel et al., 2017; Dziubek et al., 2015; Moreira et al., 2020). A simple randomization method was used. An external investigator used a computer-generated list of random numbers to allocate participants to each group. The investigators were blinded for the randomization of the groups.

The following inclusion criteria were applied: a) participants from both sexes; b) 65 years old or more; c) community dwelling; d) autonomy to move from their residence to Sertã municipal swimming pool; e) filling out the informed consent form; f) individuals with medical authorization to practice physical exercise in an aquatic environment, in cases they had some type of clinical condition or comorbidity. The following exclusion criteria were also defined: a) individuals with clinically diagnosed pathologies putting their health and others at risk while doing physical exercise in an aquatic environment; b) individuals that obtained a score of less than 9 points in the Mini-Mental States Examination (indicating severe cognitive impairment) or were clinically diagnosed with a mental illness.

The AerG, IntG and ComG groups performed physical exercise in an aquatic environment during the same period of 28 weeks (each group took part in a different exercise program). Participants from the CG group were asked to maintain their normal daily activities, without performing any type of systematic physical exercise during the same time period.

Fifty participants dropped or were excluded out from the study due to the following reasons: personal reasons (n=11); less than 50% of attendance of the exercise sessions (n=14); did not complete all the assessment tests (n=12); injury not related with the exercise intervention (n=4); disease (n=9). Consequently, 102 participants completed the entire process (AerG: n=25, 71.44±4.84 years, 80% female; IntG: n=28, 72.64±5.22 years, 89.3% female; ComG: n=29, 71.90±5.67 years, 75.9% female; CG: n=20, 73.60±5.25 years, 55% of females). Figure 7 shows the entire allocation process for the different groups.

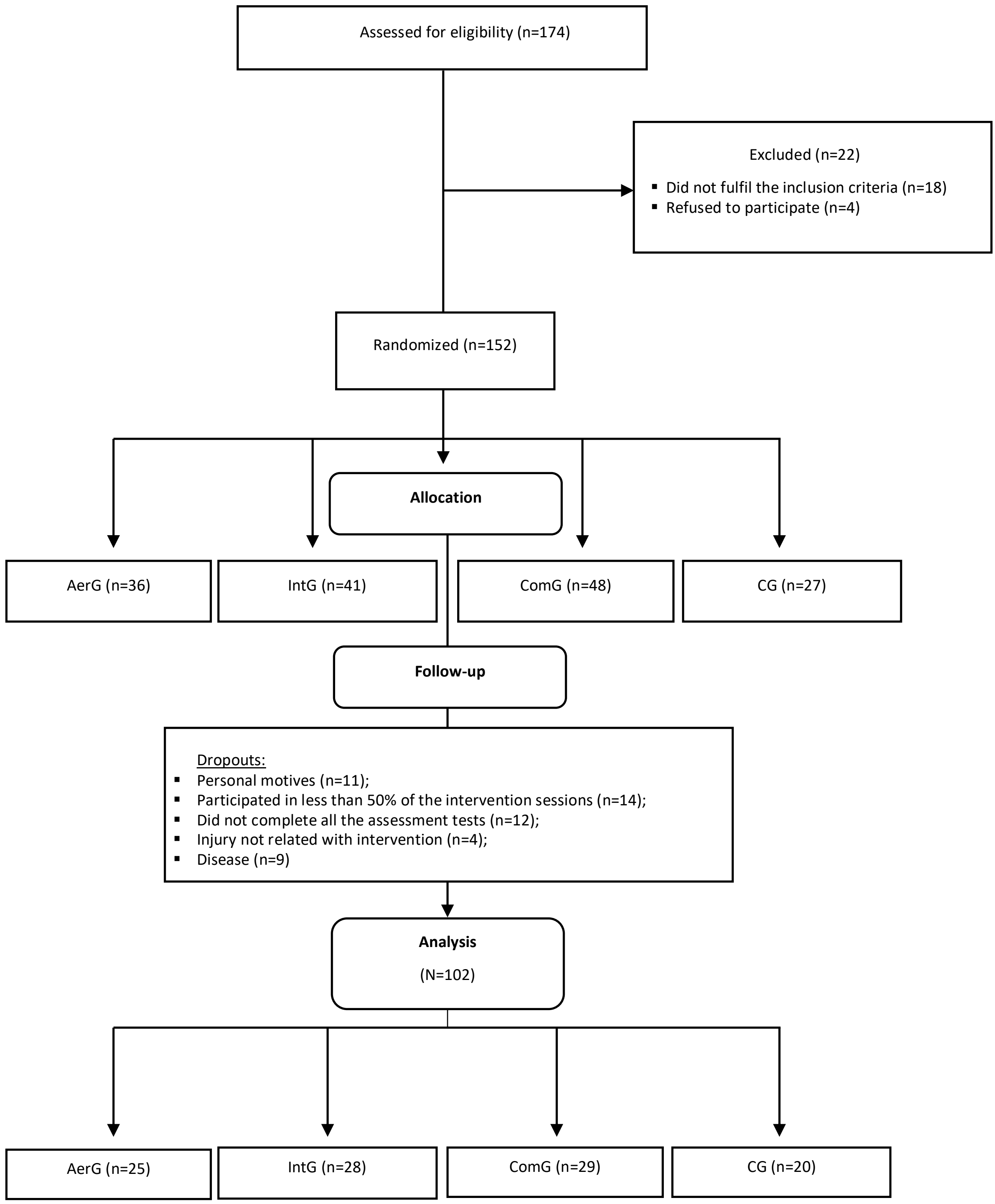

***Figure* 7** *- Allocation process for the different groups: Continuous aerobic group (AerG); Aerobic interval group (IntG); Combined group (ComG); Control group (CG).*

3.3. Outcomes measurements

3.3.1. Sample characterization

Data such as age, regular medication, allergies, alcohol consumption, smoking and drugs habits were also obtained in the first phase of data collection (M1) by completing a specific questionnaire. Values referring to anthropometry and functional fitness were also collected. Height (Hgt) was assessed using a portable Seca Bodymeter® stadiometer (model 208, Hamburg, Germany) with an accuracy of 0.1 cm. Weight (Wgt), body mass index (BMI), visceral fat (VF), fat mass (FM) and lean body mass (LBM) were evaluated using the TANITA BC-601 impedance scale (Tokyo, Japan). Functional fitness was assessed using the following tests from the Senior Fitness Test set, developed by Rikli and Jones (1999) and validated for the Portuguese population by Baptista and Sardinha (2005): muscle strength of the lower (MI) and upper (MS) limbs, with the Chair Stand test (30s-CS) and Arm Curl test (30s-AC), respectively (repetitions/30s); aerobic capacity, with the Two-Minute Step test (2min-ST) (no. of steps); the flexibility of MI and MS, with the Chair Sit and Reach test (CSR) and Back Scratch test (BS), respectively (centimeters); agility and dynamic balance, with the Timed Up and Go test (TUG) (seconds). The handgrip strength was also evaluated, with the Hang Grip test (HG), using the Jamar hand dynamometer (Lafayette Instrument Company, USA) (kg). Data are presented in table 1.

3.3.2. Biochemical Markers (interleukins and immune profile)

Participants were instructed to avoid to practice exercise physical 72 hours before blood collection. Fasting Blood samples (15ml) were collected from the ante cubital vein by a registered nurse. A complete blood count (CBC) was conducted using an automatic hematology analyzer (Coulter Act, Beckman Coulter, USA) to obtain the values for: leukocyte (LEU); lymphocytes (LI); monocytes (MO); granulocytes (GR); erythrocytes (ERI); hemoglobulin concentration (Hb); hematocrit (Hct); mean corpuscular volume (MCV); mean globular hemoglobulin (MCH); mean corpuscular hemoglobulin concentration (MCHC); erythrocyte distribution width (RDW); platelets (PL); mean platelet volume (MPV); procalcitonin (Pct); adenosine diphosphate (ADP). Next, the test tubes were centrifuged for 10 min at 3500 rotations per minute and stored in cryogenic test tubes at -80°C until further use. Levels of interleukin 1 receptor antagonist (IL-1ra), interleukin 1 beta (IL-1ß), interleukin 10 (IL-10) and tumor necrosis factor (TNF-α), were subsequently analyzed by ELISA (Invitrogen®, Alfagene, Portugal) according to the manufacturer instructions.

3.4. Intervention protocol

The exercise programs were implemented by Sport Sciences and fitness experts, with specific training in water aerobics and developed according to the exercise prescription guidelines recommended by the American College of Sport Medicine (ACSM) for the elderly (ACSM, 2018).

All exercise programs sessions had a duration of 45 min, twice a week, for 28 weeks and were performed in water environment (the water level was between 0.80 and 1.20 m with a temperature of approximately 32°C), using the music rhythm as a tool to control the intensity of the exercise. Sequences of aquatic exercises, previously defined and selected according to the objectives of each program, were applied. Water exercise sessions were organized into three different parts: initial, main and final part.

The initial part or warm-up last between 10 and 15 min, at low intensity (30-40% max HR), and were the same in the three water exercise programs. During this initial part, the aim was that participants will make an adaptation to the aquatic environment, i.e., to the water temperature, and provide muscular and metabolic stimulations to prepare the body for the main part of the session. Thus, simple exercises in water were used, such as displacements and isolated movements, with a progressive increase in complexity and intensity throughout the initial part.

The main part for each one of the three water exercise program sessions had a duration of 20 to 30 min and all were characterized as follows:

- In the water continuous aerobic program (AerG), aerobic exercises were used continuously throughout the main part of the session (20 to 30 min), with a target intensity of 60 to 70% of the maximum heart rate, according to the recommendations of the ACSM for the elderly (ACSM, 2018);

- In the water interval aerobic program (IntG), the main part of the sessions consisted of performing exercises with different intensities, such as: short duration exercises of 30 seconds, with an intensity of 70-80% of the maximal heart rate, followed by active recovery intervals of 1 min, using exercises with an intensity of 60-70% of the maximal heart rate;

- In the water combined training program (ComG), the main part of the session was divided into two phases, with equal time periods. The first phase consisted of aerobic exercises on a continuous basis, with a target intensity between 60 and 70% of the maximal heart rate. In the second phase, muscle strengthening exercises were applied (water environment): 6 to 8 different exercises, with auxiliary equipment to create more resistance to movement (e.g., “spaghetti” and dumbbells), covering muscle strengthening from trunk (e.g., rowing, inverted crunch, etc.), upper limbs (e.g., elbow flexion and extension, shoulder rotation, etc.) and lower limbs (e.g., knee flexion and extension, leg abduction and adduction, etc.). Strengthening exercise were implemented using 2 to 3 sets of 12 to 16 repetitions at moderate intensity (6-7 points in the Borg Scale).

The final part of the water exercise sessions lasted between 5 and 10 min and were the same for each of the three water exercise programs. This part consisted of two phases: return to calm, where relaxation exercises were applied to resume the participants' heart rate to values close to the resting state, and stretching exercises stimulating a greater range of motion, used to stretch the main muscle groups used throughout the sessions.

3.5. Monitoring the exercise programs intensity

For safety and intensity target control reasons, all participants randomly used heart rate monitors (Polar, R800CX) during the exercise sessions, in all three water exercise programs. Per session was used 10 monitors heart rate. Depending on the heart rate values obtained, intensity adjustments to the training plan were performed to maintain the intensity target defined for each water exercise program.

For safety and intensity target control reasons, the intensity of the different water exercise programs was predicted indirectly using the Karvonen´s formula (Karvonen et al., 1957):

Target heart rate = ((maximal heart rate - resting heart rate) × % intensity) + resting heart rate.

Additionally, and to calculate the maximal heart rate, we used the Franklin et al. (2000) formula for the elderly:

Maximal HR = 207 beats per minute - (0.7 × chronological age)

3.6. Statistical analysis

The collected data was subjected to descriptive statistical analysis where values such as maximum, minimum, mean and standard deviation were calculated for each variable in each assessment moment. Afterwards, data normality was tested by considering the response to three conditions: z-values from the Skewness and Kurtosis tests; p-values from the Shapiro-Wilk test; and visual inspection of generated histograms. All longitudinal comparisons were performed using complete case analysis. Parametric data will be analyzed using the T-Student test for independent samples to compare the different moments (M1 and M2) and the one-factor Anova test and post-hoc Tukey's test to analyze the differences between groups both at M1 and M2. Nonparametric data was analyzed using Wilcoxon test to compare the different moments (M1 and M2) and the Kruskal Wallis and Bonferroni tests to analyze differences between groups. Associations between variables were performed using Pearson's correlation coefficient values and interpreted according to Cohen (1988): $r=0.10$ to 0.29 means weak correlation; $r=0.30$ to 0.49 means moderate correlation; and $r=0.50$ to 1.0 strong correlation. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical software, version 27.0. The level of significance used was $p \leq 0.05$.

4. Results

The results from 102 community dwelling elderly participants at baseline, distributed in four different groups (AerG: n=25, 71.44±4.84 years; IntG: n=28, 72.64±5.22 years; ComG: n=29, 71.90± 5.67 years; CG: n=20, 73.60±5.25 years) that completed a 28-weeks intervention are shown in table 7.

Table 7 - Participant´s baseline characteristics by group.

	AerG (n=25) Mean (SD)	IntG (n=28) Mean (SD)	ComG (n=29) Mean (SD)	CG (n=20) Mean (SD)	p-value
Morphological parameters					
Age (years)	71.44 (4.8)	72.64 (5.2)	71.90 (5.7)	73.60 (5.3)	0.504
Stature (m)	1.58 (0.7)	1.56 (0.7)	1.57 (0.7)	1.60 (0.9)	0.331
Body mass (kg)	70.5 (8.1)	71.3 (14.3)	75.1 (11.0)	75.5 (13.3)	0.334
BMI (kg/m²)	28.20 (3.3)	29.10 (4.8)	30.80 (5.3)	29.50 (5.8)	0.272
VF (%)	11.0 (3.0)	12.0 (3.0)	13.0 (3.0)	13.0 (6.0)	0.128
FM (%)	38.9 (7.3)	41.0 (6,7)	40.3 (9.8)	34.9 (10.9)	0.134
LBM (%)	26.5 (4.3)	24.5 (3.0)	25.5 (4.3)	27.7 (4.7)	0.079
Physical fitness tests					
2min-ST (no of steps)	80.9 (17.4)	71.5 (16.5)	81.6 (19.2)	74.3 (18.9)	0.069
CSR-R (cm)	-0.5 (6.6)	-3.7 (10.6)	-3.5 (7.8)	-7.6 (9.7)	0.099
CSR-L (cm)	0.6 (7.2)	-3.9 (9.9)	-5.8 (9.9)	-3.5 (7.3)	0.054
BS-R (cm)	-9.9 (10.4)	-11.9 (11.5)	-14.3 (9.7)	-16.6 (9.9)	0.157
BS-L (cm)	-14.4 (7.2)	-17.4 (8.6)	-21.0 (10.8)	-20.6 (10.7)	0.056
TUG (s)	6.1 (1.1)	7.4 (1.8)	7.4 (3.0)	6.8 (1.7)	0.110
30s-CS (reps/30s)	15.0 (3.0)	13.0 (4.0)	13.0 (3.0)	15.0 (5.0)	0.185
30s-AC (reps/30s)	21.0 (6.0)	17.0 (7.0)	20.0 (5.0)	19.0 (6.0)	0.119
HG-R (kg)	22.0 (6.0)	21.0 (9.0)	21.0 (9.0)	24.0 (9.0)	0.411
HG-L (kg)	21.0 (6.0)	20.0 (9.0)	21.0 (9.0)	21.0 (10.0)	0.578
Health parameters	**N (%)**	**N (%)**	**N (%)**	**N (%)**	
Regular medication	23 (92%)	27 (96%)	26 (90%)	19 (95%)	0.768
No alcohol and smoking habits	22 (88%)	25 (89%)	26 (90%)	19 (95%)	0.772
Allergies	10 (40%)	9 (32%)	14 (48%)	7 (35%)	0.638

Note: : Standard deviation (SD); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG); height (Hgt); weight (Wgt); body mass index (BMI); visceral fat (VF); fat mass (FM); lean body mass (LBM); two-minute step test (2min-ST); chair sit and reach test - right (CSR-R); chair sit and reach test - left (CSR-L); back scratch test – right (BS-R); back scratch test – left (BS-L); timed up and go test (TUG); chair stand test (30s-CS); hand grip test – right (HG-R); hand grip test – left (HG-L). Regular medications include: medication to control blood pressure, cholesterol and diabetes.

Participants from all groups (AerG, IntG, ComG and CG) showed similar characteristics at baseline, with no significant statistical differences in anthropometrics, physical fitness or clinical characterization variables regarding each group.

The variation in the results of the hematologic profile when analyzed by type of aquatic exercise program, before and after the 28-weeks intervention, are shown in table 8.

Table 8 - Results of hematological variables at baseline and after 28 weeks of intervention.

	AerG			IntG			ComG			CG				
	M1 Mean (SD)	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**Group (M1)**	**Group (M2)**
LEU (x10^3/uL)	5.99 (1.17)	6.04 (1.26)	0.838	5.85 (1.22)	6.34 (1.26)	0.006*	6.37 (1.37)	6.65 (1.51)	0.044**	5.70 (1.30)	5.95 (1.44)	0.183	0.269	0.335
LI (%)	35.67 (7.57)	36.28 (8.38)	0.657	34.09 (7.54)	32.76 (9.06)	0.158	35.57 (8.19)	34.00 (8.75)	0.140	37.00 (8.23)	35.17 (7.95)	0.108	0.546	0.444
MO (%)	5.76 (2.16)	5.88 (1.80)	0.844	6.39 (2.38)	6.29 (2.06)	0.809	6.57 (1.95)	6.21 (2.25)	0.542	5.99 (2.01)	5.74 (1.79)	0.665	0.505	0.741
GR (%)	58.58 (8.11)	57.84 (8.44)	0.575	59.51 (8.45)	61.23 (9.50)	0.094	57.86 (9.12)	59.79 (8.81)	0.123	57.01 (8.97)	59.23 (9.42)	0.098	0.604	0.396
LI# (x10^3/uL)	2.13 (0.63)	2.17 (0.55)	0.707	2.00 (0.59)	2.07 (0.66)	0.213	2.26 (0.65)	2.22 (0.66)	0.554	2.06 (0.45)	2.04 (0.49)	0.741	0.529	0.676
MO# (x10^3/uL)	0.34 (0.14)	0.36 (0.14)	0.503	0.37 (0.16)	0.39 (0.17)	0.388	0.43 (0.16)	0.41 (0.16)	0.503	0.33 (0.13)	0.27 (0.12)	0.078	0.152	0.013††
GR# (x10^3/uL)	3.51 (0.86)	3.52 (1.03)	0.920	3.47 (0.87)	3.89 (1.04)	0.006*	3.71 (1.06)	4.02 (1.27)	0.046**	3.32 (1.04)	3.50 (1.16)	0.236	0.822	0.259
ERI (x10^6/uL)	4.63 (0.30)	4.53 (0.33)	0.032**	4.59 (0.40)	4.46 (0.32)	0.001**	4.64 (0.27)	4.53 (0.26)	0.003*	4.66 (0.42)	4.62 (0.55)	0.706	0.852	0.611
Hb (g/dL)	13.15 (0.80)	13.23 (0.71)	0.591	13.12 (1.07)	13.27 (0.90)	0.153	13.09 (0.77)	13.38 (1.08)	0.038**	13.22 (0.62)	13.45 (1.04)	0.285	0.793	0.852
Hct (%)	41.66 (2.19)	40.42 (2.21)	0.006*	41.65 (3.03)	40.00 (2.54)	0.000*	42.02 (2.50)	40.75 (2.56)	0.001**	42.20 (2.08)	41.91 (2.72)	0.555	0.897	0.073
MCV (fL)	90.22 (4.24)	89.34 (4.07)	0.041*	90.82 (3.81)	89.97 (3.86)	0.003*	90.75 (3.79)	89.92 (3.84)	0.006*	91.00 (5.51)	90.53 (5.03)	0.068	0.932	0.822
MCH (pg)	28.46 (1.44)	29.24 (1.60)	0.001*	28.60 (1.33)	29.86 (1.47)	0.000*	28.29 (1.43)	29.53 (1.72)	0.000*	28.54 (1.77)	29.19 (2.10)	0.092	0.868	0.477
MCHC (g/dL)	31.54 (0.84)	32.73 (0.77)	0.000**	31.50 (0.71)	33.20 (0.73)	0.000**	31.15 (0.64)	32.83 (1.13)	0.000**	31.37 (0.51)	32.09 (1.45)	0.079	0.155	0.044††
RDW (%)	12.86 (0.53)	12.73 (0.49)	0.109	12.88 (0.56)	12.71 (0.72)	0.158	13.23 (0.81)	12.89 (0.78)	0.005*	12.85 (0.50)	12.69 (0.58)	0.202	0.078	0.681
PL (x10^3/uL)	197.44 (53.91)	198.64 (63.90)	0.467	191.75 (47.50)	187.64 (46.18)	0.190	212.00 (39.28)	206.34 (42.46)	0.347	182.10 (38.28)	173.55 (38.57)	0.079	0.134	0.056
MPV (fL)	8.72 (1.03)	8.46 (0.83)	0.017*	8.70 (0.91)	8.48 (0.84)	0.002*	9.02 (0.91)	8.76 (0.75)	0.000**	8.42 (0.81)	8.28 (0.75)	0.058	0.149	0.154
Pct (%)	0.17 (0.04)	0.16 (0.04)	0.135	0.16 (0.03)	0.19 (0.15)	0.163	0.19 (0.04)	0.18 (0.04)	0.030**	0.15 (0.03)	0.15 (0.03)	0.130	0.008**	0.004††
ADP (%)	16.12 (0.45)	15.89 (0.42)	0.008*	16.09 (0.54)	15.97 (0.39)	0.237	16.09 (0.49)	15.98 (0.50)	0.296	16.17 (0.49)	16.26 (0.43)	0.403	0.928	0.042†

Note: Standard deviation (SD); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG); Leukocytes (LEU); lymphocytes - percentage (LI); monocytes – percentage (MO); granulocytes – percentage (GR); lymphocytes – gross value (LI#); monocytes – gross value (MO#); granulocytes – gross value (GR#); erythrocytes (ERI); hemoglobulin concentration (Hb); hematocrit (Hct); mean corpuscular volume (MCV); mean globular hemoglobulin (MCH); mean corpuscular hemoglobulin concentration (MCHC); erythrocytes distribution width (RDW); platelets (PL); mean platelet volume (MPV); procalcitonin (Pct); adenosine diphosphate (ADP). *Result obtained through T-Student test; **result obtained through Wilcoxon test; †results obtained through ANOVA e Tukey test; ††results obtained through Kruskal Wallis e Bonferroni test.

The general hematologic results revealed that significant differences were found between groups, before the intervention (M1), in Pct (p=0.008) between the ComG participants and the CG participants (p=0.001), as well in the IntG and the ComG (p=0.016). In both cases, the results were higher in the ComG. Significant differences also were found between group, after the intervention (M2), in MO# (p=0.013) between the CG and the IntG participants (p=0.007), as well in the CG and the ComG (p=0.002), and in the CG and the AerG (0.033). In the same way, we also found significant differences in M2 for MCHC (p=0.044) between the CG and the IntG participants (p=0.007), in Pct (p=0.004) between the CG and the IntG (p=0.039), and the CG and the ComG (p<0.001), in ADP (p=0.042) between the AerG and the CG (p=0.008), the ComG and the CG (p=0.019), and the IntG and the CG (p=0.038). In all identified cases, the results were lower in the CG, except for the ADP variable.

As a result of the intervention with aquatic exercise programs, statistically significant increase were found in the following variables: LEU in the IntG (p=0.006; Δ=8.4%) and ComG (p=0.044; Δ=4.4%); GR# in the IntG (p=0.006; Δ=12.1%) and ComG (p=0.046; Δ=8.4%); MCH in the AerG (p=0.001; Δ=2.7%), IntG (p<0.001; Δ=4.4%) and ComG (p<0.001; Δ=4.4%); MCHC in the AerG (p<0.001; Δ=3.8%), IntG (p<0.001; Δ=5.4%) and ComG (p<0.001; Δ=5.4%); and Hb in the ComG (p=0.038; Δ=2.2%).

Statistically significant reductions were also found in the following variables, as a result of the intervention with aquatic exercise programs: ERI in the AerG (p=0.032; Δ=-2.2%), IntG (p=0.001; Δ=-2.8%) and ComG (p=0.003; Δ=-2.4%); Hct in the AerG (p=0.006; Δ=-3.0%), IntG (p<0.001; Δ=-4.0%) and ComG (p =0.001; Δ=-3.0%); MCV in the AerG (p=0.041; Δ=-1.0%), IntG (p=0.003; Δ=-0.9%) and ComG (p=0.006; Δ=-0.9%); MPV in the AerG (p=0.017; Δ=-3.0%), IntG (p=0.002; Δ=-2.5%) and ComG (p<0.001; Δ=-2.9%); RDW in the ComG (p=0.005; Δ=-2.6%); Pct in the ComG (p=0.030; Δ= -5.8%); and ADP in the AerG (p=0.008; Δ=-1.4%).

The variation in the results of the cytokines analyzed by each type of aquatic exercise program, before and after the intervention, are shown in table 9.

Table 9 - Results of immune variables at baseline and after 28 weeks of intervention.

	AerG			IntG			ComG			CG				
	M1 Mean (SD)	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**M1 Mean (SD)**	**M2 Mean (SD)**	**Time (p)**	**Group (M1)**	**Group (M2)**
IL-10 (pg/ml)	18.45 (14.08)	17.67 (15.30)	0.514	28.55 (10.13)	36.70 (6.85)	0.000**	28.15 (14.12)	24.69 (10.04)	0.127	20.32 (12.87)	16.87 (9.10)	0.054	0.005††	0.000††
IL-1ra (pg/ml)	76.93 (51.79)	81.28 (58.52)	0.757	89.30 (56.68)	92.98 (69.54)	0.585	104.69 (63.82)	115.71 (67.19)	0.157	121.77 (83.56)	88.31 (59.96)	0.008**	0.179	0.220
IL-1ß (pg/ml)	30.05 (3.75)	29.50 (3.31)	0.443	35.04 (16.67)	33.25 (17.15)	0.117	27.98 (10.16)	27.00 (14.27)	0.064	25.39 (4.04)	26.49 (4.24)	0.043*	0.010††	0.007††
TNF-α (pg/ml)	37.38 (21.07)	28.65 (21.72)	0.011**	17.65 (11.13)	14.27 (9.63)	0.001**	69.01 (28.82)	52.89 (25.25)	0.005*	31.98 (16.75)	32.59 (18.96)	0.936	0.000††	0.000††
TNF-α/IL-10 ratio	5.00 (6.25)	3.85 (5.66)	0.056	0.89 (1.07)	0.45 (0.52)	0.000**	2.55 (0.75)	2.23 (0.93)	0.125	3.18 (4.16)	3.18 (3.13)	0.351	0.000††	0.000††
IL-1ß /IL-1ra ratio	0.63 (0.45)	0.75 (0.79)	0.657	0.67 (0.60)	1.01 (1.98)	0.982	0.42 (0.40)	0.36 (0.32)	0.047**	0.35 (0.33)	0.50 (0.38)	0.053	0.025††	0.097

Note: Standard deviation (SD); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG); Interleukin 10 (IL-10); interleukin 1 (IL-1ra); interleukin 1 beta (IL-1ß); tumor necrosis factor (TNF-α); racio entre tumor necrosis factor e Interleukin 10 (TNF-α/IL-10); rácio entre. interleukin 1 beta e interleukin 1 (IL-1β/IL-1ra) . *Result obtained through T-Student test; **result obtained through Wilcoxon test; †results obtained through ANOVA e Tukey test; ††results obtained through Kruskal Wallis e Bonferroni test.

Before the intervention (baseline) statistically significant differences were found between groups in the following cytokines: IL-10 (between the AerG and ComG ($p=0.011$), the AerG and IntG ($p=0.002$), and the IntG and CG ($p=0.022$)); IL-1ß (between the CG and AerG ($p=0.021$), the CG and IntG ($p=0.007$), the ComG and AerG ($p=0.045$) and the ComG and IntG ($p=0.015$)); TNF-α (between the IntG and CG ($p=0.004$), the IntG and AerG ($p<0.001$), the IntG and ComG ($p<0.001$), the CombG and CG ($p<0.001$), and the ComG and AerG ($p=0.001$)); TNF-α /IL-10 (between the IntG and CG ($p=0.001$), the IntG and AerG ($p<0.001$), and IntG and the ComG ($p<0.001$); IL-1ß /IL-1ra (between the CG and IntG ($p=0.029$), the CG and AerG ($p=0.011$), and the ComG and AerG ($p=0.035$)).

In the identified cases, the results were higher in the IntG and the ComG for IL-10, lower in the ComG and the CG for IL-1ß, lower in the IntG for TNF-α and the TNF-α /IL-10, and lower in the CG and the ComG for IL-1ß /IL-1ra.

After the intervention statistically significant differences were also found between groups in the following variables: IL-10 (between the AerG and ComG ($p=0.017$), the AerG and IntG ($p<0.001$), the CG and ComG ($p=0.042$), the CG and IntG ($p<0.001$) and the ComG and IntG ($p<0.001$)); IL-1ß (between the ComG and IntG ($p=0.001$), and the ComG and AerG ($p=0.002$)); TNF-α (between the IntG and AerG ($p=0.001$), the IntG and CG ($p<0.001$), the ComG and IntG ($p<0.001$), the ComG and AerG ($p=0.001$), and the ComG and CG ($p=0.036$)); TNF-α /IL-10 (between the IntG and AerG ($p<0.001$), the IntG and ComG ($p<0.001$), and the IntG and CG ($p<0.001$). In the identified cases, the results were higher in the IntG for IL-10 and IL-1ß/IL-1ra, lower in the ComG for IL-1ß, and lower in the IntG for TNF-α.

Due to the intervention with aquatic exercise programs, the IL-10 variable showed tendencies to decrease in all groups, except for the IntG, which showed a significant statistical increase ($p<0.01$; $\Delta=28.5\%$). The IL-1ra variable showed tendencies to increase, in all exercise groups, but not in the CG, which showed a significant decrease ($p=0.008$; $\Delta=-27.5\%$). For the IL-1ß variable, a tendency to decrease was found in all aquatic exercise groups, but not in the CG, where there was a significant increase ($p=0.043$; $\Delta=4.3\%$). In the TNF-α variable showed significant decrease in all exercise groups, due to the intervention, AerG ($p=0.011$; $\Delta=-23.4\%$), the IntG ($p=0.001$; $\Delta=-19.1\%$) and the ComG ($p=0.005$; $\Delta=-23.4\%$), but not in the CG. The results also showed a significant decrease in

TNF-α/IL-10 in IntG (p<0.001; Δ=-49.3%), as well as for IL-1ß/IL-1ra in the ComG (p=0.047; Δ=-13 .5%).

The relationships between the hematologic, immune and anthropometric variables, at both evaluation phases (M1 and M2) are presented in table 10.

Table 10 - Relationships between the hematologic, immune and anthropometric variables at baseline and after 28 weeks of intervention.

		VF		Wcir		IL-10		IL-1ß	
		M1	**M2**	**M1**	**M2**	**M1**	**M2**	**M1**	**M2**
LEU	Pearson correlation	0.199	0.217	0.293	0.273	0.242	0.226	0.136	0.098
	Sig.	0.045*	0.028*	0.003*	0.006*	0.014*	0.022*	0.172	0.325
ADP	Pearson correlation	0.235	0.214	0.138	0.200	0.18	0.011	0.142	0.117
	Sig.	0.018*	0.031*	0.167	0.044	0.07	0.909	0.155	0.243
GR	Pearson correlation	0.167	0.204	0.230	0.257	0.221	0.226	0.128	-0.011
	Sig.	0.093	0.04	0.02*	0.009*	0.025*	0.022*	0.199	0.912
PL	Pearson correlation	-0.085	-0.077	-0.11	-0.124	-0.098	0.057	-0.228	-0.344
	Sig.	0.394	0.441	0.269	0.213	0.329	0.571	0.021*	0.000*

Note: Leukocytes (LEU); adenosine diphosphate (ADP); granulocytes (GR); platelets (PL); visceral fat (VF); waist circumference (Wcir); interleukin 10 (IL-10); interleukin 1 beta (IL-1 ß). *Result obtained through Person's correlation test.

Pearson´s r results showed weak and positive correlations, in M1 and M2, between VF and LEU (r=0.22), VF and ADP (r=0.21), WCir and LEU (r=0.27), WCir and GR (r=0.26), IL-10 and LEU (r=0.23), and IL-10 and GR (r=0.23). However, results also showed a moderate and negative correlation between IL-1ß and PL (r=-0.34).

5. Discussion

The purpose of the present study was to evaluate the impact of different aquatic exercise programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) on the hematologic and immune profiles in community dwelling elderly. A preliminary systematic research revealed the innovative characteristics of the present study, since there were very few studies that had evaluated similar immune variables, in exercise performed in aquatic environment, with elderly.

Results from the hematologic variables revealed significant statistical differences in most of the variables analyzed, due to the intervention with aquatic physical exercise (AerG, IntG and ComG), but no significant differences were found in the CG. A more detailed analysis of the results revealed significant differences over time (from M1 to M2), i.e., between pre- and post-intervention, in the AerG for ERI, Hct, MCV, MCH, MCHC, MPV and ADP, in IntG for LEU, GR, ERI, Hct, MCV, MCH, MCHC and MPV, and in ComG for LEU, GR, ERI, Hb, Hct, MCV, MCH, MCHC, RDW, MPV and Pct. The variance of the mean was higher for the variable MCHC in the AerG (Δ=3.8%), for the variable GR in the IntG (Δ=12.1%), and in the ComG (Δ=8.4%).

A study carried out by Chupel et al. (2017), that tested the effectiveness of a 28 weeks land-based muscle strength exercise program using elastic bands, in elderly women, showed that exercise intervention caused changes in hematologic variables, specifically significant increases in Hb, MCV, MCH and MCHC, and significant reductions in the LEU and LI variables. In the Hct and ERI variables, no significant statistical differences were found. Similar results had already been reported in Johannsen´s et al. (2012) study using a 6-months intervention with aerobic exercise, that promoted significant reductions in the LEU and LI variables. Another recent study by Santos (2020) tested the effectiveness of an aquatic exercise program in elderly women for one year, and found a reduction in MCH, MCHC and PL, and an increase in Hct. However, those differences were not statistically significant. The Hb values remained unchanged.

The present study showed similar results to those reported by Chupel et al. (2017) in the three exercise groups (AerG, IntG and ComG), with significant increases in MCH and MCHC. The IntG and ComG groups showed a higher variance of the mean in both variables. For Hb, results showed tendencies to increase in the three exercise groups, but statistically significant only in the ComG group.

In opposition to the results found in Chupel et al. (2017) study, present results revealed a significant reduction of MCV in the three exercise groups, with the highest variation of the mean being registered in the AerG, and an increase in the number of leucocytes in the IntG and ComG groups. The IntG registered the highest variation of the mean. Regarding the Hct and ERI variables, our results showed a statistically significant reduction in all aquatic exercise groups, with the IntG registering a higher variation of the mean in both cases, in opposition to the results obtained by Chupel et al. (2017). However, in Chupel's study the participants were much older and frail.

Present results clearly reveal a positive variation regarding hematologic health, with high levels of MCV and ERI variables being associated with a higher risk of death from all causes, especially cardiovascular and infectious diseases (Dratch et al., 2019). The increase in LEU numbers has positive implications at a hematologic level, since leucocytes have an important role in tissue recovery and host defense as well as antimicrobial properties (D'asta et al., 2018). In regard to the Hct variable, the variation found in our study can be considered negative, as low levels of Hct are associated with the presence of anaemia, that can lead to the development of several chronic diseases (Suresh et al., 2021). However, despite the reduction of Hct values, they are within the reference values (36%-48% for women and 40%-54% for men). Our results when compared to those of Santos (2020), found a significant statistical increase in MCH and MCHC, in all exercise groups, and in Hb variable in the ComG. Additionally, the present study also found a non-significant increase in PL variable in the AerG. Thus, present results showed a more positive effect on hematologic health.

Although some of the present results are somehow different from those found in the literature, the values remained within the normal limits, showing that aquatic exercise contributed to the maintenance of normal hematologic variables. The differences mentioned above may be directly related to the typology of physical exercise programs applied in different studies. In our study, the impact of different aquatic exercise programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) was tested. Our research did not find any other intervention study that included these types of programs or variables, making an important contribution to this field of research.

Although leucocyte numbers have been shown to increase with ageing Hopkin et al., 2021). The total amount of LEU in the blood increases with acute physical exercise, i.e., exercise of short duration and high intensity (Gleeson et al., 2013). It is possible that our results may represent a cumulative effect of the acute bouts of exercise (both in interval aerobic exercise and combined exercise) performed regularly for 28 weeks.

Regarding the immune markers, significant differences were found for most of the analyzed variables, resulting from the aquatic physical exercise interventions. A more detailed analysis of the results revealed significant differences between the pre- and post-intervention phases: for the AerG, in TNF-α; for the IntG, in IL-10, TNF-α and TNF-α /IL-

10; for the ComG, in TNF-α and IL-1β/IL-1ra. The variance of the mean of the TNF-α variable was higher in the AerG and the ComG (Δ= -23.4% in both cases).

The results from Chupel et al. (2017) study showed significant increases in IL-10 after a land-based muscle strength program. In another study published by the same authors (Chupel et al., 2018), using a 14-weeks combined exercise program (aerobic exercise and muscle strength exercise) resulted in an increase in the anti-inflammatory markers IL-10 and IL-1ra. As for pro-inflammatory markers, results from the same study revealed a significant reduction in TNF-α and IL-1ß. Another study by Furtado et al. (2020), showed a decrease in IL-1ß after 28-weeks of intervention with a combined exercise program. In aquatic environment, a study carried out by Korb et al. (2018), that tested the effectiveness of a 12-weeks aerobic walking program, showed a reduction in IL-10, while the IL-1ß results were the same before and after the intervention. In a study by Ortega et al. (2012), that tested the effectiveness of a 8-months aquatic aerobic program, the results showed a decrease for the anti-inflammatory marker IL-10, for the pro-inflammatory markers IL-1ß and for TNF-α. The results of another study by Da Silva et al. (2018) also showed a reduction in TNF-α after a 12-weeks intervention with low-intensity aquatic exercise program.

Overall, our results show tendencies to decrease in the pro-inflammatory markers IL-1β and TNF-α in all exercising groups. The plasma levels of the anti-inflammatory IL-10 increased in the IntG, while in the other groups the values showed a trend towards a decrease. Athletes do show increased levels of plasma Il-10 in response to high training volume and intensity (Minuzzi et al., 2017). The bouts of high intensity characteristic of the interval aerobic program may explain the differences between the exercising groups for this variable. It is possible that the small decrease of IL-10 found for the AerG and ComG groups could also reflect a response to the decrease in TNF-α seen in these groups. As for the IL-1ra levels, our results showed an increasing trend in all exercise groups, whereas in the CG a significant statistical reduction was observed. Similar results had previously been found in the studies by Chupel et al. (2017) and Chupel et al. (2018), after the intervention of a land-based muscular strength and combined exercise program. Conversely, in the studies by Korb et al. (2018) and Ortega et al. (2012), the IL-10 levels were reduced with the intervention in an aquatic environment (walking exercise and aerobic exercise, respectively). For the IL-1ß variable, present results showed a trend towards a reduction in all exercise groups. However, there was a significant statistical increase in the CG. On the other end, and for the TNF-α variable there was a significant statistical reduction in all exercise groups (AerG and ComG showed a higher means variation), while in the CG the TNF-α values showed tendencies to increase. Similar results were previously found Chupel et al. (2018) and Furtado et al. (2020) after in land exercise interventions and by Ortega et al. (2012) and Silva et al. (2018) after exercise interventions in aquatic environments.

Regarding the ratios, our results showed tendencies to decreased in the TNF-α /IL-10 ratio in all aquatic exercise groups, with statistically significant differences in IntG. In the IL-1β/IL-1ra ratio a statistically significant reduction in ComG was also found. Our results confirm the results already found in the study by Chupel et al. (2018), where reductions in the TNF-α /IL-10 and IL-1β/IL-1ra ratios were also verified, after intervention with physical exercise on land. No studies were found to assess these ratios in an aquatic environment. These results suggest that physical exercise in an aquatic environment can provoke a positive response in the inflammatory profile, reflecting a less pro-inflammatory environment, contributing to the reduction in the development of cardiovascular diseases. In the study by Kumari et al. (2018), the TNF-α /IL-10 ratio was associated with the risk of coronary artery disease, suggesting that this ratio may play a vital role in the development of this type of pathologies.

This means that physical exercise caused a buffering effect, both in anti-inflammatory and in pro-inflammatory markers, since the results in the exercise groups followed a positive trend towards a less inflammatory environment, while the opposite occurred for CG where the low grade chonic inflammation increased. These results provide evidence that aquatic physical exercise programs not only have a positive effect in terms of stability, but also improve the immunologic profile.

The results of the present study suggest that the three aquatic exercise programs can effectively improve immune variables in community dwelling elderly participants. Some of the changes observed in our study are similar to those found in studies carried out on land environments. Thus, exercise in aquatic environments can be seen as a viable alternative to land-based exercise, especially when other health conditions are installed (e.g. orthopedic, rheumatological or functional limitations, circulatory disease, vertigo, etc.). As for the types of exercise programme, the AerG had a higher variation of the mean for TNF-α, the IntG had higher variation of the mean for IL-10 and IL-1ß, and the ComG had a higher variation of the mean for IL-1ra and TNF-α (the same value as AerG). However, further studies are needed to clarify the effects of exercise in aquatic environments on anti-inflammatory markers, as well as to clarify which type of exercise is the most beneficial for the improvement of the immune profile of the elderly.

As limitations of the study, we can refer to the fact that it was used the simples randomizations method, instead of using the blocked randomization method, which would guarantee the balance in the number of participants in each group, reducing sequence unpredictability.

6. Conclusions

The results of the present study allow us to conclude that the participation in physical exercise aquatic environment programs can lead to beneficial changes in the hematologic variables community dwelling elderly.

Regarding the immune variables, the present results showed that, in general, all exercise groups decreased the levels of the pro-inflammatory markers and of the inflammatory index TNF-α/IL-10, while the opposite effect was found in the CG. This means that the participation in community dwelling elderly in aquatic physical exercise programs caused a buffering effect, contributing not only to the stability, but also to an improvement in their inflammatory profile.

As for the type of aquatic program that best benefited the immune profile, the results were not totally conclusive, since different exercise programs led to improvement in different variables. However, the combined aquatic exercise program showed significant statistical improvements in a higher number of hematologic variables, while the interval aerobic aquatic exercise program showed significant statistical improvements in a greater number of immune variables.

Further studies assessing hematologic and immune variables among elderly participants in physical exercise interventions in aquatic environments are needed, aiming to better clarify the positive and most effective impact of different aquatic exercise programs in elderly.

7. References

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STUDY 5: The Impact of Aquatic Exercise Programs on the Intima-Media thickness of the Carotid Arteries, Hemodynamic Parameters, Lipid Profile and Chemokines of Community-Dwelling Older Persons: A Randomized Controlled Trial

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1. Abstract

Scientific evidence has shown that physical exercise is an effective way of improving several cardiovascular disease markers. However, few studies have tested its effectiveness when performed in aquatic environments. The purpose of this study was to test the impact of different aquatic exercise programs on the intima-media thickness of carotid arteries (IMT) and hemodynamic and biochemical markers of cardiovascular diseases in community-dwelling older persons. A total of 102 participants were randomly allocated into four groups: an aerobic exercise group (AerG) (n = 25, 71.44 ± 4.84 years); an aerobic interval group (IntG) (n = 28, 72.64 ± 5.22 years); a combined group (ComG) (n = 29, 71.90 ± 5.67 years); and a control group (CG) (n = 20, 73.60 ± 5.25 years). The AerG, IntG, and ComG participants took part in three different aquatic exercise programs for 28 weeks. The CG participants maintained their usual routines. All participants were evaluated for IMT, blood pressure, lipid profile, and MCP-1 and MIP-1α chemokines, pre- and post-intervention. Significant differences were found in the AerG for diastolic diameter (DD), in the IntG for peak systolic velocity (PSV), and in the ComG for DD and end-diastolic velocity (EDV). Regarding blood pressure, significant differences were found in AerG for systolic blood pressure (SBP) and diastolic blood pressure (DBP); in IntG for DBP; and in ComG for SBP, DBP, and heart rate (HR). Significant differences were found in the AerG and IntG for glucose (GLU). Lower plasma levels of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein (MIP-1α) were found in the AerG and in the ComG for MCP-1 after the intervention. Aquatic physical exercise appears to improve cardiovascular health, regardless of the type of the program adopted. Aerobic programs (combined and continuous aerobic exercises) seemed to have a more beneficial effect in reducing important cardiovascular risk markers.

Keywords: physical exercise; aquatic environment; hydrogymnastics; ageing; intima-media thickness of the carotid arteries; hemodynamic parameters; blood pressure; lipid profile; MDP-1α; MCP-1

2. Introduction

Cardiovascular diseases are a group of heart and blood vessel diseases that are responsible for about 31% of the world's deaths, 85% of which are caused by heart attacks and strokes [1]. According to the same source, it is estimated that 80% of these deaths could be avoided by controlling the main risk factors: smoking, unbalanced diets and physical inactivity. Evidence shows that about 150 min of moderate-intensity physical activity per week can contribute to a reduction in the risk of ischemic heart disease, risk of stroke, and hypertension by approximately 30% [1]. The ageing process is associated with cardiovascular changes that affect arterial function, leading to an increased risk of developing cardiovascular disease [2].

Cardiovascular diseases can be predicted through hemodynamic cardiovascular parameters and cardiovascular risk biomarkers. In relation to hemodynamic parameters, the intima-media thickness of the carotid arteries (IMT) is considered an early marker of coronary artery disease and also a risk factor for other types of cardiovascular disease and stroke [3]. The IMT is defined as the length between the inner layer of the carotid artery tunica intima and the inner layer of the common carotid artery tunica media. Increased IMT was positively and strongly associated with age [4], with values between 0.40 mm and 0.89

mm for IMT considered normal, thickening when values vary between 0.90 mm and 1.40 mm, and when wider than 1.40 mm, there is possibility that the carotid arteries contain plaques [5,6]. Other important markers are systolic and diastolic diameter and peak systolic and diastolic velocity. Arteries lose elasticity with ageing, thus increasing systolic blood pressure (which will consequently cause an increase in the systolic diameter) and blood flow velocity, usually leading to turbulent blood flow and loosening atheromatous plaques, which can consequently lead to obstruction of the vessels [7]. According to the same author, the systolic diameter (SD) corresponds to the highest point of each pulse, when the vessels are subjected to the highest pressure, and the diastolic diameter (DD) corresponds to the lowest arterial pressure point to which the vessels are subjected.

Concerning cardiovascular parameters, arterial hypertension is considered an early marker of the development of atherosclerotic disease, being considered a high-prevalence cardiovascular risk factor associated with endothelial dysfunction [8]. Atherosclerosis starts due to damage to the endothelial lining of large vessels triggered by pathogenic factors such as hyperlipemia and hemodynamic shear stress. It is a pathology that develops and progresses over a long period of time and consequently can lead to serious damage of the vascular tissue [9]. The development of atherosclerosis can consequently lead to ischemic heart disease, stenosis of the carotid arteries, and chronic lower limb ischemia, among others [10].

Regarding biochemical markers, ageing also leads to a deterioration of the lipid and glucose profile, increasing the levels of total cholesterol (TC), low-density lipoprotein (LDL), triglycerides (TG), and fasting blood glucose (GLU). When those elements are uncontrolled, they are directly related to cardiovascular complications [11]. Chemokines also play a fundamental role in the development of cardiovascular diseases and play a key role in the pathogenesis of inflammatory and autoimmune diseases, as well as in tumour progression [9]. The Monocyte Chemoattractant Protein-1 (MCP-1) and Macrophage inflammatory Protein (MIP-1α) play a significant role in the development of coronary artery disease [12]. Several cardiovascular diseases are associated with high levels of pro-inflammatory cytokines that promote the formation of atherosclerotic plaque through an accumulation of macrophages, lipid-laden cells, T cells, and other kinds of degenerative matter in the inner layer of blood vessel walls. Subsequently, several inflammatory molecules are released by the action of macrophages and lead to tissue damage and consequently to inflammation [12]. MCP-1 is produced by different types of cells (monocytes, macrophages, smooth muscle cells, endothelial cells, etc.); MIP-1α is induced by several pro-inflammatory agents and is negatively affected by anti-inflammatory agents [13]. Both are associated with the risk of developing inflammation, cardiovascular disease (specifically atherosclerosis), increased risk of myocardial infarction, and death [14].

According to the literature, physical activity is a very important tool for cardiovascular health. At the hemodynamic and cardiovascular level, physical exercise, mainly exercise with aerobic characteristics, can contribute to the reduction of the luminal diameter of the arteries, since it has a localized effect and/or reflects the increase in blood flow [15]. In addition, aerobic exercise is also considered a first-line strategy to prevent and treat hypertension [8]. Hypertensive individuals are encouraged to participate in aerobic exercise programs aimed at reducing systolic and diastolic blood pressure [16]. This exercise-induced reduction is also accompanied by improvements in arterial and peripheral hemodynamic factors [16]. Combined physical exercise programs (aerobic and muscle strength exercise) have also been shown to be effective in improving hemodynamic and cardiovascular parameters in the older population. A study by Son et al. [17] found that a 12-week combined exercise program provided significant changes in arterial stiffness, as well as in systolic and diastolic blood pressure, in elderly women (75 ± 2 years). However, the meta-analysis carried out by Montero et al. [18] concluded that the combined physical exercise benefits do not appear to differ significantly when compared to aerobic exercise alone.

Regular physical exercise can cause a reduction around −3.5 mmHg in systolic blood pressure and −3 mmHg in diastolic blood pressure [19]. Physical exercise in an aquatic environment, due to immersion, promotes physiological adjustments that can beneficially affect blood pressure, namely by reducing sympathetic activity and redistributing blood volume from the lower limbs and the abdominal area to the upper part of the body, thus causing a reduction in peripheral vascular resistance [19].

Regarding the biochemical markers related to cardiovascular diseases, studies have shown that physical exercise is also an effective tool to improve these factors. In Martins et al. [20], two exercise programs (aerobic and muscle strength exercise) were tested in a group of 63 sedentary elderly participants (76.0 ± 8.0 years), and both interventions produced beneficial effects on their lipidic profile (TG, TC, LDL, and HDL). According to

Gleeson [21], physical exercise can be a way of inhibiting the release of chemokines in human adipose tissue, contributing to the reduction of the pathogenesis of various cardiovascular diseases.

Physical exercise has shown evidence of being an effective way to improve cardiovascular markers, but there are few studies that test its effectiveness in aquatic environments [7]. The exercise in aquatic environment, compared to exercise on land, provides specific mechanical advantages due to the principles of buoyancy, viscosity, and drag. These advantages make the practices of exercise more pleasant and safer for the joints among the older population [22]. For this reason, the purpose of this study is to test the impact of different physical exercise programs in aquatic environments (continuous aerobic program, aerobic interval program, and combined program) on the IMT, hemodynamic parameters, and biochemical markers associated with cardiovascular diseases in community dwelling older people. Considering the low number of studies published that combine imaging technology, biochemical markers, and aquatic exercise interventions [7,18,20], we believe that this study will provide relevant information on the effect of these three exercise programs on cardiovascular risk indicators.

3. Materials and Methods

3.1. Study design

A randomized controlled trial was conducted in Beira Interior Region, Portugal. A sample of non-institutionalized older participants were submitted to a 28-week aquatic exercise intervention. The entire study protocol was previously published by Ferreira et al. [23]. Intima-media thickness of carotid arteries (IMT), blood pressure, lipid blood profile, and chemokines blood concentration (MCP-1 and MIP-1α) were measured in all participants, undergoing three different physical aquatic exercise programs: continuous aerobic (AerG), interval aerobic exercise (IntG), and combined exercise (ComG). A fourth group of participants was also selected as the control group (CG). Data were collected at two specific time moments, namely pre-intervention (baseline, M1) and post-intervention (after 28 weeks, M2). This study was carried out according to the recommendations of the Declaration of Helsinki for Human Studies. The protocol was approved by the Ethics Committee for Health of the Faculty of Sport Sciences and Physical Education, University of Coimbra (reference: CE/FCDEF-UC/00462019). Written informed consent was obtained from all participants prior to any protocol-specific procedures.

3.2. Participants and sample size

The size and statistical power of the sample were calculated using the G*Power software application (University of Dusseldorf, Dusseldorf, Germany) [24]. The following parameters were considered: F test (ANOVA); effect size, 0.25; α-level, 0.05; statistical power, 0.95; number of groups, 4; number of measures, 2 (pre and post intervention); margin of possible losses and refusals, 30%. Therefore, the initial size of the total sample was estimated at 76 participants.

Initially, 174 individuals from the community were invited to participate in the study. After the application of the inclusion and exclusion criteria, 152 individuals were randomized into the four groups mentioned above: AerG, n = 36; IntG, n = 41; ComG, n = 48 and CG, n = 27. According to the experience of the research team and previous studies, the dropout rate from exercise programs among elderly populations is high, so we gathered more participants to fulfil the sample size and compensate for potential dropouts [25,26,27]. A simple randomization method was used. An external investigator used a computer-generated list of random numbers to allocate participants to each group. The investigators were blinded for the randomization of the groups.

The following inclusion criteria were applied: (a) participants from both sexes; (b) 65 years or older; (c) non-institutionalized; (d) autonomy to travel from their residence to Sertã municipal swimming pool; (e) filling out the informed consent form; (f) individuals with medical authorization to practice physical exercise in an aquatic environment, in cases they had some type of clinical condition or comorbidity. The following exclusion criteria were also defined: (a) individuals with clinically diagnosed pathologies putting their and others' health at risk while doing physical exercise in an aquatic environment; (b) individuals that obtained a score of less than 9 points in the Mini-Mental States Examination (indicating severe cognitive impairment) or were clinically diagnosed with a mental illness; (c) an attendance of less than 50% to physical exercise sessions; (d) participants who failed to complete all proposed assessment tests.

The AerG, IntG, and ComG groups performed physical exercise in an aquatic environment during a period of 28 weeks. Participants from the CG group were asked to maintain their normal daily activities without performing any type of systematic physical exercise during the same time period. The participants in the control group were age-matched persons who did not perform any type of regular systematic exercise.

Fifty participants dropped out from the study for the following reasons: personal reasons (n = 11); less than 50% of attendance of the exercise sessions (n = 14); not completing all the assessment tests (n = 12); injury not related with the exercise intervention (n = 4); disease (n = 9). Consequently, 102 participants completed the entire process (AerG: n = 25, 71.44 ± 4.84 years old, women—80%, taking regular medication—92%, with cardiometabolic diseases—76%; IntG: n = 28, 72.64 ± 5.22 years old, women—89.3%, taking regular medication—96%, with cardiometabolic diseases—82%; ComG: n = 29, 71.90 ± 5.67 years old, women—75.9%, taking regular medication—90%, with cardiometabolic diseases—79%; CG: n = 20, 73.60 ± 5.25 years old, women—55%, taking regular medication—95%, with cardiometabolic diseases—80%). The cardiometabolic diseases included diabetes, hypertension, and hypercholesterolemia. Figure 8 shows the entire allocation process for the different groups.

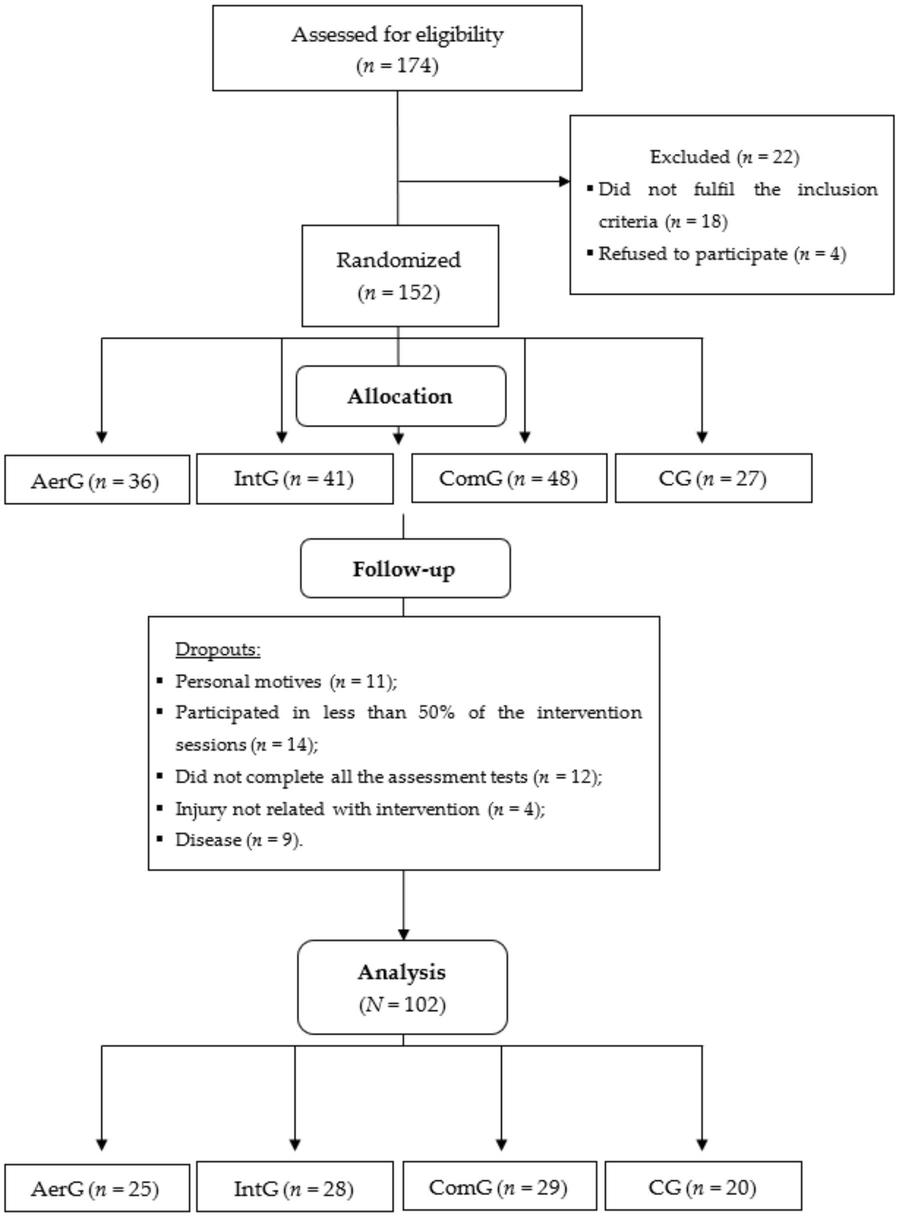

Figure 8 *- Allocation process for the different groups: Continuous aerobic group (AerG); Aerobic interval group (IntG); Combined group (ComG); Control group (CG).*

3.3. Outcomes measurements

3.3.1. Sample characterization

To characterize the sample, values related to anthropometry and physical fitness were collected. Height (Hgt) was assessed using a portable Seca Bodymeter® stadiometer (model 208, Hamburg, Germany) with an accuracy of 0.1 cm. Weight (Wgt), body mass index (BMI), visceral fat (VF), fat mass (FM), and lean body mass (LBM) were evaluated using the TANITA BC-601 impedance scale (Tokyo, Japan). Functional fitness was assessed using the following tests from the Senior Fitness Test set, developed by Rickli and Jones [28] and validated for the Portuguese population by Baptista et al. [29]: muscle strength of the lower (MI) and upper (MS) limbs, with the Chair Stand test (30 s—CS) and Arm Curl test (30 s—AC), respectively (repetitions/30 s); aerobic capacity, with the Two-Minute Step test (2 m—ST) (number of steps); the flexibility of MI and MS, with the Chair Sit and Reach test (CSR) and Back Scratch test (BS), respectively (centimetres); agility and dynamic balance, with the Timed Up and Go test (TUG) (seconds). The handgrip strength was also evaluated, with the Hang Grip test (HG), using the Jamar hand dynamometer (Lafayette Instrument Company, Lafayette, IN, USA) (kg).

3.3.2. IMT and Hemodynamic Parameters

The intima-media thickness of the carotid arteries (IMT) was evaluated with a General Electric (GE®) portable ultrasound machine, VIVID model, with a 9 L linear probe (4 to 12 MHz). All examinations were performed by a highly trained technician. The measurements and data reanalysis were interpreted by two independent technicians, with all data being recorded in digital support. All measurements were performed in the space between 10 and 20 mm before the carotid bifurcation (carotid bulb), allowing the measurement of the IMT in the most distal wall, which is the one with the best definition.

The participants performed the examination in a temperature-controlled room (22 °C to 24 °C), lying on an examination table, in a supine position, with their heads turned to the side (45°). With the use of the real-time B-mode Echo Doppler ultrasound technique, we obtained an image of the carotid artery in the longitudinal plane and transverse plane in order to confirm the IMT measurements.

The images were also used to obtain the systolic diameters (SD) and diastolic diameters (DD). The peak systolic velocity (PSV) and the endo-diastolic velocity (EDV) were measured with the Pulse-Doppler technique in association with the continuous Doppler. For higher accuracy in the velocity calculations, the insonation angle was set at 60°. This allowed us to obtain correct values with respect to the Doppler equation.

The measurements of heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were performed with the use of a digital automatic sphygmomanometer from Riester (Model Ri-ChampionN®, Jungingen, Germany). All the measurements were taken after the participants were at rest for a period of 5 min, sitting and silent, in a room with controlled temperature (22 °C to 24 °C). After this period, participants maintained their comfortable sitting position in a chair, keeping their torsos straight, and the right upper limb stretched and placed on top of a table. Then, a cuff was placed on their right upper limbs, aligned with the brachial artery, at the level of the heart, and adjusted to the perimeter of the arm. Three measurements were taken, with intervals of 1–2 min between them, to check what the average blood pressure was. The mean of the three measurements was considered according to the Guidelines for the Management of Arterial Hypertension [30] of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC).

3.3.3. Biochemical Markers

Fasting blood samples (15 mL) were collected from the ante cubital vein by a registered nurse. The blood sample was used by the clinic to assess lipidic panel values: cholesterol total (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and glucose (GLU). The atherogenic index (AI) was calculated using the TC/HDL ratio and considered normal for values less than five. Next, the test tubes were centrifuged for 10 min at 3500 rpm, and plasma and serum were retrieved and stored in cryogenic test tubes at −80 °C until further use. Levels of Monocyte Chemoattractant Protein-1 (MCP-1/CCL-2) and Macrophage Inflammatory Protein-1 alfa (MIP-1α/CCL3) were subsequently analysed by ELISA (Invitrogen®, Alfagene, Portugal) according to the manufacturer's instructions.

3.4. Intervention protocol

The exercise programs were implemented by sport science and fitness experts, with specific training in hydrogymnastics and developed according to the exercise prescription guidelines recommended by the American College of Sport Medicine (ACSM) for the elderly [31].

All exercise programs sessions had a duration of 45 min, twice a week, for 28 weeks and were performed in a water environment (the water level was between 0.80 and 1.20 m with a temperature of approximately 32 °C), using musical rhythm as a tool to control the intensity of the exercise. Sequences of aquatic exercises, previously defined and selected according to the objectives of each program, were applied. Water exercise sessions were organized into three different parts: the initial, main, and final part.

The initial part or warm-up lasted between 10 and 15 min, at low intensity (30–40% max HR), and was the same in the three water exercise programs. During this initial part, it is intended that participants will adapt to the aquatic environment, i.e., to the water temperature, and provide muscular and metabolic stimulations to prepare the body for the main part of the session. Thus, simple exercises in water were used, such as displacements and isolated movements, with a progressive increase in complexity and intensity throughout the initial part.

The main part for each one of the three water exercise program sessions had a duration of 20 to 30 min and had the following characteristics (Figure 9):

- Continuous aerobic (AerG): exercise aerobic (weeks 1–13, 60–65% maximum HR; weeks 14–28, 65–70% HR);
- Interval aerobic (IntG): exercise aerobic different intensities (weeks 1–13, 60–65% maximum HR interval to 70–75% maximum HR, weeks 14–28, 65–70% maximum HR interval to 75–80% maximum HR). In IntG, the main part of the sessions consisted of several series (1’ recovery interval with 30’’ with higher intensity);
- Combined (ComG): exercise aerobic (weeks 1–13, 60–65% maximum HR; weeks 14–28, 65–70% maximum HR) and muscle strength (weeks 1–13, 2 sets of 12 repetitions; weeks 14–28, 3 sets of 16 repetitions; 6–7 pois in the Borg Scale).

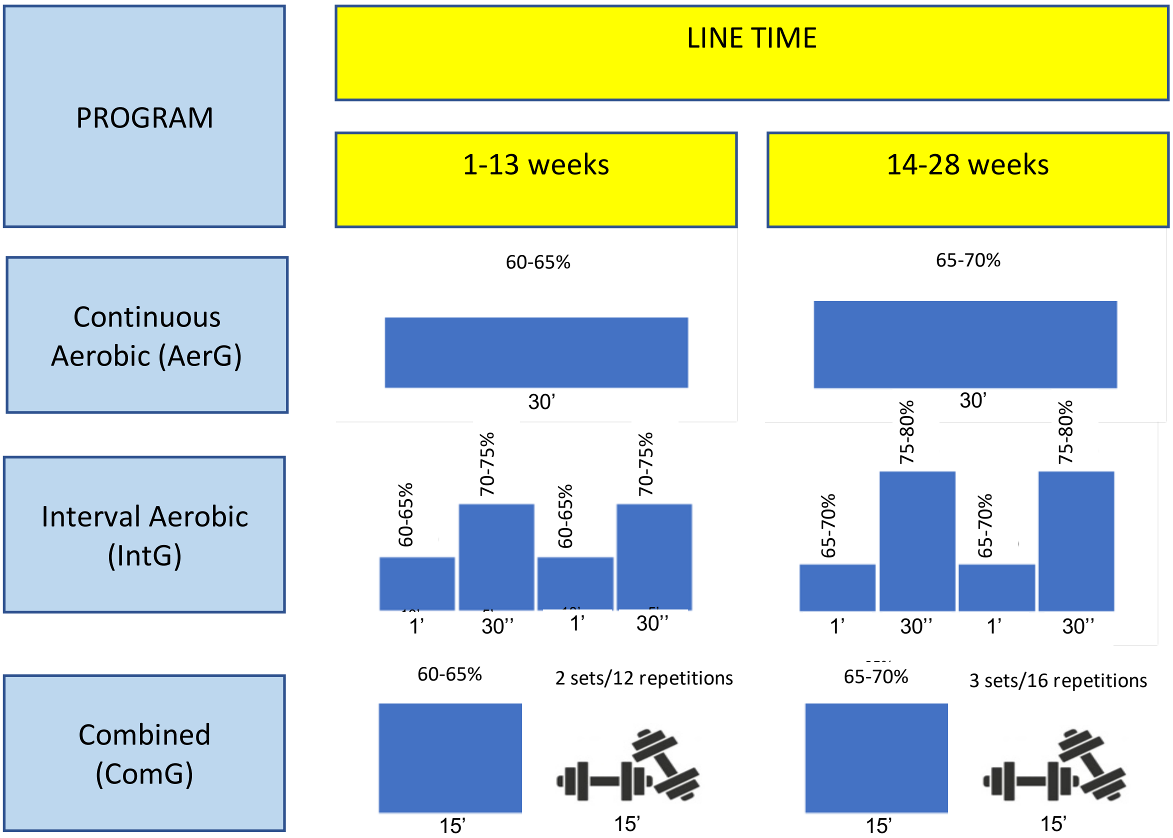

Figure 9 *- Characteristics of the main part for the three physical exercise aquatic programs (continuous aerobic, interval aerobic and combined).*

The final part of the water exercise sessions lasted between 5 and 10 min and was the same for each of the three water exercise programs. This part consisted of two phases: return to calm, where relaxation exercises were applied to bring back the participants' heart rate to values close to the resting state, and stretching exercises stimulating a greater range of motion, used to stretch the main muscle groups used throughout the sessions.

3.5. Monitoring the intensity of the exercise of programs

For safety and intensity target control reasons, all participants randomly used heart rate monitors (Polar, R800CX) (Polar Electro Oy, Professorintie 5, FI-90440 Kempele, Finland) during the exercise sessions, in all three water exercise programs. Depending on the heart rate values obtained, intensity adjustments to the training plan were performed to maintain the intensity target defined for each water exercise program.

For safety and intensity target control reasons, the intensity of the different water exercise programs was predicted indirectly using Karvonen's Formula [32]:

$$\text{Target heart rate} = ((\text{maximal heart rate} - \text{resting heart rate}) \times \%\ \text{intensity}) + \text{resting heart rate}$$

Additionally, and to calculate the maximal heart rate, we used the [33] following formula for the elderly:

$$\text{Maximal HR} = 207 \text{ beats per minute} - (0.7 \times \text{chronological age})$$

3.6. Statistical analysis

The collected data were subjected to descriptive statistical analysis where values such as maximum, minimum, mean, and standard deviation were calculated for each variable in each assessment moment. Afterwards, data normality was tested by considering the response to three conditions: z-values from the skewness and kurtosis tests; p-values from the Shapiro–Wilk test; and visual inspection of generated histograms. All longitudinal comparisons were performed using complete case analysis. Parametric data were analysed using the T-Student test for independent samples to compare the different moments (M1 and M2) and the one-factor ANOVA test and post hoc Tukey's test to analyse the differences between groups. Nonparametric data were analysed using the Wilcoxon test to compare the different moments (M1 and M2), and the Kruskal–Wallis and Bonferroni tests were used to analyse differences between groups. Associations between variables were analysed using Pearson's correlation coefficient values and interpreted as follows [34]: $r = 0.10$ to 0.29 means weak correlation; $r = 0.30$ to 0.49 means moderate correlation; and $r = 0.50$ to 1.0 means strong correlation. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical software, version 27.0 (IBM, New Orchard Road Armonk, New York, United States). The level of significance used was $p \leq 0.05$.

4. Results

The base line values characterizing of each group (mean and standard deviation) are shown in Table 11. No significant statistical differences were found between the groups before the intervention (M1), which suggests that all study groups had similar characteristics regarding anthropometry and functional fitness ($p > 0.05$). Exercise frequency rate was 75.4% in AerG, 69.3% in IntG and 77.7% in ComG.

Among the different groups of evaluated variables, intima-media thickness of the carotid arteries, blood pressure, lipid profile, fasting glucose, and MCP-1 and MIP-1α markers were analysed for correlations, in the two evaluation phases (M1 and M2) (see Figure 10). Weak and positive correlations were found between SBP and SD-L ($r = 0.28$) and DD-R ($r = 0.25$). Contrastingly, we found moderate and negative correlations between HDL and SD-L ($r = -0.30$) and weak and negative correlations between HDL and DD-R ($r = -0.20$). There were no statistically significant correlations between the fasting glucose, MCP-1 and MIP-1α markers, and the remaining variables analysed.

Table 11 - Characterization of the baseline sample (M1).

Caraterísticas	AerG (n=25) Mean (SD)	IntG (n=28) Mean (SD)	ComG (n=29) Mean (SD)	CG (n=20) Mean (SD)	Value *p*
AG (years)	71.44 (4.8)	72.64 (5.2)	71.90 (5.7)	73.60 (5.3)	0.504
Hgt (m)	1.58 (0.7)	1.56 (0.7)	1.57 (0.7)	1.60 (0.9)	0.331
WGT (kg)	70.5 (8,1)	71.3 (14.3)	75.1 (11.0)	75.5 (13.3)	0.334
BMI (kg/m²)	28.20 (3.3)	29.10 (4.8)	30.80 (5.3)	29.50 (5.8)	0.272
VF (%)	11.0 (3.0)	12.0 (3.0)	13.0 (3.0)	13.0 (6.0)	0.128
FM (%)	38.9 (7.3)	41.0 (6,7)	40.3 (9.8)	34.9 (10.9)	0.134
LBM (%)	26.5 (4.3)	24.5 (3.0)	25.5 (4.3)	27.7 (4.7)	0.079
2min-ST (no of steps)	80.9 (17.4)	71.5 (16.5)	81.6 (19.2)	74.3 (18.9)	0.069
CSR-R (cm)	-0.5 (6.6)	-3.7 (10.6)	-3.5 (7.8)	-7.6 (9.7)	0.099
CSR-L (cm)	0.6 (7.2)	-3.9 (9.9)	-5.8 (9.9)	-3.5 (7.3)	0.054
BS-R (cm)	-9.9 (10.4)	-11.9 (11.5)	-14.3 (9.7)	-16.6 (9.9)	0.157
BS-L (cm)	-14.4 (7.2)	-17.4 (8.6)	-21.0 (10.8)	-20.6 (10.7)	0.056
TUG (s)	6.1 (1.1)	7.4 (1.8)	7.4 (3.0)	6.8 (1.7)	0.110
30s-CS (reps/30s)	15.0 (3.0)	13.0 (4.0)	13.0 (3.0)	15.0 (5.0)	0.185
30s-AC (reps/30s)	21.0 (6.0)	17.0 (7.0)	20.0 (5.0)	19.0 (6.0)	0.119
HG-R (kg)	22.0 (6.0)	21.0 (9.0)	21.0 (9.0)	24.0 (9.0)	0.411
HG-L (kg)	21.0 (6.0)	20.0 (9.0)	21.0 (9.0)	21.0 (10.0)	0.578

Note: Age (AG); height (Hgt); weight (Wgt); body mass index (BMI); visceral fat (VF); fat mass (FM); lean body mass (LBM); two-minute step test (2min-ST); chair sit and reach test - right (CSR-R); chair sit and reach test - left (CSR-L); back scratch test – right (BS-R); back scratch test – left (BS-L); timed up and go test (TUG); chair stand test (30s-CS); hand grip test – right (HG-R); hand grip test – left (HG-L).

Figure 10 *- Relationships between SBP-DD-R and SBP-SD-L, and HDL-DD-R and HLD-SD-L. Notes: systolic blood pressure (SBP); diastolic diameter - right (DD-R), systolic diameter - left (SD-L), high-density lipoprotein (HDL).*

The variation in the results of the hemodynamic and cardiovascular parameters, which were analysed by type of aquatic exercise program, before and after the intervention, are shown in Table 12. An example of an image of the carotid artery of a person who participated in the two evaluation phases (M1 and M2) is presented in Figure 11. Although there were no statistically significant differences in IMT after intervention in the different exercise groups, significant reductions were observed in some participants of the exercise groups. This reduction can be explained by the high adherence to the physical exercise program, which on average was 85.6%.

Table 12 - IMT and Hemodynamic parameters results.

	AerG			IntG			ComG			CG				
	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	Time x group (M1)	Time x group (M2)
IMT-L (cm)	0.07 (0.02)	0.07 (0.02)	0.293	0.08 (0.02)	0.07 (0.02)	0.376	0.07 (0.02)	0.07 (0.02)	0.707	0.07 (0.01)	0.07 (0.02)	0.979	0.989	0.501
SD-L (cm)	0.69 (0.12)	0.67 (0.09)	0.177	0.65 (0.1)	0.65 (0.09)	0.539	0.66 (0.1)	0.64 (0.09)	0.105	0.65 (0.06)	0.66 (0.06)	0.088	0.605	0.546
DD-L (cm)	0.64 (0.12)	0.62 (0.09)	0.423	0.61 (0.09)	0.59 (0.09)	0.105	0.63 (0.09)	0.60 (0.09)	0.037**	0.61 (0.06)	0.62 (0.06)	0.558	0.640	0.264
PSV-L (cm/s)	76.79 (21.45)	80.31 (19.22)	0.242	82.51 (19.78)	77.21 (16.26)	0.104	79.38 (18.77)	78.73 (17.06)	0.315	77.28 (15.91)	80.24 (17.9)	0.097	0.508	0.835
EDV-L (cm/s)	19.28 (6.24)	19.95 (5.8)	0.397	20.8 (5.43)	20.26 (4.93)	0.581	22.38 (4.78)	19.97 (6.56)	0.027*	19.7 (5.32)	22.41 (5.62)	0.003*	0.090	0.443
IMT-R (cm)	0.08 (0.01)	0.07 (0.01)	0.808	0.08 (0.02)	0.07 (0.02)	0.055	0.08 (0.02)	0.07 (0.01)	0.205	0.08 (0.02)	0.08 (0.02)	0.308	0.654	0.698
SD-R (cm)	0.70 (0.12)	0.68 (0.09)	0.178	0.66 (0.09)	0.65 (0.09)	0.381	0.67 (0.08)	0.66 (0.09)	0.249	0.68 (0.08)	0.66 (0.08)	0.353	0.519	0.758
DD-R (cm)	0.66 (0.12)	0.63 (0.09)	0.039*	0.62 (0.08)	0.61 (0.09)	0.282	0.63 (0.09)	0.60 (0.09)	0.029*	0.62 (0.07)	0.63 (0.07)	0.813	0.316	0.543
PSV-R (cm/s)	74.6 (23.04)	74.1 (17.56)	0.904	74.63 (18)	66.23 (17.68)	0.024*	81.23 (15.74)	77.25 (12.81)	0.087	74.27 (16.9)	73.11 (16.31)	0.763	0.136	0.106
EDV-R (cm/s)	19.55 (7.29)	18.89 (5.41)	0.716	19.39 (5.44)	18.26 (4.8)	0.186	20.74 (5.29)	19.18 (6.34)	0.064	18.43 (4.63)	19.27 (5.35)	0.432	0.169	0.910
SBP (mmHg)	134 (13.0)	128 (16.0)	0.013*	135 (12.0)	132 (13.0)	0.184	138 (18.0)	133 (18.0)	0.018**	137 (15.0)	132 (18.0)	0.093	0.798	0.535
DBP (mmHg)	77 (9.0)	73 (10.0)	0.002*	76 (7.0)	73 (7.0)	0.046*	79 (8.0)	75 (8.0)	0.004*	78 (7.0)	78 (7.0)	0.950	0.399	0.099
HR (bpm)	71 (11.0)	69 (12.0)	0.279	69 (9.0)	67 (11.0)	0.115	74 (12.0)	70 (10.0)	0.010**	73 (10.0)	71 (6.0)	0.467	0.417	0.268

Note: Intima-media thickness - left (IMT-L); systolic diameter - left (SD-L); diastolic diameter - left (DD-L); peak systolic velocity – left (PSV-L); end-diastolic velocity - left (EDV-L); Intima-media thickness - right (IMT-R); systolic diameter - right (SD-R); diastolic diameter - right (DD-R); peak systolic velocity – right (PSV-R); end-diastolic velocity - right (EDV-R); Systolic blood pressure (SBP); diastolic blood pressure (DBP); heart rate (HR) *Result obtained through T-Student test; **result obtained through Wilcoxon test.

The general results of the hemodynamic parameters showed no significant differences between the groups before and after the intervention ($p > 0.05$). As a result of the intervention with the aquatic exercise program, significant differences were found between M1 and M2 in AerG for DD-R ($p = 0.039$; $\Delta = -4.5\%$), in IntG for PSV-R ($p = 0.024$; $\Delta = -11.3\%$), and in ComG for DD-L ($p = 0.037$; $\Delta = -4.8\%$), for EDV-L ($p = 0.027$; $\Delta = -10.8\%$), and DD-R ($p = 0.029$; $\Delta = -4.8\%$). In the CG, there was a significant increase for EDV-L ($p = 0.003$; $\Delta = 13.8\%$), while a significant reduction in the same variable was observed in ComG. In regard to the variables IMT-L, SD-L, PSV-L, IMT-R, SD-L, and EDV-R, no significant changes were observed after the intervention ($p > 0.05$).

Regarding the blood pressure parameters results, no significant differences were found between the groups before and after the intervention ($p > 0.05$). As a result of the intervention with the aquatic exercise programs, significant differences were found in AerG for SBP ($p = 0.013$; $\Delta = -4.5\%$) and DBP ($p = 0.002$; $\Delta = -5.2\%$), in IntG for DBP ($p = 0.046$; $\Delta = -3.9\%$), and in ComG for SBP ($p = 0.018$; $\Delta = -3.6\%$), DBP ($p = 0.004$; $\Delta =$

−5.1%) and HR ($p = 0.010$; $\Delta = -5.4\%$). In the control group, there were no significant changes ($p > 0.05$).

The variation in the results for the cardiovascular risk biomarkers, analysed by type of aquatic exercise program, before and after the intervention, are presented in Table 13.

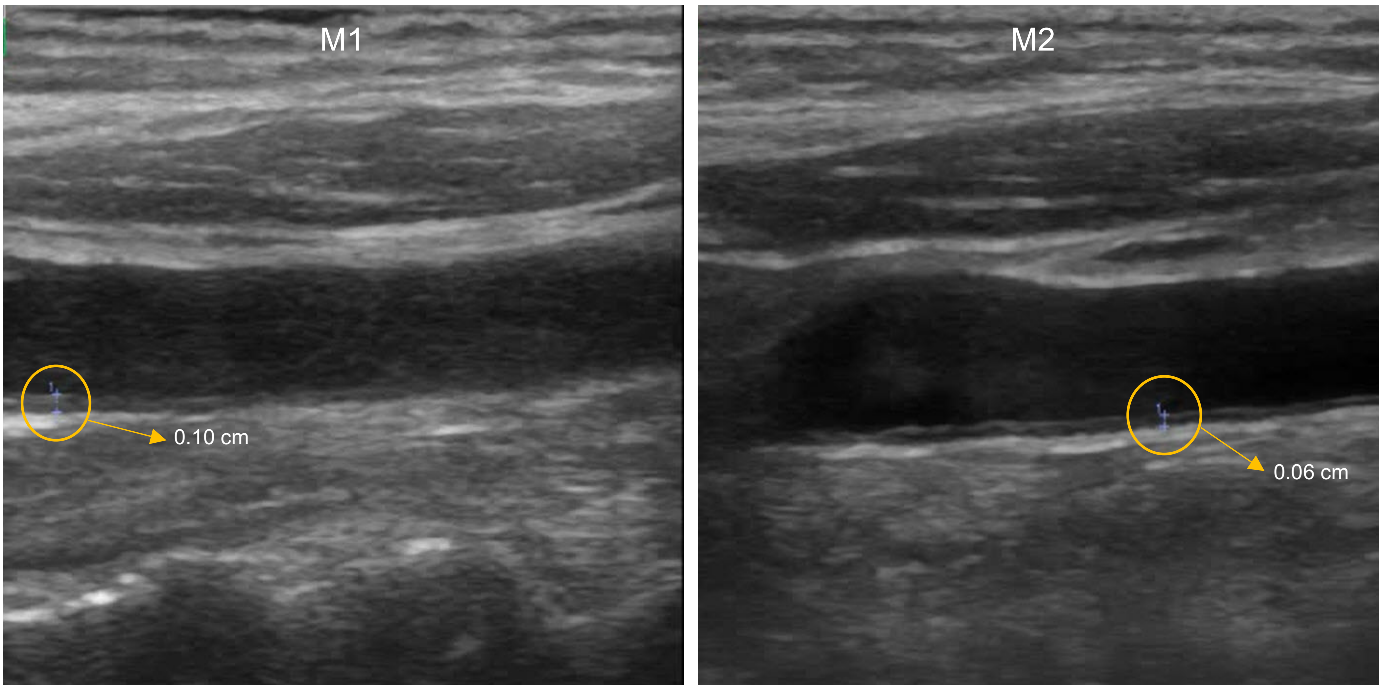

Figure 11 *- Images of the carotid artery of a study participant at different times of assessment: Before the intervention (M1), the participant had an intima and media thickness of the carotid artery (IMT) of 0.10cm; After the intervention (M2), a reduction in the IMT to 0.06cm was verified.*

Table 13 - Biochemical marker results.

	AerG			IntG			ComG			CG				
	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	Time x group (M1)	Time x group (M2)
GLU (mg/dl)	94 (27)	89 (22)	0,006**	95 (18)	90 (17)	0.041**	98 (25)	95 (24)	0.309	97 (20)	99 (18)	0.449	0.728	0.112
CLT (mg/dl)	184 (31)	185 (24)	0.935	174 (33)	172 (29)	0.650	182 (26)	178 (24)	0.346	176 (26)	177 (27)	0.757	0.512	0.381
HDL (mg/dl)	58 (14)	59 (12)	0.586	58 (15)	56 (15)	0.175	58 (11)	59 (13)	0.478	57 (11)	55 (11)	0.111	0.907	0.582
AI (mg/dl)	3.29 (0.63)	3.33 (0.49)	0.653	3.17 (0.54)	3.25 (0.6)	0.249	3.23 (0.68)	3.27 (0.71)	0.566	3.21 (0.64)	3.67 (0.52)	0.003*	0.598	0.033†
LDL (mg/dl)	106 (25)	108 (20)	0.712	95 (27)	95 (24)	0.855	103 (25)	100 (25)	0.498	97 (25)	97 (25)	0.989	0.338	0.226
TG (mg/dl)	102 (51)	101 (48)	0.903	105 (36)	104 (43)	0.682	107 (41)	104 (46)	0.503	116 (47)	119 (35)	0.478	0.393	0.191
MCP-1 (pg/ml)	141.74 (82.71)	82.45 (46.02)	0.001**	80.43 (37.15)	63.42 (42.69)	0.059	231.79 (73.36)	193.44 (94.04)	0.033**	72.76 (49.30)	54.34 (31.33)	0.093	0.000†	0.000†
MIP-1α (pg/ml)	93.09 (12.62)	91.04 (7.39)	0.009**	96.19 (1.88)	96.45 (1.29)	0.179	110.49 (9.37)	110.43 (11.77)	0.214	90.90 (15.12)	90.96 (15.40)	0.546	0.000†	0.000†

Note: Glucose (GLU); cholesterol total (CLT); high-density lipoprotein (HDL); atherogenic index (AI); low-density lipoprotein (LDL); triglycerides (TG); monocyte chemoattractant protein-1 (MPC-1); macrophage inflammatory protein (MIP-1α). *Result obtained through T-Student test; **result obtained through Wilcoxon test; †results obtained through ANOVA.

The overall lipid profile results revealed that no significant differences were found between the groups before the intervention ($p > 0.05$). After the intervention, significant differences were found for AI between IntG and CG ($p = 0.008$) and between ComG and CG ($p = 0.009$). In both cases, the CG obtained a less satisfactory result. As a result of the intervention with the aquatic exercise program, significant differences were found in AerG ($p = 0.006$; $\Delta = -5.3\%$) and IntG ($p = 0.041$; $\Delta = -5.3\%$) for GLU, and in CG for AI ($p = 0.003$; $\Delta = 14.3\%$). In regard to the variables TC, HDL, LDL, and TG, there were no significant changes ($p > 0.05$). As for the chemokines MCP-1 and MIP-1α, statistically significant differences were found between the groups, before and after the intervention ($p < 0.05$). From M1 to M2, statistically significant differences were found in AerG for MCP-1 ($p = 0.001$; $\Delta = -41.8\%$) and MIP-1α ($p = 0.009$; $\Delta = -2.2\%$) and in ComG for MCP-1 ($p = 0.033$; $\Delta = -16.5\%$). For the IntG and CG, no statistically significant differences were found in both chemokines ($p > 0.05$).

5. Discussion

The purpose of this study was to test the impact of different aquatic exercise programs (continuous aerobic exercise, aerobic exercise interval, and combined exercise) on the IMT and hemodynamic and biomarkers of cardiovascular diseases in community dwelling older people. Preliminary systematic research revealed the innovative characteristics of the present study, since there were very few studies that have evaluated similar variables with aquatic exercise interventions in older adults.

Although no changes were found in IMT or in the variables related to the hemodynamic parameters, significant reductions were observed for DD-R in AerG and ComG, for PSV-R in IntG, and for DD-L in ComG. In EDV-L, there was a significant reduction in ComG, while in CG a significant increase was observed.

A study by Santos [7] reported the effectiveness of a 10-month continuous aerobic aquatic exercise program in older women (64.05 ± 5.91 years), who were divided into two groups: older women who started practising exercise in an aquatic environment (continuous aerobic program), for the first time (beginners) and elderly women who had already practised physical exercise in an aquatic environment (continuous aerobic program) during the previous school year (trained). The results showed that in both groups there was a significant reduction in IMT, with a magnitude effect of 36.4% and that exercise had a very strong effect on the change in IMT. Regarding SD and DD, there was a significant reduction in both groups. Additionally, a reduction in PSV and EDV was reported in both groups. Such a reduction was significant in the trained group. Similarly, in the study by Park and Park [15], the effectiveness of a combined exercise program (aerobic exercise and muscle strength exercise) was tested in a group of older women (65–77 years) for 24 weeks. The results showed that these types of programs can lead to a significant reduction in IMT. In another study [35], the impact of two exercise programs (high-intensity interval exercise and continuous aerobic exercise) was tested, and the results showed that, although they did not lead to significant statistical differences, both programs resulted in a reduction of the IMT.

The results from the present study are in line with those in the existing literature. In IMT-R, although there were no significant statistical differences, a trend towards a reduction was visible in the three exercising groups, whereas in IMT-L only a reduction in IntG was visible, with the value of the mean remaining the same in the other groups. As reported by Santos [7], our results also showed a reduction in SD, DD, PSV, and EDV in all exercise groups. This reduction was significant for DD-L in ComG, for DD-R in AerG and ComG (in regard to the latter, ComG recorded a higher variation of the mean), for EDV-L in ComG, and for PSV-R in IntG. In the CG, a significant increase in EDV-L was evident. According to Homma et al. [36], there is a strong and positive correlation between advancing age and an increase in IMT (r = 0.83). In our study, although the results did not significantly decrease in all variables, in the exercise groups, they did not increase either. For this reason, we can say that all exercise programs can be considered as factors of hemodynamic balance and thus contribute to the reduction in stress on the arterial wall and reduce the risk of developing cardiovascular diseases. The combined program seems to be slightly more beneficial compared to the other ones, due to the fact that it resulted in significant differences in a higher number of variables. Additionally, compared to the study by Santos [7], our intervention lasted 7 months, while in Santos's (2020) study, the intervention lasted 10 months. These data suggest that a 7-month intervention with aquatic exercise may be sufficient to cause changes in these hemodynamic variables.

Significant reductions were also found for SBP in the AerG and ComG, as well as in the three-exercising groups for DBP, and for HR in the ComG.

A study by Park et al. [37] tested the effectiveness of a water walking program on blood pressure values in a group of women (70 ± 10.0 years), and the results showed that, after the intervention, the exercise group had a significant reduction in HR values. The SBP and DBP values were also reduced, although not significantly. In land environments, a study by Son et al. [17] reported significant reductions in SBP and DBP after intervention with a combined exercise program (muscle strength exercise and aerobic exercise) in a group of hypertensive women (75 ± 2 years old). The same results were confirmed by Park et al. [38] using the same type of program (combined exercise), which led to a decrease in SBP and DBP values. This decrease was statistically significant in SBP.

In our study, the three exercise groups showed reductions in SBP, DBP, and HR. The aerobic exercise and combined exercise program seem to be the most beneficial. Both showed similar results in SBP and DBP, with the aerobic exercise program leading to a slightly higher decrease in the mean compared to the combined exercise program. In HR, the combined exercise program was the only one where the decrease was statistically significant. We may conclude that the combined exercise program may be more beneficial for cardiovascular parameters, as in addition to having showed similar results to the aerobic program in SBP and DBP, it also led to a significant decrease in HR.

In regard to the biochemical markers, significant reductions were noticed for GLU in AerG and IntG, and a significant increase was noticed for the AI in CG (14.3%). After the intervention, significant differences were found in the AI between IntG and CG and between ComG and CG.

In a study with participants with type 2 diabetes [39], after an intervention in an aquatic environment with a moderate aerobic exercise program, the results showed a significant decrease in GLU, TC, LDL, and TG values. Another study [40] tested the effectiveness of two different aquatic exercise programs: aerobic exercise and muscle strength exercise. Both programs led to a significant decrease in the TC, LDL, and TG values and a significant increase in HDL values. Therefore, they concluded that the two types of aquatic exercise programs had identical benefits, and both could contribute to a reduction in the risk of cardiovascular events. Similar results were reported on a land study [41], where the effectiveness of two exercise programs (high-intensity interval exercise and moderate-intensity continuous aerobic exercise) was compared, with the results showing a decrease in TC, LDL, and TG values and an increase in the HDL values. In comparison with the aerobic exercise program, the interval exercise program showed results that were more beneficial.

As in the study of Cugusi et al. [39], the present study noticed a decrease in the GLU values in the three exercise groups, although only statistically significant in AerG and IntG. Our results showed that AerG and IntG are equally beneficial in reducing GLU. Regarding TC, HDL, LDL, and TG, the results did not show significant changes, and they remained within the reference values. Therefore, aquatic physical exercise may contribute to the balance of the lipid profile.

In the plasma chemokine levels, significant reductions were noticed for MCP-1 and MIP-1α in AerG, and for MCP-1 in ComG.

Among long-term intervention studies, the one conducted by Kraemer et al. [42], which consisted of carrying out a 12-week muscle strength training program with and without supplementation in young adults, MCP-1 values increased after the intervention, while the MIP-1α values decreased, regardless of the supplementation. In the study of Barry et al. [43], who tested the impact of two physical exercise programs (high-intensity interval exercise and moderate-intensity continuous exercise) in obese adults, the results showed that, although no statistically significant differences were found, the MCP-1 values were reduced in both groups after the intervention, and that the MIP-1α values increased after the intervention of the high-intensity interval program and decreased after the intervention of the moderate-intensity continuous program.

In our study, despite the fact that the intervention took place in a different context, the results partly reached the same conclusions. As in the Barry et al. [43], our results reinforce the idea that long-term continuous aerobic exercise reduces the values of MCP-1 and MIP-1α. The results of the interval aerobic program showed a reduction in MCP-1 and an increase in MIP-1α. It is possible that higher intensity exercise levels may lead to increased MIP-1α levels as a response to muscle micro-injuries. In both cases, the changes were not significant, just like in the Barry et al.'s study [43]. Finally, in the combined exercise program (continuous aerobic exercise and muscle strength exercise), the results showed a reduction in both chemokines.

MCP-1 appears to be present in the initial phase of atherosclerotic lesion formation, i.e., in the thickening of the tunica intima and fatty streaks, which suggests that this chemokine may contribute to an early influx of monocytes into blood vessels [13]. In the intimal layer of the carotid arteries, chemokines recruit monocytes that trigger the development of foam cells, leading to intimal erosion, which is caused by atherosclerosis and consequently leads to ischemia [12]. The results found in the present study suggest that aquatic physical exercise, especially exercise with aerobic characteristics, helps to prevent the development of cardiovascular diseases by contributing to the reduction in chemokines MCP-1 and MIP-1α.

Exercise in aquatic environments, due to the specific proprieties of water that make the exercise practice safer and more comfortable for the elderly population, reduces the risk of traumatic fractures and joints by giving them less stress and lower impact [44]. The viscosity of the water provides a comfortable resistance that allows the development of muscle strength [22]. The hydrostatic pressure, the force of the viscosity, and the turbulence created by the water during the physical exercise sessions provide a different proprioceptive and sensorial feedback from the land environment and provide periods of instability that must be overcome. These aspects benefit the postural control and balance system [45,46]. The practice of exercise in this environment seems to be a viable strategy, because in addition to its specific characteristics and added benefits, it seems to be equally effective for cardiovascular health when compared to exercise on land.

Study limitations included the fact that a simple randomization method was used, instead of using a blocked randomization method, which would guarantee a balance in the number of participants in each group, reducing sequence unpredictability. Another limitation was the fact that the level of physical activity of the participants in the control group was not evaluated. Although participants were not involved in regular systematic exercise, this assessment would have contributed to a stronger study. The limited number of publications with interventions in an aquatic environment in older populations was also a limitation for the discussion of results.

More intervention exercise studies in aquatic environments are needed in order to reinforce the results found in the present study and better understand the effects and effectiveness of aquatic exercise programs on markers of cardiovascular health.

6. Conclusions

Aquatic physical exercise, regardless of the type of program, seems to lead to benefits in cardiovascular variables, and this type of intervention may be a viable alternative when land-based exercise is not possible and/or desired.

In relation to the intima and average thickness of the carotid arteries, we can say that all the aquatic exercise programs studied (i.e., the continuous aerobic program, the aerobic interval program, and the combined program) can be considered as factors for hemodynamic balance and thus contribute to the reduction of stress on the arterial wall and reduce the risk of cardiovascular diseases. The combined program seems to be slightly more beneficial, compared to the other programs due to the fact that it resulted in significant differences in a higher number of variables.

As for blood pressure, the three exercise groups showed reductions in SBP, DBP, and HR. However, the aerobic and combined exercise programs proved to be more beneficial for blood pressure and present statistically significant differences for both variables (with the same mean variation). Additionally, the combined program also showed a statistically significant reduction for HR.

Regarding the metabolic profile, our results showed that AerG and IntG are equally beneficial in reducing GLU. Regarding TC, HDL, LDL, and TG, the results did not show significant changes; however, they remain within the reference values. We concluded that aquatic physical exercise may contribute to the balance of the lipid profile.

Finally, as for chemokines, the results suggest that aquatic physical exercise, especially that with aerobic characteristics, helps prevent the development of cardiovascular diseases by contributing to the reduction in chemokines MCP-1 and MIP-1α and the subsequent infiltration of monocytes and formation of atherosclerotic plaques.

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5. GLOBAL DISCUSSION

Having finished the presentation of the 4 studies that subdivide this thesis, it is necessary to understand and discuss the results taking into account the objectives initially defined (chapter 1.2). This general discussion is divided according to the objectives and describes the main results of this thesis, comparing them with the results found in the literature. A brief discussion based on the practical implications that our results have for the studied population will also be presented.

5.1. The association of between aerobic capacity, handgrip strength and cognition with risk markers for cardiometabolic diseases and mental health in community dwelling elderly (objective 1).

Aerobic capacity, handgrip strength and cognition are three components strongly affected by the ageing process, and they seem to be correlated with a large set of cardiometabolic markers. Therefore, we decided to investigate the impact of different levels of these three variables on a wide range of cardiometabolic markers, namely anthropometry, functional fitness, cognition, immunological and cardiovascular risk markers. In response to objective 1, study 2 (chapter 4) was developed. This study was divided into three specific objectives, to which we present a brief discussion for each of them.

5.1.1. The association of between aerobic capacity with risk markers for cardiometabolic diseases and mental health in community dwelling elderly.

Study 2 had the first specific objective to analyse the association between aerobic capacity with the other variables studies (anthropometry, functional fitness, cardiovascular risk markers, biochemical markers and psychological health). The results suggest a strong and positive correlation between aerobic capacity (i.e., 2min-ST) and muscular strength of the lower and upper limbs (i.e., evaluated through the 30s-CS and 30s-AC tests, respectively), as well as a correlation strong and negative difference between aerobic capacity and agility and dynamic balance (i.e., TUG). Furthermore, a moderate and positive correlation was found between aerobic capacity and the HG, EDV-D, SWLS and EQ5D-scale.

These results reinforce the results of other previously performed studies. Teodorczyk et al. (2016) also demonstrated a strong and positive correlation between aerobic capacity (2min-ST) and lower limb muscle strength, which was assessed using an isometric dynamometer armchair. Pedrosa & Holanda (2009) demonstrated a strong and negative correlation between aerobic capacity and agility/dynamic balance. Similarly, Taylor-Piliae et al. (2012) also found a moderate and positive correlation between aerobic capacity with lower limb strength, and with balance (i.e., assessed using the unipedal stance test). Katsimpris et al. (2021) demonstrated a strong and positive correlation between aerobic capacity and handgrip strength. Other studies have also verified the association between aerobic capacity and the intima and media thickness of the carotid arteries. In particular, the study by Lee et al. (2009) who demonstrated a strong and negative association between aerobic capacity and the risk of developing carotid atherosclerosis, and the study by Lee et al. (2019), who also demonstrated an inverse association between aerobic capacity and the development of carotid artery disease and with carotid artery intima and media thickness. Regarding psychological health, the studies by Marques et al. (2017) and Kouwijzer et al. (2020) also showed a moderate and positive relationship between aerobic capacity and satisfaction with life.

Our results reinforce the importance of improving aerobic capacity, which is an important component that can significantly lower cardiovascular disease risk in both the short and long term (Lin et al., 2015). According to the same authors, aerobic capacity is an indicator used in clinical practice, both in the diagnosis and prognosis of cardiovascular diseases. The results of this study have important practical implications, since aerobic capacity is related to other physical components like the ability to perform daily life activities independently, the intima and media thickness of the carotid arteries as well as with elderly people satisfaction with their own life. Therefore, it becomes important to develop effective strategies, such as training programs aimed at improving aerobic capacity in the elderly, in order to attenuate or even improve some indicators of cardiometabolic health, as well as satisfaction with life. The aquatic environment, due to the properties of water and the reduced impact on the musculoskeletal system and joints, can be an effective strategy for elderly to practice physical exercise.

5.1.2. The association of between handgrip strength with risk markers for cardiometabolic diseases and mental health in community dwelling elderly.

In another part of study 2, we analysed the association between handgrip strength with the other variables studies (anthropometry, functional fitness, cardiovascular markers, biochemical markers and psychological health). The results showed a strong and positive correlation between handgrip strength (i.e., HG) and height (Hgt). They also suggest a moderate and positive correlation between handgrip strength and lean body mass, aerobic capacity (i.e., 2min-ST), upper limb muscle strength (i.e., 30s-AC) and cognitive function (i.e., MMSE), as well as a moderate and negative correlation between handgrip strength, fat mass, agility and dynamic balance (i.e., TUG). These data suggest that handgrip strength is related to body composition, namely with height, lean body mass and fat mass and with functional fitness, mainly with aerobic capacity, muscle strength, agility and dynamic balance, as well as with cognitive function.

Other authors also corroborate our findings. Regarding body composition, Torralvo et al. (2018) demonstrated a strong and positive correlation between handgrip strength and height. However, in the study by Pizzigalli et al. (2017) this correlation was only moderate. Buehring et al. (2018) also demonstrated a moderate and positive correlation between handgrip strength and lean body mass, assessed by different methods (i.e., dual-energy X-ray absorptiometry, bioelectric impedance, and DB-CR creatine dilution), and Torralvo et al. (2018) demonstrated a strong and positive correlation between handgrip strength and lean body mass (i.e., bioelectric impedance). Torralvo et al. (2018) also demonstrated a strong and negative correlation between handgrip strength and fat mass. Regarding functional fitness, Katsimpris et al. (2021), demonstrated a strong and positive correlation between handgrip strength and aerobic capacity. Alonso et al. (2018) demonstrated a moderate and positive correlation between handgrip strength and lower limb muscle strength. Lam et al. (2016) and Alonso et al. (2018) demonstrated a weak and negative correlation between handgrip strength and agility and dynamic balance (i.e., TUG). At the level of cognitive function, Liu et al. (2021) demonstrated a weak and positive correlation between handgrip and cognition (i.e., MMSE).

The reduction in muscle strength in the elderly population is responsible for the increased difficulty in performing daily tasks, being also responsible for the significant increase in falls and loss of functional independence (Alonso et al., 2018). Handgrip strength has been considered an important clinical test used to identify the risk of morbidity and mortality (Hershkovitz et al., 2019; Wu et al., 2019). Our results have important practical implications, as handgrip strength was related to other important components of independence in the elderly, such as body composition and cognitive function. Thus, it becomes important to develop effective strategies, such as exercise programs aimed at improving muscle strength in the elderly, in order to attenuate or even improve some indicators of cardiometabolic health. In this sense, the aquatic environment, due to the properties of water and the reduced impact on the musculoskeletal system and joints, can be an effective strategy for older individuals to practice physical exercise and consequently develop muscle strength. Future research is needed to confirm the results found.

5.1.3. The association of between cognition with risk markers for cardiometabolic diseases and mental health in community dwelling elderly

Finally, in study 2, we addressed the association between cognition with the other study variables. The results suggest a moderate and positive correlation between MMSE scores and handgrip strength (i.e., HG) and quality of life (i.e., EQ5D-scale), as well as a moderate and negative correlation between cognitive function and agility and dynamic balance (i.e., TUG). These data suggest that cognitive fitness is related to physical fitness markers, namely muscle strength, agility and dynamic balance, and to psychological health markers, more precisely with quality of life.

Previously, and with regard to functional fitness, Liu et al. (2021) and Ramnath et al. (2018) also demonstrated a moderate and positive correlation between cognitive fitness and handgrip strength. Emerenziani et al. (2020) had already demonstrated a strong and positive correlation between these two variables. In the study by Chupel et al. (2017), moderate and negative correlations were also demonstrated between cognitive fitness and agility/dynamic balance (i.e. TUG). In the same sense, in the study by Borges et al. (2018) showed a weak and negative correlation between cognition and agility/dynamic balance (i.e. TUG), while in the study by Ramnath et al. (2018), there were no significant correlations between the two variables. Regarding psychological health, Babenko et al.

(2019) demonstrated a strong and positive correlation between cognitive fitness and quality of life (i.e., EQ5D). Identical results were verified in the studies by Chae et al. (2020) and Samuel et al. (2016). However, in the study by Voros et al. (2020), there were no statistically significant correlations between cognitive function and quality of life.

During the ageing process, a decline in cognition is observed. This decline stems from the expansion of the cerebrospinal fluid, the progressive deterioration of the microstructure of the white matter and other subcortical nuclei (i.e., hippocampus, cerebellum and striatum) (Cherup et al., 2018). Cognitive impairment is accompanied by reduced balance, decreased vision, gait difficulties and other factors that can lead to the occurrence of falls in the elderly population, contributing to the loss of autonomy and quality of life (Racey et al., 2021). The results of this study have important practical implications, since cognitive function is related to components of functional function, important for the autonomy of the elderly, and to psychological health, namely quality of life. Thus, it becomes important to develop effective strategies, such as training programs aimed at improving the cognitive function of the elderly. In this sense, the aquatic environment, due to the properties of water, can be an effective strategy for elderly to practice physical exercise and successively improve cognitive function, since the practice of physical exercise in this environment can contribute to the maintenance and improvement cognitive function (Kang et al., 2020).

5.2. To test the impact of different types of physical exercise programs in the aquatic environment on body composition, functional fitness and cognition in community dwelling elderly (objective 2).

With ageing there are changes in body composition, functional fitness and cognition. These changes are associated with reduced functionality, increased risk of falls, loss of autonomy and development of cardiometabolic diseases. Therefore, it is important to look for strategies that contribute to the improvement of these indicators. Furthermore, our results from study 2 showed that variables related to body composition, functional fitness and cognition may be correlated with a set of cardiometabolic markers. Thusly, for this thesis, we aimed to test the impact of different physical exercise programs in aquatic environments on body composition, functional fitness and cognition, in community dwelling elderly. In order to be able to respond to this objective, we developed study 3 (chapter 4), which was divided into three specific objectives.

5.2.1. The impact of different physical exercise programs in the aquatic environment on body composition in community dwelling elderly.

The first analysed objective in study 3 was the impact of different exercise programs, in aquatic environment, on body composition in community dwelling elderly. The results obtained showed that, regardless of the exercise program implemented, benefits in the participants' body composition were obtained. In the three programs there were statistically significant reductions in FM and a significant increase in LBM. Additionally, the continuous aerobic program also caused a statistically significant reduction in WCir. The aerobic interval program caused a statistically significant increase in BMI and a significant reduction in LCir-R. The combined program caused a statistically significant increase in WGT and a significant reduction in LCir-R and LCir-L.

These results corroborate other intervention studies in the aquatic environment, carried out by several authors. Naylor et al. (2020) demonstrated a statistically significant increase for LBM, a significant reduction for FM, and a non-significant reduction for LCir (following a 48-week water walk program intervention). Waller et al. (2016) demonstrated a statistically significant reduction for FM (after intervention with a 4-month high-intensity aquatic exercise program). Kim et al. (2021) also demonstrated a non-significant increase for LBM, and non-significant reductions for FM and WCir (after intervention with a 12-week aquatic exercise program). Ha et al. (2021) demonstrated (after intervention with a 16-week aquatic exercise program) an increased tendency for LBM and BMI and a tendency for a decrease in WCir and FM. Park et al. (2019), also demonstrated a slight increase in BMI. Sharifi et al. (2021) demonstrated a statistically significant reduction for LCir after intervention with a 24-month water walk program. Contrary to the results found in our study, the studies by Naylor et al. (2020) and Kim et al. (2021), demonstrated reduction tendency for BMI and WGT.

The statistically significant increases found in our study for BMI and WGT in both the aerobic and the combined interval programs can be explained by the increase in lean body mass percentage that was found increase in both groups. In the study by Ha et al. (2021) and Park et al. (2019), there was also an increase for BMI, which can also be explained by the increase in LBM.

Body composition changes with the ageing process, leading to the risk of developing cardiometabolic diseases (Waller et al., 2016). The results of this study have important practical implications, since exercise in an aquatic environment caused a significant improvement in indicators of body composition (i.e., in fat mass and lean body mass), which are important for a more autonomous and healthy ageing process. Body composition was associated with the risk of cardiometabolic diseases, more precisely, in the study of Araújo et al., (2017) arms circumference was associated with an increase in fasting blood glucose and waist circumference with an increase in triglycerides. Already in the study of Schorr et al. (2018), visceral fat was positively associated with cholesterol values, glucose and triglycerides, and lean body mass was negatively associated with glucose. Thusly, we can say that aquatic exercise can be considered a good strategy to improve body composition in the elderly. Regarding the hypothetical effects of different physical exercise programs on body composition, the results showed that both programs studied seem to have a similar effect on FM and LBM. However, participants in the combined program showed additional statistically significant differences for lower limb girth, evidence that suggests that combined aquatic exercise programs may have additional beneficial effects on body composition when compared with other exercise programs.

5.2.2. The impact of different physical exercise programs in the aquatic environment on functional fitness in community dwelling elderly.

Another specific objective of study 3 was to analyse the impact of different aquatic exercise programs on functional fitness in community dwelling elderly. The obtained results showed that the three physical exercise programs in water presented statistically significant improvements for lower limb muscle strength (30s-CS), and for right- and left-hand grip strength (HG-R and HG- L). Additionally, the continuous aerobic program and the aerobic interval program also caused a statistically significant improvement in aerobic capacity (2min-ST) and upper limb muscle strength (30s-AC). The aerobic interval program also caused a statistically significant improvement in upper limb flexibility (BS-R). This data suggests that physical exercise in an aquatic environment can improve functional fitness in community dwelling elderly, namely muscle strength, aerobic capacity and flexibility.

When compared with the results of other intervention studies in the aquatic environment, they reinforce the same evidence. Aidar et al. (2018) and Junior et al. (2018b) also demonstrated a statistically significant improvement for lower limb muscle strength after intervention. Dziubek et al. (2015) demonstrated a significant improvement, after intervention, for lower and upper limb muscle strength, aerobic capacity and flexibility Henwood et al. (2015) demonstrated an improvement for handgrip strength, being statistically significant in the left hand. Previously, Kim e O'sullivan (2013), had already demonstrated statistically significant improvements for muscle strength and flexibility after intervention with an aerobic exercise program in an aquatic environment.

Our results also showed a non-significant improvement for the agility/dynamic balance (TUG) component in all exercise groups. This data is in agreement with those found in the studies by Aidar et al. (2018), Dziubek et al. (2015), Junior et al., (2018b), Bento et al. (2015) and Kim e O'sullivan (2013), where significant improvements were also observed, after intervention, for agility/dynamic balance. The study by Rieping et al. (2019) demonstrated, after an intervention on land, with an aerobic exercise program with chairs, a statistically significant improvement for upper limb muscle strength and agility/dynamic balance, as well as statistically significant improvements for lower limb muscle strength, after intervention with a muscle strength program using elastic bands. These data suggest that exercise in water may be equally beneficial compared to exercise on land, in improving functional fitness, namely for muscle strength and agility/dynamic balance.

Functional fitness is one of the dimensions that undergoes the most critical changes during ageing (Daneres & Voser, 2013), being directly related to functional independence (Garatachea & Lucia, 2013) and to indicators of physical and cognitive frailty syndrome (Furtado et al., 2018),), and this process is responsible for reduced functionality, increased risk of falls, decreased cognition and loss of autonomy. The results of this study are extremely important, since exercise in the aquatic environment caused a statistically significant improvement in functional fitness indicators, essential for the physical independence of the elderly (i.e., muscle strength, aerobic capacity and flexibility). This reinforces the idea that physical exercise in an aquatic environment, regardless of the type of program, can significantly improve functional fitness in community dwelling elderly people, and can be equally effective when compared to exercise on land. Therefore, we can say that exercise in this environment can be considered a good strategy to improve functional fitness in the elderly. Regarding the possible effects of different physical exercise programs on functional fitness, the results showed that all programs studied seem to have a similar effect on muscle strength. However, the aerobic and interval programs also caused significant improvements in aerobic capacity. The aerobic interval program may be even more beneficial for functional fitness, as it additionally provided statistically significant improvements for flexibility.

5.2.3. The impact of different exercise programs in aquatic environments on cognition in community dwelling elderly.

The last specific objective to be discussed about study 3 is related to the impact of different aquatic exercise programs on cognition in community dwelling elderly. Our results showed that the aquatic combined physical exercise program promoted an improvement in the participants' cognitive function (assessed through the MMSE). Regarding the remaining exercise groups, despite showing better results after the intervention, they were not statistically significant. In the control group participants, cognitive function was unchanged.

Once again, our results confirm those of other intervention studies with physical exercise programs in the aquatic environment, carried out previously. Ayan et al. (2017) demonstrated a statistically significant improvement in cognition levels (MMSE) in healthy adult women, evidencing that aquatic exercise is able to improve cognition and quality of life, through improvements in mental health. Similarly, Kang et al. (2020), demonstrated statistically significant improvements in cognitive function and BDNF levels in elderly women. Both studies showed that aquatic exercise programs are beneficial for cognitive function and that moderate-intensity aerobic exercise programs appear to have high benefits on cognitive function in the elderly. In the same sense, Chupel et al. (2017) also demonstrated statistically significant improvements in cognition in the elderly after intervention on land with a muscle strength program using elastic bands.

In our study, the combined exercise program (aerobic and muscular strength) was the only one that showed a statistically significant improvement in the MMSE test, revealing that the combination between the two types of exercise programs can be even more beneficial to improve cognitive function in the elderly.

Cognitive decline in the elderly is an increasing problem because, with the increase in average life expectancy, the probability of memory loss and decline in the performance of cognitive tasks also increases, leading to mild cognitive impairment and, successively, to cognitive impairment, loss of quality of life and loss of autonomy (Lasierra et al., 2021). As such, we showed that the combined exercise in an aquatic environment caused an improvement in cognitive function, while in the control group the values remained unchanged after a period of 28 weeks. Therefore, we can say that combined exercise in an aquatic environment can be considered a good strategy to improve cognitive function in community dwelling elderly.

5.3. The impact of different types of physical exercise programs in the aquatic environment on the immune profile in community dwelling elderly (objective 3).

The immune profile deteriorates with ageing, with a decrease in immune system function (immunosenescence). Senescent cells accumulate in aged tissues and organs, leading to the development of disease. This process is strongly related to the increase in morbidity and mortality associated with infectious diseases in the elderly population. Thus, it is important to look for strategies that contribute to improving the immune profile of the elderly. The results obtained in study 3 showed that the different physical exercise programs in the aquatic environment caused significant changes in variables that were significantly correlated with immunological indicators (correlations verified in study 2), namely aerobic capacity, muscle strength and cognition. These data led us to believe that physical exercise in an aquatic environment could also cause changes in the immune profile in community dwelling elderly. Thus, we decided to investigate the impact of different physical exercise programs on hematological and immune variables in community dwelling elderly people, which led us to study 4, which was divided into two specific objectives.

5.3.1. The impact of different exercise programs in the aquatic environment on the hematological profile in community dwelling elderly.

The first point of study 4 focused on the analysis of the impact of different physical exercise programs in aquatic environments on the haematological profile in community dwelling elderly. The results obtained showed that physical exercise in an aquatic environment, regardless of the program, provided a statistically significant reduction in ERI, Hct, MCV and MPV, and a statistically significant increase in MCH and MCHC. In the aerobic and combined interval exercise programs, there were still statistically significant increases in LEU and GR. Additionally, the continuous aerobic program caused a statistically significant reduction in ADP, and the combined program caused a significant increase in Hb and a significant reduction in RDW and Pct.

Therefore, it is suggested that exercise in an aquatic environment can cause beneficial changes in the hematological profile in elderly participants. Chupel et al. (2017) also demonstrated a statistically significant increase for Hb, MCH and MCHC, after intervention on land with a muscle strengthening exercise program using elastic bands in elderly women. Nishiyama et al. (2016) demonstrated a statistically significant reduction for RDW and ERI, and a non-significant reduction for MCV, after intervention with a 3-week cycle stationary exercise program. El-Lithy et al. (2015) demonstrated a statistically significant increase for Hb, an increase tendency for MCH and a tendency decrease for MCV, after intervention with a 3-month aerobic exercise program. Rissanen et al. (2020) demonstrate a statistically significant increase for Hb, after intervention of a combined program (muscle strength, resistance and sauna). Juszkiewicz et al. (2019) demonstrated, after testing 2000 m on a rowing ergometer, a non-significant increase for Durmuş et al. (2021) demonstrated a statistically significant reduction for MPV after intervention with a program with aerobic exercise and relaxation. Romagnoli et al. (2014) demonstrated a non-significant reduction for Pct after maximal treadmill test in trained athletes. After a 6-day cycling intervention, Ansley et al. (2009) demonstrated an increase for Pct during the first 3 days and a reduction for Pct in the last 3 days. Martins et al. (2016) demonstrated a statistically significant reduction for ADP, after 30 weeks of intervention with physical exercise in the land environment.

However, other studies have also shown something slightly different. Chupel et al. (2017) found a statistically significant increase for MCV and an equally significant reduction for LEU, with no statistically significant changes being observed for the variables Hct and ERI. Johannsen et al. (2012) demonstrated a statistically significant reduction for LEU after intervention with aerobic exercise for 6 months. Santos (2020) after a one-year intervention with an aquatic exercise program in elderly women, demonstrated a reduction for MCH and MCHC. For Hb, no changes were found after the intervention either. Gillum et al. (2017) demonstrated a statistically significant reduction for GR after a single 45-minute running session at an intensity of 75% of VO2 max. Myette-Côté et al. (2018) demonstrated a reduction for GR after a 4-day walking intervention. El-Lithy et al. (2015) demonstrated a statistically significant increase for Hct and a tendency for decrease in MCHC.

Our data reinforces the idea that exercise in an aquatic environment, regardless of the type of program, can improve the hematological profile, in community dwelling elderly, and can be equally effective when compared to land-based exercise programs. They clearly reveal a positive variation in relation to hematological health. High levels in MCV and ERI values are associated with a higher risk of death from all causes, especially from cardiovascular and infectious diseases (Dratch et al., 2019). The increase in LEU values also has hematological implications, as leukocytes play an important role in tissue recovery and host defence, as well as antimicrobial properties (D'asta et al., 2018). Regarding the Hct variable, the variation found in our study can be considered negative, as low levels of Hct are associated with the presence of anaemia, which can lead to the development of several chronic diseases (Suresh et al., 2021). However, despite the reduction of Hct values, they are within the reference values.

The results obtained also show a more positive effect on hematological health, when compared to the intervention in an aquatic environment (Santos, 2020), as they demonstrate statistically significant changes for the variables MCH, MCHC and Hb. Although some of our results are slightly different from those found in the literature, the values of all analysed variables remain within normal limits, which shows that physical exercise in an aquatic environment can contribute to the maintenance of hematological variables. These mentioned differences may be directly related to the typology of physical exercise programs applied in the different studies. In our study, the impact of different aquatic exercise programs (continuous aerobic, aerobic interval and combined) was tested. Our search did not find any other intervention studies that included these types of programs or variables, thus making our study an important practical contribution to this field of research.

Regarding the possible effects of different physical exercise programs on the hematological profile, the results showed that the programs studied seem to have a positive effect on hematological health. However, the combined exercise program (aerobic and muscle strength exercises) was the one that showed statistically significant changes in the greatest number of variables, suggesting that this program may be more beneficial to the hematologic health of community dwelling elderly.

5.3.2. The impact of different aquatic exercise programs on the immune profile in community dwelling elderly.

Continuing with the previous objective, in study 4, we also analysed the impact of different physical exercise programs in aquatic environments on the immune profile in community dwelling elderly. The results obtained showed that all exercise groups (AerG, IntG and ComG) caused a decrease in the pro-inflammatory markers IL-1ß and TNF-α. For IL-1ß, there was a tendency to decrease in all exercise groups. However, there was a statistically significant increase in the CG. For the variable TNF-α, there was a statistically significant reduction in all exercise groups, while in the CG the values increased. As for the anti-inflammatory markers, a statistically significant increase was observed in the plasma levels of the anti-inflammatory IL-10 in IntG, while in the other groups the values showed a downward trend. This increase verified in IntG in IL-10 values can be justified by the increase in intensity that was verified in the exercise sessions. In the study by Minuzzi et al. (2017), an increase in IL-10 levels was observed in response to increased training volume and intensity in athletes. As for IL-1ra levels, our results showed an increasing trend in all exercise groups, while in the CG a statistically significant reduction was observed.

Regarding the ratios, our results showed a reduction in the TNF-α /IL-10 ratio in all exercise groups, with statistically significant differences in IntG. For the IL-1ß /IL-1ra ratio, there were also statistically significant reductions in ComG. These data suggest that physical exercise in an aquatic environment can provoke a positive response in the inflammatory profile, reflecting in a less pro-inflammatory environment, which contributes to the reduction of the development of cardiovascular diseases. In the study by Kumari et al. (2018) the TNF-α /IL-10 ratio was associated with the risk of coronary artery disease, suggesting that this marker may play a vital role in the development of this type of pathologies.

These data support previous conclusions of other authors. In interventions carried out on land, with a muscle strength program, Chupel et al. (2017) also demonstrated a statistically significant increase for IL-10. In another study by the same authors, Chupel et al. (2018), after intervention with a 14-week combined exercise program (aerobic and muscle strength), the results demonstrated a statistically significant increase for IL-10, IL-1ra and a statistically significant decrease for TNF-α and IL-1ß , and TNF-α /IL-10 and IL-1ß /IL-1ra ratios. Furtado et al. (2020) also demonstrated a statistically significant reduction for IL-1ß following intervention with a 28-week combined program. Regarding the interventions carried out in an aquatic environment, Ortega et al. (2012) also demonstrated a statistically significant reduction for the pro-inflammatory markers IL-1ß and TNF-α, after intervention with an 8-month aerobic aquatic program. Silva et al. (2018), after intervention with a 12-week low-intensity aquatic exercise program, also demonstrated a statistically significant reduction for TNF-α.

However, other studies also demonstrate something slightly different. Ortega et al. (2012) and Korb et al. (2018) found a statistically significant reduction in the anti-inflammatory marker IL-10. In the study by Korb et al. (2018) the values of the pro-inflammatory marker IL-1ß remained unchanged after intervention with a 12-week water walking program.

Our results suggest that physical exercise in an aquatic environment can improve the immune profile in community dwelling elderly. This exercise caused a “buffer” effect on both anti-inflammatory and pro-inflammatory markers, as the results in the exercise groups followed a positive trend in contributing to a less inflammatory environment. In the control group, the opposite occurred, with an increase in the inflammatory environment. These data therefore provide evidence that physical exercise programs in an aquatic environment not only have a positive effect in terms of stability, but also improve the immune profile. As for the different types of programs, AerG had a greater mean variation for TNF-α, IntG had a greater mean variation for IL-10 and IL-1ß, and ComG had a greater mean variation for IL-1ra and TNF- α (same value as AerG). However, further studies are needed to clarify which type of program is the most beneficial to improve the immune system in the elderly.

5.4. The impact of different types of physical exercise programs in aquatic environments on cardiovascular risk markers in community dwelling elderly (objective 4).

Cardiovascular diseases are responsible for 31% of deaths worldwide, and it is essential to find strategies that contribute to improve the cardiovascular health of the elderly. These diseases can be predicted through markers, such as the intima and media thickness of the carotid arteries and the chemokines MCP-1 and MIP-1α. Both are strongly associated with the development of atherosclerotic disease and coronary artery disease and are also directly correlated with the ageing process. The results of study 4 showed that different physical exercise programs in an aquatic environment can cause significant changes in the immune profile, contributing to the development of a less pro-inflammatory environment, which will consequently reduce the probability of developing cardiovascular diseases. Thus, we believe that exercise in an aquatic environment can also contribute to a significant improvement in cardiovascular risk markers in community dwelling elderly. This belief led us to the development of study 5, which was divided into 4 specific objectives.

5.4.1. The impact of different water-based exercise programs on the intima and media thickness of the carotid arteries in community dwelling elderly.

The first specific objective analysed in study 5 is related to the impact of different aquatic exercise programs on the intima and media thickness of the carotid arteries in community dwelling elderly. The results obtained showed that in all the variables analysed, trends towards reduction were verified, as a result of the intervention of the different programs. In more detail, statistically significant reductions were found in AerG for DD-R, in IntG for PSV-R, and in ComG for DD-L, EDV-L and DD-R.

These data suggest that physical exercise in an aquatic environment provided positive changes in the intima and mean thickness of the carotid arteries in community dwelling elderly people, thus reinforcing the results obtained in the study by Santos (2020), where statistically significant reductions were observed in the variables IMT, DS, DD, PSV and EDV, after intervention with a 10-month aquatic exercise program in elderly women. With regard to programs carried out on land, Park & Park (2017) demonstrated a statistically significant reduction for IMT, after an intervention with a combined exercise program (muscle and aerobic strength exercise). Similarly, Farahati et al. (2020) also demonstrated a statistically significant reduction for IMT after an intervention with two exercise programs (high-intensity interval and continuous aerobic exercise). That said, we can conclude that exercise in an aquatic environment can be equally beneficial compared to exercise on land, for IMT.

Our results showed a trend towards the improvement of IMT. For IMT-R, there was a slight reduction in all exercise groups and for IMT-L there was a reduction in IntG. The other groups remained unchanged. These data corroborate those found in the study by Santos (2020), Park & Park (2017) and Farahati et al. (2020), where statistically significant reductions for IMT were found after their interventions.

Our results thus reinforce the idea that physical exercise in an aquatic environment can significantly improve the intima and media thickness of the carotid arteries in non-community dwelling. According to Homma et al. (2001), there is a strong and positive correlation between advancing age and increasing IMT (r=0.83). In our study, although the results did not significantly decrease in all variables in the exercise groups, they showed a tendency to do so. Thus, we can say that physical exercise in an aquatic environment may have caused a balance/improvement in the hemodynamic system, contributing to the reduction of stress on the arterial walls, and to reducing the risk of developing cardiovascular diseases. Regarding the effects of different exercise programs on the intima and mean thickness of the carotid arteries, the results showed that all programs studied appear to have a similar effect. However, the combined program appears to be slightly more effective as it showed statistically significant reductions in a greater number of variables.

5.4.2. The impact of different aquatic exercise programs on blood pressure and heart rate in community dwelling elderly.

Following the previous objective, we analysed the impact of different physical exercise programs in aquatic environments on blood pressure and heart rate in community dwelling elderly. With the results obtained, we found that the different programs of physical exercise in the aquatic environment caused a reduction in the values of blood pressure and heart rate, namely statistically significant reductions in AerG for SBP and DBP, in IntG for DBP, and in ComG for SBP, DBP and HR.

Our results are in accordance with those found in other intervention studies. The study by Park et al. (2019), after intervention with a water walking program, also demonstrated reductions for SBP, DBP and HR (being only statistically significant for HR). With regard to programs carried out on land, Park et al. (2020) and Son et al. (2017), demonstrated a statistically significant reduction for SBP and BPD, after an intervention with combined exercise programs that included muscle strength and aerobic capacity exercises. These data suggest that exercise in water may have similar effects to land-based exercise on blood pressure.

With ageing, arteries lose their elasticity, increasing not only the systolic pressure (which will consequently cause an increase in the systolic diameter), but also the blood flow velocity, which can lead to turbulent blood flow and respective atheromatous plaques. These events can lead to vessel obstruction or occlusion (Santos, 2020). The results of our study have important practical implications, as they reinforce the idea that exercise in an aquatic environment can improve, in a significant way, blood pressure and heart rate, which is very important for the prevention of the development of cardiovascular diseases.

Regarding the effects of different exercise programs on blood pressure and heart rate, the results showed that both programs can have benefits. Although, in our study, the three exercise programs caused a reduction in SBP and BPD, the continuous and combined aerobic exercise programs appear to be the most beneficial. Both showed identical results in SBP and DBP, with the aerobic exercise program showing a slightly greater reduction in the mean compared to the combined exercise program. Additionally, the combined exercise program was the only one that showed a statistically significant reduction in HR. Thus, we can conclude that the combined exercise program may be more beneficial, as in addition to having presented identical results to the aerobic program for SBP and BPD, it also presented a significant decrease in HR.

5.4.3. The impact of different aquatic exercise programs on the metabolic profile in community dwelling elderly.

The next point of article 5 consisted of the analysis of the impact of different exercise programs in the aquatic environment on the metabolic profile. The results obtained showed a statistically significant reduction in GLU for AerG and IntG. Regarding the variable AI, there were no significant changes in the physical exercise groups. However, in the control group a statistically significant increase was visible. As for the variables CLT, HDL, LDL and TG, our results did not show significant changes. For CLT, the values tended to decrease in IntG and ComG. HDL showed a tendency to increase in AerG and ComG. LDL had a tendency to decrease in ComG, and TG showed a tendency to decrease in all exercise groups.

These data suggest that exercise in an aquatic environment, in addition to having contributed to a balance in the metabolic profile, provided a significant improvement in blood glucose levels in community dwelling elderly, thus reinforcing the results found in other intervention studies. Cugusi et al. (2015) demonstrated, after intervention with a moderate aerobic exercise program, performed in an aquatic environment, a statistically significant reduction for GLU, CLT, LDL, TG and a trend towards a reduction for HDL. Costa et al. (2019) also demonstrated a statistically significant reduction for CLT, LDL, TG and a statistically significant increase for HDL, after intervention with two different programs (aerobic and muscle strength exercise). Regarding programs carried out on land, Keyhani et al. (2020) demonstrated a statistically significant reduction for CLT, LDL and TG and a statistically significant increase for HDL, after an intervention with two different programs (high-intensity interval and moderate-intensity continuous aerobic exercise). Dieli-Conwright et al. (2018) also demonstrated a statistically significant reduction for GLU, CLT, LDL and TG and a significant increase for HDL, after an intervention with a combined exercise program (aerobic and muscle strength exercise). These data suggest that, also with regard to the metabolic profile, exercise in an aquatic environment may be equally beneficial as exercise in a land-based environment.

Ageing leads to a deterioration of the metabolic profile, and when not controlled, this deterioration is directly related to cardiovascular and metabolic complications (Fittipaldi et al., 2020). The results of this study bring important practical applications insofar as exercise in an aquatic environment, in addition to causing stability in indicators of the metabolic profile, also significantly improved blood glucose levels. Thus, we can say that exercise in this environment can be considered a good strategy to improve the metabolic profile in the elderly. Regarding the effects of different physical exercise programs in aquatic environments on the metabolic profile, continuous aerobic and aerobic interval exercise programs showed that they are equally effective in reducing blood glucose levels. With regard to the remaining variables analysed, the results were very similar between the exercise groups, and further studies are needed to better clarify the differences between the various types of programs.

5.4.4. The impact of different exercise programs in the aquatic environment on chemokines (MCP-1 and MIP-1α) in community dwelling elderly.

Finally, the last specific objective analysed in study 5 was the impact of different physical aquatic exercise programs on chemokines MCP-1 and MIP-1α. The results obtained showed a statistically significant reduction for MCP-1 in AerG and ComG and for and MIP-1α in AerG, thus suggesting that exercise in an aquatic environment can contribute to the reduction of the values of the chemokines analysed.

Our results partially agree with those found in other intervention studies. Barry et al. (2017) tested the effectiveness of two different land-based programs (high-intensity interval and continuous aerobic exercise) in obese adults, and the results showed a significant reduction in the continuous aerobic exercise program for MCP-1 and MIP-1α. In another study carried out by Kraemer et al. (2014), the effectiveness of a muscle strength program (on land) was tested in young adults, and the results showed an increase for MCP-1 and a reduction for MIP-1α.

Our results, despite having occurred in a different context and with a different sample from the studies found in the literature, showed partially similar conclusions. As in the study by Barry et al. (2017), our results from the continuous aerobic exercise program also showed a statistically significant reduction for both chemokines, thus reinforcing the idea that continuous aerobic programs contribute to the reduction of MCP-1 and MIP-1α. However, in our study's combined exercise program (aerobic and muscle strength exercise), the results showed a statistically significant reduction for MCP-1 and also a trend for the reduction of MIP-1α, suggesting that combined programs may be more effective in reducing chemokines, compared to muscle strength exercise programs applied in isolation.

The results of our study reinforce the notion that aerobic exercise programs can contribute to the reduction of chemokines, and also present the innovative idea that combined exercise programs can be more effective in reducing chemokines than muscle strength exercise programs alone.

Similar to what happened in the previous points, these data suggest that exercise in an aquatic environment have similar effects to exercise in a land environment.

Elevated levels of pro-inflammatory cytokines are associated with the development of several cardiovascular diseases. These cytokines trigger the formation of atheromatous plaques through the accumulation of macrophages and other cells and materials that affect the inner layer of blood vessels (Amin et al., 2020). In the intima and media layer of the carotid arteries, chemokines recruit monocytes that trigger the development of foam cells, leading to erosion of the intima, which is caused by atherosclerosis and consequently leads to ischemia (Amin et al., 2020). The results of this study are very important, as they prove that physical exercise in an aquatic environment, namely aerobic exercise, can help prevent the development of diseases related to atherosclerotic lesion, through the reduction of MCP-1 chemokines. and MIP-1α. Thus, exercise aerobic can contribute to the reduction of inflammation by reducing chemokines. As for the effects of the different exercise programs, the continuous aerobic exercise program proved to be more effective in reducing the plasma levels of the analysed chemokines, when compared to the other programs.

6. CONCLUSIONS

Our study found that individuals with higher performance in aerobic capacity (2min-ST) and handgrip strength (HG) tests present better results in variables related to physical function, cardiovascular system, biochemical markers and mental health. Additionally, individuals with better results in the cognitive function test (MMSE) also showed more beneficial results in variables related to physical function and mental health. This suggests that the levels of aerobic capacity, handgrip strength and cognitive level may be significantly associated with variables related to cardiometabolic diseases.

Regarding the impact of physical exercise in an aquatic environment, our results provided evidence of the beneficial effects of physical exercise in this environment, on body composition, functional fitness (mainly on lower and upper limb muscle strength, handgrip strength and aerobic capacity), and on cognitive function in community dwelling elderly. The combined program was also shown to be more effective in improving variables related to body composition and cognitive function, while the continuous aerobic and interval aerobic programs were more effective in functional fitness.

Regarding the immune profile, the results of the present study allowed us to conclude that the practice of physical exercise in an aquatic environment (regardless of the types of programs) can lead to beneficial changes in hematological variables in elderly participants. As for the immunological variables in general, all exercise programs caused a reduction in the levels of pro-inflammatory markers and of the inflammatory index TNF-α /IL-10, while in the CG there was an opposite effect. This means that the practice of exercise in an aquatic environment causes a buffering effect, contributing not only to stability, but also to the improvement of the inflammatory profile of the participants. As for the most effective type of program, the results were inconclusive, as different programs led to improvements in different variables. However, the combined program showed statistically significant improvements in a greater number of hematologic variables, and the aerobic interval program showed statistically significant improvements in a greater number of immune variables.

As for cardiovascular markers, the results showed that exercise in water, regardless of the program, seems to provide beneficial changes. Regarding the intima and media thickness of the carotid arteries, we can conclude that both programs can provide a hemodynamic balance, contributing to the reduction of stress on the arterial wall. Here, the combined program seems to be slightly more beneficial as it showed significant improvements in a greater number of variables. As for blood pressure and heart rate, the three programs showed reductions in SBP, DBP and HR after 28 weeks of practice. However, the combined exercise program seems to be more beneficial since in addition to presenting identical results to the continuous aerobic program for SBP and DBP, it was the only one where there was a statistically significant reduction for HR.

In the metabolic profile, the results showed that the continuous aerobic program and aerobic interval are equally beneficial in reducing blood glucose levels. Regarding the variables CLT, HDL, LDL and TG, the results did not show significant changes, but the values remained within the clinical reference parameters. Thus, we conclude that physical exercise in an aquatic environment can contribute to the balance of the metabolic profile. Finally, for MCP-1 and MIP-1α, the results suggest that exercise in an aquatic environment, namely programs with aerobic characteristics, provide a reduction in the plasma levels of these chemokines, thus contributing to the prevention of the development of diseases related to atherosclerosis.

In general, we can say that exercise in the aquatic environment provided beneficial changes in most of the analysed variables. Thus, the practice of exercise in this environment can be considered an effective tool for preventing the development of cardiometabolic diseases in community dwelling elderly. Our results, in addition to corroborating results previously found in the literature, also added new knowledge to this scientific area, namely because this is the only study that compared the impact of three different exercise programs in an aquatic environment (continuous aerobic, interval aerobic and combined exercise) using several methodologies (functional tests, questionnaires, imaging ultrasound, blood testing) on an ample array of variables, in community-dwelling elderly. Most of the results found in the present study are identical to those found in other studies with intervention in land-based environments. This means that exercising in an aquatic environment can be seen as a viable alternative to land-based exercise, especially when there are health conditions that make exercising on land difficult (for example: orthopaedic, rheumatological or functional limitations, circulatory diseases, vertigo, etc.).

FINAL CONSIDERATIONS

General limitations

Some limitations were observed while carrying the present study, namely at a methodological level. The fact that a simple randomization method was used, instead of a block randomization method that would guarantee a balance in the number of participants in each of the groups, thus reducing the unpredictability of the sequence, was the main limitation. Another limitation of the study was that the levels of daily physical activity of the elderly were not evaluated, nor the nutritional pattern, variables that may influence the variables analyzed.

Suggestions for future studies

As a suggestion for future studies, we consider that more intervention studies in aquatic environments are necessary, in order to further corroborate the benefits that the practice of physical exercise in this environment would have in an ageing population. Namely, correlation studies that analyse the impact of exercise in an aquatic environment on immune and cardiometabolic markers, since our results did not show statistically significant association between some of the variables analysed. There is also a need for more intervention studies that seek to compare physical exercise in aquatic environments and exercise in land environments, in order to reinforce the evidence found in our study, that exercise in aquatic environment has identical benefits to exercise in a land-based environment, and could be considered a viable alternative, when the exercise in land-based environment is not feasible or desired. Further intervention studies with different aquatic exercise programs are also needed, in order to clarify the effects of different programs on cardiometabolic markers and to understand whether there are programs that are more beneficial than others. In our study, this comparison was not conclusive, since each of the programs studied had differentiated impacts in the variables studied. To the best of our knowledge, this was the only study that tested the impact of three different aquatic exercise programs (continuous aerobic, interval aerobic, and combined aerobic exercise program). It would also be interesting to understand the impact of these three programs in a shorter intervention period (e.g., 4 months) and also in a longer intervention period (e.g., 24 months). Studies that compare other types of programs in the aquatic environment (e.g., walking, relaxation, cycling, circuit, etc.) would also be of interest. Finally, we suggest carrying out intervention studies with programs in the aquatic environment with other age groups (e.g., adolescents, young adults, adults, etc.), or special groups (e.g. obesity, type II diabetics, cancer recovering patients, etc) in order to understand the benefits that the practice of aquatic exercise could have in these groups.

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ANNEXES

Annex 1: Consent forms

CONSENTIMENTO INFORMADO, ESCLARECIDO E LIVRE PARA PARTICIPAÇÃO EM ESTUDOS DE INVESTIGAÇÃO

(de acordo com a Declaração de Helsínquia e a Convenção de Oviedo)

<u>Título do estudo:</u>

Estudo de intervenção.

<u>Enquadramento:</u>

Testar o impacto de diferentes programas de exercício físico em meio aquático, em marcadores de risco de doenças cardiometabólicas e na cognição.

Estudo realizado no âmbito do Doutoramento em Ciências do Desporto no Ramo de Atividade Física e Saúde, da Faculdade de Ciências do Desporto e Educação Física da Universidade de Coimbra, sob coordenação do Professor Doutor José Pedro Ferreira, Professora Doutora Ana Maria Teixeira e, co-coordenação do Professor Doutor João Júlio Serrano.

<u>Explicação do estudo:</u>

Os participantes serão distribuídos aleatoriamente em 4 grupos: grupo nº1, nº2 e nº3 irão participar em diferentes programas de exercício físico em meio aquático, durante 28 semanas. O grupo nº4 será constituído por participantes que não irão estar envolvidos em nenhum programa específico de exercício físico (grupo de controlo). Após 14 semanas de práticas, os participantes serão submetidos a um programa de desenvolvimento cognitivo paralelamente com o programa de exercício físico.

Todos os participantes serão avaliados em três momentos: no início do estudo, após 14 semanas de prática e, no final do estudo (após 28 semanas). Os participantes serão avaliados nas instalações da Piscina Municipal da Sertã, sendo os mesmos informados das datas e respetivos horários com antecedência.

Serão recolhidos os seguintes dados:

- <u>Antropométricos</u>: balança e fita métrica
- <u>Aptidão física</u>: bateria de testes (Functional Fitness Test)
- <u>Variabilidade da frequência cardíaca</u>: monitor de frequência cardíaca
- <u>Saúde psicológica</u>: questionários
- <u>Nível cognitivo</u>: questionários
- <u>Espessura das camadas íntima e média das artérias carótidas</u>: ultra-sonagrafia com Doppler
- <u>Marcadores bioquímicos</u>: recolha de amostras de sangue em jejum

<u>Condições e financiamento:</u>

O presente estudo não implica quaisquer deslocações adicionais, nem envolve quaisquer contrapartidas ou pagamentos aos investigadores ou aos utentes que voluntariamente aceitem participar.

A participação no estudo é de carater voluntário, não havendo qualquer tipo de prejuízos assistenciais ou outros pela não participação.

Este estudo mereceu Parecer favorável da Comissão de Ética para a Saúde da Faculdade de Ciências do Desporto e Educação física da Universidade de Coimbra.

<u>Confidencialidade e anonimato:</u>

Todos os dados recolhidos ao longo do estudo serão confidenciais e utilizados exclusivamente para fins de investigação científica.

Os dados recolhidos serão anónimos, cumprindo com o Regulamento de Proteção de Dados, em vigor a partir de 25 de maio de 2018.

Foi solicitada e obtida autorização da Comissão Nacional de Proteção de Dados, garantindo, em qualquer caso, que a identificação dos participantes nunca será tornada pública e que, os contactos realizados entre a equipa de investigação e os participantes, serão realizados em ambiente de privacidade.

Agradecemos a vossa autorização, que nos concede para a recolha de dados e sua utilização para fins de investigação científica.

<u>Identificação do investigador:</u>

Nome: Carlos Manuel Nunes Farinha

Local de trabalho: Faculdade de Ciências do Desporto e Educação Física da Universidade de Coimbra

Contacto telefónico: 924 167 929

Contacto eletrónico: cmnfarinha@gmail.com

Por favor, leia com atenção a seguinte informação. Se achar que algo está incorreto ou que não está claro, não hesite em solicitar mais informações. Se concorda com a proposta que lhe foi feita, queira assinar este documento.

Assinatura/s de quem pede consentimento:

____

Declaro ter lido e compreendido este documento, bem como as informações verbais que me foram fornecidas pela/s pessoa/s que acima assina/m. Foi-me garantida a possibilidade de, em qualquer altura, recusar participar neste estudo sem qualquer tipo de consequências. Desta forma, aceito participar neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade e anonimato que me são dadas pelo/a investigador/a.

Nome:

____

____

Assinatura: ____

Data: _____/_____/_______

SE NÃO FOR O PRÓPRIO A ASSINAR POR IDADE OU INCAPACIDADE

(se o menor tiver discernimento deve também assinar em cima, se consentir)

Nome:

____

____

BI/CC N.º: ____ Data ou validade ______/_____ /_______

Grau de parentesco ou tipo de representação:

____

Assinatura:

____

ESTE DOCUMENTO É COMPOSTO DE DUAS PÁGINA/S E FEITO EM DUPLICADO:

UMA VIA PARA O/A INVESTIGADOR/A, OUTRA PARA A PESSOA QUE CONSENTE.

Annex 2: Test batteries

MINI MENTAL STATE EXAMINATION (MMSE)

Código: ____ Idade: ______

Data da avaliação: _____/_____/_____

1- Orientação (1 ponto por cada resposta correta)

1.1- Em que ano estamos? _____
1.2- Em que mês estamos? _____
1.3- Em que dia do mês estamos? _____
1.4- Em que dia da semana estamos? _____
1.5- Em que estação do ano estamos? _____
1.6- Em que país vive? _____
1.7- Em que distrito vive? _____
1.8- Em que terra vive? _____
1.9- Em que casa estamos? _____
1.10- Em que andar estamos? _____

Pontos: _____

2- Retenção (1 ponto por cada palavra corretamente repetida)

"Vou dizer três palavras, queria que as repetisse, mas só depois de eu as dizer todas. Tente decora-las."

Pera _____ Gato _____ Bola _____

Pontos: _____

3- Atenção e Cálculo (1 ponto por cada resposta correta)

Se der uma resposta errada mas depois continuar a subtrair bem, consideram-se as seguintes como correctas. Parar ao fim de 5 respostas.

"Agora peço-lhe que me diga quantos são 30 menos 3 e depois, ao número encontrado, volte a tirar 3 e repete assim até eu lhe dizer para parar."

27 _____
24 _____
21 _____
18 _____
15 _____

Pontos: _____

4- Evocação (1 ponto por cada resposta correta)

"Veja se consegue dizer as três palavras que pedi há pouco para decorar."

Pera _____

Gato _____

Bola _____

Pontos: ________

5- <u>Linguagem</u> (1 ponto por cada resposta correta)

a. "Como se chama isto?" Mostrar os objectos:

Relógio _____

Lápis _____

Pontos: _____

b. "Repita a frase que eu vou dizer: O RATO ROEU A ROLHA"

Pontos: _____

c. "Quando eu lhe der esta folha de papel, pegue nela com a mão direita, dobre-a ao meio e ponha sobre a mesa." (dar a folha segurando com as duas mãos)

Pega com a mão direita _____

Dobra ao meio _____

Coloca onde deve _____

Pontos: _____

d. "Leia o que está neste cartão e faça o que lá diz." Mostrar um cartão com a frase bem legível, "FECHE OS OLHOS". Sendo analfabeto, lê-se a frase.

Fechou os olhos _____

Pontos: _____

e. "Escreva uma frase inteira aqui." (Deve ter sujeito e verbo e fazer sentido; os erros gramaticais não prejudicam a pontuação)

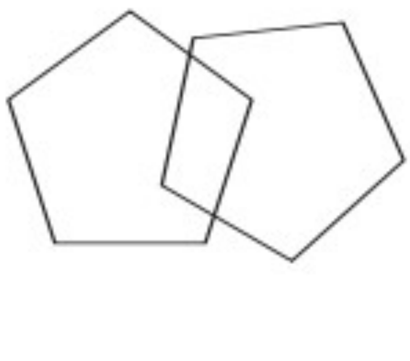
____
_____

Pontos: _____

6- <u>Habilidade Construtiva</u> (1 ponto pela cópia correta)

Deve copiar um desenho. Dois pentágonos parcialmente sobrepostos, cada um deve ficar com 5 lados, dois dos quais intersectados. Não valorizar tremor ou rotação.

DESENHO: CÓPIA:



Pontos: ________

TOTAL (Máximo 30 pontos):________

UNIVERSIDADE DE COIMBRA

Faculdade de Ciências do Desporto e Educação Física

(Doutoramento em Ciências do Desporto – Atividade Física e Saúde)

N.º processo: ______________

Bateria de testes para avaliação do Nível Cognitivo e Saúde Psicológica

Versão Portuguesa

(Utilização sujeita a autorização prévia)

jpferreira@fcdef.uc.pt

cmnfarinha@gmail.com

Estes questionários destinam-se à realização de um projeto de investigação na área das Ciências do Desporto – Atividade Física e Saúde.

Trata-se de um instrumento que envolve a recolha de **informação confidencial**, pelo que **nunca**, no decorrer deste projeto será divulgada a identificação dos indivíduos nele intervenientes.

Ao responderem às questões, façam-no de uma forma sincera e, por favor, não deixem qualquer questão por responder, pois disso, **dependerá** o **rigor científico deste projeto.**

Obrigado pela sua colaboração!

Para cada item, faça uma cruz sobre o rectângulo que corresponde à concepção de valor que tem por si próprio(a):

	Concordo Completamente	Concordo	Discordo	Discordo Completamente
1. No geral, estou satisfeito(a) comigo mesmo(a).	☐	☐	☐	☐
2. Por vezes penso que não sou nada bom (a).	☐	☐	☐	☐
3. Sinto que tenho um bom número de qualidades.	☐	☐	☐	☐
4. Estou apto(a) para fazer coisas tão bem como a maioria das pessoas	☐	☐	☐	☐
5. Sinto que não tenho muito de que me orgulhar.	☐	☐	☐	☐
6. Sinto-me por vezes inútil.	☐	☐	☐	☐
7. Sinto que sou uma pessoa de valor, pelo menos num plano de igualdade com os outros.	☐	☐	☐	☐
8. Gostava de ter mais respeito por mim mesmo(a).	☐	☐	☐	☐
9. Em termos gerais estou inclinado(a) a sentir que sou um(a) falhado (a).	☐	☐	☐	☐
10. Eu tomo uma atitude positiva perante mim mesmo(a).	☐	☐	☐	☐

Estas são afirmações que permitem às pessoas descrever-se a si mesmas. Para cada linha:

1. Decida **qual** das duas afirmações o descreve melhor (a afirmação da esquerda **ou** a da direita)
2. Assinale com uma **CRUZ** o lado que merece a sua maior concordância (quase verdade para mim **ou** realmente verdade para mim).
3. Por favor, não assinale com uma CRUZ em ambos os lados.

Realmente verdade para mim	**Quase verdade para mim**	**EXEMPLO**			**Quase verdade para mim**	**Realmente verdade para mim**
☐	☐	Algumas pessoas são muito competitivas	MAS	Outras não são assim tão competitivas	☒	☐
☐	☐	Algumas pessoas conseguem ser fisicamente activas no seu dia-a-dia	MAS	Outras acham difícil a actividade física diária	☐	☐
☐	☐	Algumas pessoas acham que, quando comparadas com a maioria, a sua saúde física não é a melhor	MAS	Outras acham que, quando comparadas com a maioria, têm uma saúde física muito boa	☐	☐
☐	☐	Algumas pessoas sentem que não são muito boas a praticar desporto	MAS	Outras sentem que são mesmo boas em qualquer desporto	☐	☐
☐	☐	Algumas pessoas sentem que, comparadas com a maioria da sua idade, têm um corpo atraente	MAS	Outras sentem que, comparadas com a maioria da sua idade, não têm um corpo propriamente atraente	☐	☐
☐	☐	Algumas pessoas sentem que são fisicamente fortes para a sua idade	MAS	Outras sentem que lhes falta força física quando comparadas com pessoas da sua idade	☐	☐
☐	☐	Algumas pessoas sentem-se extremamente satisfeitas pelo que são fisicamente	MAS	Outras sentem-se um pouco insatisfeitas pelo que são fisicamente	☐	☐
☐	☐	Algumas pessoas sentem-se confiantes quanto à sua capacidade de desempenhar as suas actividades e tarefas do dia-a-dia	MAS	Outras sentem-se menos capazes de desempenhar as suas actividades e tarefas do dia-a-dia	☐	☐

Realmente verdade para mim	Quase verdade para mim				Quase verdade para mim	Realmente verdade para mim
☐	☐	Algumas pessoas sentem que têm de visitar frequentemente o seu médico por causa da sua saúde física	MAS	Outras são fisicamente saudáveis e raramente visitam o seu médico	☐	☐
☐	☐	Algumas pessoas sentem que são capazes de ter um bom desempenho em actividades desportivas	MAS	Outras sentem que não têm um bom desempenho no desporto	☐	☐
☐	☐	Algumas pessoas sentem que o seu físico ou figura são por vezes admirados	MAS	Outras raramente sentem que são admiradas pela aparência do seu físico ou figura	☐	☐
☐	☐	Algumas pessoas sentem falta de confiança na sua força física	MAS	Outras sentem-se muito confiantes relativamente à sua força física	☐	☐
☐	☐	Algumas pessoas sentem-se extremamente orgulhosas face à forma como são e àquilo que são capazes de fazer em termos físicos	MAS	Outras raramente sentem orgulho na forma como são fisicamente	☐	☐
☐	☐	Algumas pessoas sentem-se confiantes quanto à capacidade de se manterem regularmente activos e em forma	MAS	Outras não se sentem confiantes quanto à capacidade de se manterem regularmente activos e em forma	☐	☐
☐	☐	Algumas pessoas são mais lentas do que a maioria, no que diz respeito à aprendizagem de gestos técnicos desportivos	MAS	Outras parecem ser mais rápidas na aprendizagem de gestos técnicos desportivos	☐	☐
☐	☐	Algumas pessoas sentem que o seu corpo tem uma boa aparência para a idade que têm	MAS	Outras sentem que o seu corpo não apresenta uma aparência tão boa quanto desejariam	☐	☐
☐	☐	Algumas pessoas não se sentem muito confiantes quanto à capacidade de se manterem fisicamente saudáveis	MAS	Outras sentem-se bastante confiantes quanto à capacidade de se manterem fisicamente saudáveis	☐	☐

Realmente verdade para mim	**Quase verdade para mim**				**Quase verdade para mim**	**Realmente verdade para mim**
☐	☐	Algumas pessoas sentem que não são tão boas a lidar com situações que requerem força física	MAS	Outras sentem que frequentemente se saem bem quando envolvidas em situações que requerem força física	☐	☐
☐	☐	Algumas pessoas têm sempre sentimentos positivos acerca da sua parte física	MAS	Outras por vezes não têm sentimentos positivos acerca da sua parte física	☐	☐

Indique, por favor, para cada uma das cinco afirmações, a que se aproxima mais do modo como se tem sentido nas últimas duas semanas. Note que os números maiores indicam maior bem-estar.

Exemplo: Se ao longo das últimas duas semanas se sentiu alegre e bem disposto/a durante mais de metade do tempo, coloque uma cruz no quadrado com o número 3

	Durante as últimas duas semanas	Todo o tempo	A maior parte do tempo	Mais de metade do tempo	Menos de metade do tempo	Algumas vezes	Nunca
1	Senti-me alegre e bem disposto/a	☐	☐	☐	☐	☐	☐
2	Senti-me calmo/a e tranquilo/a	☐	☐	☐	☐	☐	☐
3	Senti-me ativo/a e enérgico/a	☐	☐	☐	☐	☐	☐
4	Acordei a sentir-me fresco/a e repousado/a	☐	☐	☐	☐	☐	☐
5	O meu dia-a-dia tem sido preenchido com coisas que me interessam	☐	☐	☐	☐	☐	☐

Esta escala compreende cinco fases com as quais poderá concordar ou discordar.
Utilize a escala de 1 a 5 e marque uma cruz (x) no quadro que melhor indica a sua resposta).

	Discordo muito (1)	**Discordo um pouco (2)**	**Nem concordo, nem discordo (3)**	**Concordo um pouco (4)**	**Concordo muito (5)**
1. A minha vida parece-se, em quase tudo, com o que eu desejaria que fosse.					
2. As minhas condições de vida são muito boas.					
3. Estou satisfeito com a minha vida.					
4. Até agora, tenho conseguido as coisas importantes da vida, que eu desejaria.					
5. Se eu pudesse recomeçar a minha vida, não mudaria quase nada.					

Assinale com uma cruz (assim X), um quadrado de cada um dos seguintes grupos, indicando qual das afirmações descreve melhor o seu estado de saúde hoje.

Mobilidade

Não tenho problemas em andar ❑1
Tenho alguns problemas em andar ❑2
Tenho de estar na cama ❑3

Cuidados Pessoais

Não tenho problemas em cuidar de mim ❑1
Tenho alguns problemas a lavar-me ou vestir-me ❑2
Sou incapaz de me lavar ou vestir sozinho/a ❑3

Actividades Habituais (ex. trabalho, estudos, actividades domésticas, actividades em família ou de lazer)

Não tenho problemas em desempenhar as minhas actividades habituais ❑1
Tenho alguns problemas em desempenhar as minhas actividades habituais ❑2
Sou incapaz de desempenhar as minhas atividades habituais ❑3

Dor / Mal Estar

Não tenho dores ou mal estar ❑1
Tenho dores ou mal estar moderados ❑2
Tenho dores ou mal estar extremos ❑3

Ansiedade / Depressão

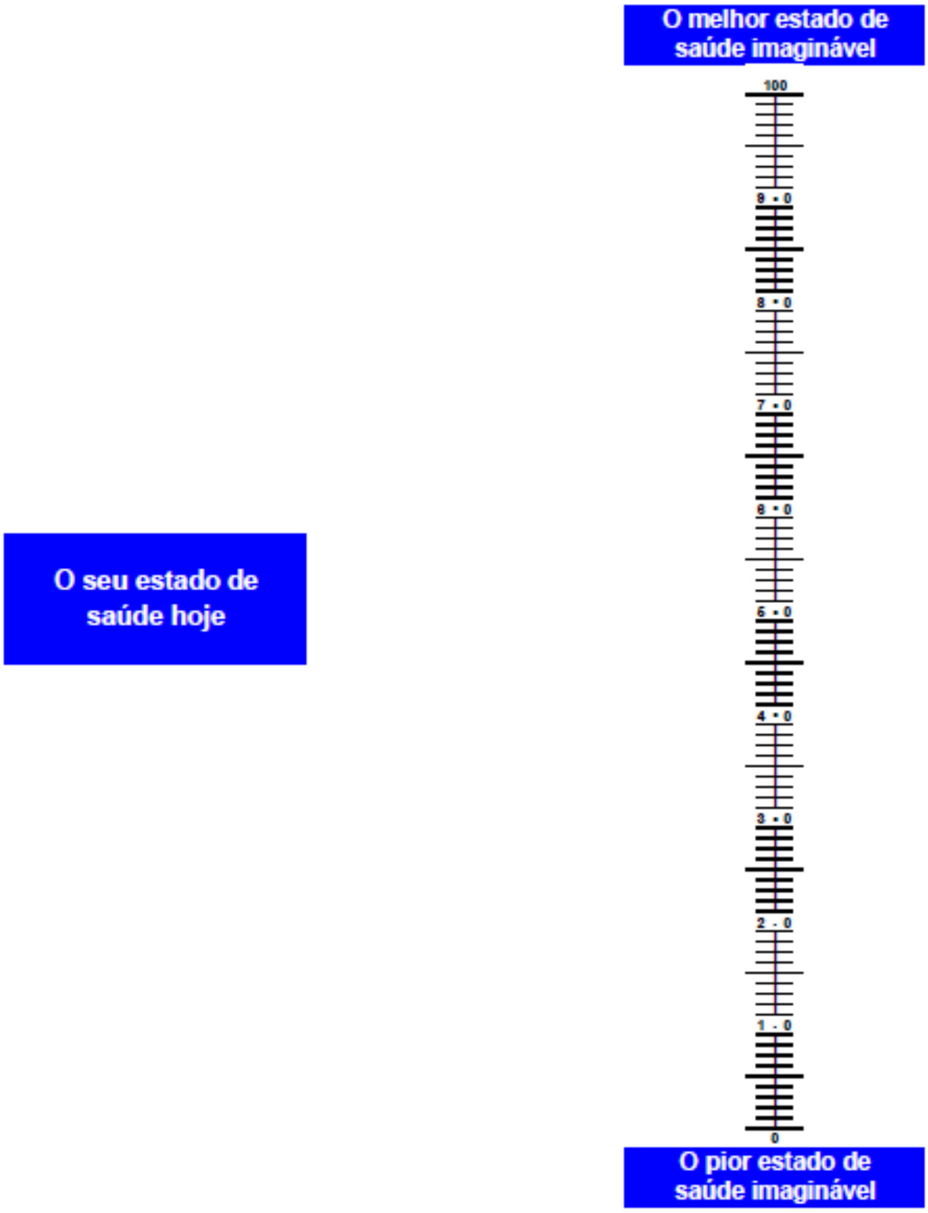
Não estou ansioso/a ou deprimido/a ❑1
Estou moderadamente ansioso/a ou deprimido/a ❑2
Estou extremamente ansioso/a ou deprimido/a ❑3

Comparado com o meu nível geral de saúde durante os últimos 12 meses, o meu estado de saúde hoje é:

Melhor ❑1
O mesmo ❑2
Pior ❑3

Para ajudar as pessoas a classificarem o seu estado de saúde, desenhámos uma escala (semelhante a um termómetro) na qual o melhor estado de saúde que possa imaginar é marcado por 100 e o pior estado de saúde que possa imaginar é marcado por 0.

Gostaríamos que indicasse nesta escala qual é hoje, na sua opinião, o seu estado de saúde. Por favor, desenhe uma linha a partir do rectângulo que se encontra à esquerda, até ao ponto da escala que melhor classifica o seu estado de saúde hoje.



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1. Está satisfeito (a) com sua vida?	Não ☐	Sim ☐
2. Diminuiu a maior parte de suas atividades e interesses?	Sim ☐	Não ☐
3. Sente que a vida está vazia?	Sim ☐	Não ☐
4. Aborrece-se com frequência?	Sim ☐	Não ☐
5. Sente-se de bem com a vida na maior parte do tempo?	Não ☐	Sim ☐
6. Teme que algo ruim lhe possa acontecer?	Sim ☐	Não ☐
7. Sente-se feliz a maior parte do tempo?	Não ☐	Sim ☐
8. Sente-se frequentemente desamparado (a)?	Sim ☐	Não ☐
9. Prefere ficar em casa a sair e fazer coisas novas?	Sim ☐	Não ☐
10. Acha que tem mais problemas de memória que a maioria?	Sim ☐	Não ☐
11. Acha que é maravilhoso estar vivo agora?	Não ☐	Sim ☐
12. Vale a pena viver como vive agora?	Não ☐	Sim ☐
13. Sente-se cheio(a) de energia?	Não ☐	Sim ☐
14. Acha que sua situação tem solução?	Não ☐	Sim ☐
15. Acha que tem muita gente em situação melhor?	Sim ☐	Não ☐

Instrução: Para cada questão, pedimos que indique com que frequência se sentiu ou pensou de determinada maneira, durante o último mês. Apesar de algumas perguntas serem parecidas, existem diferenças entre elas e deve responder a cada uma como perguntas separadas. Responda de forma rápida e espontânea. Para cada questão indique, com uma cruz (X), a alternativa que melhor se ajusta à sua situação.

	Nunca	Quase Nunca	Algumas vezes	Frequentemente	Muito Frequente
	0	1	2	3	4
1. No último mês, com que frequência esteve preocupado(a) por causa de alguma coisa que aconteceu inesperadamente?					
2. No último mês, com que frequência se sentiu incapaz de controlar as coisas importantes da sua vida?					
3. No último mês, com que frequência se sentiu nervoso(a) e em stresse?					
4. No último mês, com que frequência sentiu confiança na sua capacidade para enfrentar os seus problemas pessoais?					
5. No último mês, com que frequência sentiu que as coisas estavam a correr à sua maneira?					
6. No último mês, com que frequência sentiu que não aguentava com as coisas todas que tinha para fazer?					
7. No último mês, com que frequência foi capaz de controlar as suas irritações?					
8. No último mês, com que frequência sentiu ter tudo sob controlo?					
9. No último mês, com que frequência se sentiu furioso(a) por coisas que ultrapassaram o seu controlo?					
10. No último mês, com que frequência sentiu que as dificuldades se estavam a acumular tanto que não as conseguia ultrapassar?					
	0	1	2	3	4

Senior Fitness Test – SFT

1. Levantar e sentar na cadeira

Objetivo: avaliar a força e resistência dos membros inferiores.
Instrumentos: cronômetro, cadeira com encosto e sem braços, com altura de assento de aproximadamente 43 cm.
Organização dos instrumentos: por razões de segurança, a cadeira deve ser colocada contra uma parede, ou estabilizada de qualquer outro modo, evitando que se mova durante o teste.
Posição do avaliado: sentado na cadeira com as costas encostadas no encosto e pés apoiados no chão.
Posição do avaliador: próximo ao avaliado, segurando a cadeira.
Procedimento: o participante cruza os braços com o dedo médio em direção ao acrômio. Ao sinal o participante ergue-se e fica totalmente em pé e então retorna a posição sentada. O participante é encorajado a completar tantas ações de ficar totalmente em pé e sentar quanto possível em 30 segundos. O analisador deverá realizar uma vez para demonstrar o teste para que o participante tenha uma aprendizagem apropriada. O teste deverá ser realizado uma vez.
Pontuação: a pontuação é obtida pelo número total de execuções corretas num intervalo de 30 segundos. Se o participante estiver no meio da elevação no final dos 30 segundos, deve-se contar esta como uma execução.
Observação:

2. Flexão de antebraço

Objetivo: avaliar a força e resistência do membro superior.
Instrumentos: cronômetro, ou relógio de pulso ou qualquer outro que tenha ponteiro de segundos. Cadeira com encosto e sem braços e halteres de mão (2,3 kg para mulheres e 3,6 kg para homens). Já foram validados para o Brasil 2 kg para mulheres e 4 kg para homens. Será utilizado 2 Kg e 4 Kg.
Organização dos instrumentos: o participante senta em uma cadeira com as costas retas, os pés no chão e o lado dominante do corpo próximo à borda da cadeira. Ele segura o halter com a mão dominante, utilizando uma empunhadura de aperto de mão.
Posição do avaliado: o participante senta em uma cadeira com as costas retas, os pés no chão e o lado dominante do corpo próximo à borda da cadeira. Ele segura o halter com a mão dominante, utilizando uma empunhadura de aperto de mão. O teste começa com o braço estendido perto da cadeira, perpendicular ao chão.
Posição do avaliador: o avaliador ajoelha-se (ou senta em uma cadeira) próximo ao avaliado no lado do braço dominante, colocando seus dedos no meio do braço da pessoa

para estabilizar a parte superior do braço e pra garantir que uma flexão total seja feita (o antebraço do avaliado deve apertar os dedos do avaliador. É importante que a região superior do braço do avaliado permaneça parada durante todo o teste.

O avaliador pode também precisar posicionar sua outra mão atrás do cúbito do avaliado para ajudar a medir quando a extensão total tenha sido alcançada e para impedir um movimento de balanço para trás do braço.

Procedimento: O teste começa com o braço estendido perto da cadeira e perpendicular ao chão. Ao sinal indicativo, o participante gira sua palma para cima enquanto flexiona o braço em amplitude total de movimento e então retorna o braço para uma posição completamente estendida. Na posição inicial, o peso deve retornar para a posição de empunhadura de aperto de mão. O avaliado é encorajado a executar tantas repetições quanto possível em 30 segundos. Após a demonstração, faça uma ou duas repetições para verificar a forma apropriada, seguida do teste. Deverá ser executado o teste uma vez.

Pontuação: a pontuação é obtida pelo número total de flexões corretas realizadas num intervalo de 30 segundos. Se no final dos 30 segundos o antebraço estiver em meia flexão, conta-se como uma flexão total.

3. Sentado e Alcançar

Objetivo: avaliar a flexibilidade dos membros inferiores.

Instrumentos: cadeira com encosto e sem braços a uma altura de, aproximadamente, 43 cm, até o assento e uma régua de 45 cm.

Organização dos instrumentos: Por razões de segurança deve-se colocar a cadeira contra uma parede de forma a que se mantenha estável (não deslize para frente) quando o participante se sentar na respectiva extremidade.

Posição do avaliado: o ponto aproximado entre a linha inguinal e os glúteos deve estar paralelo ao assento da cadeira. Mantenha uma perna flexionada e o pé do chão, os joelhos paralelos, voltados para frente, o participante estende a outra perna (a perna preferida) à frente do quadril, com o calcanhar no chão e dorsiflexão plantar a aproximadamente 90°.

Posição do avaliador: próximo ao avaliado.

Procedimento: com a perna estendida (porém não superestendida), o participante inclina-se lentamente para a frente, mantendo a coluna o mais ereta possível e a cabeça alinhada com a coluna. O avaliado tenta tocar os dedos dos pés escorregando as mãos, uma em cima da outra, com as pontas dos dedos médios, na perna estendida. A posição deve ser mantida por dois segundos. Se o joelho estendido começar a flexionar, peça ao avaliado para sentar de volta lentamente até que o joelho esteja estendido. Lembre o avaliado de expirar á medida que se inclina para a frente, evitando saltos ou movimentos forçados rápidos e nunca alongando ao ponto de sentir dor. Seguindo a demonstração, faça que o avaliado determine sua perna preferida – a perna que produz o melhor escore. Dê então ao avaliado duas tentativas (alongamento) nesta perna, seguidas por duas provas de teste.

Pontuação: usando uma régua de 45 cm, o avaliador registra a distância (cm) até os dedos dos pés (resultado mínimo) ou a distância (cm) que se consegue alcançar para além dos dedos dos pés (resultado máximo). O meio do dedo grande do pé na extremidade do sapato representa o ponto zero. Registrar ambos os valores encontrados com a aproximação de 1 cm, e fazer um círculo sobre o melhor resultado. O melhor resultado é usado para avaliar o desempenho.

Observação:

4. Sentado, caminhar 2,44m e voltar a sentar

Objetivo: avaliar a mobilidade física – velocidade, agilidade e equilíbrio dinâmico.

Instrumentos: cronômetro, fita métrica, cone (ou outro marcador) e cadeira com encosto a uma altura de aproximadamente 43 cm, até o assento.

Organização dos instrumentos: a cadeira deve ser posicionada contra a parede ou de forma que garanta a posição estática durante o teste. A cadeira deve também estar numa zona desobstruída, em frente coloca-se um cone (ou outro marcador), à distância de 2,44 m (medição desde a ponta da cadeira até a parte anterior do marcador, cone). Deverá haver pelo menos 1,22 m de distância livre à volta do cone, permitindo ao participante contornar livremente o cone.

Posição do avaliado: o avaliado começa em uma posição sentada na cadeira com uma postura ereta, mãos nas coxas e os pés no chão com um pé levemente na frente do outro.

Posição do avaliador: o avaliador deve servir como um marcador, ficando no meio do caminho entre a cadeira e o cone, pronto para auxiliar o avaliado em caso de perda de equilíbrio.

Procedimento: ao sinal indicativo, o avaliado levanta da cadeira (pode dar um impulso nas coxas ou na cadeira), caminha o mais rapidamente possível em volta do cone, retorna para a cadeira e senta. Para uma marcação confiável, o avaliador deve acionar o cronômetro no movimento do sinal, quer a pessoa tenha ou não começado a se mover, e parar o cronômetro no instante exato que a pessoa sentar na cadeira.

Após a demonstração, o avaliado deve ensaiar o teste uma vez para praticar e, então, realizar duas tentativas. Lembre ao avaliado que o cronômetro não será parado até que ele esteja completamente sentado na cadeira.

Pontuação: o resultado corresponde ao tempo decorrido entre o sinal de "partida" até o momento em que o participante está sentado na cadeira. Registram-se dois escores do teste para o décimo de segundo mais próximo. O melhor escore (menor tempo) será o escore utilizado para avaliar o desempenho.

Observação: lembre ao avaliado que este é um teste de tempo e que o objetivo é caminhar o mais rapidamente possível (sem correr) em volta do cone e voltar para a cadeira.

5. Alcançar atrás das costas

Objetivo: avaliar a flexibilidade dos membros superiores (ombro).
Instrumentos: régua de 45,7 cm.
Organização dos instrumentos:
Posição do avaliado: em pé próximo ao avaliador.
Posição do avaliador: atrás do avaliado.
Procedimento: em pé, o avaliado coloca a mão preferida sobre o mesmo ombro, a palma aberta e os dedos estendidos, alcançando o meio das costas tanto quanto possível (cúbito apontado para cima). A mão do outro braço está colocada atrás das costas, a palma para cima, alcançando para cima o mais distante possível na tentativa de tocar ou sobrepor os dedos médios estendidos de ambas as mãos. Sem mover as mãos de avaliado, o avaliador ajuda a verificar se os dedos médios de cada mão estão direcionados um ao outro. Não é permitido ao avaliado agarrar seus dedos unidos e puxar.

Seguindo a demonstração, o avaliado determina a mão preferida e são feitas duas tentativas de aprendizagem, seguidas pelo teste (2 tentativas).
Pontuação: à distância da sobreposição, ou a distância entre as pontas dos dedos médios é a medida ao cm mais próximo. Os resultados negativos (-) representam a distância mais curta entre os dedos médios; os resultados positivos (+) representam a medida da sobreposição dos dedos médios. Registram-se as duas medidas. O "melhor" valor é usado para medir o desempenho. Certifique-se de marcar os sinais (-) e (+) na ficha de pontuação.
Observação:

Classificação do teste levantar e sentar na cadeira (Homens)

Classificação: (homens)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ 13	≤ 11	≤ 11	≤ 10	≤ 9	≤ 7	≤ 7
Fraco	14-15	12-14	12-13	11-13	10-11	8-10	8-9
Regular	16-17	15-16	14-16	14-15	12-13	11-12	9-11
Bom	18-20	17-19	17-18	16-18	14-16	13-15	11-13
Muito bom	≥ 21	≥ 20	≥ 19	≥ 19	≥ 17	≥ 16	≥ 14

Classificação do teste levantar e sentar na cadeira (Mulheres)

Classificação: (mulheres)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ 12	≤ 12	≤ 11	≤ 10	≤ 10	≤ 9	≤ 8
Fraco	13-15	13-14	12-13	11-13	11-12	10-11	9-10
Regular	16-17	15-16	14-16	13-15	13-14	12-13	11-12
Bom	18-20	17-19	17-18	16-18	15-16	14-15	12-15
Muito bom	≥ 21	≥ 20	≥ 19	≥ 19	≥ 17	≥ 16	≥ 15

Classificação do teste flexão do antebraço (Homens)

Classificação: (homens)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ 15	≤ 14	≤ 13	≤ 12	≤ 12	≤ 10	≤ 9
Fraco	16-18	15-17	14-16	13-15	13-15	11-13	10-11
Regular	19-20	18-20	17-19	16-17	15-17	14-15	12-13
Bom	21-23	21-23	20-22	18-20	18-20	16-17	14-15
Muito bom	≥ 24	≥ 24	≥ 23	≥ 21	≥ 21	≥ 18	≥ 16

Classificação do teste flexão do antebraço (Mulheres)

Classificação: (mulheres)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ 13	≤ 11	≤ 11	≤ 10	≤ 9	≤ 7	≤ 6
Fraco	14-15	12-14	12-14	11-13	10-11	8-10	7-9
Regular	16-18	15-17	15-16	14-16	12-14	11-13	10-11
Bom	19-21	18-20	17-19	17-18	15-17	14-16	12-14
Muito bom	≥ 22	≥ 21	≥ 20	≥ 19	≥ 18	≥ 17	≥ 15

Classificação do teste sentado e alcançar (Homens)

Classificação: (homens)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ -3,4	≤ -3,9	≤ -3,9	≤ -5,0	≤ -6,2	≤ -5,9	≤ -7,2
Fraco	-3,3_-0,6	-3,8_-1,1	-3,8_-1,2	-4,9_-2,3	-6,1_-3,2	-5,8_-3,5	-7,1_-4,7
Regular	-0,5_-1,8	-1,0_1,1	-1,1_1,1	-2,2_0,1	-3,1_-0,8	-3,4_-1,3	-4,6_-2,5
Bom	1,9_4,6	1,2_3,9	1,2_3,8	0_2,8	-0,7_2,2	-1,2_1,1	-2,4_0
Muito bom	≥ 4,7	≥ 4,0	≥ 3,9	≥ 2,9	≥ 2,3	≥ 1,2	≥ 0,1

Classificação do teste sentado e alcançar (Mulheres)

Classificação: (mulheres)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ -1,3	≤ -1,0	≤ -1,7	≤ -2,0	≤ -2,6	≤ -3,2	≤ -5,1
Fraco	-1,2_1,1	-0,9_1,1	-1,6_0,5	-1,9_0,2	-2,5_-0,4	-3,1_-1,0	-5,0_-2,7
Regular	1,2_3,1	1,2_2,9	0,6_2,3	0,3_2,1	-0,3_1,4	-0,9_0,8	-2,6_-0,7
Bom	3,2_5,5	3,0_5,0	2,4_4,5	2,2_4,4	1,5_3,6	0,9_3,0	-0,6_1,7
Muito bom	≥ 5,6	≥ 5,1	≥ 4,6	≥ 4,5	≥ 3,7	≥ 3,1	≥ 1,8

Classificação do teste alcançar atrás das costas (Homens)

Classificação: (homens)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ -7,4	≤ -8,2	≤ -8,6	≤ -9,9	≤ -10,5	≤ -10,2	≤ -11,2
Fraco	-7,3_-4,6	-8,1_-5,3	-8,5_-5,7	-9,8_-6,9	-10,4_-7,1	-10_-7,4	-11,1_-8,4
Regular	-4,5_-2,2	-5,2_-2,9	-5,6_-3,3	-6,8_-4,3	-7,0_-4,3	-7,3_-5,0	-8,3_-6,0
Bom	-2,1_0,6	-2,8_0	-3,2_-0,4	-4,2_-1,3	-4,2_-1,2	-4,9_-2,2	-5,9_-3,2
Muito bom	≥ 0,7	≥ 0,1	≥ -0,3	≥ -1,2	≥ -1,1	≥ -2,1	≥ -3,1

Classificação do teste alcançar atrás das costas (Mulheres)

Classificação: (mulheres)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ -3,6	≤ -4,3	≤ -4,9	≤ -5,5	≤ -6,1	≤ -7,7	≤ -8,9
Fraco	-3,5_-1,6	-4,2_-2,1	-4,8_-2,6	-5,4_-3,1	-6,0_-3,7	-7,6_-5,0	-8,8_-5,8
Regular	-1,5_0,2	-2,0_-0,3	-2,5_-0,8	-3,0_-1,1	-3,6_-1,6	-4,9_-2,8	-5,7_-3,2
Bom	0,3_1,9	-0,2_1,9	-0,7_1,5	-1,0_1,3	-1,5_0,9	-2,7_-0,1	-3,1_-0,1
Muito bom	≥ 2,0	≥ 2,0	≥ 1,6	≥ -1,4	≥ -1,0	≥ -0,0	≥ -0,0

Classificação do teste sentado, caminhar 2.44 metros e voltar a sentar (Homens)

Classificação: (homens)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ 5,8	≤ 6,1	≤ 6,4	≤ 7,5	≤ 7,9	≤ 9,4	≤ 10,5
Fraco	5,7-5,0	6,0-5,4	6,3-5,6	7,4-6,4	7,8-6,9	9,3-7,9	10,4-8,8
Regular	4,9-4,4	5,3-4,8	5,5-5,0	6,3-5,4	6,8-6,0	7,8-6,5	8,7-7,4
Bom	4,3-3,6	4,7-4,1	4,9-4,2	5,3-4,3	5,9-4,9	6,4-5,0	7,3-5,7
Muito bom	≥ 3,5	≥ 4,0	≥ 4,1	≥ 4,2	≥ 4,8	≥ 4,9	≥ 5,6

Classificação do teste sentado, caminhar 2.44 metros e voltar a sentar (Mulheres)

Classificação: (mulheres)	60-64 anos de idade	65-69 anos de idade	70-74 anos de idade	75-79 anos de idade	80-84 anos de idade	85-89 anos de idade	90-94 anos de idade
Muito fraco	≤ 6,2	≤ 6,6	≤ 7,3	≤ 7,6	≤ 9,0	≤ 10	≤ 12,1
Fraco	6,1-5,5	6,5-5,9	7,2-6,4	7,5-6,7	8,9-7,8	9,9-8,5	12-10,2
Regular	5,4-4,9	5,8-5,3	6,3-5,6	6,6-5,6	7,7-6,7	8,4-7,3	10,1-8,6
Bom	4,8-4,2	5,2-4,6	5,5-4,7	5,8-5,0	6,6-5,4	7,2-5,8	8,5-6,7
Muito bom	≥ 4,1	≥ 4,5	≥ 4,6	≥ 4,9	≥ 5,3	≥ 5,7	≥ 6,6

2 minute Step Test
(Rikli, Jones 1999)

1. Take resting vital signs
2. Have patient/client stand next to a wall. Measure the height of the iliac crest and patella and mark it on the wall. Then place a piece of tape on the wall half the distance between the two.
3. On the signal "go" the patient/client begins stepping (not running) in place, raising each knee to the mark on the wall, for as many times as possible in the 2 minute period.
4. Only count the number of times the right knee reaches the required height. That is the score.
5. If the proper knee height cannot be maintained, ask the participant to slow down, or to stop until they can regain the proper form, but keep the stopwatch running.
6. At the end of the test, provide a cool down by asking the patient/client to walk slowly for a minute.
7. A person with impaired balance may use the back of a chair as a touch-hold for stability. Note this modification in your documentation
8. One trial.
9. Take post exercise vital signs.

Range of scores between the 25% and 75% percentiles

Age	Number of steps – Women	Number of steps – Men
60 - 64	75-107	87-115
65 - 79	73-107	86-116
70 - 74	68-101	80-110
75 - 79	68-100	73-109
80 - 84	60-90	71-103
85 - 90	55-85	59-91
90 - 95	44-72	52-86

Scores less than 65 were associated with lower levels of functional ability

HANDGRIP STRENGTH TEST PROCEDURES

Supplies

- Hydraulic Hand Dynamometer

Definition & Purpose

Handgrip strength is a simple and commonly used test of a person's general strength level.

Measurement

1. Have participant sit comfortably with the shoulder adducted and neutrally rotated, with the elbow towards/against the body and flexed at 90 degrees, and the forearm and wrist in a neutral position.

2. Place the hand dynamometer in the participant's hand, while you use the wrist safety strap and gently support the base to prevent accidental dropping and damage to the instrument.

3. Let the participant arrange the instrument so that it fits comfortably in the hand. Adjust the handle if necessary for a comfortable grip. Make sure that the handle clip is located at the lower (furthest) post from the gauge. If the handle is not in the correct position, results will be inaccurate.

4. Reset the indicator needle by rotating it to zero

5. Request that the participant squeeze with maximum strength. The needle will automatically record the highest force exerted. Grip force should be applied smoothly, without rapid wrenching or jerking motion. Minimal wrist extension (30 degrees or less) is permissible as maximum grip is achieved. Wrist extension greater than 30 degrees should be noted with results.

6. Test each hand twice and record the best effort rating (i.e., Excellent, Very Good, etc.) of each on the participant's handout and on the aggregate form. Do not forget to reset the indicator needle before each and every effort.

Interpreting Results

Rating	**Males (kg)**	**Females (kg)**
Excellent	> 64	> 38
Very Good	56 – 64	34 – 38
Above Average	52 – 56	30 – 34
Average	48 – 52	26 – 30
Below Average	44 – 48	22 -26
Poor	40 – 44	20 – 22
Very Poor	< 40	< 20

Annex 3: Published articles

International Journal of
Environmental Research and Public Health

MDPI

Study Protocol

Impact of Aquatic-Based Physical Exercise Programs on Risk Markers of Cardiometabolic Diseases in Older People: A Study Protocol for Randomized-Controlled Trials

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Abstract: Cardiometabolic diseases are one of the primary causes of mortality and morbidity worldwide and sedentary lifestyles are contributing factors to these pathologies. Physical exercise has been recognized as an important tool in the prevention and treatment of these diseases. However, there are still some doubts about the efficacy of certain type of physical exercise programs for older participants. The main goal of this study is to assess the impact of different aquatic-based physical exercise programs on risk markers of cardiometabolic diseases in older people. The study group will consist of non-institutionalized individuals, within the age group of 65 or older. The sample will be randomly divided into four groups, three experimental groups (EG) and one control group (CG). Participants from the EGs will be exposed to three physical aquatic-based exercise programs for a period of 28 weeks (continuous aerobic, interval aerobic and combined). The evaluated parameters include anthropometry, physical functions, mental health, cognitive function, carotid arteries intima-media thickness, heart rate variability and biochemical markers. The results will allow an interpretation of the impact of different aquatic-based physical exercise programs on cardiometabolic diseases markers and can also be used as a tool for professionals to prescribe adequate and more efficient physical exercise programs.

Keywords: exercise; aquatic-based; hydro gymnastics; elderly; cardiometabolic diseases

1. Introduction

Ageing is considered a progressive and inevitable phenomenon, where the reduction in various physical and metabolic functions occurs [1]. As they age, humans become increasingly sensitive to certain pathologies such as cardiometabolic diseases. These diseases are considered to be the main cause of mortality and morbidity worldwide and have attracted great interest in various fields of scientific investigation. The World Health Organization (WHO) reports that cardiometabolic diseases are responsible for 63% of 57 million annual deaths, with a significant correlation of 6 to 10% of these deaths due to physical inactivity [2].

Nowadays, more than 60% of the elderly population live a sedentary lifestyle [3]. This contributes to physical and mental frailty. Physical frailty is characterized by a reduction in physical activity levels, unintended weight loss, fatigue, reduction in handgrip strength, and a decrease in walking speed [4]. The same authors characterize cognitive frailty as the decline in all functional mental abilities that regulate the lifestyle of an individual. These mental activities range from simple to complex. Both types of frailty are associated to the process of physical and mental decline, with a deterioration in physical and mental capabilities that may lead to a serious decrease in health conditions, loss of autonomy, institutionalization and mortality [5]. To counteract sedentary lifestyles, WHO has developed strategies for a more active and healthy society. These strategies include the creation of guidelines that state the frequency, duration, intensity, type and amount of physical activity that are recommended for different age groups. Such guidelines target the prevention of non-transmissible chronic diseases [6]. Simultaneously, there has been an increase in research using a set of variables that are related to these types of pathologies, such as body composition [7], functional fitness [8], and variables related to the cardiovascular [9], cognitive [4] and immune systems [10].

Physical exercise is considered an effective tool during the process of ageing, as it helps to stabilize the loss of physical and metabolic capacities, mitigates the overall progress of ageing and enables more autonomy. Various studies indicate that regular physical exercise plays an important role in the prevention and treatment of cardiometabolic and cognitive diseases [10–14].

Different studies have been conducted recently to verify the efficacy of various physical exercise programs (aerobic, muscular strength and combined) on variables related to these pathologies. Such studies have analyzed the impact of exercise on cognition, cardiovascular, metabolic, immunological, functional and mental levels [15–18]. However, most of these studies have focused on land-based exercise and have not involved specific aquatic exercise programs, which are popular and successful among older participants.

The present investigation and its specific experimental design using different types of aquatic-based exercise programs aims to assess the impact of different aquatic-based physical exercise programs on risk markers of cardiometabolic diseases in older people.

2. Materials and Methods

2.1. Design

This investigation will be based on a randomized intervention, using three different aquatic-based exercise programs conducted in parallel for 28 weeks. The three programs include a continuous aerobic program, an interval aerobic program and a combined (aerobic and muscular strength) program. Variables related to cardiometabolic diseases will be evaluated, i.e., cardiovascular, mental health, and cognitive variables and biochemical markers.

All exercise programs will be conducted at the Piscina Muncipal da Sertã, two times a week (non-consecutive days), with 45-min sessions. All variables related to cardiometabolic diseases will be evaluated at two specific assessment moments: M1, before implementation of the physical exercise programs (baseline) and M2, after the conclusion of the 28 weeks aquatic-based physical exercise programs (post-intervention).

Each evaluation will be divided into three phases: Phase 1—Anthropometry evaluation, physical functions, cognitive and mental health levels; Phase 2—Evaluation of carotid arteries' intima-media thickness and heart rate variability; and Phase 3—Evaluation of biochemical markers.

The blood collection for biochemical analysis will be conducted by a specialized and certified lab. The measurement of the carotid arteries intima-media thickness will be conducted by a specialist in the area of cardiology. All the remaining data will be collected and organized by the research team members.

2.2. Participants

The participants were recruited in the central area of Portugal, more specifically in the region of Sertã. The sample was recruited by the non-probabilistic method for convenience. One hundred and fifty individuals from the community were personally invited, but only 102 agreed to participate in the study (mean age of 72.32 ± 5.2 and BMI 29.47 ± 4.85). Participants will be selected according to the following inclusion criteria: individual of both genders; age equal to or above 65 years of age; non-institutionalized individuals; they give permission to be part of the study and if the participant presents with a clinical condition or comorbidity, it must be stable and enable participation in aquatic-based exercises classes as approved by local medical staff. The exclusion criteria are: individuals less than 65 years of age; individuals with medical diagnosed pathologies that jeopardize their health while preforming aquatic based exercises, participants that attend less than 50% of all the sessions and participants who cannot complete all of the proposed tests.

The participants will be distributed randomly into different exercise groups based on their registration for one of the schedules offered for the hydro-gymnastics sessions. There will be three distinct schedules (9.00, 9.50 and 10.40), with the constraint being that the participants must attend the same schedule for the whole year. The 9.00 session includes the continuous aerobic program, the 9.50 session includes the interval aerobic program, and the 10.40 session includes the combined exercise program. This information will not be provided to participants before they register for the sessions so it will not be possible to establish any association between the schedule and the different exercises programs. Such information will be communicated later in the study. The control group will be randomly recruited from the community and will include those individuals that have not been involved in any kind of physical exercise during the last year.

Before each assessment moment, participants will be taken to a testing room, where the assessment tests will be performed. The room will be large and isolated, and the temperature will be controlled, and each assessment stages should be organized to provide maximum comfort and privacy to the participants during the tests. The research team will give the participants information about the tests they will perform for data collection, explain the purpose of each test, and explain the order and duration of the tests. Participants will be able to question researchers about any doubts that they may have regarding the tests and any possible consequences. During the assessment, participants may pause the evaluation and continue on another day if they feel very fatigued or if they are not able to complete all the tests at that time. In this situation, a new date will be scheduled to continue the tests.

2.3. Protocols

The percentage of adherence to the physical exercise programs, for each program, will be calculated considering the total attended sessions: (S × 100)/T, where "S" indicates the number of sessions that have been attended by the participant during the study and "T" indicates the total number (56) of physical exercise sessions. The participants' attendance will be recorded in a database. If a participant has two consecutive absences, they will be contacted and given motivational reinforcement to incentivize them to resume their physical exercise sessions.

During the study period, all adverse effects or health problems attributed to the physical exercise sessions or evaluation tests will be reported. Parameters such as muscle pain, excessive fatigue and general pain will also be reported and inserted in a database. Exercise technicians and researchers will be responsible for data collection as well as for gathering and communicating all relevant data.

Three physical exercise programs will be conducted for a time period of 28 weeks, two times per week (non-consecutive days) and have the following common characteristics: all sessions will have a duration of 45 min, taking into account previous studies [10] that suggest sessions of this duration seem to be sufficient to provide changes in several parameters in the elderly population; they will be aquatic-based (the water level will be between 0.80 and 1.20 m with a temperature of approximately 32 °C); and they will be conducted to the rhythm of music (bpm) that can be adjusted to achieve the target HR. Sessions are divided into three parts: the initial part, main part and final part, with common exercises in the initial and final part of the three programs. The initial part or warm-up has a duration of 10 min and the purpose is to assist participants to adapt to the water environment, more specifically, for participants' to acclimatize and prepare for muscular and metabolic stimulation. Simple aquatic-based exercises will be conducted, e.g., displacement and isolated movements. The exercises increase in complexity and intensity during this initial phase. The final part has a duration of 5 min. This part will be divided into two phases: return to calm (relaxation) where relaxing exercises are conducted with the purpose of returning the participants' heart rate (HR) value to a resting level. The second phase is composed of stretching routines that stretch the most exercised muscle groups stimulated in the main part of the session and reduce the level of lactic acid and the occurrence of post-exercise pain. The main part is different in the three physical exercise programs and their characteristics are described in Table 1. All physical exercise programs will be planned and implemented according to the recommendations of the American College of Sports Medicine [19] and conducted by specialized physical exercise technicians (with a degree in sports science) with specialization in hydro-gymnastics (instructor course—level 1).

Table 1. Characteristics of the three physical exercise programs applied for 28 weeks (continuous aerobic, interval aerobic and combined).

Program	Description	Intensity (Week 1–13)	Intensity (Week 14–28)	Exercises
Continuous Aerobic	30 min exercise aerobic (moderate intensity)	60–65% maximum HR	65–70% maximum HR	Basic hydro-gymnastics exercise, with some variations: running, bounce, kicking, pendulum jumping, skiing, twister and horse.
Interval Aerobic	10 min exercise aerobic (moderate intensity)	60–65% maximum HR	65–70% maximum HR	Basic hydro-gymnastics exercise, with some variations: running, bounce, kicking, pendulum jumping, skiing, twister and horse.
	5 min exercise aerobic (high intensity)	70–75% maximum HR	75–80% maximum HR	
	10 min exercise aerobic (moderate intensity)	60–65% maximum HR	65–70% maximum HR	
	5 min exercise aerobic (high intensity)	70–75% maximum HR	75–80% maximum HR	
Combined	15 min exercise aerobic (moderate intensity)	60–65% maximum HR	65–70% maximum HR	Basic hydro-gymnastics exercise, with some variations: running, bounce, kicking, pendulum jumping, skiing, twister and horse.
	15 min muscular strengthening exercises	2 steps 12 repetitions	3 steps 16 repetitions	Exercises with auxiliary equipment (dumbbells, pool noodles, etc.): elbow extension/flexion; shoulder extension/flexion; shoulder abduction/adduction; hip abduction/adduction; hip flexion/extension; knee flexion/extension; dorsal and plantar flexion of the ankle.

HR monitors (Polar V800) will be used on the participants during all sessions of the different physical exercise program. The intensity of each exercise program will be monitored using the data provided by the Polar V800 and adjusted accordingly. As a precaution and safety measure, the intensity will be indirectly calculated using the following equation [20]:

$$Target\ HR = ((Maximum\ HR - Resting\ HR)\ \%\ intensity) + Resting\ HR \quad (1)$$

Maximum HR is calculated with the following equation for senior populations [21]:

$$Maximum\ HR = 207 - (0.7 \times age) \quad (2)$$

The control group consists of non-institutionalized individual participants who have not partaken in any physical exercise during the preceding year. These participants will be encouraged to conduct their daily activities as usual, except for the data collection (M1 and M2) organized by the researchers.

2.4. Instruments

2.4.1. Individual Characterization

In the first assessment moment (M1), all participants will fill in a clinical survey to help with the clinical characterization of each participant. This document includes the following information: civil status, regular medication, infections, allergies, diseases, annual doctor consultations, average hours of daily sleep, supplement use, latest blood panel, information on dietary habits, smoking habits and drug use.

2.4.2. Environmental Characteristics

The physical exercise programs are aquatic-based, and will take place in water at temperatures that follow regional health guidelines (30–32 degrees Celsius) in the swimming pool complex of Piscina Municipal da Sertã (indoor pool), Portugal. During the study, daily monitoring of parameters such as the pool water temperature, free chlorine, combined chlorine, pH, relative humidity and pier external temperature will be conducted and the results will be inserted in a database by two facility staff members. An external certified company will conduct a bi-weekly analysis of the following parameters: pH, conductivity, free chlorine, total chlorine, temperature and, bacteriological tests (total germs, total coliforms, Escherichia coli, fecal enterococci, total staphylococci, coagulase-producing staphylococci, and pseudomonas aeruginosa).

2.4.3. Anthropometry

Anthropometric measurements will be conducted by a certified investigator by FCDEF-UC. The following parameters will be evaluated: (i) stature, using a portable stadiometer, Seca Bodymeter® (model 208, Hamburg, Germany) with a precision of 0.1 cm; (ii) weight, body mass index (BMI), visceral fat, percentage of fat and muscle mass using a portable scale from Seca® (model 770, Hamburg, Germany) with 0.1 kg accuracy; and (iii) waist circumference, arms and legs using a retractable fiberglass tape (model Hoechst mass-Rollfix®, Sulzbach, Germany) with an accuracy of 0.1 cm.

2.4.4. Physical Function

Physical function will be assessed using the Senior Fitness Test, developed and reviewed by Rikli and Jones [22] and validated for the Portuguese population [23]. It is composed of the following test items:

- Chair stand, assesses lower body strength and consists of the maximum number of full stands that can be concluded in 30 s. Necessary equipment: chair and stopwatch.
- Arm curl, assesses upper body strength and consists of the maximum number of bicep curls that can be completed in 30 s while holding a hand weight. Necessary equipment: 2.27 kg hand weight for women and 3.63 kg for men, chair and stopwatch.
- 2-min step, assesses aerobic endurance and consists of maximum number of full steps completed in 2 min, a full step is recorded when each knee reaches the point midway between the patella (kneecap) and iliac crest (top hip bone). Necessary equipment: stopwatch, sticky-tape and ruler.
- Chair sit and reach, assesses lower body flexibility and is conducted from a sitting position where one of the participant's legs is extended while the other is flexed and where hands are reaching towards the toes. This test is assessed in cm and is positive (+) if the extended fingers pass the tip of the toes or negative (−) if the extended fingers do not pass the tip of the toes. Necessary equipment: chair and ruler.
- Back scratch, assesses upper body flexibility and is conducted with one hand reaching over the shoulder in the direction of the floor and the other hand up the middle of the back in the direction of the head. This test is assessed in cm and is positive (+) if both hands overlap and is negative (−) if overlapping does not occur. Necessary equipment: ruler.

- Timed up and go, assesses agility and dynamic balance and is conducted from a starting sitting position where the participant stands up and walks, as fast as possible, to and from a distance 2.44 m (marked by a cone). Necessary equipment: chair, cone and stopwatch.
- Hand grip, assesses hand grip strength and consists of asking the participant to grip a dynamometer with maximum achievable force, the output value of the device is then registered. Necessary equipment: Lafayette hydraulic manual dynamometer (model J00105).

2.4.5. Cognitive Function

Cognitive function will be assessed with the Portuguese version of the Mini Mental State Examination (MMSE) [24]. The MMSE, evaluates the following cognitive areas: orientation, short term memory, attention and calculation capacities, long term memory and language capabilities. The final score has a maximum of 30 points, and scores below 24 can be used as an aid in the assessment of dementia. The test will be used as an instrument to create a cognitive profile with the following criterium [25]: severe cognitive impairment (scores between 1 and 9 points); moderate cognitive impairment (scores between 10 and 18 points); mild impairment (score between 19 and 24 points), normal cognitive profile (scores between 25 and 30 points).

2.4.6. Mental Health

Mental health will be assessed using the following scales and questionnaires validated for the Portuguese population: the Rosenberg Self-Esteem Scale (RSES) [26]; Physical Self-Perception Profile for Clinical Populations (CPSPP) [27]; World Health Organization Well-Being Index (WHO-5) [28]; Satisfaction With Life Scale (SWLS) [29]; EuroQol (EQ-5D) [30]; Geriatric Depression Scale (GDS) [31] and; Perceived Stress Scale (PSS) [32].

- RSES, assesses global self-esteem and is composed of 10 items that are answered using a 4-point Likert scale, the answers vary from "I totally agree" to "I totally disagree". In items 1, 2, 4, 6 and 7 the score is reversed. Global self-esteem is represented by the summation of all individual scores, providing a final score ranging from 10 to 40 points, where higher scores indicate higher self-esteem.
- CPSPP, is an instrument designed to provide a self-assessment summary of the physical characteristics of elderly groups in clinical and rehabilitation settings. A scale is defined by six subscales of three items that evaluate the following subdomains: functionality, physical health, sports competence, physical attractiveness, physical strength and physical self-worth. Answers to the items are displayed in an alternative structured format that is designed to eliminate social desirability bias. The score can vary between 3 to 12, with higher scores representing better performance.
- WHO-5, is an instrument that assesses psychological well-being. It is a self-administrated short questionnaire composed of 5 items with positive words, these words are related to a positive mood (good mood, relaxation), vitality (being active and waking up fresh and rested) and general interests. Each item is classified on a 5-point Likert scale, ranging from 0 (not present) to 5 (constantly present). The scores are summed, with the final score ranging from 0 to 25 points. The final score is then converted to a scale of 0 to 100 (by multiplying by 4), where higher scores represent a higher level of well-being and better quality of life. A final score equal to or below 50 points represents poor well-being but does not necessarily mean depression. A final score equal to or below 28 possibly indicates clinical depression.
- SWLS, assesses global cognitive parameters of life satisfaction. It is composed of 5 items with a 7-point Likert scale. The answers indicate the level of agreement the participant feels with each item. The final score ranges from 1 to 35 points, where higher final scores indicate higher satisfaction with life.
- EQ-5D, is an instrument that assesses general health status. It consists of two parts: the EQ-5D health descriptive system and the EQ visual analogue scale. The descriptive system consists of

five dimensions (mobility, personal care, usual activities, pain/discomfort and anxiety/depression). The participant is asked to indicate their health status by selecting the most appropriate options in the five dimensions. The visual analogue is self-assessed and is conducted in a scale from the lowest rate (0) "the worst health you can imagine" to the highest rate (100) "the best health you can imagine".

- GDS, assesses life satisfaction, interruptions in activities, annoyances, isolation, energy, joy and memory problems. It consists of fifteen easy to understand questions and has a binary answer system (0 or 1 point) for answers of "no" and "yes", respectively. A participant who obtains a final score between 0 and 5 points is considered healthy; scores between 6 and 10 points indicate signs of mild to moderate depression; scores between 11 and 15 points indicate signs of severe depression.
- PSS, is an instrument to measure perceptions of stress. It is composed of 14 items, where 7 items are considered as positive aspects while the rest are considered as negative aspects. The questions are about feelings and thoughts during the last month. A point reversal is conducted on items 4, 5, 6, 7, 9, 10 and 13. The final score may vary between 14 and 70 points and a higher score indicates higher stress levels.

2.4.7. Assessment of Carotid Arteries Intima-Media Thickness

The carotid arteries intima-media thickness assessment takes place with the participant lying down in a dorsal position. Then, the following parameters are evaluated using a sphygmomanometer from Riester (Model RI-championN®, Jungingen, Germany): heart rate (HR), systolic blood pressure (SP) and diastolic blood pressure (DP). The intima-media thickness of the right and left carotid arteries are measured with a Doppler two-dimensional ultrasound and are assessed with the AIRC study protocol [33]. The following values are then recorded through a portable ultrasound from General Electric® (VIDe, Vancouver, Canada) with probe linear 11 L: Intima-media thickness (IMT); systolic diameter (SD), diastolic diameter (DD), peak systolic velocity (PSV) and end-diastolic velocity (EDV).

2.4.8. Heart Rate Variability (HRV) Measurement

HRV will be assessed according to the procedures of Abad et al. [34] using Polar V800 heart rate monitors. Participants will place the sensor, which is synchronized with the V800 clock, on their chest beneath their pectoral muscles. Then, the participants will be asked to lie down in a dorsal position, in silence, with open eyes and with a calm respiration. The test will have a duration of 10 min in a calm, silent and low-light environment. After the conclusion of the test, HRV measurement data are downloaded from the Polar Flow Web Service.

2.4.9. Biochemical Markers

Blood samples will be drawn via venipuncture from fasting participants. For each participant a total of 18.5 mL of blood will be drawn. Of the 18.5 mL, 3.5 mL will be used by the clinic to assess lipid panel values (HDL, LDL, glucose, triglycerides). The remaining 15 mL, processed by the college laboratory, will be divided into three tubes: two serum separator tubes and one ethylenediaminetetraacetic acid (EDTA) tube. Once the test tubes arrive at the university laboratory a complete blood count (CBC) will be conducted using an automatic hematology analyzer Coulter Act Diff, Beckman Coulter, USA. Next, the test tubes are centrifuged for10 min at 3500× *g* rotations per minute and stored in cryogenic test tubes. Levels of HbA1C, IL-1, IL-1ra, IL-6, IL-10, TNF-alpha, Adiponectin, Leptin, MIP-1alpha, MCP-1, SOD, MMP-9, are subsequently analyzed with ELISA Invitrogen® CA kits (Bender MedSystems GmbH, Vienna, Austria).

2.5. Ethical Aspects

The researcher will be responsible for the data integrity and validity during the entire study. All data collected will be confidential and used exclusively for scientific purposes. Anonymization procedures will be conducted under the Data Protection Regulation of 25 May 2018. Data anonymization will be implemented by attributing a code to each participant. Each participant will receive a unique identification code that will correspond to their process; this code will be visually accessible with the use of "code cards". These "code cards" will be used by the participants during data collection. Once the data collection is finalized the participants will be asked to destroy their "code cards", thus finalizing the anonymization process.

Data will be stored in an Excel Microsoft Office 2016 database. Access to this database will be protected with a password and will be restricted to the main researcher responsible for the data collection. Data backups will be carried out regularly by the main researcher.

Participation in this study is purely voluntary, with no reprisals for non-participation. All subjects will be asked for their informed consent before they start participating in the study. The study will be conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee for Health of the Faculty of Sport Sciences and Physical Education of the University of Coimbra (reference: CE/FCDEF-UC/00462019).

The results from this study will be disseminated through publication in various scientific journals. The participants, if desired, will receive a copy of the main results and publications.

2.6. Statistical Analysis

The size and statistical power of the sample will be calculated using the G*Power software application [35]. The following parameters will be considered: F test (ANOVA); effect size: 0.25; α-level: 0.05; statistical power: 0.95; number of groups: 4; number of measures: 2 (pré and post intervention); a 30% margin for possible losses and refusals. Therefore, the initial size of the total sample was estimated at 76 participants.

The collected data will be subjected to descriptive statistical analysis where values such as maximum, minimum, mean and standard deviation will be calculated for each variable in each assessment moment. Afterwards, data normality will be tested by considering the response to three conditions: z-values from Skewness and Kurtosis tests; p-value from Shapiro-Wilk test; and visual inspection of generated histograms. Parametric data will be analyzed using the Student's t-test for independent samples to compare the groups and two paired samples to compare the different moments (M1 and M2). Nonparametric data will be analyzed using the Mann–Whitney U test to compare the groups and the Wilcoxon test to compare the different moments (M1 and M2). Statistical analysis will be performed using the Statistical Package for the Social Sciences (SPSS) statistical software, version 25.0. The level of significance used will be $p \leq 0.05$.

3. Expected Results/Discussion

The main aim of this study is to assess the impact of different aquatic exercise programs (a continuous aerobic program, aerobic interval program and combined program) on risk markers of cardiometabolic diseases in older people. The study is guided by scientific-based evidence on the practice of physical exercise in the elderly population [11,36].

The practice of regular physical exercise is considered as the optimum tool in the prevention and treatment of various types of cardiometabolic diseases. Thus, it is important that research on this topic continues; by doing so, new intervention methods can be identified and their efficacy validated. The offer of aquatic physical exercise programs is highly successful among the elderly because they transform physical exercise into something more pleasant and suitable for this particular population. However, the high costs of maintaining aquatic environments and the additional difficulties associated with assessment methodologies and specific equipment needed to monitor exercise in aquatic environments, means this topic not yet been sufficiently explored in the literature.

After completion of the data collection and analysis, the participants in the experimental groups are expected to show positive developments with regard to the anthropometric level, physical function, the intima-media thickness of the carotid arteries, heart rate variability, cognitive function, mental health and a number of biochemical markers. It is also expected that statistically significant differences will be found between the exercise groups for some of the variables. In the control group, no changes are expected in the analyzed variables. It is believed that the expected results can be attributed to the physical and physiological effects of the aquatic environment associated with the different proposed exercise protocols. The results will be published after the study is completed.

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Declaração

Para os devidos efeitos, e na qualidade de editora da publicação "Disability & Inclusion Issues", declaro que o capítulo de livro intitulado ***"Association between aerobic capacity, grip strength and cognition function with cardiometabolic diseases risk markers and mental health in the non-institutionalized old adults: a cross-sectional analysis"***, da autoria de Carlos Farinha, Ana Maria Teixeira, João Serrano, Hélder Santos, Fernanda M. Silva, Márcio Cascante-Rusenhack, Paulo Luís e José Pedro Ferreira, foi aceite para publicação, no próximo número deste periódico, que será lançado durante o ano de 2022.

Coimbra, em 28 de março de 2022

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Effects of physical fitness and cognition in old people´s cardiometabolic disease risk markers

Association between aerobic capacity, grip strength and cognition function with cardiometabolic diseases risk markers and mental health in the non-institutionalized old adults: a cross-sectional analysis/ *Associação entre a capacidade aeróbia, força de preensão manual e cognição com marcadores de risco de doenças cardiometabólicas e saúde mental, em idosos não institucionalizados: uma análise transversal*

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Abstract: The elderly population is constantly growing worldwide. One of the characteristics of aging is the decrease in functional fitness and cognitive function, leading to the appearance of cardiometabolic disorders. Methodology: The aim of this study is to verify the association between aerobic capacity, handgrip strength and cognition with risk markers for cardiometabolic diseases and mental health in community dwelling elderly. The study consists of a cross-sectional analysis of baseline data from a 28-week randomized controlled trial, with a sample of 102 participants (mean age 72.32 ± 5.25 years). The sample was evaluated for anthropometry, functional fitness, heart rate variability, carotid artery intima and mean thickness (IMT), cognitive function, mental health and biochemical markers. Correlations were evaluated using Pearson's statistical analysis and interpreted according to Cohen's (1988). Results: Statistically significant correlations were found between aerobic capacity (2m-ST) and markers of functional, cardiovascular, biochemical, cognitive function and mental health fitness. Handgrip strength (HG) was statistically significantly correlated with anthropometric measurements, various indicators of functional fitness, biochemical markers, cognitive function, and mental health variables. Finally, cognitive function (MMSE) was correlated with anthropometric measures, functional fitness, cardiovascular and biochemical markers, and mental health. These data suggest that aerobic capacity, handgrip strength and cognitive function may be hypothetically associated with cardiovascular disease risk markers.
Keywords: Aerobic capacity; hand strength; cognition; cardiometabolic risk factors; Elderly.

Resumo: A população idosa está em constante crescimento a nível mundial. Uma das caraterísticas do envelhecimento é a diminuição da aptidão funcional e função cognitiva, levando ao aparecimento de distúrbios cardiometabólicos. Metodologia: O objetivo deste estudo é verificar a associação entre a capacidade aeróbia, força de preensão manual e cognição com marcadores de risco de doenças cardiometabólicas e saúde mental, em idosos da comunidade. O estudo consiste na análise transversal dos dados de linha base de um estudo randomizado controlado de 28 semanas, sendo a amostra constituída por 102 participantes (média de idade de 72,32 ± 5,25 anos). A amostra avaliada quanto à antropometria, aptidão funcional, variabilidade da frequência cardíaca, espessura íntima e média das artérias carótidas (IMT), função cognitiva, saúde mental e marcadores bioquímicos. As correlações foram avaliadas através da análise estatística de Pearson e interpretadas de acordo com Cohen's (1988). Resultados: Foram verificadas correlações estatisticamente significativas entre a capacidade aeróbia (2m-ST) e marcadores de aptidão funcional, cardiovasculares, bioquímicos, função cognitiva e saúde mental. A força de preensão manual (HG) foi correlacionada de forma estatisticamente significativa com medidas antropométricas, vários indicadores de aptidão funcional, marcadores bioquímicos, função cognitiva e variáveis de saúde mental. Finalmente a função cognitiva (MMSE) foi correlacionada com medidas antropométricas, aptidão funcional, marcadores cardiovasculares e bioquímicos, e saúde mental. Estes dados sugerem que a capacidade aeróbia, força de preensão manual e função cognitiva podem estar hipoteticamente associadas a marcadores de risco de doenças cardiovasculares.
Palavras-chave: Capacidade aeróbia; preensão manual; cognição; fatores de risco cardiometabolico, idosos.

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INTRODUCTION

The elderly population is in constant growth. Estimates indicate that the percentage of world population with more than 60 years of age will nearly double between 2015 and 2050, from 12% to 22% (OMS, 2018). This ageing pattern is advancing at a large rate compared to what has been previously verified. Countries now face an enormous challenge in the preparation of social and health systems to meet the needs of this demographic change (OMS, 2018).

During aging, individuals are more prone to molecular and cellular damage, which leads to the gradual advance of physical and psychological disorders. Aging also increases the occurrence of various types of diseases, such as cardiometabolic diseases, and finally to death (Lira et al., 2020; Morgan et al., 2016). A decrease in cognition levels is also present during aging, specifically in executive functions (Kirk-Sanchez & McGough, 2013). Physical function is likewise influenced by aging, more specifically with a relevant decrease in aerobic capacity (Garatachea et al., 2015), muscular strength (Dugan et al., 2018) and balance (Borges et al., 2014; Olchowik et al., 2015).

Aerobic capacity is one of the key indicators of cardiorespiratory fitness and is directly influenced by aging (Garatachea et al., 2015). The average rate of decline in the VO2 max, in the elderly population per decade, is equal to or greater than 4-5 ml/kg/min. Progressive muscular aging contributes to a reduction in the capacity of oxygen usage, which in turn is caused by: reduction of lean body mass (LBM), reduction in muscle capillary density, endothelial dysfunction, changes in skeletal muscle microcirculation, and reduced muscle oxidative capacity (Garatachea et al., 2015).

In elderly, LBM is also affected, a progressive decline in this parameter is observed after the age of 25 to 30 (Nieuwpoort et al., 2018), causing problems of functional health, independence, and reduced well-being (Stojanović et al., 2021). A progressive reduction in muscular strength can be caused by the quantitative loss of the cross-sectional area of the muscles (Garatachea et al., 2015), that is, the skeletal muscles atrophy and become progressively weaker (McPhee et al., 2016). Hand grip strength has often been applied as a general metric for whole body strength assessment (Hershkovitz et al., 2019). Lower values of hand grip strength are indicators of cardiometabolic diseases, morbidity and early mortality (Alonso et al., 2018). Reduced hand grip strength is also associated with the reduced ability to perform daily activities (Hershkovitz et al., 2019), this reduced ability leads to an increased risk of falling and to mental and depressive health problems (Fukumori et al., 2015).

Cognitive functions decline with senescence, this decline is notable in executive functions (difficulties with daily activities, slower response times, reduced information comprehension speed, decline in the realization of tasks involving exchanges of attention and decreased inhibitory control capacity) (Kirk-Sanchez & McGough, 2013). Mild cognitive impairment is considered to be the transition between a normal cognitive profile and dementia and is characterized by a greater than expected cognitive decline, without significantly affecting daily activities. In its turn dementia is characterized by a progressive and severe decline which causes loss of ability to perform activities of daily living (Karssemeijer et al., 2017).

Studies have identified correlations between aerobic capacity, muscular strength, cognition and cardiometabolic risk markers. Chong et al. (2020) verified the existence of correlations between hand grip strength, systolic and diastolic blood pressure values, BMI, protein C and diabetes levels. Other authors (Mainous et al., 2015), correlated hand grip strength with diabetic and hypertension values. Another study (Rebollo-Ramos et al., 2020), tested the correlation between aerobic fitness with different levels of adiposity, blood pressure, lipid profile, inflammatory profile (IL-6 and TNF-α) and lifestyles, in a sample with ages

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between 18 and 40 years. Regarding cognition Yeh et al. (2015) correlated cognitive performance with cardiometabolic variables and hyper white matter intensities, in individuals aged between 50 and 85 years. Furtado et al. (2020), tested the association between frailty and several geriatric characteristics in a group of institutionalized women aged between 75 and 85 years. In the study (Chupel et al., 2017), significant correlations were found between the Mini Mental State Examination (MMSE) test score and the 8-foot up and go test (8-foot) and chair stand test (30s-CS) in a group of old women (82.7 ± 5.7 years) with moderate cognitive impairment. In the same direction, the study (Furtado et al., 2017), correlations were found between indicators of physical fragility and the 8-foot (positive strong), the 30s-CS and arm curl test (30s-AC) (negative moderate) and no correlation was found between frailty indicators and body mass index (BMI) in a sample of 119 women (81.96 ± 7.89 years).

Despite being a topic that is frequently addressed in current literature, a lack of studies of the aforementioned associations in the community dwelling elderly is notable (Aparicio et al., 2017; Hernandez-Martinez et al., 2019; Lee et al., 2016; Tay et al., 2019). Therefore, the necessity of conducting more studies that use as a basis community dwelling elderly is of importance. These studies aid in discovering evidence that support the efficiency of using aerobic capacity, grip strength and cognition in preventing cardiometabolic diseases.

The purpose of this study is to investigate the association between the aerobic capacity, hand grip strength and cognition with a wide range of cardiometabolic markers (anthropometry, physical function, immunological markers and cardiovascular markers) and mental health, in community dwelling elderly.

METHODS

Study design

Participants in this study are part of the sample of the study protocol published by Ferreira et al. (2020). The present study consists of a cross-sectional analysis of baseline data collected from 28 weeks randomized and controlled intervention study with physical exercise in aquatic environment with elderly aged equal to or above 65 years old, living in Sertã region, Centre of Portugal. All participants attended a community aquatic physical exercise program ran by the Municipality of Sertã in the local swimming pool. This was an exploratory case-control, local, population-based survey (Pearce, 2012) that collected information on the effects of different aquatic exercise programs on anthropometrics, physical function, cognitive function, mental health, heart rate variability, carotid arteries intima-media thickness, and biochemical markers in community dwelling elderly. In addition, this study was designed to provide information on the trends and results expected when using a representative probabilistic sample of this population in future studies.

Sample selection criteria

The participants were recruited with a non-probability convenience sampling method in the central area of Portugal (Sertã). The sample size was calculated using G-Power software (Effect size: 0.25; α-level: 0.05; power: 0.95; n=76). The following eligibility criteria was used to form the sample: a) both female and male individuals; b) age equal to or above 65 years; c) community dwelling elderly; d) ability of participants to autonomously travel from there residence to Sertã municipal pool. Furthermore, ineligible criteria were also defined: a) individuals with medically diagnosed pathologies that jeopardize their health while preforming aquatic based exercises; b) severe cognitive impairment, that is, a score below 9 in the Mini

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Mental States Examination or a clinically diagnosed mental illness. After applying the previously mentioned eligibility and ineligible criteria, the final sample was composed of 102 individuals (24 male and 78 female) with a average age of 72,32 ± 5,25 years.

Ethical aspects

All participants or their legal representatives signed consent forms. The study protocol was approved by the Ethical Committee of the Faculty of Sport Science and Physical Education at University of Coimbra (Reference code CE/FCDEF-UC/00462019) and previously published (Ferreira et al., 2020). The study protocol respected the Portuguese Resolution (Art. 4th; Law nº 12/2005, 1st series) and complied with research guidelines in humans of the Helsinki Declaration (Petrini, 2014).

Data collected is confidential and used exclusively for scientific investigation purposes, obeying all the anonymization procedures defined by the Data Projection Regulation, applied since May 25, 2018.

Assessment instruments

Blood samples were drawn at a professional clinic using certified methods (Clinic-Affidea Sertã). The carotid arteries intima-media thickness assessment was conducted by a cardiology specialist. The rest of the data was collected and organized by members of the research team. Data quality was assessed using internal consistency reliability (ICR) measure.

Anthropometry measurements

Anthropometry measurements were conducted by a certified investigators by FCDEF-UC. The following parameters were assessed: height (Hgt), evaluated with a portable stadiometer, Seca Bodymeter® (model 208, Germany), with a precision of 0.1 cm; weight (Wgt), body mass index (BMI), visceral fat (VF), percentage of fat mass (FM) and muscle body mass (LBM) using a portable scale TANITA BC-601 with a precision of 0.1 cm with 0.1 kg accuracy; and waist circumference (WCir), arms (ACir) and legs (LCir) were measured using a retractable fiberglass tape (model Hoechstmass-Rollfix®, Germany) with an accuracy of 0.1 cm.

Physical Function

Physical Function was assessed with the Senior Fitness Test Battery (Rikli & Jones, 1999), validated for the Portuguese population by Baptista and Sardinha (2005). Lower body muscular strength was evaluated with the chair stand test (30s-CS) (repetitions/30s); Upper body strength was evaluated with the arm curl test (30s-AC) (repetitions/30s); aerobic capacity was assessed with the two-minutes step test (2m-ST) (repetitions /2min); Lower body flexibility was measured with the chair sit and reach (CSR) (centimetres) and upper body flexibility was assessed with the back scratch test (BS) (centimetres); agility and dynamic balance was assessed via the timed up and go test (TUG) (seconds); and hand grip strength was evaluated with the hand grip test (HG) (kg).

Cognitive function and mental health

Cognitive function was calculated with the Portuguese version of the Mini Mental State Examination - MMSE (Morgado et al., 2009), which evaluates the following areas of cognition: orientation, short term memory, attention and calculation capacities, and long term memory and language aptitudes. The final score has a maximum of 30 points, and scores below 24 can be used as an aid in the assessment of

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dementia. The test will be used as an instrument to create a cognitive profile with the following criterium (Mungas, 1991): severe cognitive impairment (scores between 1 and 9 points); moderate cognitive impairment (scores between 10 and 18 points); mild impairment (score between 19 and 24 points), normal cognitive profile (scores between 25 and 30 points).

Mental health was assessed with following questionnaires and scales validated for the Portuguese population: Rosenberg Self-Esteem Scale – RSES (Neto, 1996), which assess global self-esteem; Physical Self-Perception Profile For Clinical Populations – CPSPP (Ferreira et al., 2017), which is a self-assessment of physical characteristics in elderly groups and is composed of the following dimensions: functionality, physical health, sports competence, body attraction, physical strength and physical self-worth; World Health Organization Well-Being Index - WHO5 (Canavarro et al., 2009), which gauges psychological well-being; Satisfaction With Life Scale – SWLS (Neto & Oliveira, 2004), which evaluates global cognitive parameters of life satisfaction; EuroQol – EQ5D (Ferreira et al., 2013), which assess the general health of the participants; Geriatric Depression Scale - GDS (Apóstolo et al., 2014), assess life satisfaction, interruptions in activities, annoyances, isolation, energy, joy and memory problems; Perceived Stress Scale - PSS (Trujillo & Cabrera, 2007), used to measure perception of stress.

The carotid arteries intima-media thickness (IMT)

The (right and left) IMT was measured with a portable Doppler ultrasound of make and model General Electric VIDe® with an 11L probe, using the AIRC Study protocol (Stein et al., 2008). The measurements consisted of the following parameters: Intima-media thickness (IMT); systolic diameter (SD); diastolic diameter (DD); peak systolic velocity (PSV); end-diastolic velocity (EDV). Heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were also evaluated with a RI-Champion N® sphygmomanometer.

Heart rate variability (HRV)

HRV was assessed by following the procedures devised by Abad et al. (2017). Polar V800 (Polar Electro Oy, Finland) heart rate monitors were used, for 10 minutes, with the participants in a calm, silent and low light environment. The following values will be recorded: minimum R-R interval (RRmin); maximum R-R interval (RRmax); mean R-R range (RRmean); Root mean square of the successive normal sinus R-R-interval difference (RMSSD).

Biochemical markers

Blood samples were drawn via venepuncture, with 12 hour fasting participants. The following parameters were evaluated: HDL cholesterol (HDL), LDL cholesterol (LDL), cholesterol total (CLT), glucose (GLU), triglycerides (TRI), leukocytes (LEU), lymphocytes (LI), monocytes (Mo), red blood cells (RBCs), erythrocytes (ERI), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean globular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocytes distribution width (RDW), platelets (PL), mean platelet volume (MPV), procalcitonin (Pct), adenosine diphosphate (ADP), using an automatic hematologic analyser Coulter Act Diff, Beckman Coulter, USA; Levels of interleukin 10 (IL-10), interleukin 1 (IL-1ra), interleukin 1 beta (IL-1β) and tumor necrosis factor (TNF-α) were also analysed with ELISA Invitrogen® CA kits.

Data analysis

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The collected data was subject to descriptive statistical analysis, were values such as maximum, minimum, mean and standard deviation were calculated for each variable. Afterward, data normality was tested considering the response to 3 conditions: z-values from Skewness and Kutosis tests; p value from Shapiro-Wilk test; and histogram redundancy. Statistical tests, Pearson's r-moment and Spearman's r-product correlation tests were applied to the data to establish the relationship between the different variables under study. Relationship between the different variables were interpreted according to Cohen's (1988) cutoff values which consider that r = -/+.10 to -/+ .29 means weak correlation, r = -/+.30 to -/+ .49 means moderate correlation and r = -/+.50 to -/+ 1.0 means strong correlation.

The whole statistical analysis was performed using version 26.0 of the statistical software, Statistical Package for the Social Sciences (SPSS), with a level of significance of $p \leq 0.05$.

RESULTS

The sample was composed of 102 community dwelling elderly, with a mean age of 72.32 ± 5.25 years, 24 males (mean age 74.00 ± 4.39) and 78 females (mean age 71.81 ± 5.40). The short query answers revealed that 95 % of the participants used medication (MED) in a regular basis, 61% mentioned not to have any allergies (ALL), 41% responded that they attended a doctor (VDY) in average 3 or more times a year, 49% classified their sleep (SQ) as being good. The participant´s anthropometric characteristics are presented in Table 1.

Table 1 - Participant´s anthropometric profile characterization analyzed by gender

	All sample (N=102)							
	Female (N=78)				Male (N=24)			
	Minimum	Maximum	Mean	Std. Dev.	Minimum	Maximum	Mean	Std. Dev.
Age	65	86	71.81	5.40	65	82	74.00	4.39
Hgt (cm)	1.47	1.66	1.54	0.04	1.59	1.76	1.67	0.05
Wgt (kg)	49.4	104.3	71.23	11.96	63.9	97.7	78.82	9.97
BMI (kg/m²)	20.7	42.9	29.92	5.07	20.5	38.0	27.98	3.76
VF (%)	6	18	11.59	2.90	14	28	14.58	5.20
FM (%)	27.4	57.1	42.73	6.26	11.5	33.8	27.22	4.73
LBM (%)	18.4	41.0	24.32	3.33	26.8	37.5	30.97	2.15

Note: Height (HGT); Weight (Wgt); Body mass index (BMI); Visceral fat (VF); Fat mass (FM); Lean body mass (LBM).

In terms of aerobic capacity, the final mean result for the study sample was 77,22. The relationship between aerobic capacity and the different variables assessed in the present study (anthropometry, physical function, cognitive function, mental health, immunological markers and cardiovascular markers) showed a strong and positive correlation between the 2m-ST variable and the 30s-CS (r=.562; n=102; *p*<0.001); 30s-AC (r=.513; n=102; *p*<0.001); and a strong and negative correlation with the TUG variable (r=-.591;

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n=102; *p*<0.001). There was a moderate and positive correlation between the 2m-ST variable and the HG (r=.426; n=102; *p*<0.001), EDV–R (r=.326; n=102; *p*=.001), SWLS (r=.314; n=102; *p*=.001), and EQ5D – scale (r=.394; n=102; *p*<0.001). Finally, there was a weak and positive correlation between the aerobic capacity variable and CSR–L (r=.198; n=102; *p*=.046); BS–R (r=.212; n=102; *p*=.032); BS–L (r=.230; n=102; *p*=.020); EDV–L (r=.232; n=102; *p*=.019); PSV–R (r=.248; n=102; *p*=.012); TNF-α (r=.210; n=102; *p*=.034); LI (r=.286; n=102; *p*=.004); and MMSE (r=.275; n=102; *p*=.005). There was a weak and negative correlation between the 2m-ST variable and the IMT–R (r=-.271; n=102; *p*=.006), GR (r=-.287; n=102; *p*=.003) and GDS (r=-.291; n=102; *p*=.003). These results are presented in Figure 1 and 2.

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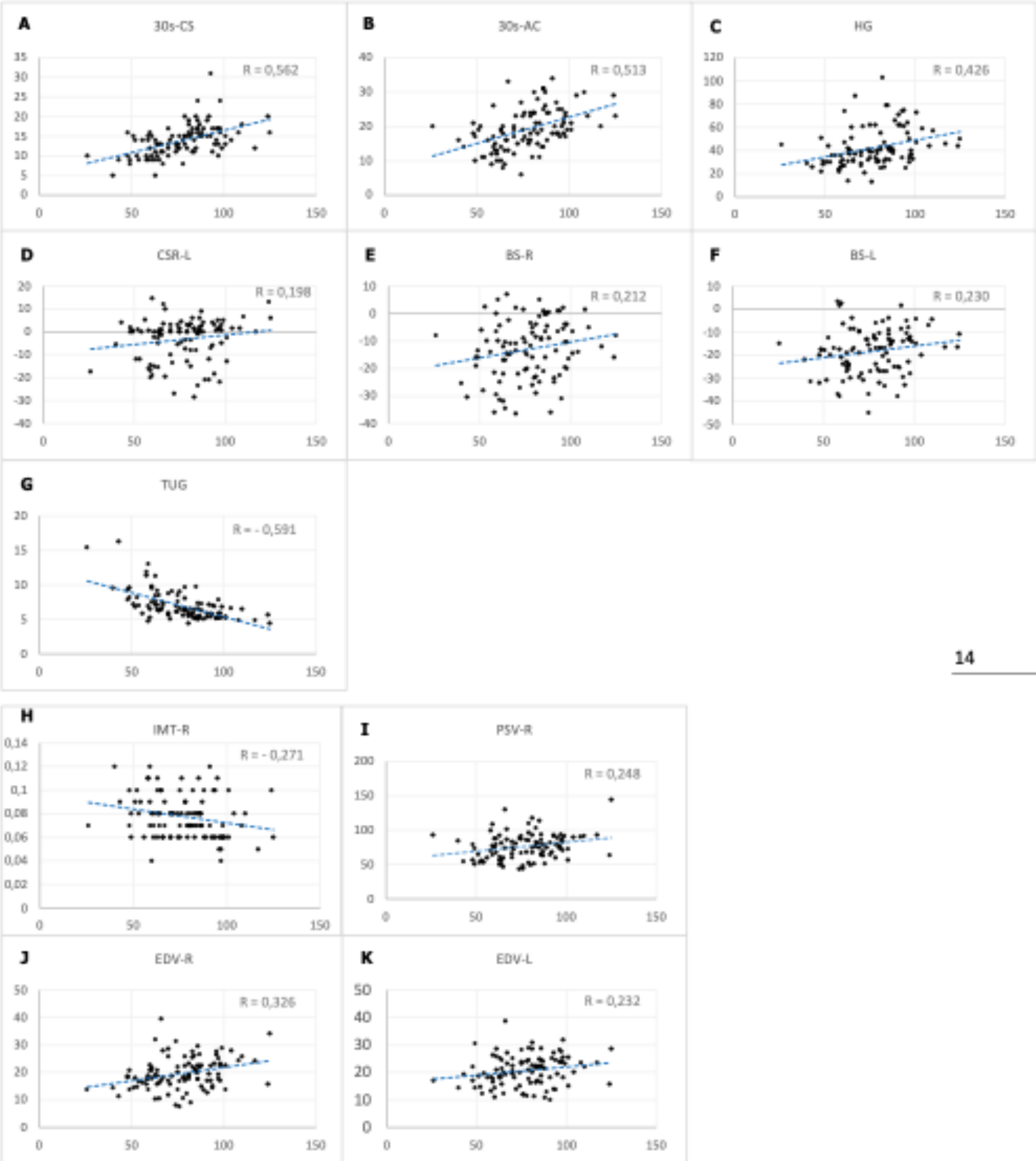

Figure 1 - Relationship between aerobic capacity variable assessed through the 2m-ST and chair stand test (30s-CS) (A), arm curl test (30s-AC) (B), hand grip test (HG) (C), chair sit and reach test (CSR) (D), back scratch test - right (BS-R) (E), back scratch test – left (BS-L) (F), timed up and go test (TUG) (G), intima-media thickness (IMT) (H),peak systolic velocity (PSV) (I), end-diastolic velocity – right (EDV-R) (J) and end-diastolic velocity – left (EDV-L) (K).

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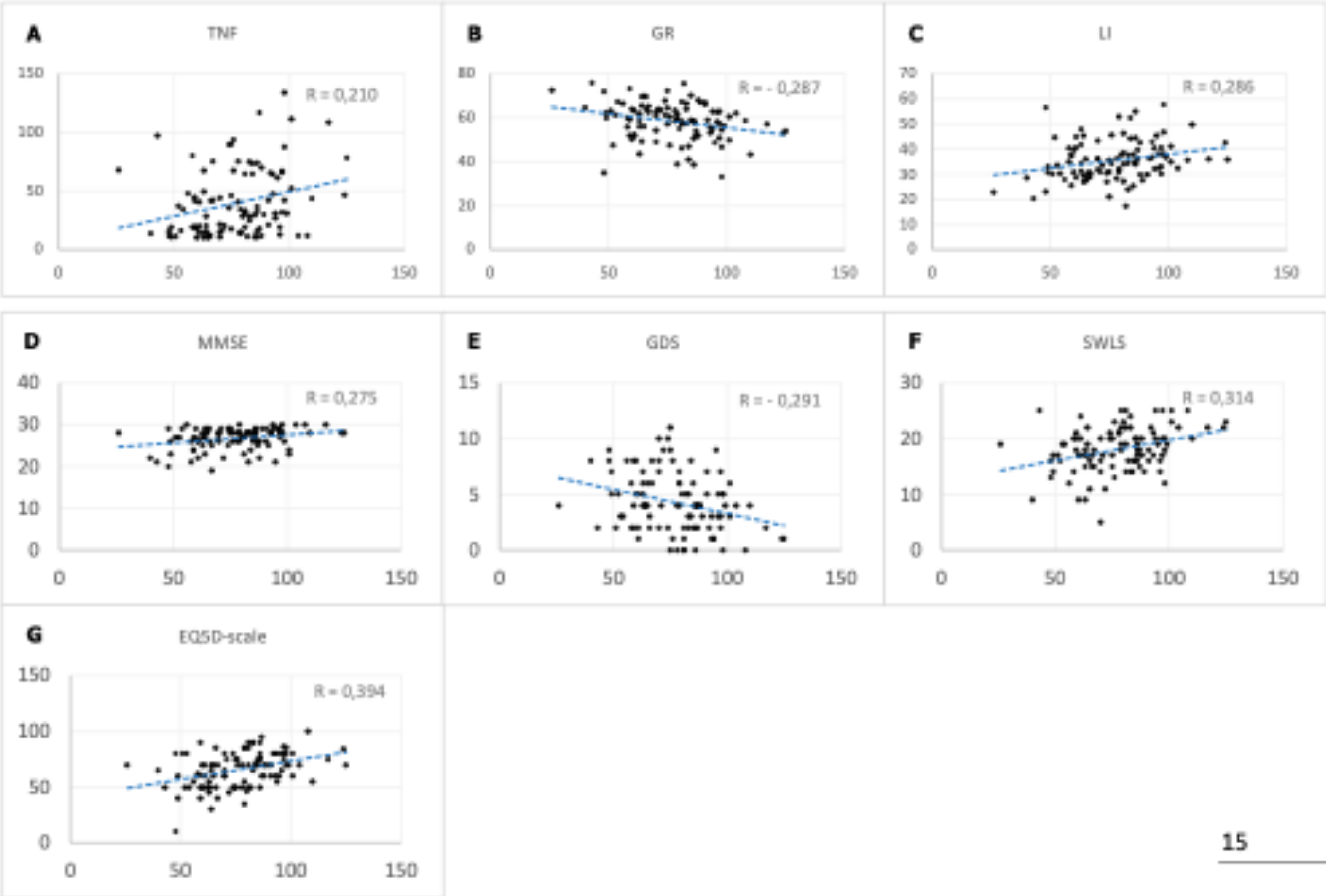

Figure 2 - Relationship between aerobic capacity variable assessed through the 2m-ST and tumor necrosis factor (TNF-α) (A), granulocytes (GR) (B), lymphocytes (LI) (C), mini mental state examination (MMSE) (D), geriatric depression scale (GDS) (E), satisfaction with life scale (SWLS) (F) and euroqol (EQ5D) (G).

In relation to handgrip strength, the final mean result for the study sample was 42kg. Value was calculated according to the sum of the two best results from the HG from both hands (ACSM, 2018). The relationship between hand grip test (HG) and the different variables assessed in the present study (anthropometry, physical function, cognitive function, mental health, immunological markers and cardiovascular markers) showed a strong and positive correlation between the HG variable and the Hgt (r=.522; n=102; p<0.001). There was a moderate and positive correlation between the HG variable and LBM (r=.381; n=102; p<0.001), 2m-ST (r=.426; n=102; p<0.001), 30s-AC (r=.365; n=102; p<0.001), and MMSE (r=.342; n=102; p<0.001). There was a moderate and negative correlation between the HG variable and FM (r=-.348; n=102; p<0.001) and TUG (r=-.403; n=102; p<0.001). Finally, there was a weak and positive correlation between the HG variable and Wgt (r=.253; n=102; p=.010); 30s-CS (r=.289; n=102; p=.003); ERI (r=.233; n=102; p=.018); Hb (r=.270; n=102; p=.006); Hct (r=.275; n=102; p=.005); RSES (r=.233; n=102; p=.019); and EQ5D–scale (r=.217; n=102; p=.028). There was a weak and negative correlation between GLU (r=-.197; n=102; p=.047); CLT (r=-.255; n=102; p=.010); HDL (r=-.285; n=102; p=.004); GDS (r=-.290; n=102; p=.003); and PSS (r=-.239; n=102; p=.015). These results are presented in Figure 3 and 4.

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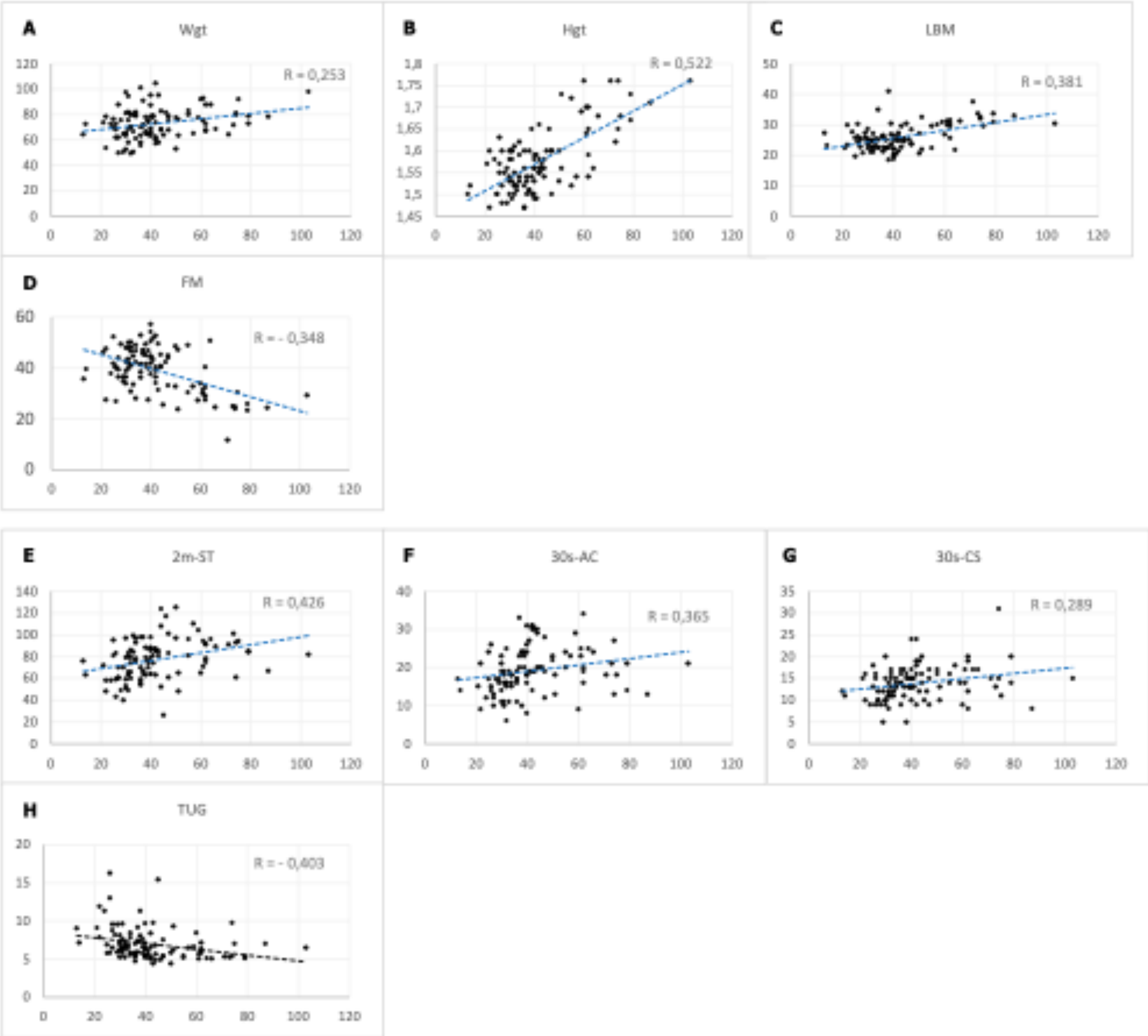

Figure 3 - Relationship between hand grip strength assessed through the HG and weight (Wgt) (A), height (Hgt) (B), muscle mass (LBM) (C), fat mass (FM) (D), two-minutes step test (2m-ST) (E), arm curl test (30s-AC) (F), chair stand test (30s-CS) (G) and timed up and go test (TUG) (H).

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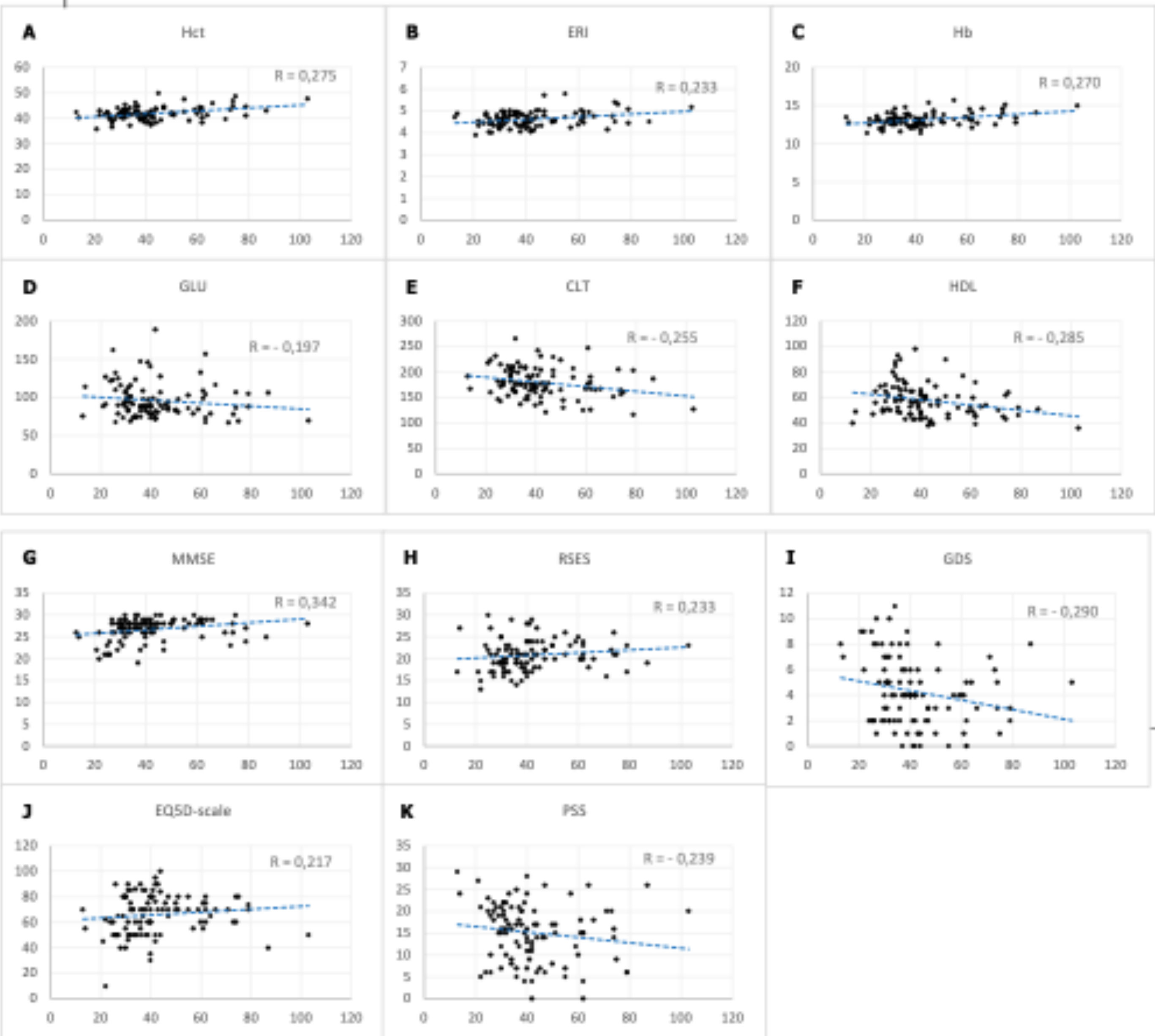

Figure 4 - Relationship between hand grip strength assessed through the HG and hematocrit (Hct) (A), erythrocytes (ERI) (B), hemoglobin concentration (Hb) (C), glucose (GLU) (D), cholesterol total (CLT) (E), high-density lipoprotein (HDL) (F), mini mental state examination (MMSE) (G), rosenberg self-esteem scale (RSES) (H), geriatric depression scale (GDS) (I), euroqol (EQ5D) (J) and perceived stress scale (PSS) (K).

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Finally, cognitive function was assessed using the MMSE, with the total sample achieving an average score equivalent of 26.71 points, being considered as a normal cognitive profile (Mungas, 1991). The relationship between cognitive function assessed using the MMSE score and the different variables assessed in the present study (anthropometry, physical function, cognitive function, mental health, immunological markers and cardiovascular markers) revealed a moderate and positive correlation between HG (r=.342; n=102; *p*<0.001), EQ5D–scale (r=.307; n=102; p=.002), and a moderate and negative between TUG (r=-.300; n=102; p=.002). Finally, there was a weak and positive correlation between LCir–R (r=.247; n=102; p=.012); LCir–L (r=.222; n=102; p=.025); 2m-ST (r=.275; n=102; p=.005); BS–R (r=.210; n=102; p=.035); 30s-CS (r=.194; n=102; p=.050); and 30s-AC (r=.253; n=102; p=.010). There was also a weak and negative correlation between IMT–L (r=-.213; n=102; p=.031), ADP (r=-.208; n=102; p=.036) and GDS (r=-.201; n=102; p=.043). These results are presented in Figure 5.

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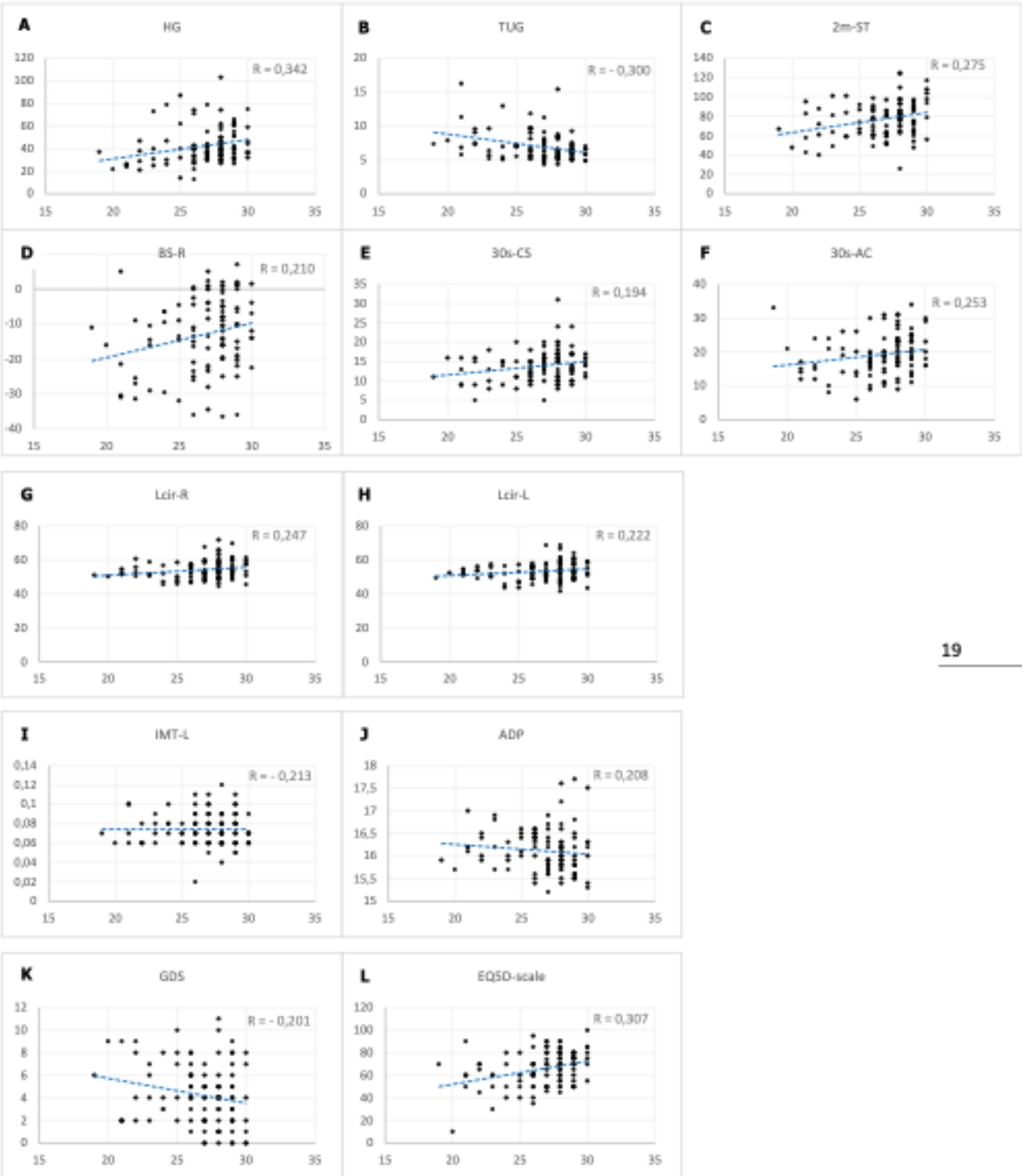

Figure 5 - Relationship between cognitive function assessed through the MMSE and hand grip test (HG) (A), timed up and go test (TUG) (B), two-minutes steo test (2m-ST) (C), back scratch test – right (BS-R) (D), chair stand test (30s-CS) (E), arm curl test (30s-AC) (F), legs circumference – right (LCir-R) (G), legs circumference – left (LCir-L) (H), intima-media thickness (IMT) (I), adenosine diphosphate (ADP) (J), geriatric depression scale (GDS) (K) and euroqol (EQ5D) (L).

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DISCUSSION

The purpose of this study was investigate the association between the aerobic capacity, hand grip strength and cognition with a wide range of cardiometabolic markers (anthropometry, physical function, immunological markers and cardiovascular markers) and mental health, in community dwelling elderly.

The results of this study suggesting a strong positive correlation between aerobic fitness (assessed by 2m-ST) and 30s-CS and 30s-AC, and a strong negative correlation between TG. A moderate positive correlation between 2m-ST and HG, EVD-R, SWLS and EQ5D-scale. A weak positive correlation between 2m-ST and CSR-L, BS-R, BS-L, EDV-L, PSV-R, TNF-α, LI and MMSE, and a weak negative correlation between IMT-R, GR and GDS.

Our results are in agreement with those found in other studies. Regarding physical function, Teodorczyk et al. (2016) also demonstrated a strong and positive correlation between aerobic capacity and lower limb muscle strength, assessed using an isometric dynamometer armchair. Likewise, in the study of Kaymaz et al. (2018), positive and moderate correlations were also found between aerobic capacity (i.e., assessed through the incremental back-and-forth walk test and through the resistance walk test) and upper limb muscle strength (i.e., assessed through the one-repetition maximal test for the upper limb strength). Pedrosa and Holanda (2009) demonstrated a strong and negative correlation between aerobic capacity and agility/dynamic balance. Similarly, Taylor-Piliae et al. (2012) also found a moderate and positive correlation between aerobic capacity with lower limb strength, and with balance (i.e., assessed using the unipedal stance test). Katsimpris et al. (2021) demonstrated a strong and positive correlation between aerobic capacity and handgrip strength. Regarding flexibility, in the study of Chang et al. (2019) a moderate and positive correlation was verified between aerobic capacity and flexibility (back-scratch test). However, in studies of Santos et al. (2018) e Huang et al. (2018), no statistically significant correlations were found between these two variables.

Other studies have also verified the association between aerobic capacity and the intima and media thickness of the carotid arteries. In particular, the study of Lee et al. (2009) which demonstrated a strong and negative association between aerobic capacity and the risk of developing carotid atherosclerosis, and the study of J. Lee et al. (2019), which also demonstrated an inverse association between aerobic capacity and the development of carotid artery disease and with the intima and medium thickness of the carotid arteries.

As for biochemical markers, in the study of Lacedonia et al. (2016) there was also a moderate and negative correlation between aerobic capacity (6-minute walking test) and granulocyte levels. Contrary to the results found in the present study, Pérez & Núñez (2018), found a weak and negative correlation between aerobic capacity and the TNF-α marker. In the study of Neves et al. (2021), there was also a moderate and negative correlation between these two variables. Likewise, the results of Okan (2020) showed a negative correlation between aerobic capacity (6-minute walking test) and the neutrophil/lymphocyte ratio.

Regarding psychological health, studies of Marques et al. (2017) e Kouwijzer et al. (2020), also showed a moderate and positive relationship between aerobic capacity and life satisfaction. In the study of Kim et al. (2019) a moderate and positive correlation was found between aerobic capacity and quality of life. And in the study of Baldasseroni et al. (2014), a moderate and negative correlation was found between aerobic capacity and the geriatric depression scale. As for the cognitive level, the study of Yazar et al. (2018),

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showed a moderate and positive correlation between aerobic capacity (6-minute walking test) and the MMSE.

The 2m ST is considered an adequate tool to assess aerobic fitness, as it has minimal requirements that make it easy to apply to different study scenarios (Ricci et al., 2019). Our results reinforce the importance of improving aerobic capacity, which is an important component that can significantly improve the predictive ability of cardiovascular disease risk in both the short and long term (Lin et al., 2015). According to the same authors, aerobic capacity is an indicator used in clinical practice, both in the diagnosis and prognosis of cardiovascular diseases.

Regarding muscle strength, we verified the strong positive correlation between HG and Hgt. A moderate positive correlation between HG and LBM, 2m-ST, 30s-AC and MMSE, and a moderate negative correlation between HG and FM and TUG. A weak positive correlation between HG and Wgt, 30s-CS, ERI, Hb, Hct, RSES and EQ5D, and weak negative correlation between GLU, CLT, HDL, GDS and PSS.

The obtained results are aligned with those found in the literature. Regarding body composition, Torralvo et al. (2018) also demonstrated a strong and positive correlation between handgrip strength and height. However, in the study of Pizzigalli et al. (2017) this correlation was moderate and positive. Buehring et al. (2018) also demonstrated a moderate and positive correlation between handgrip strength and muscle mass, assessed by different methods (i.e., dual-energy X-ray asborptiometry, bioelectric impedance and creatine dilution DB-CR), e Torralvo et al. (2018) demonstrated a strong and positive correlation between handgrip strength and muscle mass (i.e., bioelectric impedance). Torralvo et al. (2018) also demonstrated a strong and negative correlation between handgrip strength and fat mass. However, in the study of Bentes et al. (2017), there were no statistically significant correlations between handgrip strength and weight.

Regarding functional fitness, Katsimpris et al. (2021) also demonstrated a strong and positive correlation between handgrip strength and aerobic capacity. Alonso et al. (2018) demonstrated a moderate and positive correlation between handgrip strength and lower limb muscle strength. C. Liu et al. (2017), also demonstrated a moderate and positive relationship between HG and upper limb muscle strength. (30s-AC). Lam et al. (2016) e Alonso et al. (2018) demonstrated a weak and negative correlation between handgrip strength and agility and dynamic balance (i.e., TUG).

At the immunological level, in the study of Belury et al. (2021), ERI values were also positively associated with handgrip strength. Hu et al. (2018) demonstrated a positive correlation between handgrip strength and Hb levels. in the same sense, Sayre et al. (2017) demonstrated a positive association between HG and Hb and Hct levels. In the lipid profile, blood glucose values were negatively associated with HG, that is, participants with higher blood glucose levels had a lower result in HG (Åström et al., 2021; Liang et al., 2020) and Pedrero-Chamizo et al. (2020) found a weak and negative correlation between HG and total cholesterol. In HDL levels, the same authors found a weak and positive correlation with HG, contradicting the results verified in the present study.

In terms of cognitive function and mental health, our study found a statistically significant correlation between HG and cognitive function, quality of life, level of depression and perceived stress. In the study of Y. Liu et al. (2021), the results also showed a weak and positive correlation between handgrip and cognitive level (i.e., MMSE). However, such association was not found in previous studies (Chupel et al., 2017) assessing the same variables in elderly participants. in the study of Hu et al. (2018), the results also showed a positive association between handgrip strength and quality of life. Ozer et al. (2021) showed a weak and negative correlation between the HG and the Geriatric Depression Scale, in participants aged over 60 years. in the study of Poornima et al. (2014), the results also showed a weak and negative correlation between

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handgrip strength and perceived stress (assessed using the PSS). Additionally, in our study, a statistically significant correlation was found between HG and self-storage (assessed using the Rosenberg scale). No other studies were found where this correlation was verified.

These results are relevant since reduced muscular strength, in the elderly, impacts everyday tasks. A significant increase in the risk of falling and loss of functional independence is also associated with muscular strength reduction (Alonso et al., 2018). Hand grip strength is fast and simple to assess. It has been considered as an important clinical test to be used to identify increased risk of morbidity and mortality (Hershkovitz et al., 2019; Wu et al., 2019). Hand grip strength is often used in the detection of deteriorations related to aging and is observed in the LBM and strength (McGrath et al., 2018) parameters.

Regarding cognition function, we verified the moderate positive correlation between MMSE and HG and EQ5D-scale, and a moderate negative correlation between MMSE and TUG. A weak positive correlation between MMSE and LCir-R, LCir-L, 2m-ST, BS-R, 30s-CS and 30s-AC, and weak negative correlation between IMT-L, ADP and GDS.

The results obtained are in line with those found in other studies. With regard to functional fitness, Y. Liu et al. (2021) and Ramnath et al. (2018) also demonstrated a moderate and positive correlation between cognitive fitness and handgrip strength. However, Emerenziani et al. (2020) had already demonstrated a strong and positive correlation between these two variables. n the study of Chupel et al. (2017), moderate and negative correlations were also demonstrated between cognitive aptitude and agility/dynamic balance. In the same vein, in the study of Borges et al. (2018) a weak and negative correlation was shown between cognition and agility/dynamic balance while, in the study of Ramnath et al. (2018), there were no significant correlations between the two variables. Still in functional fitness, Chupel et al. (2017), also found moderate positive associations between MMSE and upper and lower strength (30s-CS and 30s-AC), and cardiorespiratory fitness (2m-ST), in institutionalized elderly. According to the authors, these associations can be explained by ascertaining that physical fitness tests involve, in addition to physical requirements, some attention, concentration and understanding from the perspective of the participants. Similarly, Yang et al. (2018) presented the correlation that lower and upper limb strength, lower limb flexibility, dynamic agility/balance and aerobic capacity are independently associated with cognitive function. According to the authors, little is known about the mechanisms between cognitive and physical functions. However, physical fitness tests require refined brain control mechanisms to start tasks, in addition to motor unit recruitment and motor coordination. Similarly, Yang et al. (2018) also showed that participants with cognitive impairment scored lower on several tests of functional fitness (30s-CS, 30s-AC, 2m-ST, CSR and BS).

In relation to the intima and mean thickness of the carotid arteries, our results are in agreement with those found in the study by Yue et al. (2016), where they demonstrated that participants with cognitive impairment had a higher IMT value, compared to participants with a normal cognitive level. This result reinforces the results of the study by Gorgone et al. (2009), that found a moderate and negative correlation between the MMSE and the IMT.

Regarding the relationship between cognitive functions and mental health, our results are supported by the study by Chae et al. (2020), where a lower score on the Quality of Life questionnaire was associated with a cognitive decline (MMSE), suggesting that quality of life metrics should be used as a tool to detect cognitive changes in the elderly, and thus prevent or delay cognitive decline. Similarly, Samuel et al. (2016) concluded that compromised cognitive function, in the elderly, has a negative effect on health-related quality of life metrics, regardless of gender, education or the existence of chronic illnesses. On the other hand, in the study by Voros et al. (2020), no significant associations were found between elderly cognitive function

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(assessed using the MMSE) and quality of life (Older People Quality of Life questionnaire). As in our study, Ozer et al. (2021) also demonstrated a weak and negative correlation between the MMSE and the GDS.

Additionally, in our study, a statistically significant correlation was found between MMSE and the variables ADP, LCir-R and LCir-L. No other studies were found where these correlations were verified. These data suggest that cognitive function can also influence the immune profile and body composition, more precisely the perimeters of the lower limbs.

Cognitive function assessment has considerable scientific value. With aging, a decline in cognition is observed. This decline originates in the expansion of cerebral spinal fluid, in the progressive deterioration of the microstructure of white matter and other subcortical nuclei (hippocampus, cerebellum and striatum) (Cherup et al., 2018).

The results found in the present study showed that aerobic capacity (2m-ST), handgrip strength (HG) and cognitive function (MMSE) can be correlated with a wide range of cardiometabolic markers. More studies are needed to corroborate the evidence found. It is also relevant to develop more studies that test interventions that can improve these markers, such as exercise in an aquatic environment.

CONCLUSION

Our results suggest that aerobic capacity (assessed using 2m-ST) may be related to functional fitness, cardiovascular, biochemical, cognition and mental health markers. They also suggest that handgrip strength (assessed using the GH) may be correlated with anthropometric measurements, functional and biochemical fitness markers, with cognitive function and mental health. Regarding cognitive function (MMSE), the results suggested that it may be associated with anthropometric measures, functional fitness, cardiovascular and biochemical markers, and also with mental health. These data show that aerobic capacity, handgrip strength and cognitive function may hypothetically be related to risk markers for cardiometabolic diseases.

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Impact of Different Aquatic Exercise Programs on Body Composition, Functional Fitness and Cognitive Function of Non-Institutionalized Elderly Adults: A Randomized Controlled Trial

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Abstract: Aquatic physical exercise programs have become progressively more popular among elderly people. Some of the major physical exercise program disadvantages on land are minimized due to the specific properties of the aquatic environment. The purpose of the present randomized controlled study is to verify the effects of different aquatic physical exercise programs on body composition, functional fitness and cognitive function in non-institutionalized elderly people. For this study, 102 elderly individuals were randomly allocated into four different groups: AerG (n = 25, 71.44 ± 4.84 years); IntG (n = 28, 72.64 ± 5.22 years); ComG (n = 29, 71.90 ± 5.67 years) and CG (n = 20, 73.60 ± 5.25 years). Individuals from the groups AerG, IntG and ComG participated in three different aquatic physical exercise programs for a period of 28 weeks. The CG participants kept to their usual routines. All participants were evaluated for body composition, functional fitness and cognitive function at two time moments, i.e., pre- (M1) and post-intervention (M2). Significant differences for body composition were found between M1 and M2 for FM ($p < 0.001$), LBM ($p < 0.001$) and WCir ($p < 0.01$) in the AerG, for BMI ($p < 0.05$), FM ($p < 0.05$), LBM ($p < 0.001$) and LCir-R ($p < 0.05$) in the IntG, and for WGT ($p < 0.01$), FM ($p < 0.05$), LBM ($p < 0.01$), LCir-R ($p < 0.05$) and LCir-L ($p < 0.01$) in the ComG groups. For functional fitness, differences were found between M1 and M2 for 2m-ST ($p < 0.000$), 30s-CS ($p < 0.000$), 30s-AC ($p < 0.05$), HG-T-R ($p < 0.000$) and HG-T-L ($p < 0.000$) in the AerG, for 2m-ST ($p < 0.05$), BS-R ($p < 0.05$), 30s-CS ($p < 0.000$), 30s-AC($p < 0.01$), HG-T-R ($p < 0.000$) and HG-T-L ($p < 0.000$) in the IntG, and for 30s-CS ($p < 0.000$), HG-T-R ($p < 0.000$) and HG-T-L ($p < 0.000$) in the ComG groups. The present study evidenced the beneficial effects of physical exercise in an aquatic environment on body composition, functional fitness and cognitive function in non-institutionalized elderly adults. The ComG water-based exercise program showed more beneficial effects in the improvement of body composition and cognitive function variables, while the IntG and AerG programs were more effective in the improvement of functional fitness.

Keywords: physical exercise; water-based exercise; aging; elderly

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1. Introduction

Regular physical activity is a recognized cost-effective intervention for public health and is associated with an ever-widening constellation of health, economic, and other benefits, playing an important role in the prevention and management of many major chronic conditions. Thus, the implementation of programs, practices, and policies to facilitate more physical activity and limit sedentary behavior could result in significant health improvements and other benefits, as well as reducing the burden and cost of chronic disease to healthcare systems [1]. In this respect, sedentary behavior is associated with negative changes in the neuromuscular systems of healthy older adults, resulting in a decrease in physical functioning [2]. Body composition, functional fitness and cognition are variables that undergo negative changes during aging, and these changes can lead to the development of cardiometabolic diseases [3].

Evidence reveals that physical exercise on land has been identified as an effective method for improving body composition [4] and functional capacity [5], and reducing the cognitive impairment associated with aging [6]. However, exercise in an aquatic environment has progressively gained popularity, particularly in the elderly population, as it minimizes or overcomes some existing disadvantages from land-based exercise programs due to specific water properties, such as buoyancy and water viscosity. Such properties help to reduce strain on the joints and offer additional support for balance and other problems associated with a lack of strength, as is very frequent in frailty, providing a safer and more protective environment for physical exercise. In addition, aquatic exercise programs seem to be at least as or even more effective as land-based exercise programs [7] for improving elderly people´s health and well-being.

A recent intervention study [8] that tested the impact of two walking programs (land-walking versus water-walking), concluded that the water program can be considered safer and is a preferred activity for elderly people, providing benefits in body composition that are similar or higher than those achieved in land-based exercise programs. Results showed that older participants benefit more from the lower impact forces and decreased risk of falls associated with water-walking, without compromising improvements in their cardiorespiratory fitness.

A meta-analysis performed by Waller et al. [3] analyzed the effects of water-based exercise programs on functional fitness in healthy older adults compared to physical exercise programs performed on land. The authors concluded that physical exercise in aquatic environments seems to be effective in the maintenance and improvement of functional fitness in healthy older people. Moreover, when compared to exercise on land, exercise in an aquatic environment seems to be at least as effective and can be used as an alternative type of exercise, when exercise on land is not feasible or desirable [3].

Regarding cognitive function, another intervention study [8] tested the effectiveness of an aquatic exercise program in a group of older women, aged between 60 and 80 years old, and reported that aquatic exercises can contribute to the maintenance and improvement of cognitive function.

For all these reasons, aquatic exercise programs appear to be a viable alternative to exercise on land, and it is crucial to continue exploring the potential of this environment in order to clarify its benefits and the effects of different water-based exercise programs. Thus, the aim of this randomized controlled trial is twofold. Firstly, to perceive the impact of different water-based exercise programs (continuous aerobic, aerobic interval and combined) on body composition, functional fitness and cognitive function in non-institutionalized older people. Secondly, to perceive which of the three water-based exercise programs will be more effective in improving function in non-institutionalized older people. According to the existing literature [3,5,8–11] we predicted that differences would be found in the experimental groups, as opposed to the CG, and that the ComG program would be more effective than the AerG and IntG programs.

2. Materials and Methods

2.1. Study Design

A randomized controlled trial was conducted in the central region of Portugal, between October 2018 and July 2020. The study included non-institutionalized older adults participating in three different aquatic physical exercise programs for a period of 28 weeks. A complete study protocol was previously published in [12]. Participants were recruited in order to analyze the impact of three aquatic exercise programs (over 28 weeks), namely: continuous aerobic exercise, aerobic interval exercise and combined exercise. Data regarding body composition, functional fitness and cognition were collected at two specific moments, pre-intervention (M1) and post-intervention (M2).

This study conformed to the Declaration of Helsinki guidelines and the protocol was approved by the Faculty of Sports Science and Physical Education, University of Coimbra Ethics Committee (reference: CE/FCDEF-UC/00462019). All participants provided written informed consent prior to participation.

2.2. Participants and Sample Size

The size and statistical power of the sample were calculated using the G*Power software application [13]. The following parameters were considered: F test (ANOVA); effect size: 0.25; α-level: 0.05; statistical power: 0.95; number of groups: 4; number of measures: 2 (pre- and post-intervention); margin for possible losses and refusals: 30%. Therefore, the initial size of the total sample was estimated at 76 participants.

Initially, 174 individuals from the community were personally invited to participate in the study. After applying the inclusion and exclusion criteria, 152 individuals were randomized into four different groups: the continuous aerobic exercise group (AerG, $n = 36$), aerobic interval exercise group (IntG, $n = 41$), combined exercise group (ComG, $n = 48$), and control group (CG, $n = 27$). According to the experience of the research team and according to previous studies [6,7,14], the rate of dropping out from exercise programs in the elderly population is high, so we gathered more participants. An external researcher used a computer-generated list of random numbers to allocate participants to each group in a ratio of 1:1:1:1. Researchers were blinded to ensure group randomization.

The inclusion criteria were the following: (a) participants of both sexes; (b) 65 years of age or older; (c) non-institutionalized older people; (d) having the autonomy to move from their residence to the municipal swimming pool; (e) individuals who gave permission to take part in the study by signing the consent form; (f) individuals with medical authorization to practice physical exercise in an aquatic environment, in cases where they have some type of clinical condition or comorbidity. The exclusion criteria were the following: (a) individuals with clinically diagnosed pathologies that put their health and that of others at risk during the practice of physical exercise in an aquatic environment; (b) having severe cognitive impairment, that is, a score lower than 9 points in the MMSE or mental illness that has been clinically diagnosed; (c) participants who attended less than 50% of the physical exercise sessions; (d) participants who failed to complete all of the proposed assessment tests.

All three experimental groups (AerG, IntG and ComG) performed different types of water-based exercise simultaneously for 28 weeks. Participants from the control group (CG) were asked to maintain their normal daily activities, including not performing any type of systematic physical exercise during the same time period.

Figure 1 shows the entire allocation process for the different groups. Fifty participants were excluded from the study due to the following reasons: personal reasons ($n = 11$); the participant attended less than 50% of the exercise program sessions ($n = 14$); the participant did not complete all assessment tests ($n = 12$); injury not related to the intervention program ($n = 4$); and disease ($n = 9$).

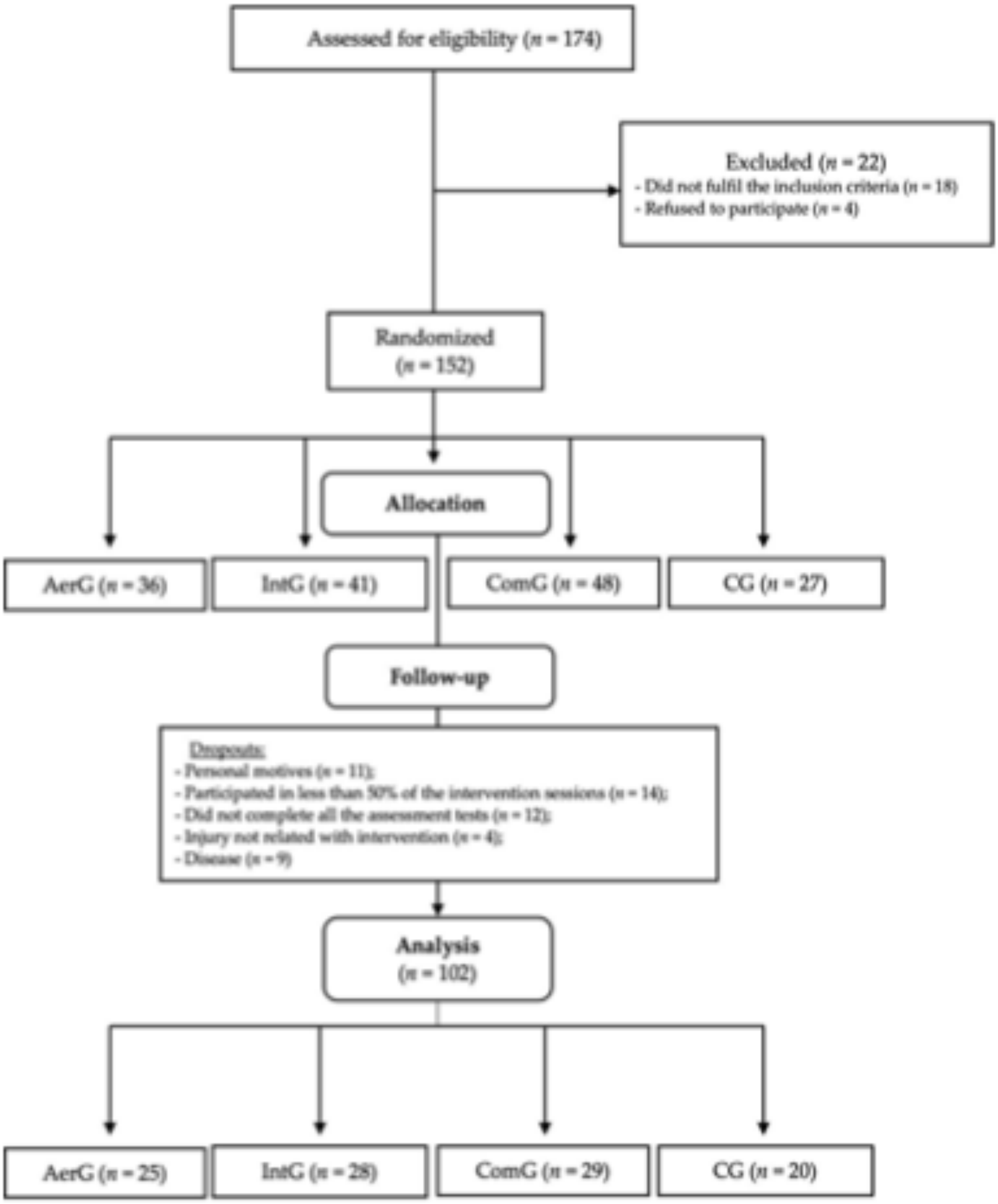

Figure 1. Allocation process for the different groups: continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG).

2.3. *Outcomes Measurements*

2.3.1. Clinical and Demographic Characteristics

Data regarding age (AG), marital status (MS), regular medication (RMED), allergies (ALL), disease (DIS), annual medical appointments (AMA), sleep quality (SQ), alcohol, tobacco and drugs consumption (ATD) were assessed at baseline (M1) using a specific questionnaire developed for this purpose.

2.3.2. Anthropometry

Anthropometric measurements were conducted by two certified investigators by FCDEF-UC. The following parameters of anthropometry were evaluated: height (HGT), using a portable stadiometer, Seca Bodymeter® (model 208, Hamburg, Germany) with a precision of 0.1 cm; weight (WGT); body mass index (BMI); visceral fat (VF); percentage of fat mass (FM) and muscle body mass (LBM) using a portable scale TANITA BC-601 with a precision of 0.1 cm with 0.1 kg accuracy; and waist circumference (WCir); arm circumference (ACir) and leg circumference (LCir), using a retractable fiberglass tape (model Hoechst mass-Rollfix®, Sulzbach, Germany) with an accuracy of 0.1 cm.

2.3.3. Physical Function

Physical function was assessed using the senior fitness test, developed by Rikli and Jones [15] and validated for the Portuguese population [16]: the strength of the lower and upper limbs was assessed using the chair stand test (30 s-CS) and arm curl test (30 s-AC), respectively (repetitions/30 s); aerobic capacity was assessed using the two-minute step test (2m-ST) (number of steps); flexibility of lower and upper limbs was tested with the chair sit and reach test (CSR) and back scratch test (BS), respectively (centimeters); and agility and dynamic balance through the timed up and go test (TUG) (seconds). Hand strength was assessed through the handgrip test (HG-T) using the Jamar hand dynamometer (Lafayette Instrument Company, Lafayette, IN, USA) (kg).

2.3.4. Cognitive Function

Cognitive function was assessed with the Portuguese version of the mini-mental state examination (MMSE) [17]. The MMSE evaluates the following cognitive areas: orientation, short-term memory, attention and calculation capacities, long-term memory, and language capabilities. The final score has a maximum of 30 points, and scores below 24 can be used as an aid in the assessment of dementia. The test was used as an instrument to create a cognitive profile with the following criteria [18]: severe cognitive impairment (scores between 1 and 9 points); moderate cognitive impairment (scores between 10 and 18 points); mild impairment (score between 19 and 24 points), normal cognitive profile (scores between 25 and 30 points).

2.4. Intervention Protocol

The exercise programs were implemented by sport sciences and fitness experts with specific training in water aerobics and were developed according to the exercise prescription guidelines recommended by the American College of Sport Medicine (ACSM) for the elderly [19].

All exercise program sessions had a duration of 45 min, twice a week, for 28 weeks and were performed in a water environment (the water level was between 0.80 and 1.20 m, with a temperature of approximately 32 °C), using the rhythm of the music as a tool to control the intensity level of exercise. Each session had a maximum limit of 16 participants, with a ratio of 1 exercise coach for every 16 participants. Water exercise sessions were organized into three different sections: initial, main and final parts.

The initial part, or the warm-up, lasted between 10 and 15 min, at low intensity (30–40% max HR), and were the same in the three water exercise programs. During this initial part, it was intended that the participants would adapt to the aquatic environment, i.e., to the water temperature, and provide muscular and metabolic stimulations to prepare the body for the main part of the session. Thus, simple exercises in water were used, such as displacements and isolated movements, with a progressive increase in complexity and intensity throughout the initial part.

The main part for each one of the three water exercise program sessions had a duration of 20 to 30 min and all were characterized as follows:

1. In the water-based continuous aerobic program, aerobic exercises were used continuously throughout the main part of the session (20 to 30 min), with a target intensity of 60 to 70% of the maximum heart rate, according to the recommendations of the ACSM for the elderly [19];

2. In the water-based interval aerobic program, the main part of the session consisted of performing exercises with different intensities, such as: short duration exercises of 30 seconds, with an intensity of 70–80% of the maximal heart rate, followed by active recovery intervals of 1 minute, using exercises with an intensity of 60–70% of the maximal heart rate;
3. In the water-based combined training program (continuous aerobic and muscular strength), the main part of the session was divided into two phases, with equal time periods. The first phase consisted of aerobic exercises on a continuous basis, with a target intensity of between 60 and 70% of the maximal heart rate. In the second phase, muscle-strengthening exercises were applied (water environment): 6 to 8 different exercises, with auxiliary equipment to create more resistance to movement (e.g., "spaghetti" and dumbbells), covering muscle strengthening from trunk (e.g., rowing, inverted crunches, etc.), upper limbs (e.g., elbow flexion and extension, shoulder rotation, etc.) and lower limbs (e.g., knee flexion and extension, leg abduction and adduction, etc.). Strengthening exercises were implemented using 2 to 3 sets of 12 to 16 repetitions at a moderate intensity (6–7) on the Borg scale.

The final part of the water exercise sessions lasted between 5 and 10 min and was the same for each of the three water exercise programs. This part consisted of two phases: return to calm, where relaxation exercises were applied to resume the participants' heart rate to values close to the resting state, and stretching, where exercises using a greater range of motion were used to stretch the main muscle groups used throughout the sessions.

2.5. Monitoring the Intensity of the Physical Exercise

For safety and intensity target control reasons, all participants were randomly using heart rate monitors (Polar, RS800CX, Finland) during the exercise sessions, in all three water exercise programs. Depending on the heart rate values obtained, adjustments were performed to maintain the target intensity established for each water exercise program.

The intensity of the different water exercise programs was predicted indirectly using the Karvonen formula [20]:

Target heart rate = ((maximal heart rate − resting heart rate) × % intensity) + resting heart rate.

Additionally, and to calculate the maximal heart rate, the formula created by [21] for the elderly was used:

Maximal HR = 207 beats per minute − (0.7 × chronological age).

2.6. Statistical Analysis

The collected data was subject to descriptive statistical analysis, where values such as maximum, minimum, mean and standard deviation were calculated for each variable in each assessment moment. Afterward, data normality was tested by considering the response to three conditions: z-values from the Skewness and Kurtosis tests; p-values from the Shapiro–Wilk test; and visual inspection of generated histograms. All longitudinal comparisons were performed using complete case analysis. Parametric data were analyzed using Student's t-test for independent samples to compare the different moments (M1 and M2), and an ANOVA of one factor and post hoc Tukey's test to analyze the differences between groups. Nonparametric data was analyzed using the Wilcoxon test to compare the different moments (M1 and M2), and the Kruskal–Wallis and Bonferroni tests to analyze differences between groups. Statistical analysis was performed using the statistical package for the social sciences (SPSS) statistical software, version 27.0. The level of significance used was $p \leq 0.05$.

3. Results

The final 102 participants completed the entire selection process (AerG: n = 25, 71.44 ± 4.84 years, 80% female; IntG: n = 28, 72.64 ± 5.22 years, 89.3% female; ComG: n = 29, 71.90 ± 5.67 years, 75.9% female; CG: n = 20, 73.60 ± 5.25 years, 55% female).

Despite there being no inclusion/exclusion criteria based on gender, the sample was unintentionally formed mostly of female participants, mainly as a result of the higher number of female elderly participants who usually attend community aquatic exercise programs, compared to males. No adverse event was reported during the intervention, showing that the practice of physical exercise in an aquatic environment is a safe modality for the elderly population. Individual characteristics for each group at baseline are presented in Table 1.

Table 1. Major sample characteristics at baseline.

Characteristics	AerG (n = 25)	IntG (n = 28)	ComG (n = 29)	CG (n = 20)	Value p
AG (years)	71.44 ± 4.84	72.64 ± 5.22	71.90 ± 5.67	73.60 ± 5.25	0.504
MMSE	27.00 ± 2.00	27.00 ± 2.00	26.00 ± 3.00	26.00 ± 3.00	0.489
BMI (kg/m^2)	28.20 ± 3.30	29.10 ± 4.80	30.80 ± 5.30	29.50 ± 5.80	0.272
RMED	n (%)	n (%)	n (%)	n (%)	
Yes (%)	23 (92%)	27 (96.4%)	26 (89.7)	19 (95%)	0.768
No (%)	2 (8%)	1 (3.6)	3 (10.3%)	1 (5%)	
MS	n (%)	n (%)	n (%)	n (%)	
Married (%)	23 (92%)	20 (71.4%)	21 (72.4%)	19 (95.0%)	
Divorced (%)	0 (0.0%)	2 (7.1%)	0 (0.0%)	0 (0.0%)	
Widow (%)	2 (8.0%)	6 (21.4%)	6 (20.7%)	1 (5.0%)	0.116
Single (%)	0 (0.0%)	0 (0.0%)	2 (6.9%)	0 (0.0%)	
ALL	n (%)	n (%)	n (%)	n (%)	
Yes (%)	10 (40.0%)	9 (32.8%)	14 (48.3%)	7 (35.0%)	0.638
No (%)	15 (60.0%)	19 (67.9%)	15 (51.7%)	13 (65.0%)	
DIS	n (%)	n (%)	n (%)	n (%)	
No disease (%)	6 (24.0%)	5 (17.9%)	6 (20.7%)	3 (15.0%)	
Cardiovascular (%)	4 (16.0%)	7 (25.0%)	4 (13.8%)	5 (25.0%)	0.794
Metabolic (%)	6 (24.0%)	5 (17.9%)	5 (17.2%)	7 (35.0%)	
Cardiovascular and metabolic (%)	9 (36.0%)	11 (39.3%)	14 (48.3%)	5 (25.0%)	
ATD	n (%)	n (%)	n (%)	n (%)	
None (%)	22 (88.0%)	25 (89.3%)	26 (89.7%)	19 (95.0%)	
Alcohol (%)	0 (0.0%)	0 (0.0%)	1 (3.4%)	0 (0.0%)	
Tabaco (%)	3 (12.0%)	3 (10.7%)	1 (3.4%)	1 (5.0%)	0.772
Both (%)	0 (0.0%)	0 (0.0%)	1 (3.4%)	0 (0.0%)	

Note: Age (AG); mini mental state examination (MMSE); body mass index (BMI); regular medication (RMED); marital status (MS); allergies (ALL); diseases (DIS); alcohol, tobacco and drugs consumption (ATD); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG).

Group characteristics were very similar between groups at baseline. as no significant statistical differences were found for AG, MMSE, BMI, RMED, MS, ALL, DIS and ATD variables among participants from the AerG, IntG, ComG and CG groups. Most participants were taking medication intended to control their cholesterol, blood pressure and diabetes values. Body composition results were analyzed by type of aquatic exercise program before and after the intervention, and are presented in Table 2.

Global body composition results revealed that no significant statistical differences were found between groups, before and after intervention (Table 2). As a result of intervention with aquatic exercise, an increase in WGT was found in all exercise groups, with significant statistical differences on ComG (p = 0.007; Δ = 1.2%), but not in the CG. A similar increase was found for BMI in all aquatic exercise groups, with significant statistical differences in IntG (p = 0.041; Δ = 3.4%), but not in the CG.

Table 2. Body composition results variation analysed by type of aquatic exercise program before and after intervention.

	AerG			IntG			ComG			CG				
	M1 Mean (SD)	M2 Mean (SD)	Time (*p*)	M1 Mean (SD)	M2 Mean (SD)	Time (*p*)	M1 Mean (SD)	M2 Mean (SD)	Time (*p*)	M1 Mean (SD)	M2 Mean (SD)	Time (*p*)	Time × Group (M1)	Time × Group (M2)
WGT (kg)	70.5 (8.1)	71.0 (7.6)	0.150	71.3 (14.3)	71.9 (14.5)	0.097	75.1 (11.0)	76.0 (11.5)	0.007 *	75.5 (13.3)	75.2 (13.5)	0.589	0.334	0.350
BMI (kg/m^2)	28.2 (3.3)	28.5 (3.0)	0.360	29.1 (4.8)	30.1 (6.2)	0.041 **	30.8 (5.3)	31.0 (5.1)	0.677	29.5 (5.8)	29.4 (5.9)	0.489	0.272	0.476
VF (%)	11.0 (3.0)	11.0 (3.0)	0.388	12.0 (3.0)	12.0 (3.0)	0.096	13.0 (3.0)	13.0 (3.0)	0.839	13.0 (6.0)	13.0 (6.0)	1.000	0.128	0.267
FM (%)	38.9 (7.3)	37.1 (8.4)	0.009 **	41.0 (6.7)	40.0 (6.7)	0.016 *	40.3 (9.8)	39.4 (9.8)	0.031 **	34.9 (10.9)	34.6 (10.7)	0.310	0.134	0.145
LBM (%)	26.5 (4.3)	27.2 (3.8)	0.007 **	24.5 (3.0)	25.4 (3.0)	0.001 **	25.5 (4.3)	26.3 (4.3)	0.002 **	27.7 (4.7)	27.9 (4.7)	0.188	0.079	0.160
WCir (cm)	99.7 (8.7)	96.5 (9.0)	0.018 *	102.2 (11.4)	101.1 (11.8)	0.216	105.1 (11.5)	103.4 (10.1)	0.109	103.1 (15.8)	102.5 (17.0)	0.652	0.409	0.181
ACir-R (cm)	31.5 (2.2)	31.5 (2.2)	0.729	32.7 (4.1)	32.2 (4.5)	0.107	32.7 (3.2)	32.4 (3.2)	0.331	31.7 (3.5)	31.6 (3.2)	0.745	0.659	0.734
ACir-L (cm)	31.6 (2.5)	31.3 (2.0)	0.381	32.3 (4.2)	31.7 (4.3)	0.850	32.4 (3.3)	32.2 (3.5)	0.475	31.6 (3.2)	31.4 (3.3)	0.278	0.861	0.616
LCir-R (cm)	54.0 (4.6)	53.5 (4.9)	0.443	54.9 (4.2)	53.8 (5.0)	0.019 *	54.6 (6.2)	53.2 (6.2)	0.025 *	52.5 (6.2)	51.8 (4.7)	0.247	0.093	0.375
LCir-L (cm)	53.7 (4.0)	53.1 (4.5)	0.266	53.8 (3.8)	53.3 (4.7)	0.265	54.3 (6.4)	52.6 (6.7)	0.008 *	51.0 (5.9)	50.6 (4.6)	0.466	0.153	0.312

Note: * *t*-Student test results; ** Wilcoxon test results; Correlation is significant at 0.05 level. Weight (WGT); body mass index (BMI); visceral fat (VF); fat mass (FM); muscular mass (LBM); waist circumference (WCir); right upper limb perimeter (ACir-R); left upper limb perimeter (ACir-L); right lower limb perimeter (LCir-R); left lower limb perimeter (LCIr-L); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG).

FM variable results revealed a significant statistical reduction in all aquatic exercise groups as a result of the intervention AerG ($p = 0.009$; $\Delta = -4.6\%$), IntG ($p = 0.016$; $\Delta = -2.4\%$) and ComG ($p = 0.031$; $\Delta = -2.2\%$), but not in the CG. LBM variable showed a significant increase in all aquatic exercise groups AerG ($p = 0.007$; $\Delta = 2.6\%$), IntG ($p = 0.001$; $\Delta = 3.7\%$) and ComG ($p = 0.002$; $\Delta = 3.1\%$), but not in the CG. Anthropometric results also showed a significant statistical reduction for WCir in the AerG ($p = 0.018$; $\Delta = -3.2\%$), for LCir-R in the IntG ($p = 0.019$; $\Delta = -2.0\%$) and in the ComG ($p = 0.025$; $\Delta = 2.6\%$), and for LCir-L in the ComG ($p = 0.008$; $\Delta = -3.1\%$).

For functional fitness variables (Table 3), there were no significant statistical differences between groups before intervention ($p > 0.05$). However, we found significant statistical differences for 2 m-ST ($p = 0.05$) between participants from AerG and IntG, after intervention, as well as for CSR-R ($p = 0.006$), CSR-L ($p = 0.004$) and 30 s-CS ($p = 0.018$) variables, between participants from the AerG and CG, after intervention. In all identified cases, the results were higher in the AerG. Considering the time effects due to the intervention (M1 and M2), we found significant statistical differences for 2 m-ST in AerG ($p = 0.000$; $\Delta = 16.8\%$) and in the IntG ($p = 0.015$; $\Delta = 10.8\%$). For the BS-R variable, there was a global trend showing a reduction in the performance level in all groups but was just statistically significant for IntG ($p = 0.026$; $\Delta = 15.1\%$). For the 30 s-CS variable, we found a significant average increase in all three aquatic exercise groups: AerG ($p = 0.000$; $\Delta = 20.0\%$), IntG ($p = 0.000$; $\Delta = 15.4\%$) and ComG ($p = 0.000$; $\Delta = 23.1\%$), and an average reduction in the CG. For the 30 s-AC variable, we found an increase in average for all groups, and it was statistically significant in the AerG group ($p = 0.010$; $\Delta = 9.5\%$) and IGnt ($p = 0.005$; $\Delta = 11.8\%$). For the HG-T variable, we found a statistically significant increase in average in the three aquatic exercise groups, for both the right hand—AerG ($p = 0.000$; $\Delta = 27.3\%$), IntG ($p = 0.000$; $\Delta = 23.8\%$) and ComG ($p = 0.000$; $\Delta = 23.8\%$), and the left hand—AerG ($p = 0.000$; $\Delta = 19.0\%$), IntG ($p = 0.000$; $\Delta = 20.0\%$) and ComG ($p = 0.000$; $\Delta = 23.8\%$), but not for the CG.

Table 4 shows the cognitive results variation, analyzed according to the type of aquatic exercise program, before and after intervention.

Regarding the cognitive function variable, no significant statistical differences were found between groups before and after the intervention (see Table 4). However, after the intervention, we found an increase in the MMSE average scores in the three aquatic exercise groups (between M1 and M2), with significant statistical differences in the ComG group ($p = 0.008$; $\Delta = 3.8\%$), but not in the CG.

Table 3. Functional fitness results variation analysed by type of aquatic exercise program before and after intervention.

	AerG			IntG			ComG			CG				
	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	Time × Group (M1)	Time × Group (M2)
2m-ST (no of steps)	80.9 (17.4)	94.5 (21.2)	0.000 **	71.5 (16.5)	79.2 (20.5)	0.015 *	81.6 (19.2)	85.4 (22.7)	0.250	74.3 (18.9)	79.4 (21.7)	0.147	0.069	0.048 †
CSR-R (cm)	−0.5 (6.6)	1.2 (8.7)	0.182	−3.7 (10.6)	−2.5 (10.5)	0.174	−3.5 (7.8)	−3.3 (8.1)	0.819	−7.6 (9.7)	−7.6 (7.0)	0.954	0.099	0.013 †
CSR-L (cm)	0.6 (7.2)	1.1 (8.8)	0.467	−3.9 (9.9)	−2.7 (11.6)	0.190	−5.8 (9.9)	−3.6 (8.6)	0.073	−3.5 (7.3)	−6.8 (8.2)	0.113	0.054	0.039 ††
BS-R (cm)	−9.9 (10.4)	−11.3 (11.4)	0.387	−11.9 (11.5)	−13.7 (12.8)	0.026 **	−14.3 (9.7)	−15.6 (9.7)	0.245	−16.6 (9.9)	−17.1 (10.2)	0.195	0.157	0.361
BS-L (cm)	−14.4 (7.2)	−15.9 (8.8)	0.098	−17.4 (8.6)	−18.1 (12.1)	0.631	−21.0 (10.8)	−21.5 (9.4)	0.369	−20.6 (10.7)	−21.2 (10.1)	0.197	0.056	0.241
TUG (s)	6.1 (1.1)	5.9 (0.9)	0.201	7.4 (1.8)	7.4 (2.2)	0.546	7.4 (3.0)	7.4 (2.9)	0.702	6.8 (1.7)	6.6 (1.9)	0.443	0.110	0.065
30s-CS (reps/30s)	15.0 (3.0)	18.0 (5.0)	0.000 *	13.0 (4.0)	15.0 (4.0)	0.000 *	13.0 (3.0)	16.0 (4.0)	0.000 **	15.0 (5.0)	14.0 (4.0)	0.229	0.185	0.016 †
30s-AC (reps/30s)	21.0 (6.0)	23.0 (6.0)	0.010 *	17.0 (7.0)	19.0 (6.0)	0.005 **	20.0 (5.0)	22.0 (6.0)	0.053	19.0 (6.0)	20.0 (6.0)	0.192	0.119	0.109
HG-T-R (kg)	22.0 (6.0)	28.0 (6.0)	0.000 **	21.0 (9.0)	26.0 (9.0)	0.000 **	21.0 (9.0)	26.0 (9.0)	0.000 **	24.0 (9.0)	25.0 (11.0)	0.259	0.411	0.317
HG-T-L (kg)	21.0 (6.0)	25.0 (6.0)	0.000 *	20.0 (9.0)	24.0 (9.0)	0.000 **	21.0 (9.0)	26.0 (8.0)	0.000 **	21.0 (10.0)	22.0 (10.0)	0.175	0.578	0.271

Note: * *t*-Student test results; ** Wilcoxon test results; † ANOVA and Turkey test results; †† Kruskal Wallis and Bonferroni test results. Correlation is significant at the 0.05 level. Two-minute step test (2m-ST); chair sit and reach test – right (CSR-R); chair sit and reach test – left (CSR-L); back scratch test – right (BS-R); back scratch test – left (BS-L); timed up and go test (TUG); chair stand test (30s-CS); arm curl test (30s-AC); hand grip test – rigth (HG-T-R); hand grip test – left (HG-T-L); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG).

Table 4. Cognitive function results variation analysed by type of aquatic exercise program before and after intervention.

	AerG			IntG			ComG			CG				
	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	Time × Group (M1)	Time × Group (M2)
MMSE	27.0 (2.0)	28.0 (2.0)	0.294	27.0 (2.0)	28.0 (2.0)	0.138	26.0 (3.0)	27.0 (2.0)	0.008 **	26.0 (3.0)	26.0 (3.0)	0.651	0.489	0.255

Note: ** Wilcoxon test results. Correlation is significant at the 0.05 level. Mini mental state examination (MMSE); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG).

4. Discussion

The main purpose of the present study was to assess the impact of different aquatic exercise programs on the body composition, functional fitness and cognitive function of non-institutionalized elderly people. A preliminary systematic search revealed the innovative characteristics of the present study, as no other study was identified as assessing similar variables in an aquatic environment with elderly participants.

For body composition, global results revealed that significant statistical differences were found for most of the variables analyzed, as a result of the intervention with aquatic exercise (AerG, IntG and ComG), while no differences were found in the CG. A more detailed analysis of the results revealed significant statistical differences over time (from M1 to M2), i.e., between pre- and post-intervention time moments in the AerG for the FM, LBM and WCir variables, in IntG for the BMI, FM, LBM and LCIr-R variables, and in the ComG group for the WGT, FM, LBM, LCir-R and LCir-L variables. The variance of the mean was higher for the FM variable in the AerG ($\Delta = -4.6\%$), for the LBM variable in the IntG ($\Delta = 3.7\%$), and for the LCir-D variable in the ComG ($\Delta = -2.6\%$). The increase of the WGT in all aquatic exercise groups may be associated with the significant statistical increase of LBM found in all exercise groups.

A study conducted by [8] that tested the effectiveness of two walking programs at moderate intensity (both aquatic and land environment) on the body composition of elderly participants showed that both programs had beneficial effects on body composition. Like our results, [8] revealed that exercise in an aquatic environment provided a significant reduction in abdominal fat. In spite of there being no significant statistical differences for VF in our study, Wcir was reduced in all aquatic exercise groups, and that reduction was statistically significant in the AerG group. Additionally, in the same study, significant differences were found for LBM in the aquatic walking group. Similar results were found in the present study for all three aquatic exercise groups, with the IntG group obtaining the highest mean variation from all analyzed groups. The study by [8] also found significant differences for the LBM variable in the aquatic walking group. Similar results were found in the three aquatic exercise groups in our study, with the IntG group obtaining the highest mean variation when compared with the other two groups. However, in the study by [8], no significant statistical differences were found for BMI. In our study, the BMI increased in all aquatic exercise groups, and differences were statistically significant in the IntG group.

Another study, conducted by [3], tested the effectiveness of a high-intensity aquatic resistance program in a group of postmenopausal women, before and after 4 months of intervention. Significant statistical differences in FM were reported. The present study also found significant statistical differences for FM in all aquatic exercise groups, with the AerG group reporting the highest mean value.

Previous results show that physical aquatic exercise seems to have a beneficial impact on body composition. Regarding the hypothetical effects of different types of aquatic exercise programs on body composition, functional fitness and cognitive function, results show that they all seem to have a similar positive effect on the FM and BMI variables, but the ComG group also showed significant statistical differences in the LCir variables, providing additional evidence to confirm that combined aquatic exercise programs may have a more positive effect on body composition.

Global results regarding functional fitness showed significant statistical differences in all aquatic exercise groups (AerG, IntG and ComG), but not in the CG. A more comprehensive analysis of the results showed that significant statistical differences were found between AerG and IntG for the 2 m-ST variable, and between AerG and CG for CSR-R, CSR-L and 30 s-CS variables, as a result of the intervention with aquatic exercise. Additionally, significant statistical differences were also found in the AerG group for 2 m-ST, 30 s-CS, 30 s-AC, HG-T-R and HG-T-L variables, in the IntG for 2 m-ST, BS-D, 30 s-CS, 30 s-AC, HG-T-R and HG-T-L variables and in ComG for 30 s-CS, HG-T-R and HG-T-L variables before and after intervention (M1 versus M2). Delta (Δ) variation of the mean was higher for 2 m-ST (Δ = 16.8%) and HG-T-D (Δ = 27.3%) variables in the AerG, for 30 s-AC (Δ = 11.8%) variable in the IntG, and for 30 s-CS (Δ = 23.1%) and HG-T-L (Δ = 23.8%) variables in the ComG group.

A study run by [22] assessed functional fitness in 13 individuals before and after a 12-week aquatic exercise intervention program. Sessions consisted of warm-up exercises on land, walking in the water, aquatic exercises with different materials, and swimming. Results showed significant statistical differences in agility, balance and the muscle strength of the lower limbs. A similar study conducted by [14] analyzed the impact of a 3-month aquatic exercise program with music and found significant statistical differences in upper- and lower-limb muscular strength, flexibility, aerobic capacity agility and dynamic balance. A third study conducted by [23], based on a 24-week high-intensity jumping exercise program, showed improvements in agility, dynamic balance, and lower limb muscular strength. Furthermore, [5] developed a 14-week land aerobic chair-based exercise intervention program and found significant statistical differences for upper-limb muscular strength, agility and dynamic balance. In the same study, a strength-training program using elastic bands showed significant statistical differences for lower-limb strength as a result of the intervention.

As evidenced in the studies above, the results of the present study also showed significant statistical differences in the muscle strength of upper and lower limbs and in hand-grip strength in all three aquatic exercise groups. Regarding aerobic capacity, significant differences were also reported between AerG and IntG, with AerG showing a higher mean variation. On the other hand, no significant statistical differences were found regarding flexibility, agility and dynamic balance. Thus, the present results support the idea that aquatic exercise programs seem to have positive effects on functional fitness, mainly in variables related to upper- and lower-limb strength. In spite of there being no evidence found in the present study, it seems that aquatic exercise programs also tend to have a positive impact on agility and dynamic balance [24,25] in older people. Such results are in agreement with those presented by [5] when comparing elderly institutionalized women participating in a chair-based aerobic exercise walking program, versus a chair-based elastic-band muscle strength program.

Finally, and regarding cognitive function, we found a global trend for positive improvements in cognitive function (i.e., through MMSE instrument) as a result of intervention with aquatic exercise in all exercise groups, but not in the CG. A previous study by [26] tested the efficacy of a water-based exercise program on physical fitness improvement and cognitive function stimulation in adult healthy women. Results showed significant improvements in participants' cognitive function and the mental health domain, regardless of the group in which they were initially included, providing evidence that water-based exercise is capable of enhancing cognitive function and quality of life through improvements in mental health in healthy adult women. Similar results were reported by [9] when testing the efficacy of a 16-week moderate-intensity aquatic exercise program on elderly women´s BDNF levels and cognitive function. Thus, most studies show that aquatic exercise programs are beneficial to cognitive function and that moderate-intensity aerobic exercise seems to have high benefits on elderly people´s cognition levels.

However, the present study also tested the efficacy of a combined aquatic exercise program (aerobic and muscle strength) on elderly non-institutionalized participants. The positive effects of strength-training exercise programs on elderly women´s cognition have previously been reported by [6]. The authors found significant statistical differences in older women´s levels of cognition after their participation in a 28-week elastic-band strength-training intervention program, supporting the idea that strength training is also an effective type of exercise program to be used by groups with cognitive impairment. In our study, combined exercise (aerobic and muscular strength) was responsible for a significant increase in MMSE results ($p = 0.008$) in older participants from M1 to M2, revealing that the combination between the two types of exercise programs may be even more beneficial for improving cognitive function in elderly people.

As regards the limitations of the study, we refer to the fact that it used a simple randomization method, instead of using a blocked randomization method, which would guarantee a balance in the number of participants in each group, reducing sequence unpredictability. Another limitation was in measurements: inter-observer reliability was not measured; however, the evaluators jointly trained the measurers to have the same methods and both evaluators were the same at the evaluation moments (M1 and M2).

Further research is needed, using different types of aquatic exercise programs, to confirm this hypothesis, as the limited number of studies published in the literature regarding aquatic exercise in older people was a limitation when trying to discuss the present results and for clarifying which type of exercise program is more effective in the elderly adult population.

5. Conclusions

Results from the present study provided evidence for the beneficial effects of physical exercise in an aquatic environment on body composition, functional fitness (namely in lower- and upper-limb muscle strength, handgrip strength and aerobic capacity), and cognitive function in non-institutionalized older adults. The present study also revealed that different types of aquatic exercise programs may have different impacts on body composition, functional fitness and cognitive function. ComG was more effective in the improvement of body composition and cognitive function variables, while IntG and AerG were more effective in the improvement of functional fitness.

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Data Availability Statement: All data are shown in the manuscript.

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frontiers

The impact of aquatic exercise programs on the immunologic profile of community dwelling older persons: A Randomized Controlled Trial

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Author contribution statement

C.F., H.S., F.M.S. and M.C.-R. participated in data collection and data organization. C.F. and B.O. analyzed the data and C.F. wrote the manuscript. J.S. and H.S. made relevant contributions in their fields. J.P.F. A.M.T. and F.M.S. reviewed the manuscript. J.P.F., A.M.T. and J.S. coordinated the research. All authors have read and agreed to the published version of the manuscript.

Keywords

physical exercise, aquatic environment, Hydrogymnastics, Ageing, Interleukins, inflammageing

Abstract

Word count: 306

Evidence shows that physical exercise is important in maintaining an efficient immune system during ageing. However, there are few studies that test the impact of aquatic exercise programs on the immune system. This study aims to analyze the impact of different physical exercise programs in aquatic environments on the immune profile of non-institutionalized older persons. One hundred and two older individuals were randomly allocated into four groups: a continuous aerobic exercise group (AerG) (n=25, 71.44±4.84 years); an interval aerobic exercise group (IntG) (n=28, 72.64±5.22 years); a combined exercise group (ComG) (n=29, 71.90±5.67 years); a control group (CG) (n=20, 73.60±5.25 years). The AerG, IntG and ComG participants took part in three different aquatic exercise programs over a 28-weeks period. The CG participants maintained their usual routines during the same time period. Blood samples were collected from all participants in order to access hematologic indicators, by means of cell count, and the immune profile by ELISA. After 28 weeks, significant differences were found for several hematologic variables in the AerG, IntG and ComG with increases in mean corpuscular hemoglobulin (MCH), mean corpuscular hemoglobulin concentration (MCHC), and hemoglobulin (Hb). In terms of immune variables, statistically significant differences were found in the AerG for IL-10, in the IntG for TNF-α and TNF-α /IL-10 ratio, and in the ComG for TNF-α and IL-1ß/IL-1ra ratio. The present study suggests that aquatic exercise programs were able to improve the immune profile of the participants. Those in the exercise intervention groups showed a shift towards a lower pro-inflammatory profile while the non-exercising group showed the opposite behaviour. The IntG and ComG aquatic exercise programs appeared to be more effective than the AerG program in decreasing chronic low-grade inflammation by mediating the production of higher levels of anti-inflammatory cytokines. However, the differences found between the exercising groups were small and may not have clinical significance.

Contribution to the field

Evidence shows that physical exercise is important in maintaining an efficient immune system during ageing. However, there are few studies that test the impact of aquatic exercise programs on the immune system. The purpose of the present study was to evaluate the impact of different aquatic exercise programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) on the hematologic and immune profiles of non-institutionalized older participants. Our research did not find any other intervention study that included these types of programs or variables, making an important contribution to this field of research. Regarding the hematologic variables, the results of the present study allow us to conclude that the participation in physical exercise aquatic environment programs can lead to beneficial changes in the hematologic variables of non-institutionalized older persons. As for the immune variables, the present results showed that, in general, all exercise groups decreased the levels of the pro-inflammatory markers and of the inflammatory index TNF-α/IL-10, while the opposite effect was found in the CG. This means that the participation of non-institutionalized older adults in aquatic physical exercise programs caused a buffering effect, contributing not only to the stability, but also to an improvement in their inflammatory profile.

Ethics statements

Studies involving animal subjects

Generated Statement: No animal studies are presented in this manuscript.

Studies involving human subjects

Generated Statement: The studies involving human participants were reviewed and approved by Ethics Committee for Health of the Faculty of Sport Sciences and Physical Education, University of Coimbra (reference: CE/FCDEF-UC/00462019). The patients/participants provided their written informed consent to participate in this study.

Inclusion of identifiable human data

Generated Statement: No potentially identifiable human images or data is presented in this study.

Data availability statement

Generated Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

The impact of aquatic exercise programs on the immunologic profile of community dwelling older persons: A Randomized Controlled Trial

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Keywords: physical exercise; aquatic environment; hydrogymnastics; ageing; interleukins; inflammageing.

Abstract

Evidence shows that physical exercise is important in maintaining an efficient immune system during ageing. However, there are few studies that test the impact of aquatic exercise programs on the immune system. This study aims to analyze the impact of different physical exercise programs in aquatic environments on the immune profile of non-institutionalized older persons. One hundred and two older individuals were randomly allocated into four groups: a continuous aerobic exercise group (AerG) (n=25, 71.44±4.84 years); an interval aerobic exercise group (IntG) (n=28, 72.64±5.22 years); a combined exercise group (ComG) (n=29, 71.90±5.67 years); a control group (CG) (n=20, 73.60±5.25 years). The AerG, IntG and ComG participants took part in three different aquatic exercise programs over a 28-weeks period. The CG participants maintained their usual routines during the same time period. Blood samples were collected from all participants in order to access hematologic indicators, by means of cell count, and the immune profile by ELISA. After 28 weeks, significant differences were found for several hematologic variables in the AerG, IntG and ComG with increases in mean corpuscular hemoglobulin (MCH), mean corpuscular hemoglobulin concentration (MCHC), and hemoglobulin (Hb). In terms of immune variables, statistically significant differences were found in

the AerG for IL-10, in the IntG for TNF-α and TNF-α /IL-10 ratio, and in the ComG for TNF-α and IL-1ß/IL-1ra ratio. The present study suggests that aquatic exercise programs were able to improve the immune profile of the participants. Those in the exercise intervention groups showed a shift towards a lower pro-inflammatory profile while the non-exercising group showed the opposite behaviour. The IntG and ComG aquatic exercise programs appeared to be more effective than the AerG program in decreasing chronic low-grade inflammation by mediating the production of higher levels of anti-inflammatory cytokines. However, the differences found between the exercising groups were small and may not have clinical significance.

1- Introduction

The immune system is characterized as a complex network of cells and molecules that operate to protect the body from disease, prevent the entry of invading microorganisms and facilitate wound healing (Simpson et al., 2015).

Ageing is associated with immunosenescence, i.e., a progressive decline in the immune function, which causes changes in innate and adaptive immunity. These changes are related to increased morbidity from infectious causes, leading to the appearance of several age-related diseases, including cardiovascular and metabolic diseases (Sellami et al., 2018). Immunosenescence is characterized by the following aspects: decreased response to new invading infectious agents; unsupported memory T cell response; a higher susceptibility to autoimmune diseases; low-grade chronic inflammation (inflammation) (Rodriguez et al., 2020).

The ageing process is characterized by a decrease in naive T cells and the consequent increase in memory T cells (Weltevrede et al., 2016). Its reduction is considered to be a crucial factor in decreasing the ability to recognize the pathogen, which increases the probability of infection (Spielmann et al., 2011). T cells are subject to continuous remodeling, that results from their interaction with different stressors from the internal and external environment. This interaction leads to the decrease of T cells, which is accompanied by a decline in the T cell receptor clonal diversity and consequent increase in the memory cell subpopulation, with an accumulation of terminally differentiated T cells that are dysfunctional or depleted (Rodriguez et al., 2020).

Regular physical exercise is associated with a reduction of different types of chronic diseases associated with ageing, by means of changes in immune function, specifically in the decreasing of immune senescence, the increasing of the innate immune function and the decreasing of chronic inflammation (Abd El-Kader & Al-Shreef, 2018). In contrast, a sedentary lifestyle leads to accumulation of visceral fat, which is infiltrated by macrophages and pro-inflammatory T cells. This causes pro-inflammatory macrophages to predominate and the inflamed adipose tissue to release adipokines and TNF-α, which leads to a state of systemic inflammation (causing insulin resistance, tumor growth, neurodegeneration and atherosclerosis). In comparison, because physical exercise reduces visceral fat, it will stimulate a decrease in the production and release of adipokines, causing an anti-inflammatory environment during each exercise session (Gleeson et al., 2013). In addition to the reduction of visceral fat, physical exercise, more specifically skeletal muscle contractions, directly stimulates the production of IL6, that will produce anti-inflammatory cytokines, such as IL-10, IL-1ra, and cortisol secretion (Pedersen & Fischer, 2007). A study by Chupel et al. (2017) concluded that a 28-weeks exercise intervention with chair muscular strength exercise program supported the increase of anti-inflammatory balance in a sample of elderly women. Similar results were observed by Furtado et al. (2020), after an intervention with two different physical exercise programs (a combined exercise program, with muscle strength exercise using elastic bands and a chair-based combined exercise). In another study by Werner et al. (2019), the results showed an increase in leukocytes, granulocytes and lymphocytes after a 26-weeks intervention with aerobic exercise and interval aerobic exercise. Contrastingly, the muscle strength exercise did not produce the same increase. However, Kapasi et al. (2003) concluded that an 8-month intervention with combined exercise (aerobic exercise and muscle strength exercise) did not induce changes in lymphocyte subpopulations. Likewise, Shimizu´s et al. (2011) study showed that the number of leukocytes, lymphocytes and monocytes did not change after a 12-weeks muscle strength intervention program.

The studies mentioned above have shown that physical exercise can be a powerful tool to improve the immune profile of elderly participants. However, it is still not clear which type of exercise is the most effective. Additionally, few studies have analyzed the impact of physical exercise programs within these indicators, in an aquatic environment (Bansi et al., 2013; Santos, 2020). Considering the limitations previously mentioned, the purpose of this study is to compare the impact of different physical exercise aquatic programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) on the immunologic profiles of non-institutionalized elderly people, by determining which of the three aquatic exercise programs used was the most effective.

2- Materials and methods

2.1- Study design

A randomized controlled trial was conducted in the Beira Interior region, Portugal. A sample of non-institutionalized elderly participants were submitted to 28-weeks aquatic exercise intervention. The entire study protocol was previously published (Ferreira et al., 2020). To analyzed the impact of different aquatic exercise programs on the immunologic profile of elderly participants, three different physical exercise programs were used: continuous aerobic (AerG), interval aerobic exercise (IntG) and combined exercise (ComG). Blood samples were collected at two specific time moments, namely: pre-intervention (baseline, M1) and post-intervention (after 28 weeks, M2). This study was carried out according to the recommendations of the Declaration of Helsinki for Human Studies. The protocol was approved by the Ethics Committee for Health of the Faculty of Sport Sciences and Physical Education, University of Coimbra (reference: CE/FCDEF-UC/00462019). Written informed consent was obtained from all participants prior to any protocol-specific procedures.

2.2- Participants and sample size

The size and statistical power of the sample were calculated using the G*Power software application (Franz et al., 2007). The following parameters were considered: F test (ANOVA); effect size: 0.25; α-level: 0.05; statistical power: 0.95; number of groups: 4; number of measures: 2 (pre and post intervention); margin of possible losses and refusals: 30%. Therefore, the initial size of the total sample was estimated at 76 participants.

Initially, 174 individuals from the community were invited to participate in the study. After the application of the inclusion and exclusion criteria, 152 individuals were randomized into four groups: continuous aerobic exercise group (AerG, n=36); interval aerobic exercise group (IntG, n=41); combined exercise group (ComG, n=48); control group (CG, n=27). According to the experience of the research team and previous studies, the dropout rate from exercise programs among elderly population is high, so we gathered more participants to fulfil the sample size and compensate potential dropouts (Chupel et al., 2017b; Dziubek et al., 2015; Moreira et al., 2020). A simple randomization method was used. An external investigator used a computer-generated list of random numbers to allocate participants to each group. The investigators were blinded for the randomization of the groups.

The following inclusion criteria were applied: a) participants from both sexes; b) 65 years old or more; c) non-institutionalized; d) autonomy to move from their residence to Sertã municipal swimming pool; e) filling out the informed consent form; f) individuals with medical authorization to practice physical exercise in an aquatic environment, in cases they had some type of clinical condition or comorbidity. The following exclusion criteria were also defined: a) individuals with clinically diagnosed pathologies putting their health and others at risk while doing physical exercise in an aquatic environment; b) individuals that obtained a score of less than 9 points in the Mini-Mental States Examination (indicating severe cognitive impairment) or were clinically diagnosed with a mental illness; c) an attendance of less than 50% to physical exercise sessions; d) participants who failed to complete all proposed assessment tests.

The AerG, IntG and ComG groups performed physical exercise in an aquatic environment during the same period of 28 weeks (each group took part in a different exercise program). Participants from the CG group were asked to maintain their normal daily activities, without performing any type of systematic physical exercise during the same time period.

Fifty participants droped out from the study due to the following reasons: personal reasons (n=11); less than 50% of attendance of the exercise sessions (n=14); did not complete all the assessment tests (n=12); injury not related with the exercise intervention (n=4); disease (n=9). Consequently, 102 participants completed the entire process (AerG: n=25, 71.44±4.84 years, 80% female; IntG: n=28, 72.64±5.22 years, 89.3% female; ComG: n=29, 71.90±5.67 years, 75.9% female; CG: n=20, 73.60±5.25 years, 55% of females). Figure 1 shows the entire allocation process for the different groups.

FIGURE 1

2.3- Outcomes measurements

2.3.1- Sample characterization

Data such as age, regular medication, allergies, alcohol consumption, smoking and drugs habits were also obtained in the first phase of data collection (M1) by completing a specific questionnaire. Values referring to anthropometry and functional fitness were also collected. Height (Hgt) was assessed using a portable Seca Bodymeter® stadiometer (model 208, Hamburg, Germany) with an accuracy of 0.1 cm. Weight (Wgt), body mass index (BMI), visceral fat (VF), fat mass (FM) and muscle mass (LBM) were evaluated using the TANITA BC-601 impedance scale (Tokyo, Japan). Functional fitness was assessed using the following tests from the Senior Fitness Test set, developed by Rikli and Jones (1999) and validated for the Portuguese population by Baptista and Sardinha (2005): muscle strength of the lower (MI) and upper (MS) limbs, with the Chair Stand test (30s-CS) and Arm Curl test (30s-AC), respectively (repetitions/30s); aerobic capacity, with the Two-Minute Step test (2m-ST) (no. of steps); the flexibility of MI and MS, with the Chair Sit and Reach test (CSR) and Back Scratch test (BS), respectively (centimeters); agility and dynamic balance, with the Timed Up and Go test (TUG) (seconds). The handgrip strength was also evaluated, with the Hang Grip test (HG), using the Jamar hand dynamometer (Lafayette Instrument Company, USA) (kg). Values/data are presented in table 1.

2.3.2- Biochemical Markers (interleukins and immune profile)

Fasting Blood samples (15ml) were collected from the ante cubital vein by a registered nurse. A complete blood count (CBC) was conducted using an automatic hematology analyzer (Coulter Act, Beckman Coulter, USA) to obtain the values for: leukocyte (LEU); lymphocytes (LI); monocytes (MO); granulocytes (GR); erythrocytes (ERI); hemoglobulin concentration (Hb); hematocrit (Hct); mean corpuscular volume (MCV); mean globular hemoglobulin (MCH); mean corpuscular hemoglobulin concentration (MCHC); erythrocyte distribution width (RDW); platelets (PL); mean platelet volume (MPV); procalcitonin (Pct); adenosine diphosphate (ADP). Next, the test tubes were centrifuged for 10 min at 3500x g rotations per minute and stored in cryogenic test tubes at -80°C until further use. Levels of interleukin 1 (IL-1ra), interleukin 1 beta (IL-1ß), interleukin 10 (IL-10) and tumor necrosis factor (TNF-α), were subsequently analyzed by ELISA (Invitrogen®, Alfagene, Portugal) according to the manufacturer instructions.

2.4- Intervention protocol

The exercise programs were implemented by Sport Sciences and fitness experts, with specific training in water aerobics and developed according to the exercise prescription guidelines recommended by the American College of Sport Medicine (ACSM) for the elderly (ACSM, 2018).

All exercise programs sessions had a duration of 45 min, twice a week, for 28 weeks and were performed in water environment (the water level was between 0.80 and 1.20 m with a temperature of approximately 32°C), using the music rhythm as a tool to control the intensity of the exercise. Sequences of aquatic exercises, previously defined and selected according to the objectives of each program, were applied. Water exercise sessions were organized into three different parts: initial, main and final part.

The initial part or warm-up last between 10 and 15 min, at low intensity (30-40% max HR), and were the same in the three water exercise programs. During this initial part, it is intended that participants will make an adaptation to the aquatic environment, i.e., to the water temperature, and provide muscular and metabolic stimulations to prepare the body for the main part of the session. Thus, simple exercises in water were used, such as displacements and isolated movements, with a progressive increase in complexity and intensity throughout the initial part.

The main part for each one of the three water exercise program sessions had a duration of 20 to 30 min and all were characterized as follows:

- In the water continuous aerobic program (AerG), aerobic exercises were used continuously throughout the main part of the session (20 to 30 min), with a target intensity of 60 to 70% of the maximum heart rate, according to the recommendations of the ACSM for the elderly (ACSM, 2018);

- In the water interval aerobic program (IntG), the main part of the sessions consisted of performing exercises with different intensities, such as: short duration exercises of 30 seconds, with an intensity of 70-80% of the maximal heart rate, followed by active recovery intervals of 1 min, using exercises with an intensity of 60-70% of the maximal heart rate;

- In the water combined training program (ComG), the main part of the session was divided into two phases, with equal time periods. The first phase consisted of aerobic exercises on a continuous basis, with a target intensity between 60 and 70% of the maximal heart rate. In the second phase, muscle strengthening exercises were applied (water environment): 6 to 8 different exercises, with auxiliary equipment to create more resistance to movement (e.g., "spaghetti" and dumbbells), covering muscle strengthening from trunk (e.g., rowing, inverted crunch, etc.), upper limbs (e.g., elbow flexion and extension, shoulder rotation, etc.) and lower limbs (e.g., knee flexion and extension, leg abduction and adduction, etc.). Strengthening exercise were implemented using 2 to 3 sets of 12 to 16 repetitions at moderate intensity (6-7 points in the Borg Scale).

The final part of the water exercise sessions lasted between 5 and 10 min and were the same for each of the three water exercise programs. This part consisted of two phases: return to calm, where relaxation exercises were applied to resume the participants' heart rate to values close to the resting state, and stretching exercises stimulating a greater range of motion, used to stretch the main muscle groups used throughout the sessions.

2.5- Monitoring the exercise programs intensity

For safety and intensity target control reasons, all participants randomly used heart rate monitors (Polar, R800CX) during the exercise sessions, in all three water exercise programs. Depending on the heart rate values obtained, intensity adjustments to the training plan were performed to maintain the intensity target defined for each water exercise program.

For safety and intensity target control reasons, the intensity of the different water exercise programs was predicted indirectly using the Karvonen´s formula (Karvonen et al., 1957):

$$Target\ heart\ rate = \big((maximal\ heart\ rate - resting\ heart\ rate)\ X\ \%\ intensity\big) + resting\ heart\ rate$$

Additionally, and to calculate the maximal heart rate, we used the Franklin et al. (2000) formula for the elderly:

$$Maximal\ HR = 207\ beats\ per\ min. - (0.7\ X\ chronological\ age)$$

2.6- Statistical analysis

The collected data was subjected to descriptive statistical analysis where values such as maximum, minimum, mean and standard deviation were calculated for each variable in each assessment moment. Afterwards, data normality was tested by considering the response to three conditions: z-values from the Skewness and Kurtosis tests; p-values from the Shapiro-Wilk test; and visual inspection of generated histograms. All longitudinal comparisons were performed using complete case analysis. Parametric data will be analyzed using the Student's t-test for independent samples to compare the different moments (M1 and M2) and the one-factor Anova test and post-hoc Tukey's test to analyze the differences between groups. Nonparametric data was analyzed using Wilcoxon test to compare the different moments (M1 and M2) and the Kruskal Wallis and Bonferroni tests to analyze differences between groups. Associations between variables were performed using Pearson's correlation coefficient values and interpreted according to Cohen (1988): r=0.10 to 0.29 means weak correlation; r=0.30 to 0.49 means moderate correlation; and r=0.50 to 1.0 strong correlation. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical software, version 27.0. The level of significance used was $p \leq 0.05$.

3- Results

The results from 102 non-institutionalized elderly participants at baseline, distributed in four different groups (AerG: n=25, 71.44±4.84 years; IntG: n=28, 72.64±5.22 years; ComG: n=29, 71.90± 5.67 years; CG: n=20, 73.60±5.25 years) that completed a 28-weeks intervention are shown in table 1.

Table 1 – Participant´s baseline characteristics by group.

	AerG (n=25) Mean (SD)	IntG (n=28) Mean (SD)	ComG (n=29) Mean (SD)	CG (n=20) Mean (SD)	*p*-value
Morphological parameters					
AG (years)	71.44 (4.8)	72.64 (5.2)	71.90 (5.7)	73.60 (5.3)	0.504
Stature (m)	1.58 (0.7)	1.56 (0.7)	1.57 (0.7)	1.60 (0.9)	*0.331*
Body mass (kg)	70.5 (8.1)	71.3 (14.3)	75.1 (11.0)	75.5 (13.3)	*0.334*
BMI (kg/m²)	28.20 (3.3)	29.10 (4.8)	30.80 (5.3)	29.50 (5.8)	0.272
VF (%)	11.0 (3.0)	12.0 (3.0)	13.0 (3.0)	13.0 (6.0)	*0.128*
FM (%)	38.9 (7.3)	41.0 (6,7)	40.3 (9.8)	34.9 (10.9)	*0.134*
LBM (%)	26.5 (4.3)	24.5 (3.0)	25.5 (4.3)	27.7 (4.7)	*0.079*
Physical fitness tests					
2m-ST (no of steps)	80.9 (17.4)	71.5 (16.5)	81.6 (19.2)	74.3 (18.9)	*0.069*
CSR-R (cm)	-0.5 (6.6)	-3.7 (10.6)	-3.5 (7.8)	-7.6 (9.7)	*0.099*
CSR-L (cm)	0.6 (7.2)	-3.9 (9.9)	-5.8 (9.9)	-3.5 (7.3)	*0.054*
BS-R (cm)	-9.9 (10.4)	-11.9 (11.5)	-14.3 (9.7)	-16.6 (9.9)	*0.157*
BS-L (cm)	-14.4 (7.2)	-17.4 (8.6)	-21.0 (10.8)	-20.6 (10.7)	*0.056*
TUG (s)	6.1 (1.1)	7.4 (1.8)	7.4 (3.0)	6.8 (1.7)	*0.110*
30s-CS (reps/30s)	15.0 (3.0)	13.0 (4.0)	13.0 (3.0)	15.0 (5.0)	*0.185*
30s-AC (reps/30s)	21.0 (6.0)	17.0 (7.0)	20.0 (5.0)	19.0 (6.0)	*0.119*
HG-R (kg)	22.0 (6.0)	21.0 (9.0)	21.0 (9.0)	24.0 (9.0)	*0.411*
HG-L (kg)	21.0 (6.0)	20.0 (9.0)	21.0 (9.0)	21.0 (10.0)	*0.578*
Health parameters	**N (%)**	**N (%)**	**N (%)**	**N (%)**	
Regular medication	23 (92%)	27 (96%)	26 (90%)	19 (95%)	0.768
No alcohol and smoking habits	22 (88%)	25 (89%)	26 (90%)	19 (95%)	0.772
No allergies	15 (60%)	19 (68%)	15 (52%)	13 (65%)	0.638

Note: *Standard deviation (SD); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG); Age (AG); height (Hgt); weight (Wgt); body mass index (BMI); visceral fat (VF); fat mass (FM); muscle mass (LBM); two-minute step test (2m-ST); chair sit and reach test - right (CSR-R); chair sit and reach test - left (CSR-L); back scratch test – right (BS-R); back scratch test – left (BS-L); timed up and go test (TUG); chair stand test (30s-CS); hand grip test – right (HG-R); hand grip test – left (HG-L).*

Participants from all groups (AerG, IntG, ComG and CG) showed similar characteristics at baseline, with no significant statistical differences in anthropometrics, physical fitness or clinical characterization variables regarding each group.

The variation in the results of the hematologic profile when analyzed by type of aquatic exercise program, before and after the 28-weeks intervention, are shown in table 2.

Table 2 – Results of hematological variables at baseline and after 28 weeks of intervention.

	AerG			**IntG**			**ComG**			**CG**				
	M1 Mean (SD)	M2 Mean (SD)	*Time (p)*	M1 Mean (SD)	M2 Mean (SD)	*Time (p)*	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	*Time (p)*	*Time x group (M1)*	*Time x group (M2)*
LEU	5.99 (1.17)	6.04 (1.26)	0.838	5.85 (1.22)	6.34 (1.26)	**0.006***	6.37 (1.37)	6.65 (1.51)	**0.044****	5.70 (1.30)	5.95 (1.44)	0.183	0.269	0.335
LI (%)	35.67 (7.57)	36.28 (8.38)	0.657	34.09 (7.54)	32.76 (9.06)	0.158	35.57 (8.19)	34.00 (8.75)	0.140	37.00 (8.23)	35.17 (7.95)	0.108	0.546	0.444
MO (%)	5.76 (2.16)	5.88 (1.80)	0.844	6.39 (2.38)	6.29 (2.06)	0.809	6.57 (1.95)	6.21 (2.25)	0.542	5.99 (2.01)	5.74 (1.79)	0.665	0.505	0.741
GR (%)	58.58 (8.11)	57.84 (8.44)	0.575	59.51 (8.45)	61.23 (9.50)	0.094	57.86 (9.12)	59.79 (8.81)	0.123	57.01 (8.97)	59.23 (9.42)	0.098	0.604	0.396
LI# (x10^3/ul)	2.13 (0.63)	2.17 (0.55)	0.707	2.00 (0.59)	2.07 (0.66)	0.213	2.26 (0.65)	2.22 (0.66)	0.554	2.06 (0.45)	2.04 (0.49)	0.741	0.529	0.676
MO# (x10^3/ul)	0.34 (0.14)	0.36 (0.14)	0.503	0.37 (0.16)	0.39 (0.17)	0.388	0.43 (0.16)	0.41 (0.16)	0.503	0.33 (0.13)	0.27 (0.12)	0.078	0.152	**0.013††**
GR# (x10^3/ul)	3.51 (0.86)	3.52 (1.03)	0.920	3.47 (0.87)	3.89 (1.04)	**0.006***	3.71 (1.06)	4.02 (1.27)	**0.046****	3.32 (1.04)	3.50 (1.16)	0.236	0.822	0.259
ERI	4.63 (0.30)	4.53 (0.33)	**0.032****	4.59 (0.40)	4.46 (0.32)	**0.001****	4.64 (0.27)	4.53 (0.26)	**0.003***	4.66 (0.42)	4.62 (0.55)	0.706	0.852	0.611
Hb	13.15 (0.80)	13.23 (0.71)	0.591	13.12 (1.07)	13.27 (0.90)	0.153	13.09 (0.77)	13.38 (1.08)	**0.038****	13.22 (0.62)	13.45 (1.04)	0.285	0.793	0.852
Hct	41.66 (2.19)	40.42 (2.21)	**0.006***	41.65 (3.03)	40.00 (2.54)	**0.000***	42.02 (2.50)	40.75 (2.56)	**0.001****	42.20 (2.08)	41.91 (2.72)	0.555	0.897	0.073
MCV	90.22 (4.24)	89.34 (4.07)	**0.041***	90.82 (3.81)	89.97 (3.86)	**0.003***	90.75 (3.79)	89.92 (3.84)	**0.006***	91.00 (5.51)	90.53 (5.03)	0.068	0.932	0.822
MCH	28.46 (1.44)	29.24 (1.60)	**0.001***	28.60 (1.33)	29.86 (1.47)	**0.000***	28.29 (1.43)	29.53 (1.72)	**0.000***	28.54 (1.77)	29.19 (2.10)	0.092	0.868	0.477
MCHC	31.54 (0.84)	32.73 (0.77)	**0.000****	31.50 (0.71)	33.20 (0.73)	**0.000****	31.15 (0.64)	32.83 (1.13)	**0.000****	31.37 (0.51)	32.09 (1.45)	0.079	0.155	**0.044††**
RDW	12.86 (0.53)	12.73 (0.49)	0.109	12.88 (0.56)	12.71 (0.72)	0.158	13.23 (0.81)	12.89 (0.78)	**0.005***	12.85 (0.50)	12.69 (0.58)	0.202	0.078	0.681

PL	197.44 (53.91)	198.64 (63.90)	0.467	191.75 (47.50)	187.64 (46.18)	0.190	212.00 (39.28)	206.34 (42.46)	0.347	182.10 (38.28)	173.55 (38.57)	0.079	0.134	0.056
MPV	8.72 (1.03)	8.46 (0.83)	**0.017***	8.70 (0.91)	8.48 (0.84)	**0.002***	9.02 (0.91)	8.76 (0.75)	**0.000****	8.42 (0.81)	8.28 (0.75)	0.058	0.149	0.154
Pct	0.17 (0.04)	0.16 (0.04)	0.135	0.16 (0.03)	0.19 (0.15)	0.163	0.19 (0.04)	0.18 (0.04)	**0.030****	0.15 (0.03)	0.15 (0.03)	0.130	**0.008****	**0.004††**
ADP	16.12 (0.45)	15.89 (0.42)	**0.008***	16.09 (0.54)	15.97 (0.39)	0.237	16.09 (0.49)	15.98 (0.50)	0.296	16.17 (0.49)	16.26 (0.43)	0.403	0.928	**0.042†**

Note: *Standard deviation (SD); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG); Leukocytes (LEU); lymphocytes - percentage (LI); monocytes – percentage (MO); granulocytes – percentage (GR); lymphocytes – gross value (LI#); monocytes – gross value (MO#); granulocytes – gross value (GR#); erythrocytes (ERI); hemoglobulin concentration (Hb); hematocrit (Hct); mean corpuscular volume (MCV); mean globular hemoglobulin (MCH); mean corpuscular hemoglobulin concentration (MCHC); erythrocytes distribution width (RDW); platelets (PL); mean platelet volume (MPV); procalcitonin (Pct); adenosine diphosphate (ADP). *Result obtained through T-Student test; **result obtained through Wilcoxon test; †results obtained through ANOVA e Tukey test; ††results obtained through Kruskal Wallis e Bonferroni test.*

The general hematologic results revealed that significant differences were found between groups in Pct (p=0.008) between the ComG participants and the CG participants (p=0.001), as well as between the IntG and the ComG (p=0.016) before the intervention. In both cases, the results were higher in the ComG. However, we found significant differences for MO# (p=0.013) between the CG and the IntG participants (p=0.007), between the CG and the ComG (p=0.002), and between the CG and the AerG (0.033), after the intervention. We also found significant differences for MCHC (p=0.044) between the CG and the IntG participants (p=0.007), in Pct (p=0.004) between the CG and the IntG (p=0.039), and between the CG and the ComG (p<0.001), in ADP (p=0.042) between the AerG and the CG (p=0.008), between the ComG and the CG (p=0.019), and between the IntG and the CG (p=0.038). In all identified cases, the results were lower in the CG, except for the ADP variable.

As a result of the intervention with aquatic exercise programs, statistically significant increase were found in the following variables: LEU in the IntG (p=0.006; Δ=8.4%) and ComG (p=0.044; Δ=4.4%); GR# in the IntG (p=0.006; Δ=12.1%) and ComG (p=0.046; Δ=8.4%); MCH in the AerG (p=0.001; Δ=2.7%), IntG (p<0.001; Δ=4.4%) and ComG (p<0.001; Δ=4.4%); MCHC in the AerG (p<0.001; Δ=3.8%), IntG (p<0.001; Δ=5.4%) and ComG (p<0.001; Δ=5.4%); and Hb in the ComG (p=0.038; Δ=2.2%).

Statistically significant reductions were also found in the following variables, as a result of the intervention with aquatic exercise programs: ERI in the AerG (p=0.032; Δ=-2.2%), IntG (p=0.001; Δ=-2.8%) and ComG (p=0.003; Δ=-2.4%); Hct in the AerG (p=0.006; Δ=-3.0%), IntG (p<0.001; Δ=-4.0%) and ComG (p =0.001; Δ=-3.0%); MCV in the AerG (p=0.041; Δ=-1.0%), IntG (p=0.003; Δ=-0.9%) and ComG (p=0.006; Δ=-0.9%); MPV in the AerG (p=0.017; Δ=-3.0%), IntG (p=0.002; Δ=-2.5%) and ComG (p<0.001; Δ=-2.9%); RDW in the ComG (p=0.005; Δ=-2.6%); Pct in the ComG (p=0.030; Δ= -5.8%); and ADP in the AerG (p=0.008; Δ=-1.4%).

The variation in the results of the immune variables analyzed by each type of aquatic exercise program, before and after the intervention, are shown in table 3.

Table 3 – Results of immune variables at baseline and after 28 weeks of intervention.

	AerG			IntG			ComG			CG				
	M1 Mean (SD)	M2 Mean (SD)	*Time (p)*	M1 Mean (SD)	M2 Mean (SD)	*Time (p)*	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	*Time (p)*	*Time x group (M1)*	*Time x group (M2)*
IL-10 (pg/ml)	18.45 (14.08)	17.67 (15.30)	0.514	28.55 (10.13)	36.70 (6.85)	***0.000*****	28.15 (14.12)	24.69 (10.04)	*0.127*	20.32 (12.87)	16.87 (9.10)	0.054	***0.005††***	***0.000††***
IL-1ra (pg/ml)	76.93 (51.79)	81.28 (58.52)	0.757	89.30 (56.68)	92.98 (69.54)	*0.585*	104.69 (63.82)	115.71 (67.19)	*0.157*	121.77 (83.56)	88.31 (59.96)	***0.008*****	0.179	0.220
IL-1ß (pg/ml)	30.05 (3.75)	29.50 (3.31)	0.443	35.04 (16.67)	33.25 (17.15)	*0.117*	27.98 (10.16)	27.00 (14.27)	0.064	25.39 (4.04)	26.49 (4.24)	***0.043****	***0.010††***	***0.007††***
TNF-α (pg/ml)	37.38 (21.07)	28.65 (21.72)	***0.011*****	17.65 (11.13)	14.27 (9.63)	***0.001*****	69.01 (28.82)	52.89 (25.25)	***0.005****	31.98 (16.75)	32.59 (18.96)	0.936	***0.000††***	***0.000††***
TNF-α/IL-10 ratio	5.00 (6.25)	3.85 (5.66)	0.056	0.89 (1.07)	0.45 (0.52)	***0.000*****	2.55 (0.75)	2.23 (0.93)	0.125	3.18 (4.16)	3.18 (3.13)	0.351	***0.000††***	***0.000††***
IL-1β /IL-1ra ratio	0.63 (0.45)	0.75 (0.79)	0.657	0.67 (0.60)	1.01 (1.98)	0.982	0.42 (0.40)	0.36 (0.32)	***0.047*****	0.35 (0.33)	0.50 (0.38)	0.053	***0.025††***	0.097

*Note: Standard deviation (SD); continuous aerobic group (AerG); aerobic interval group (IntG); combined group (ComG); control group (CG); Interleukin 10 (IL-10); interleukin 1 (IL-1ra); interleukin 1 beta (IL-1β); tumor necrosis factor (TNF-α); racio entre tumor necrosis factor e Interleukin 10 (TNF-α/IL-10); rácio entre. interleukin 1 beta e interleukin 1 (IL-1β/IL-1ra) . *Result obtained through T-Student test; **result obtained through Wilcoxon test; †results obtained through ANOVA e Tukey test; ††results obtained through Kruskal Wallis e Bonferroni test.*

Before the intervention (baseline) statistically significant differences were found between groups in the following variables immunes: IL-10 (between the AerG and ComG (p=0.011), the AerG and IntG (p=0.002), and the IntG and CG (p=0.022)); IL-1ß (between the CG and AerG (p=0.021), the CG and IntG (p=0.007), the ComG and AerG (p =0.045) and the ComG and IntG (p=0.015)); TNF-α (between the IntG and CG (p=0.004), the IntG and AerG (p<0.001), the IntG and ComG (p<0.001), the CombG and CG (p<0.001), and the ComG and AerG (p=0.001)); TNF-α /IL-10 (between the IntG and CG (p=0.001), the IntG and AerG (p<0.001), and IntG and the ComG (p<0.001); IL-1ß /IL-1ra (between the CG and IntG (p=0.029), the CG and AerG (p =0.011), and the ComG and AerG (p=0.035)).

In the identified cases, the results were higher in the IntG and the ComG for IL-10, lower in the ComG and the CG for IL-1ß, lower in the IntG for TNF-α and the TNF-α /IL-10, and lower in the CG and the ComG for IL-1ß /IL-1ra.

After the intervention statistically significant differences were also found between groups in the following variables: IL-10 (between the AerG and ComG (p=0.017), the AerG and IntG (p<0.001), the CG and ComG (p=0.042), the CG and IntG (p<0.001) and the ComG and IntG (p<0.001)); IL-1ß (between the ComG and IntG (p=0.001), and the ComG and AerG (p=0.002)); TNF-α (between the IntG and AerG (p=0.001), the IntG and CG (p<0.001), the ComG and IntG (p<0.001), the ComG and AerG (p=0.001), and the ComG and CG (p=0.036)); TNF-α /IL-10 (between the IntG and AerG (p<0.001), the IntG and ComG (p<0.001), and the IntG and CG (p<0.001). In the identified cases, the results were higher in the IntG for IL-10 and IL-1ß/IL-1ra, lower in the ComG for IL-1ß, and lower in the IntG for TNF-α.

Due to the intervention with aquatic exercise programs, the IL-10 variable showed a reduction in all groups, except for the IntG, which showed a significant statistical increase ($p<0.01$; $\Delta=28.5\%$). Further increases were found for the IL-1ra variable, in all exercise groups, but not in the CG, which showed a significant reduction ($p=0.008$; $\Delta=-27.5\%$). Furthermore, and for the IL-1ß variable, a significant reduction was found in all aquatic exercise groups, but not in the CG, where there was a significant increase ($p=0.043$; $\Delta=4.3\%$). A similar reduction was found in TNF-α, with significant differences in all exercise groups, due to the AerG intervention ($p=0.011$; $\Delta=-23.4\%$), the IntG ($p=0.001$; $\Delta=-19.1\%$) and the ComG ($p=0.005$; $\Delta=-23.4\%$), but not in the CG. The results also showed a significant reduction in TNF-α/IL-10 in IntG ($p<0.001$; $\Delta=-49.3\%$), as well as for IL-1ß/IL-1ra in the ComG ($p=0.047$; $\Delta=-$ 13 .5%).

The relationships between the hematologic, immune and anthropometric variables, at both evaluation phases (M1 and M2) are presented in table 4.

Table 4 – Relationships between the hematologic, immune and anthropometric variables at baseline and after 28 weeks of intervention.

		VF		Wcir		IL-10		IL-1ß	
		M1	M2	M1	M2	M1	M2	M1	M2
LEU	Pearson correlation	0.199	0.217	0.293	0.273	0.242	0.226	0.136	0.098
	Sig.	***0.045****	***0.028****	***0.003****	***0.006****	***0.014****	***0.022****	0.172	0.325
ADP	Pearson correlation	0.235	0.214	0.138	0.200	0.18	0.011	0.142	0.117
	Sig.	***0.018****	***0.031****	0.167	0.044	0.07	0.909	0.155	0.243
GR	Pearson correlation	0.167	0.204	0.230	0.257	0.221	0.226	0.128	-0.011
	Sig.	0.093	0.04	***0.02****	***0.009****	***0.025****	***0.022****	0.199	0.912
PL	Pearson correlation	-0.085	-0.077	-0.11	-0.124	-0.098	0.057	-0.228	-0.344
	Sig.	0.394	0.441	0.269	0.213	0.329	0.571	***0.021****	***0.000****

Note: *Leukocytes (LEU); adenosine diphosphate (ADP); granulocytes (GR); platelets (PL); visceral fat (VF); waist circumference (Wcir); interleukin 10 (IL-10); interleukin 1 beta (IL-1 ß). *Result obtained through Person's correlation test.*

Pearson´s r results showed weak and positive correlations, in M1 and M2, between VF and LEU (r=0.22), VF and ADP (r=0.21), WCir and LEU (r=0.27), WCir and GR (r=0.26), IL-10 and LEU (r=0.23), and IL-10 and GR (r=0.23). However, results also showed a moderate and negative correlation between IL-1ß and PL (r=-0.34).

4- Discussion

The purpose of the present study was to evaluate the impact of different aquatic exercise programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) on the hematologic and immune profiles of non-institutionalized older participants. A preliminary systematic research revealed the innovative characteristics of the present study, since there were very few studies that had evaluated similar immune variables, in exercise performed in aquatic environment, with older adults.

Results from the hematologic variables revealed significant statistical differences in most of the variables analyzed, due to the intervention with aquatic physical exercise (AerG, IntG and ComG), but no significant differences were found in the CG. A more detailed analysis of the results revealed significant differences over time (from M1 to M2), i.e., between pre- and post-intervention, in the AerG for ERI, Hct, MCV, MCH, MCHC, MPV and ADP, in IntG for LEU, GR, ERI, Hct, MCV, MCH, MCHC and MPV, and in ComG for LEU, GR, ERI, Hb, Hct, MCV, MCH, MCHC, RDW, MPV and Pct. The variance of the mean was higher for the variable MCHC in the AerG (Δ=3.8%), for the variable GR in the IntG (Δ=12.1%), and in the ComG (Δ=8.4%).

A study carried out by Chupel et al. (2017), that tested the effectiveness of a 28 weeks land-based muscle strength exercise program using elastic bands, in elderly women, for, showed that exercise intervention caused changes in hematologic variables, specifically significant increases in Hb, MCV, MCH and MCHC, and significant reductions in the LEU and LI variables. In the Hct and ERI variables, no significant statistical differences were found. Similar results had already been reported in Johannsen´s et al. (2012) study using a 6-months intervention with aerobic exercise, that promoted significant reductions in the LEU and LI variables. Another recent study by Santos (2020) tested the effectiveness of an aquatic exercise program in elderly women for one year, and found a reduction in MCH, MCHC and PL, and an increase in HCT. However, those differences were not statistically significant. The Hb values remained unchanged.

The present study showed similar results to those reported by Chupel et al. (2017) in the three exercise groups (AerG, IntG and ComG), with significant increases in MCH and MCHC. The IntG and ComG groups showed a higher variance of the mean in both variables. For Hb, results showed an increase in the three exercise groups, but statistically significant only in the ComG group.

In opposition to the results found in Chupel et al. (2017) study, present results revealed a significant reduction of MCV in the three exercise groups, with the highest variation of the mean being registered in the AerG, and an increase in the number of leucocytes in the IntG and ComG groups. The IntG registered the highest variation of the mean. Regarding the Hct and ERI variables, our results showed a statistically significant reduction in all aquatic exercise groups, with the IntG registering a higher variation of the mean in both cases, in opposition to the results obtained by Chupel et al. (2017). However, in Chupel's study the participants were much older and frail.

Present results clearly reveal a positive variation regarding hematologic health, with high levels of MCV and ERI variables being associated with a higher risk of death from all causes, especially cardiovascular and infectious diseases (Dratch et al., 2019). The increase in LEU numbers has positive implications at a hematologic level, since leucocytes have an important role in tissue recovery and host defense as well as antimicrobial properties (D'asta et al., 2018). In regard to the Hct variable, the variation found in our study can be considered negative, as low levels of Hct are associated with the presence of anaemia, that can lead to the development of several chronic diseases (Suresh et al., 2021). However, despite the reduction of Hct values, they are within the reference values (36%-48% for women and 40%-54% for men). The present study when compared with the study by Santos (2020), found a significant statistical increase in MCH and MCHC, in all exercise groups, and in Hb variable in the ComG. Additionally, the present study also found a non-significant increase in PL variable in the AerG. Thus, present results showed a more positive effect on hematologic health.

Although some of the present results are somehow different from those found in the literature, the values remained within the normal limits, showing that aquatic exercise contributed to the maintenance of normal hematologic variables. The differences mentioned above may be directly related to the typology of physical exercise programs applied in different studies. In our study, the impact of different aquatic exercise programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) was tested. Our research did not find any other intervention study that included these types of programs or variables, making an important contribution to this field of research.

Although leucocyte numbers have been shown to increase with ageing (Hopkin et al., 2021). The total amount of LEU in the blood increases with acute physical exercise, i.e., exercise of short duration and high intensity (Gleeson et al., 2013). It is possible that our results may represent a cumulative effect of the acute bouts of exercise (both in interval aerobic exercise and combined exercise) performed regularly for 28 weeks.

Regarding the immune markers, significant differences were found for most of the analyzed variables, resulting from the aquatic physical exercise interventions. A more detailed analysis of the results revealed significant differences between the pre- and post-intervention phases: for the AerG, in TNF-α; for the IntG, in IL-10, TNF-α and TNF-α /IL-10; for the ComG, in TNF-α and IL-1β/IL-1ra. The variance of the mean of the TNF-α variable was higher in the AerG and the ComG (Δ= -23.4% in both cases).

The results from Chupel et al. (2017) study showed significant increases in IL-10 after a land-based muscle strength program. In another study published by the same authors (Chupel et al., 2018), using a 14-weeks combined exercise program (aerobic exercise and muscle strength exercise) resulted in an increase in the anti-inflammatory markers IL-10 and IL-1ra. As for pro-inflammatory markers, results from the same study revealed a significant reduction in TNF-α and IL-1ß. Another study by Furtado et al. (2020), showed a decrease in IL-1ß after 28-weeks of intervention with a combined exercise program. In aquatic environment, a study carried out by Korb et al. (2018), that tested the effectiveness of a 12-weeks aerobic walking program, showed a reduction in IL-10, while the IL-1ß results were the same before and after the intervention. In a study by Ortega et al. (2012), that tested the effectiveness of a 8-months aquatic aerobic program, the results showed a decrease for the anti-inflammatory marker IL-10, for the pro-inflammatory markers IL-1ß and for TNF-α. The results of another study by Da Silva et al. (2018) also showed a reduction in TNF-α after a 12-weeks intervention with low-intensity aquatic exercise program.

Overall, our results show a decrease in the pro-inflammatory markers IL-1β and TNF-α in all exercising groups. The plasma levels of the anti-inflammatory IL-10 increased in the IntG, while in the other groups the values showed a trend towards a decrease. Athletes do show increased levels of plasma Il-10 in response to increased training volume and intensity (Minuzzi et al., 2017). The increased bouts of intensity characteristic of the interval aerobic program may explain the differences between the exercising groups for this variable. It is possible that the small decrease of IL-10 found for the AerG and ComG groups could also reflect a response to the decrease in TNF-α seen in these groups. As for the IL-1ra levels, our results showed an increasing trend in all exercise groups, whereas in the CG a significant statistical reduction was observed. Similar results had previously been found in the studies by Chupel et al. (2017) and Chupel et al. (2018), after the intervention of a land-based muscular strength and combined exercise program. Conversely, in the studies by Korb et al. (2018) and Ortega et al. (2012), the IL-10 levels were reduced with the intervention in an aquatic environment (walking exercise and aerobic exercise, respectively). For the IL-1ß variable, present results showed a trend towards a reduction in all exercise groups. However, there was a significant statistical increase in the CG. On the other end, and for the TNF-α variable there was a significant statistical reduction in all exercise groups (AerG and ComG showed a higher means variation), while in the CG the TNF-α values increased. Similar results were previously found Chupel et al. (2018) and Furtado et al. (2020) after in land exercise interventions and by Ortega et al. (2012) and Silva et al. (2018) after exercise interventions in aquatic environments.

Regarding the ratios, our results showed a reduction in the TNF-α /IL-10 ratio in all aquatic exercise groups, with statistically significant differences in IntG. In the IL-1β/IL-1ra ratio a statistically significant reduction in ComG was also found. Our results confirm the results already found in the study by Chupel et al. (2018), where reductions in the TNF-α /IL-10 and IL-1β/IL-1ra ratios were also verified, after intervention with physical exercise on land. No studies were found to assess these ratios in an aquatic environment. These results suggest that physical exercise in an aquatic environment can provoke a positive response in the inflammatory profile, reflecting a less pro-inflammatory environment, contributing to the reduction in the development of cardiovascular diseases. In the study by Kumari et al. (2018), the TNF-α /IL-10 ratio was associated with the risk of coronary artery disease, suggesting that this marker may play a vital role in the development of this type of pathologies.

This means that physical exercise caused a buffering effect, both in anti-inflammatory and in pro-inflammatory markers, since the results in the exercise groups followed a positive trend towards a less inflammatory environment, while in the opposite occurred for CG where the low grade chonic inflammation increased. These results provide evidence that aquatic physical exercise programs not only have a positive effect in terms of stability, but also improve the immunologic profile.

The results of the present study suggest that the three aquatic exercise programs can effectively improve immune variables in non-institutionalized elderly participants. Some of the changes observed in our study are similar to those found in studies carried out on land environments. Thus, exercise in aquatic environments can be seen as a viable alternative to land-based exercise, especially when other health conditions are installed (e.g. orthopedic, rheumatological or functional limitations, circulatory disease, vertigo, etc.). As for the types of exercise programme, the AerG had a higher variation of the mean for TNF-α, the IntG had higher variation of the mean for IL-10 and IL-1ß, and the ComG had a higher variation of the mean for IL-1ra and TNF-α (the same value as AerG). However, further studies are needed to clarify the effects of exercise in aquatic environments on anti-inflammatory markers, as well as to clarify which type of exercise is the most beneficial for the improvement of the immune profile of the elderly.

5- Conclusions

The results of the present study allow us to conclude that the participation in physical exercise aquatic environment programs can lead to beneficial changes in the hematologic variables of non-institutionalized older persons.

Regarding the immune variables, the present results showed that, in general, all exercise groups decreased the levels of the pro-inflammatory markers and of the inflammatory index TNF-α/IL-10, while the opposite effect was found in the CG. This means that the participation of non-institutionalized older adults in aquatic physical exercise programs caused a buffering effect, contributing not only to the stability, but also to an improvement in their inflammatory profile.

As for the type of aquatic program that best benefited the immune profile, the results were not totally conclusive, since different exercise programs led to improvement in different variables. However, the combined aquatic exercise program showed significant statistical improvements in a higher number of hematologic variables, while the interval aerobic aquatic exercise program showed significant statistical improvements in a greater number of immune variables.

Further studies assessing hematologic and immune variables among elderly participants in physical exercise interventions in aquatic environments are needed, aiming to better clarify the positive and most effective impact of different aquatic exercise programs in older populations.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

C.F., H.S., F.M.S. and M.C.-R. participated in data collection and data organization. C.F. and B.O. analyzed the data and C.F. wrote the manuscript. J.S. and H.S. made relevant contributions in their fields. J.P.F. A.M.T. and F.M.S. reviewed the manuscript. J.P.F., A.M.T. and J.S. coordinated the research. All authors have read and agreed to the published version of the manuscript.

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Figure 1.JPEG

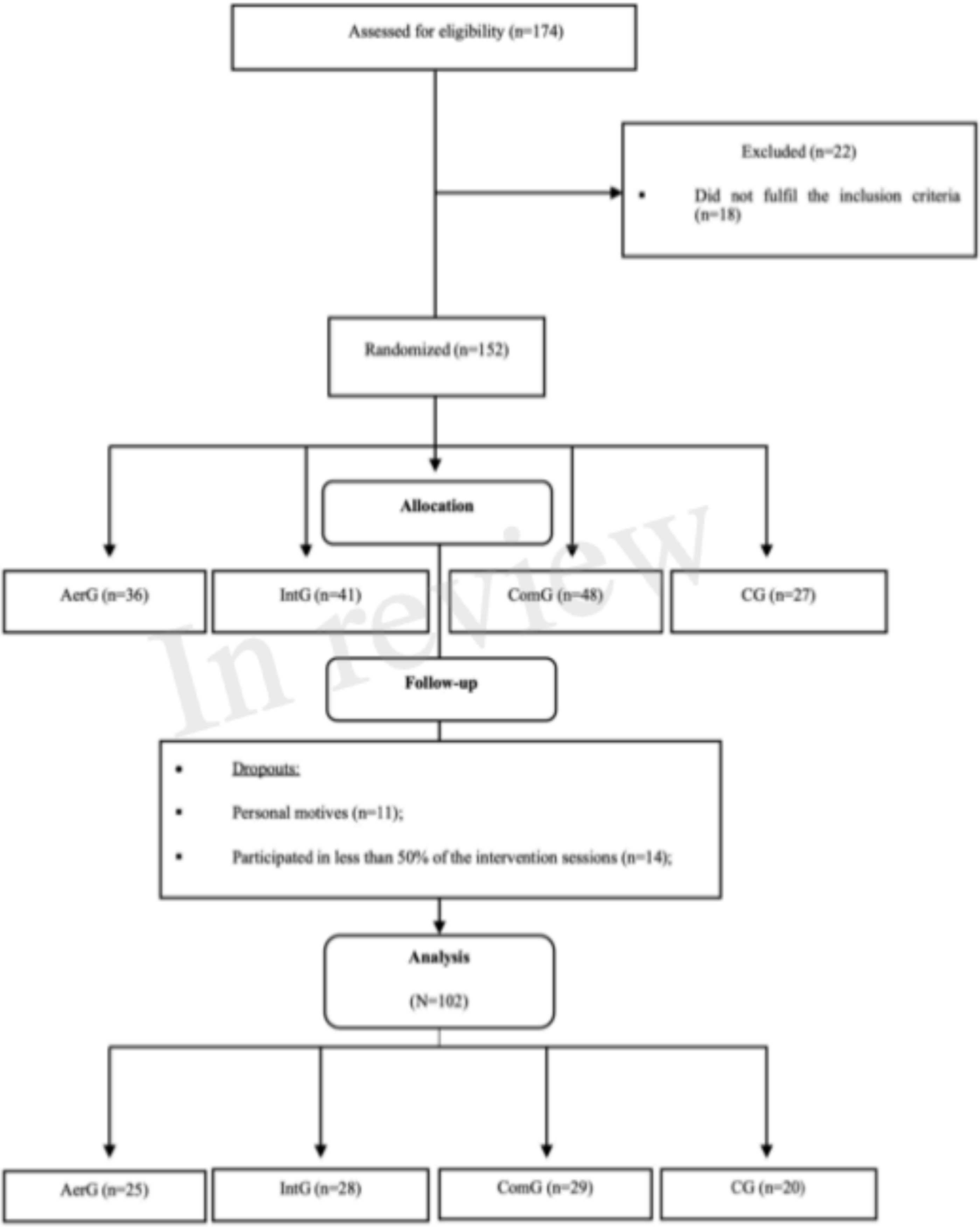

Figure 1. Allocation process for the different groups: Continuous aerobic group (AerG); Aerobic interval group (IntG); Combined group (ComG); Control group (CG).

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Article

The Impact of Aquatic Exercise Programs on the Intima-Media thickness of the Carotid Arteries, Hemodynamic Parameters, Lipid Profile and Chemokines of Community-Dwelling Older Persons: A Randomized Controlled Trial

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Abstract: Scientific evidence has shown that physical exercise is an effective way of improving several cardiovascular disease markers. However, few studies have tested its effectiveness when performed in aquatic environments. The purpose of this study was to test the impact of different aquatic exercise programs on the intima-media thickness of carotid arteries (IMT) and hemodynamic and biochemical markers of cardiovascular diseases in community-dwelling older persons. A total of 102 participants were randomly allocated into four groups: an aerobic exercise group (AerG) ($n = 25$, 71.44 ± 4.84 years); an aerobic interval group (IntG) ($n = 28$, 72.64 ± 5.22 years); a combined group (ComG) ($n = 29$, 71.90 ± 5.67 years); and a control group (CG) ($n = 20$, 73.60 ± 5.25 years). The AerG, IntG, and ComG participants took part in three different aquatic exercise programs for 28 weeks. The CG participants maintained their usual routines. All participants were evaluated for IMT, blood pressure, lipid profile, and MCP-1 and MIP-1α chemokines, pre- and post-intervention. Significant differences were found in the AerG for diastolic diameter (DD), in the IntG for peak systolic velocity (PSV), and in the ComG for DD and end-diastolic velocity (EDV). Regarding blood pressure, significant differences were found in AerG for systolic blood pressure (SBP) and diastolic blood pressure (DBP); in IntG for DBP; and in ComG for SBP, DBP, and heart rate (HR). Significant differences were found in the AerG and IntG for glucose (GLU). Lower plasma levels of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein (MIP-1α) were found in the AerG and in the ComG for MCP-1 after the intervention. Aquatic physical exercise appears to improve cardiovascular health, regardless of the type of the program adopted. Aerobic programs (combined and continuous aerobic exercises) seemed to have a more beneficial effect in reducing important cardiovascular risk markers.

Keywords: physical exercise; aquatic environment; hydrogymnastics; ageing; intima-media thickness of the carotid arteries; hemodynamic parameters; blood pressure; lipid profile; MDP-1α; MCP-1

1. Introduction

Cardiovascular diseases are a group of heart and blood vessel diseases that are responsible for about 31% of the world's deaths, 85% of which are caused by heart attacks and strokes [1]. According to the same source, it is estimated that 80% of these deaths could be avoided by controlling the main risk factors: smoking, unbalanced diets and physical inactivity. Evidence shows that about 150 min of moderate-intensity physical activity per week can contribute to a reduction in the risk of ischemic heart disease, risk of stroke, and hypertension by approximately 30% [1]. The ageing process is associated with cardiovascular changes that affect arterial function, leading to an increased risk of developing cardiovascular disease [2].

Cardiovascular diseases can be predicted through hemodynamic cardiovascular parameters and cardiovascular risk biomarkers. In relation to hemodynamic parameters, the intima-media thickness of the carotid arteries (IMT) is considered an early marker of coronary artery disease and also a risk factor for other types of cardiovascular disease and stroke [3]. The IMT is defined as the length between the inner layer of the carotid artery tunica intima and the inner layer of the common carotid artery tunica media. Increased IMT was positively and strongly associated with age [4], with values between 0.40 mm and 0.89 mm for IMT considered normal, thickening when values vary between 0.90 mm and 1.40 mm, and when wider than 1.40 mm, there is possibility that the carotid arteries contain plaques [5,6]. Other important markers are systolic and diastolic diameter and peak systolic and diastolic velocity. Arteries lose elasticity with ageing, thus increasing systolic blood pressure (which will consequently cause an increase in the systolic diameter) and blood flow velocity, usually leading to turbulent blood flow and loosening atheromatous plaques, which can consequently lead to obstruction of the vessels [7]. According to the same author, the systolic diameter (SD) corresponds to the highest point of each pulse, when the vessels are subjected to the highest pressure, and the diastolic diameter (DD) corresponds to the lowest arterial pressure point to which the vessels are subjected.

Concerning cardiovascular parameters, arterial hypertension is considered an early marker of the development of atherosclerotic disease, being considered a high-prevalence cardiovascular risk factor associated with endothelial dysfunction [8]. Atherosclerosis starts due to damage to the endothelial lining of large vessels triggered by pathogenic factors such as hyperlipemia and hemodynamic shear stress. It is a pathology that develops and progresses over a long period of time and consequently can lead to serious damage of the vascular tissue [9]. The development of atherosclerosis can consequently lead to ischemic heart disease, stenosis of the carotid arteries, and chronic lower limb ischemia, among others [10].

Regarding biochemical markers, ageing also leads to a deterioration of the lipid and glucose profile, increasing the levels of total cholesterol (TC), low-density lipoprotein (LDL), triglycerides (TG), and fasting blood glucose (GLU). When those elements are uncontrolled, they are directly related to cardiovascular complications [11]. Chemokines also play a fundamental role in the development of cardiovascular diseases and play a key role in the pathogenesis of inflammatory and autoimmune diseases, as well as in tumour progression [9]. The Monocyte Chemoattractant Protein-1 (MCP-1) and Macrophage inflammatory Protein (MIP-1α) play a significant role in the development of coronary artery disease [12]. Several cardiovascular diseases are associated with high levels of pro-inflammatory cytokines that promote the formation of atherosclerotic plaque through an accumulation of macrophages, lipid-laden cells, T cells, and other kinds of degenerative matter in the inner layer of blood vessel walls. Subsequently, several inflammatory molecules are released by the action of macrophages and lead to tissue damage and consequently to inflammation [12]. MCP-1 is produced by different types of cells (monocytes, macrophages, smooth muscle cells, endothelial cells, etc.); MIP-1α is induced by several pro-inflammatory agents and is negatively affected by anti-inflammatory agents [13]. Both are associated with the risk of developing inflammation, cardiovascular disease (specifically atherosclerosis), increased risk of myocardial infarction, and death [14].

According to the literature, physical activity is a very important tool for cardiovascular health. At the hemodynamic and cardiovascular level, physical exercise, mainly exercise with aerobic characteristics, can contribute to the reduction of the luminal diameter of the arteries, since it has a localized effect and/or reflects the increase in blood flow [15]. In addition, aerobic exercise is also considered a first-line strategy to prevent and treat hypertension [8]. Hypertensive individuals are encouraged to participate in aerobic exercise programs aimed at reducing systolic and diastolic blood pressure [16]. This exercise-induced reduction is also accompanied by improvements in arterial and peripheral hemodynamic factors [16]. Combined physical exercise programs (aerobic and muscle strength exercise) have also been shown to be effective in improving hemodynamic and cardiovascular parameters in the older population. A study by Son et al. [17] found that a 12-week combined exercise program provided significant changes in arterial stiffness, as well as in systolic and diastolic blood pressure, in elderly women (75 ± 2 years). However, the meta-analysis carried out by Montero et al. [18] concluded that the combined physical exercise benefits do not appear to differ significantly when compared to aerobic exercise alone.

Regular physical exercise can cause a reduction around −3.5 mmHg in systolic blood pressure and −3 mmHg in diastolic blood pressure [19]. Physical exercise in an aquatic environment, due to immersion, promotes physiological adjustments that can beneficially affect blood pressure, namely by reducing sympathetic activity and redistributing blood volume from the lower limbs and the abdominal area to the upper part of the body, thus causing a reduction in peripheral vascular resistance [19].

Regarding the biochemical markers related to cardiovascular diseases, studies have shown that physical exercise is also an effective tool to improve these factors. In Martins et al. [20], two exercise programs (aerobic and muscle strength exercise) were tested in a group of 63 sedentary elderly participants (76.0 ± 8.0 years), and both interventions produced beneficial effects on their lipidic profile (TG, TC, LDL, and HDL). According to Gleeson [21], physical exercise can be a way of inhibiting the release of chemokines in human adipose tissue, contributing to the reduction of the pathogenesis of various cardiovascular diseases.

Physical exercise has shown evidence of being an effective way to improve cardiovascular markers, but there are few studies that test its effectiveness in aquatic environments [7]. The exercise in aquatic environment, compared to exercise on land, provides specific mechanical advantages due to the principles of buoyancy, viscosity, and drag. These advantages make the practices of exercise more pleasant and safer for the joints among the older population [22]. For this reason, the purpose of this study is to test the impact of different physical exercise programs in aquatic environments (continuous aerobic program, aerobic interval program, and combined program) on the IMT, hemodynamic parameters, and biochemical markers associated with cardiovascular diseases in community dwelling older people. Considering the low number of studies published that combine imaging technology, biochemical markers, and aquatic exercise interventions [7,18,20], we believe that this study will provide relevant information on the effect of these three exercise programs on cardiovascular risk indicators.

2. Materials and Methods

2.1. Study Design

A randomized controlled trial was conducted in Beira Interior Region, Portugal. A sample of non-institutionalized older participants were submitted to a 28-week aquatic exercise intervention. The entire study protocol was previously published by Ferreira et al. [23]. Intima-media thickness of carotid arteries (IMT), blood pressure, lipid blood profile, and chemokines blood concentration (MCP-1 and MIP-1α) were measured in all participants, undergoing three different physical aquatic exercise programs: continuous aerobic (AerG), interval aerobic exercise (IntG), and combined exercise (ComG). A fourth group of participants was also selected as the control group (CG). Data were collected at two specific time moments, namely pre-intervention (baseline, M1) and post-intervention (after 28 weeks, M2). This study was carried out according to the recommendations of the Declaration of Helsinki for Human Studies. The protocol was approved by the Ethics Committee for Health of the Faculty of Sport Sciences and Physical Education, University of Coimbra (reference: CE/FCDEF-UC/00462019). Written informed consent was obtained from all participants prior to any protocol-specific procedures.

2.2. Participants and Sample Size

The size and statistical power of the sample were calculated using the G*Power software application (University of Dusseldorf, Dusseldorf, Germany) [24]. The following parameters were considered: F test (ANOVA); effect size, 0.25; α-level, 0.05; statistical power, 0.95; number of groups, 4; number of measures, 2 (pre and post intervention); margin of possible losses and refusals, 30%. Therefore, the initial size of the total sample was estimated at 76 participants.

Initially, 174 individuals from the community were invited to participate in the study. After the application of the inclusion and exclusion criteria, 152 individuals were randomized into the four groups mentioned above: AerG, $n = 36$; IntG, $n = 41$; ComG, $n = 48$ and CG, $n = 27$. According to the experience of the research team and previous studies, the dropout rate from exercise programs among elderly populations is high, so we gathered more participants to fulfil the sample size and compensate for potential dropouts [25–27]. A simple randomization method was used. An external investigator used a computer-generated list of random numbers to allocate participants to each group. The investigators were blinded for the randomization of the groups.

The following inclusion criteria were applied: (a) participants from both sexes; (b) 65 years or older; (c) non-institutionalized; (d) autonomy to travel from their residence to Sertã municipal swimming pool; (e) filling out the informed consent form; (f) individuals with medical authorization to practice physical exercise in an aquatic environment, in cases they had some type of clinical condition or comorbidity. The following exclusion criteria were also defined: (a) individuals with clinically diagnosed pathologies putting their and others' health at risk while doing physical exercise in an aquatic environment; (b) individuals that obtained a score of less than 9 points in the Mini-Mental States Examination (indicating severe cognitive impairment) or were clinically diagnosed with a mental illness; (c) an attendance of less than 50% to physical exercise sessions; (d) participants who failed to complete all proposed assessment tests.

The AerG, IntG, and ComG groups performed physical exercise in an aquatic environment during a period of 28 weeks. Participants from the CG group were asked to maintain their normal daily activities without performing any type of systematic physical exercise during the same time period. The participants in the control group were age-matched persons who did not perform any type of regular systematic exercise.

Fifty participants dropped out from the study for the following reasons: personal reasons ($n = 11$); less than 50% of attendance of the exercise sessions ($n = 14$); not completing all the assessment tests ($n = 12$); injury not related with the exercise intervention ($n = 4$); disease ($n = 9$). Consequently, 102 participants completed the entire process (AerG: $n = 25$, 71.44 ± 4.84 years old, women—80%, taking regular medication—92%, with cardiometabolic diseases—76%; IntG: $n = 28$, 72.64 ± 5.22 years old, women—89.3%, taking regular medication—96%, with cardiometabolic diseases—82%; ComG: $n = 29$, 71.90 ± 5.67 years old, women—75.9%, taking regular medication—90%, with cardiometabolic diseases—79%; CG: $n = 20$, 73.60 ± 5.25 years old, women—55%, taking regular medication—95%, with cardiometabolic diseases—80%). The cardiometabolic diseases included diabetes, hypertension, and hypercholesterolemia. Figure 1 shows the entire allocation process for the different groups.

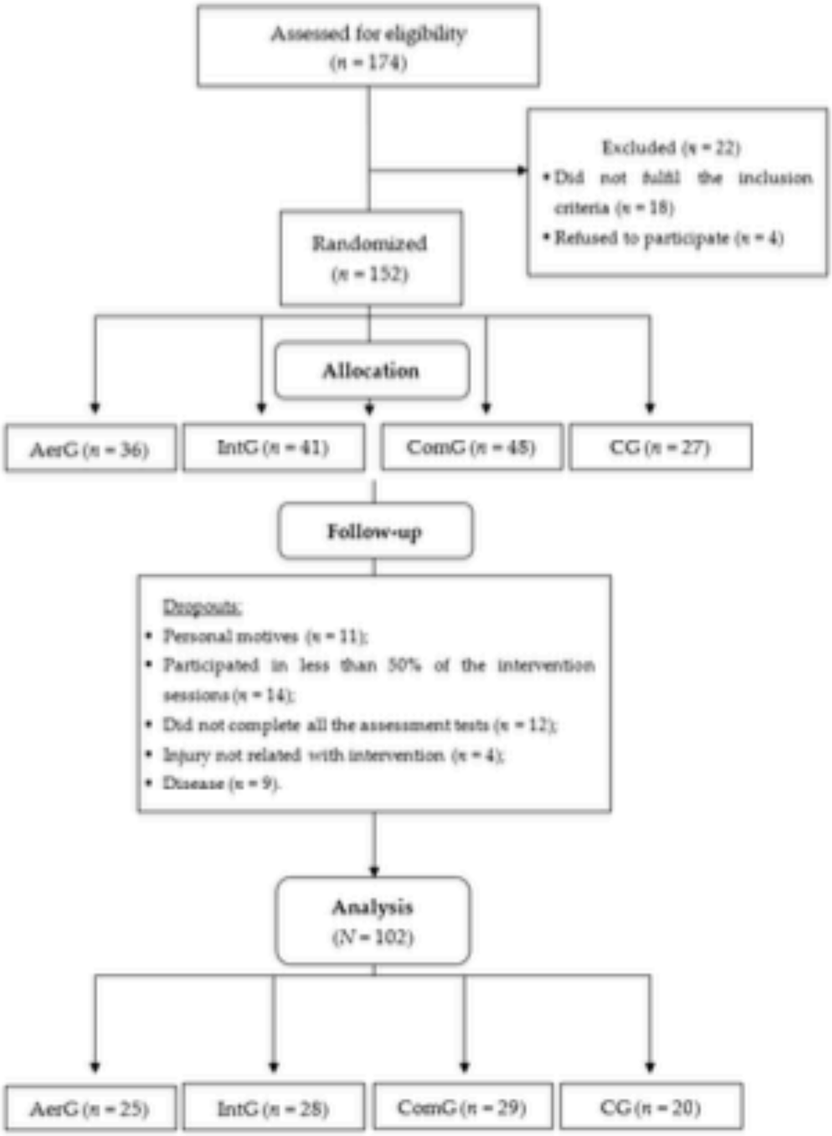

Figure 1. Allocation process for the different groups: Continuous aerobic group (AerG); Aerobic interval group (IntG); Combined group (ComG); Control group (CG).

2.3. Outcomes Measurements

2.3.1. Sample Characterization

To characterize the sample, values related to anthropometry and physical fitness were collected. Height (Hgt) was assessed using a portable Seca Bodymeter® stadiometer (model 208, Hamburg, Germany) with an accuracy of 0.1 cm. Weight (Wgt), body mass index (BMI), visceral fat (VF), fat mass (FM), and lean body mass (LBM) were evaluated using the TANITA BC-601 impedance scale (Tokyo, Japan). Functional fitness was assessed using the following tests from the Senior Fitness Test set, developed by Rickli and Jones [28] and validated for the Portuguese population by Baptista et al. [29]: muscle strength of the lower (MI) and upper (MS) limbs, with the Chair Stand test (30 s—CS) and Arm Curl test (30 s—AC), respectively (repetitions/30 s); aerobic capacity, with the Two-Minute Step test (2 m—ST) (number of steps); the flexibility of MI and MS, with the Chair Sit and Reach test (CSR) and Back Scratch test (BS), respectively (centimetres); agility and dynamic balance, with the Timed Up and Go test (TUG) (seconds). The handgrip strength was also evaluated, with the Hang Grip test (HG), using the Jamar hand dynamometer (Lafayette Instrument Company, Lafayette, IN, USA) (kg).

2.3.2. IMT and Hemodynamic Parameters

The intima-media thickness of the carotid arteries (IMT) was evaluated with a General Electric (GE®) portable ultrasound machine, VIVID model, with a 9 L linear probe (4 to 12 MHz). All examinations were performed by a highly trained technician. The measurements and data reanalysis were interpreted by two independent technicians, with all data being recorded in digital support. All measurements were performed in the space between 10 and 20 mm before the carotid bifurcation (carotid bulb), allowing the measurement of the IMT in the most distal wall, which is the one with the best definition.

The participants performed the examination in a temperature-controlled room (22 °C to 24 °C), lying on an examination table, in a supine position, with their heads turned to the side (45°). With the use of the real-time B-mode Echo Doppler ultrasound technique, we obtained an image of the carotid artery in the longitudinal plane and transverse plane in order to confirm the IMT measurements.

The images were also used to obtain the systolic diameters (SD) and diastolic diameters (DD). The peak systolic velocity (PSV) and the endo-diastolic velocity (EDV) were measured with the Pulse-Doppler technique in association with the continuous Doppler. For higher accuracy in the velocity calculations, the insonation angle was set at 60°. This allowed us to obtain correct values with respect to the Doppler equation.

The measurements of heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were performed with the use of a digital automatic sphygmomanometer from Riester (Model Ri-ChampionN®, Jungingen, Germany). All the measurements were taken after the participants were at rest for a period of 5 min, sitting and silent, in a room with controlled temperature (22 °C to 24 °C). After this period, participants maintained their comfortable sitting position in a chair, keeping their torsos straight, and the right upper limb stretched and placed on top of a table. Then, a cuff was placed on their right upper limbs, aligned with the brachial artery, at the level of the heart, and adjusted to the perimeter of the arm. Three measurements were taken, with intervals of 1–2 min between them, to check what the average blood pressure was. The mean of the three measurements was considered according to the Guidelines for the Management of Arterial Hypertension [30] of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC).

2.3.3. Biochemical Markers

Fasting blood samples (15 mL) were collected from the ante cubital vein by a registered nurse. The blood sample was used by the clinic to assess lipidic panel values: cholesterol total (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and glucose (GLU). The atherogenic index (AI) was calculated using the TC/HDL ratio and considered normal for values less than five. Next, the test tubes were centrifuged for 10 min at 3500 rpm, and plasma and serum were retrieved and stored in cryogenic test tubes at −80 °C until further use. Levels of Monocyte Chemoattractant Protein-1 (MCP-1/CCL-2) and Macrophage Inflammatory Protein-1 alfa (MIP-1α/CCL3) were subsequently analysed by ELISA (Invitrogen®, Alfagene, Portugal) according to the manufacturer's instructions.

2.4. Intervention Protocol

The exercise programs were implemented by sport science and fitness experts, with specific training in hydrogymnastics and developed according to the exercise prescription guidelines recommended by the American College of Sport Medicine (ACSM) for the elderly [31].

All exercise programs sessions had a duration of 45 min, twice a week, for 28 weeks and were performed in a water environment (the water level was between 0.80 and 1.20 m with a temperature of approximately 32 °C), using musical rhythm as a tool to control the intensity of the exercise. Sequences of aquatic exercises, previously defined and selected according to the objectives of each program, were applied. Water exercise sessions were organized into three different parts: the initial, main, and final part.

The initial part or warm-up lasted between 10 and 15 min, at low intensity (30–40% max HR), and was the same in the three water exercise programs. During this initial part, it is intended that participants will adapt to the aquatic environment, i.e., to the water temperature, and provide muscular and metabolic stimulations to prepare the body for the main part of the session. Thus, simple exercises in water were used, such as displacements and isolated movements, with a progressive increase in complexity and intensity throughout the initial part.

The main part for each one of the three water exercise program sessions had a duration of 20 to 30 min and had the following characteristics (Figure 2):

- Continuous aerobic (AerG): exercise aerobic (weeks 1–13, 60–65% maximum HR; weeks 14–28, 65–70% HR);
- Interval aerobic (IntG): exercise aerobic different intensities (weeks 1–13, 60–65% maximum HR interval to 70–75% maximum HR, weeks 14–28, 65–70% maximum HR interval to 75–80% maximum HR). In IntG, the main part of the sessions consisted of several series (1′ recovery interval with 30″ with higher intensity);
- Combined (ComG): exercise aerobic (weeks 1–13, 60–65% maximum HR; weeks 14–28, 65–70% maximum HR) and muscle strength (weeks 1–13, 2 sets of 12 repetitions; weeks 14–28, 3 sets of 16 repetitions; 6–7 pois in the Borg Scale).

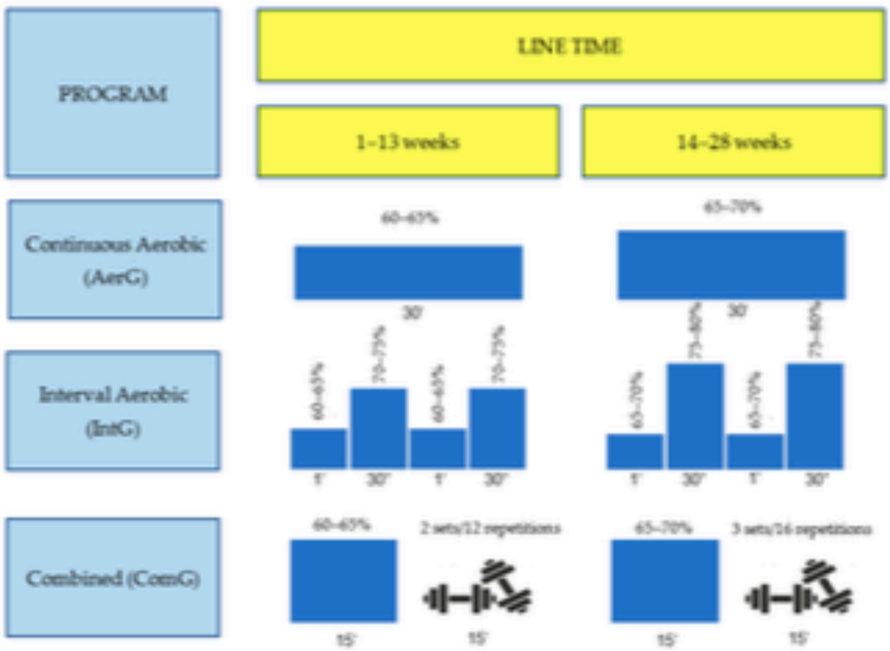

Figure 2. Characteristics of the main part for the three physical exercise aquatic programs (continuous aerobic, interval aerobic, and combined).

The final part of the water exercise sessions lasted between 5 and 10 min and was the same for each of the three water exercise programs. This part consisted of two phases: return to calm, where relaxation exercises were applied to bring back the participants' heart rate to values close to the resting state, and stretching exercises stimulating a greater range of motion, used to stretch the main muscle groups used throughout the sessions.

2.5. Monitoring the Intensity of the Exercise of Programs

For safety and intensity target control reasons, all participants randomly used heart rate monitors (Polar, R800CX) (Polar Electro Oy, Professorintie 5, FI-90440 Kempele, Finland) during the exercise sessions, in all three water exercise programs. Depending on the heart rate values obtained, intensity adjustments to the training plan were performed to maintain the intensity target defined for each water exercise program.

For safety and intensity target control reasons, the intensity of the different water exercise programs was predicted indirectly using Karvonen's Formula [32]:

$$\text{Target heart rate} = ((\text{maximal heart rate} - \text{resting heart rate}) \times \%\ \text{intensity}) + \text{resting heart rate}$$

Additionally, and to calculate the maximal heart rate, we used the [33] following formula for the elderly:

$$\text{Maximal HR} = 207\ \text{beats per minute} - (0.7 \times \text{chronological age})$$

2.6. Statistical Analysis

The collected data were subjected to descriptive statistical analysis where values such as maximum, minimum, mean, and standard deviation were calculated for each variable in each assessment moment. Afterwards, data normality was tested by considering the response to three conditions: z-values from the skewness and kurtosis tests; p-values from the Shapiro–Wilk test; and visual inspection of generated histograms. All longitudinal comparisons were performed using complete case analysis. Parametric data were analysed using the Student's t-test for independent samples to compare the different moments (M1 and M2) and the one-factor ANOVA test and post hoc Tukey's test to analyse the differences between groups. Nonparametric data were analysed using the Wilcoxon test to compare the different moments (M1 and M2), and the Kruskal–Wallis and Bonferroni tests were used to analyse differences between groups. Associations between variables were analysed using Pearson's correlation coefficient values and interpreted as follows [34]: $r = 0.10$ to 0.29 means weak correlation; $r = 0.30$ to 0.49 means moderate correlation; and $r = 0.50$ to 1.0 means strong correlation. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical software, version 27.0 (IBM, New Orchard Road Armonk, New York, United States). The level of significance used was $p \leq 0.05$.

3. Results

The base line values characterizing of each group (mean and standard deviation) are shown in Table 1. No significant statistical differences were found between the groups before the intervention (M1), which suggests that all study groups had similar characteristics regarding anthropometry and functional fitness ($p > 0.05$). Exercise frequency rate was 75.4% in AerG, 69.3% in IntG and 77.7% in ComG.

Among the different groups of evaluated variables, intima-media thickness of the carotid arteries, blood pressure, lipid profile, fasting glucose, and MCP-1 and MIP-1 markers were analysed for correlations, in the two evaluation phases (M1 and M2) (see Figure 3). Weak and positive correlations were found between SBP and SD-L ($r = 0.28$) and DD-R ($r = 0.25$). Contrastingly, we found moderate and negative correlations between HDL and SD-L ($r = -0.30$) and weak and negative correlations between HDL and DD-R ($r = -0.20$). There were no statistically significant correlations between the fasting glucose, MCP-1 and MIP-1 markers, and the remaining variables analysed.

Table 1. Characterization of the baseline sample (M1).

Characteristic	AerG (n = 25)	IntG (n = 28)	ComG (n = 29)	CG (n = 20)	p Value
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
AG (years)	71.44 (4.8)	72.64 (5.2)	71.90 (5.7)	73.60 (5.3)	0.504
Hgt (m)	1.58 (0.7)	1.56 (0.7)	1.57 (0.7)	1.60 (0.9)	0.331
WGT (kg)	70.5 (8.1)	71.3 (14.3)	75.1 (11.0)	75.5 (13.3)	0.334
BMI (kg/m^2)	28.20 (3.3)	29.10 (4.8)	30.80 (5.3)	29.50 (5.8)	0.272
VF (%)	11.0 (3.0)	12.0 (3.0)	13.0 (3.0)	13.0 (6.0)	0.128
FM (%)	38.9 (7.3)	41.0 (6.7)	40.3 (9.8)	34.9 (10.9)	0.134
LBM (%)	26.5 (4.3)	24.5 (3.0)	25.5 (4.3)	27.7 (4.7)	0.079
2 m-ST (no of steps)	80.9 (17.4)	71.5 (16.5)	81.6 (19.2)	74.3 (18.9)	0.069
CSR-R (cm)	−0.5 (6.6)	−3.7 (10.6)	−3.5 (7.8)	−7.6 (9.7)	0.099
CSR-L (cm)	0.6 (7.2)	−3.9 (9.9)	−5.8 (9.9)	−3.5 (7.3)	0.054
BS-R (cm)	−9.9 (10.4)	−11.9 (11.5)	−14.3 (9.7)	−16.6 (9.9)	0.157
BS-L (cm)	−14.4 (7.2)	−17.4 (8.6)	−21.0 (10.8)	−20.6 (10.7)	0.056
TUG (s)	6.1 (1.1)	7.4 (1.8)	7.4 (3.0)	6.8 (1.7)	0.110
30 s-CS (reps/30 s)	15.0 (3.0)	13.0 (4.0)	13.0 (3.0)	15.0 (5.0)	0.185
30 s-AC (reps/30 s)	21.0 (6.0)	17.0 (7.0)	20.0 (5.0)	19.0 (6.0)	0.119
HG-R (kg)	22.0 (6.0)	21.0 (9.0)	21.0 (9.0)	24.0 (9.0)	0.411
HG-L (kg)	21.0 (6.0)	20.0 (9.0)	21.0 (9.0)	21.0 (10.0)	0.578

Note: Age (AG); height (Hgt); weight (Wgt); body mass index (BMI); visceral fat (VF); fat mass (FM); lean body mass (LBM); two-minute step test (2 m-ST); chair sit and reach test—right (CSR-R); chair sit and reach test—left (CSR-L); back scratch test—right (BS-R); back scratch test—left (BS-L); timed up and go test (TUG); chair stand test (30 s-CS); hand grip test—right (HG-R); hand grip test—left (HG-L).

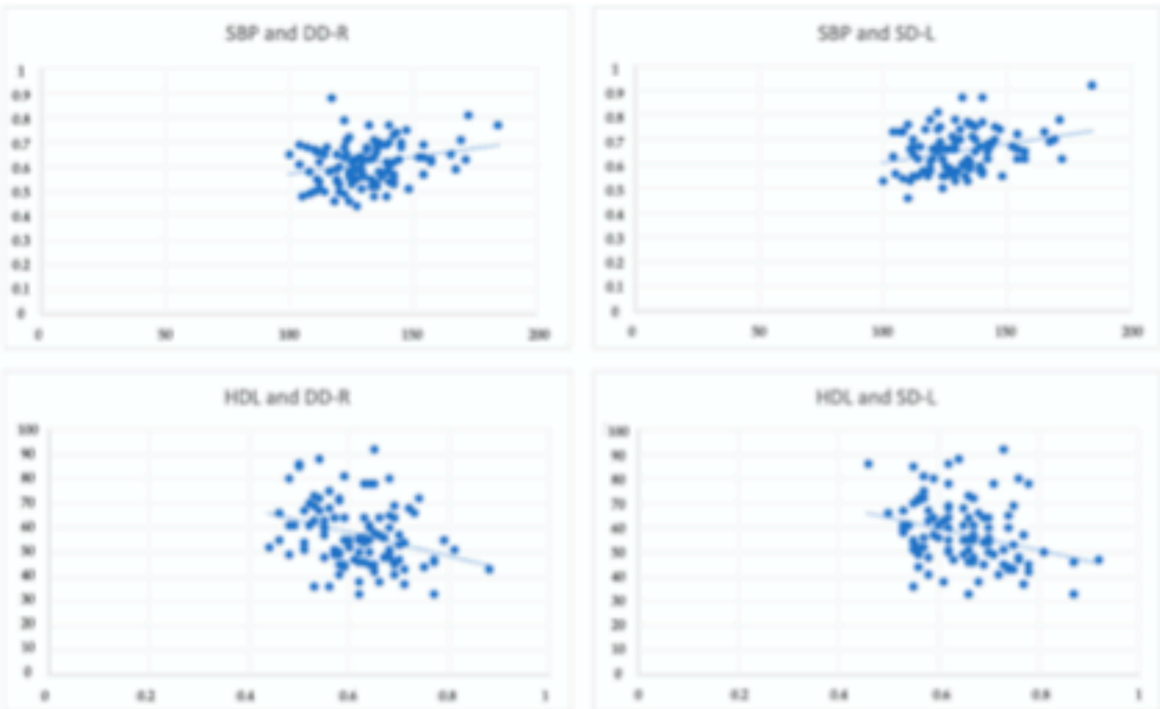

Figure 3. Relationships between SBP-DD-R and SBP-SD-L, and HDL-DD-R and HLD-SD-L. Notes: systolic blood pressure (SBP), diastolic diameter—right (DD-R), systolic diameter—left (SD-L), high-density lipoprotein (HDL).

The variation in the results of the hemodynamic and cardiovascular parameters, which were analysed by type of aquatic exercise program, before and after the intervention, are shown in Table 2. An example of an image of the carotid artery of a person who participated in the two evaluation phases (M1 and M2) is presented in Figure 4. Although there were no statistically significant differences in IMT after intervention in the different exercise groups, significant reductions were observed in some participants of the exercise groups. This reduction can be explained by the high adherence to the physical exercise program, which on average was 85.6%.

Table 2. IMT and Hemodynamic parameters results.

	AerG			IntG			ComG			CG			Time × Group (M1)	Time × Group (M2)
	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M2 Mean (SD)	M1 Mean (SD)	Time (p)		
IMT-L (cm)	0.07 (0.02)	0.07 (0.02)	0.293	0.08 (0.02)	0.07 (0.02)	0.376	0.07 (0.02)	0.07 (0.02)	0.707	0.07 (0.01)	0.07 (0.02)	0.979	0.989	0.501
SD-L (cm)	0.69 (0.12)	0.67 (0.09)	0.177	0.65 (0.1)	0.65 (0.09)	0.539	0.66 (0.1)	0.64 (0.09)	0.105	0.65 (0.06)	0.66 (0.06)	0.088	0.605	0.546
DD-L (cm)	0.64 (0.12)	0.62 (0.09)	0.423	0.61 (0.09)	0.59 (0.09)	0.105	0.63 (0.09)	0.60 (0.09)	0.037 **	0.61 (0.06)	0.62 (0.06)	0.558	0.640	0.264
PSV-L (cm/s)	76.79 (21.45)	80.31 (19.22)	0.242	82.51 (19.78)	77.21 (16.26)	0.104	79.38 (18.77)	78.73 (17.06)	0.315	77.28 (15.91)	80.24 (17.9)	0.097	0.508	0.835
EDV-L (cm/s)	19.28 (6.24)	19.95 (5.8)	0.397	20.8 (5.43)	20.26 (4.93)	0.581	22.38 (4.78)	19.97 (6.56)	0.027 *	19.7 (5.32)	22.41 (5.62)	0.003 *	0.090	0.443
IMT-R (cm)	0.08 (0.01)	0.07 (0.01)	0.808	0.08 (0.02)	0.07 (0.02)	0.055	0.08 (0.02)	0.07 (0.01)	0.205	0.08 (0.02)	0.08 (0.02)	0.308	0.654	0.698
SD-R (cm)	0.70 (0.12)	0.68 (0.09)	0.178	0.66 (0.09)	0.65 (0.09)	0.381	0.67 (0.08)	0.66 (0.09)	0.249	0.68 (0.08)	0.66 (0.08)	0.353	0.519	0.758
DD-R (cm)	0.66 (0.12)	0.63 (0.09)	0.039 *	0.62 (0.08)	0.61 (0.09)	0.282	0.63 (0.09)	0.60 (0.09)	0.029 *	0.62 (0.07)	0.63 (0.07)	0.813	0.316	0.543
PSV-R (cm/s)	74.6 (23.04)	74.1 (17.56)	0.904	74.63 (18)	66.23 (17.68)	0.024 *	81.23 (15.74)	77.25 (12.81)	0.087	74.27 (16.9)	73.11 (16.31)	0.763	0.136	0.106
EDV-R (cm/s)	19.55 (7.29)	18.89 (5.41)	0.716	19.39 (5.44)	18.26 (4.8)	0.186	20.74 (5.29)	19.18 (6.34)	0.064	18.43 (4.63)	19.27 (5.35)	0.432	0.169	0.910
SBP (mmHg)	134 (13.0)	128 (16.0)	0.013 *	135 (12.0)	132 (13.0)	0.184	138 (18.0)	133 (18.0)	0.018 **	137 (15.0)	132 (18.0)	0.093	0.798	0.535
DBP mmHg)	77 (9.0)	73 (10.0)	0.002 *	76 (7.0)	73 (7.0)	0.046 *	79 (8.0)	75 (8.0)	0.004 *	78 (7.0)	78 (7.0)	0.950	0.399	0.099
HR (bpm)	71 (11.0)	69 (12.0)	0.279	69 (9.0)	67 (11.0)	0.115	74 (12.0)	70 (10.0)	0.010 **	73 (10.0)	71 (6.0)	0.467	0.417	0.268

Note: Intima–media thickness—left (IMT-L); systolic diameter—left (SD-L); diastolic diameter—left (DD-L); peak systolic velocity—left (PSV-L); end-diastolic velocity—left (EDV-L); Intima-media thickness—right (IMT-R); systolic diameter—right (SD-R); diastolic diameter—right (DD-R); peak systolic velocity—right (PSV-R); end-diastolic velocity—right (EDVR); systolic blood pressure (SBP); diastolic blood pressure (DBP); heart rate (HR) * Result obtained through T-Student test; ** result obtained through Wilcoxon test.

The general results of the hemodynamic parameters showed no significant differences between the groups before and after the intervention ($p > 0.05$). As a result of the intervention with the aquatic exercise program, significant differences were found between M1 and M2 in AerG for DD-R ($p = 0.039$; $\Delta = -4.5\%$), in IntG for PSV-R ($p = 0.024$; $\Delta = -11.3\%$), and in ComG for DD-L ($p = 0.037$; $\Delta = -4.8\%$), for EDV-L ($p = 0.027$; $\Delta = -10.8\%$), and DD-R ($p = 0.029$; $\Delta = -4.8\%$). In the CG, there was a significant increase for EDV-L ($p = 0.003$; $\Delta = 13.8\%$), while a significant reduction in the same variable was observed in ComG. In regard to the variables IMT-L, SD-L, PSV-L, IMT-R, SD-L, and EDV-R, no significant changes were observed after the intervention ($p > 0.05$).

Regarding the blood pressure parameters results, no significant differences were found between the groups before and after the intervention ($p > 0.05$). As a result of the intervention with the aquatic exercise programs, significant differences were found in AerG for SBP ($p = 0.013$; $\Delta = -4.5\%$) and DBP ($p = 0.002$; $\Delta = -5.2\%$), in IntG for DBP ($p = 0.046$; $\Delta = -3.9\%$), and in ComG for SBP ($p = 0.018$; $\Delta = -3.6\%$), DBP ($p = 0.004$; $\Delta = -5.1\%$)

and HR ($p = 0.010$; $\Delta = -5.4\%$). In the control group, there were no significant changes ($p > 0.05$).

The variation in the results for the cardiovascular risk biomarkers, analysed by type of aquatic exercise program, before and after the intervention, are presented in Table 3.

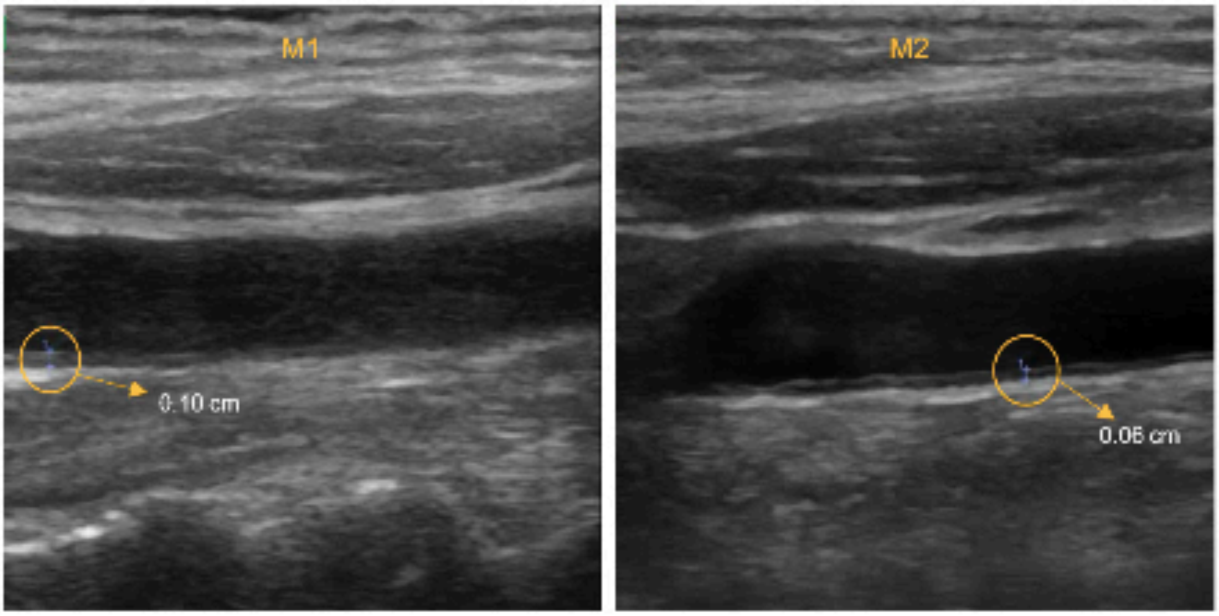

Figure 4. Images of the carotid artery of a study participant at different times of assessment: before the intervention (M1), the participant had an intima and media thickness of the carotid artery (IMT) of 0.10 cm; after the intervention (M2), a reduction in the IMT to 0.06 cm was verified.

Table 3. Biochemical marker results.

	AerG			IntG			ComG			CG			Time × Group (M1)	Time × Group (M2)
	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)	M1 Mean (SD)	M2 Mean (SD)	Time (p)		
GLU (mg/dL)	94 (27)	89 (22)	0.006 **	95 (18)	90 (17)	0.041 **	98 (25)	95 (24)	0.309	97 (20)	99 (18)	0.449	0.728	0.112
TC (mg/dL)	184 (31)	185 (24)	0.935	174 (33)	172 (29)	0.650	182 (26)	178 (24)	0.346	176 (26)	177 (27)	0.757	0.512	0.381
HDL (mg/dL)	58 (14)	59 (12)	0.586	58 (15)	56 (15)	0.175	58 (11)	59 (13)	0.478	57 (11)	55 (11)	0.111	0.907	0.582
AI (mg/dL)	3.29 (0.63)	3.33 (0.49)	0.653	3.17 (0.54)	3.25 (0.6)	0.249	3.23 (0.68)	3.27 (0.71)	0.566	3.21 (0.64)	3.67 (0.52)	0.003 *	0.598	0.033 †
LDL (mg/dL)	106 (25)	108 (20)	0.712	95 (27)	95 (24)	0.855	103 (25)	100 (25)	0.498	97 (25)	97 (25)	0.989	0.338	0.226
TG (mg/dL)	102 (51)	101 (48)	0.903	105 (36)	104 (43)	0.682	107 (41)	104 (46)	0.503	116 (47)	119 (35)	0.478	0.393	0.191
MCP-1 (pg/mL)	141.74 (82.71)	82.45 (46.02)	0.001 **	80.43 (37.15)	63.42 (42.69)	0.059	231.79 (73.36)	193.44 (94.04)	0.033 **	72.76 (49.30)	54.34 (31.33)	0.093	0.000 †	0.000 †
MIP-1α (pg/mL)	93.09 (12.62)	91.04 (7.39)	0.009 **	96.19 (1.88)	96.45 (1.29)	0.179	110.49 (9.37)	110.43 (11.77)	0.214	90.90 (15.12)	90.96 (15.40)	0.546	0.000 †	0.000 †

Note: Glucose (GLU); cholesterol total (TC); high-density lipoprotein (HDL); atherogenic index (AI); low-density lipoprotein (LDL); triglycerides (TG); monocyte chemoattractant protein-1 (MPC-1); macrophage inflammatory protein (MIP-1α). * Result obtained through T-Student test; ** result obtained through Wilcoxon test; † results obtained through ANOVA.

The overall lipid profile results revealed that no significant differences were found between the groups before the intervention ($p > 0.05$). After the intervention, significant differences were found for AI between IntG and CG ($p = 0.008$) and between ComG and CG ($p = 0.009$). In both cases, the CG obtained a less satisfactory result. As a result of the intervention with the aquatic exercise program, significant differences were found in

AerG (p = 0.006; Δ = −5.3%) and IntG (p = 0.041; Δ = −5.3%) for GLU, and in CG for AI (p = 0.003; Δ = 14.3%). In regard to the variables TC, HDL, LDL, and TG, there were no significant changes ($p > 0.05$). As for the chemokines MCP-1 and MIP-1α, statistically significant differences were found between the groups, before and after the intervention ($p < 0.05$). From M1 to M2, statistically significant differences were found in AerG for MCP-1 (p = 0.001; Δ = −41.8%) and MIP-1α (p = 0.009; Δ = −2.2%) and in ComG for MCP-1 (p = 0.033; Δ = −16.5%). For the IntG and CG, no statistically significant differences were found in both chemokines ($p > 0.05$).

4. Discussion

The purpose of this study was to test the impact of different aquatic exercise programs (continuous aerobic exercise, aerobic exercise interval, and combined exercise) on the IMT and hemodynamic and biomarkers of cardiovascular diseases in community dwelling older people. Preliminary systematic research revealed the innovative characteristics of the present study, since there were very few studies that have evaluated similar variables with aquatic exercise interventions in older adults.

Although no changes were found in IMT or in the variables related to the hemodynamic parameters, significant reductions were observed for DD-R in AerG and ComG, for PSV-R in IntG, and for DD-L in ComG. In EDV-L, there was a significant reduction in ComG, while in CG a significant increase was observed.

A study by Santos [7] reported the effectiveness of a 10-month continuous aerobic aquatic exercise program in older women (64.05 ± 5.91 years), who were divided into two groups: older women who started practising exercise in an aquatic environment (continuous aerobic program), for the first time (beginners) and elderly women who had already practised physical exercise in an aquatic environment (continuous aerobic program) during the previous school year (trained). The results showed that in both groups there was a significant reduction in IMT, with a magnitude effect of 36.4% and that exercise had a very strong effect on the change in IMT. Regarding SD and DD, there was a significant reduction in both groups. Additionally, a reduction in PSV and EDV was reported in both groups. Such a reduction was significant in the trained group. Similarly, in the study by Park and Park [15], the effectiveness of a combined exercise program (aerobic exercise and muscle strength exercise) was tested in a group of older women (65–77 years) for 24 weeks. The results showed that these types of programs can lead to a significant reduction in IMT. In another study [35], the impact of two exercise programs (high-intensity interval exercise and continuous aerobic exercise) was tested, and the results showed that, although they did not lead to significant statistical differences, both programs resulted in a reduction of the IMT.

The results from the present study are in line with those in the existing literature. In IMT-R, although there were no significant statistical differences, a trend towards a reduction was visible in the three exercising groups, whereas in IMT-L only a reduction in IntG was visible, with the value of the mean remaining the same in the other groups. As reported by Santos [7], our results also showed a reduction in SD, DD, PSV, and EDV in all exercise groups. This reduction was significant for DD-L in ComG, for DD-R in AerG and ComG (in regard to the latter, ComG recorded a higher variation of the mean), for EDV-L in ComG, and for PSV-R in IntG. In the CG, a significant increase in EDV-L was evident. According to Homma et al. [36], there is a strong and positive correlation between advancing age and an increase in IMT (r = 0.83). In our study, although the results did not significantly decrease in all variables, in the exercise groups, they did not increase either. For this reason, we can say that all exercise programs can be considered as factors of hemodynamic balance and thus contribute to the reduction in stress on the arterial wall and reduce the risk of developing cardiovascular diseases. The combined program seems to be slightly more beneficial compared to the other ones, due to the fact that it resulted in significant differences in a higher number of variables. Additionally, compared to the study by Santos [7], our intervention lasted 7 months, while in Santos's (2020) study, the intervention lasted 10 months. These data suggest that a 7-month intervention with aquatic exercise may be sufficient to cause changes in these hemodynamic variables.

Significant reductions were also found for SBP in the AerG and ComG, as well as in the three-exercising groups for DBP, and for HR in the ComG.

A study by Park et al. [37] tested the effectiveness of a water walking program on blood pressure values in a group of women (70 ± 10.0 years), and the results showed that, after the intervention, the exercise group had a significant reduction in HR values. The SBP and DBP values were also reduced, although not significantly. In land environments, a study by Son et al. [17] reported significant reductions in SBP and DBP after intervention with a combined exercise program (muscle strength exercise and aerobic exercise) in a group of hypertensive women (75 ± 2 years old). The same results were confirmed by Park et al. [38] using the same type of program (combined exercise), which led to a decrease in SBP and DBP values. This decrease was statistically significant in SBP.

In our study, the three exercise groups showed reductions in SBP, DBP, and HR. The aerobic exercise and combined exercise program seem to be the most beneficial. Both showed similar results in SBP and DBP, with the aerobic exercise program leading to a slightly higher decrease in the mean compared to the combined exercise program. In HR, the combined exercise program was the only one where the decrease was statistically significant. We may conclude that the combined exercise program may be more beneficial for cardiovascular parameters, as in addition to having showed similar results to the aerobic program in SBP and DBP, it also led to a significant decrease in HR.

In regard to the biochemical markers, significant reductions were noticed for GLU in AerG and IntG, and a significant increase was noticed for the AI in CG (14.3%). After the intervention, significant differences were found in the AI between IntG and CG and between ComG and CG.

In a study with participants with type 2 diabetes [39], after an intervention in an aquatic environment with a moderate aerobic exercise program, the results showed a significant decrease in GLU, TC, LDL, and TG values. Another study [40] tested the effectiveness of two different aquatic exercise programs: aerobic exercise and muscle strength exercise. Both programs led to a significant decrease in the TC, LDL, and TG values and a significant increase in HDL values. Therefore, they concluded that the two types of aquatic exercise programs had identical benefits, and both could contribute to a reduction in the risk of cardiovascular events. Similar results were reported on a land study [41], where the effectiveness of two exercise programs (high-intensity interval exercise and moderate-intensity continuous aerobic exercise) was compared, with the results showing a decrease in TC, LDL, and TG values and an increase in the HDL values. In comparison with the aerobic exercise program, the interval exercise program showed results that were more beneficial.

As in the study of Cugusi et al. [39], the present study noticed a decrease in the GLU values in the three exercise groups, although only statistically significant in AerG and IntG. Our results showed that AerG and IntG are equally beneficial in reducing GLU. Regarding TC, HDL, LDL, and TG, the results did not show significant changes, and they remained within the reference values. Therefore, aquatic physical exercise may contribute to the balance of the lipid profile.

In the plasma chemokine levels, significant reductions were noticed for MCP-1 and MIP-1α in AerG, and for MCP-1 in ComG.

Among long-term intervention studies, the one conducted by Kraemer et al. [42], which consisted of carrying out a 12-week muscle strength training program with and without supplementation in young adults, MCP-1 values increased after the intervention, while the MIP-1α values decreased, regardless of the supplementation. In the study of Barry et al. [43], who tested the impact of two physical exercise programs (high-intensity interval exercise and moderate-intensity continuous exercise) in obese adults, the results showed that, although no statistically significant differences were found, the MCP-1 values were reduced in both groups after the intervention, and that the MIP-1α values increased after the intervention of the high-intensity interval program and decreased after the intervention of the moderate-intensity continuous program.

In our study, despite the fact that the intervention took place in a different context, the results partly reached the same conclusions. As in the Barry et al. [43], our results reinforce the idea that long-term continuous aerobic exercise reduces the values of MCP-1 and MIP-1α. The results of the interval aerobic program showed a reduction in MCP-1 and an increase in MIP-1α. It is possible that higher intensity exercise levels may lead to increased MIP-1α levels as a response to muscle micro-injuries. In both cases, the changes were not significant, just like in the Barry et al.'s study [43]. Finally, in the combined exercise program (continuous aerobic exercise and muscle strength exercise), the results showed a reduction in both chemokines.

MCP-1 appears to be present in the initial phase of atherosclerotic lesion formation, i.e., in the thickening of the tunica intima and fatty streaks, which suggests that this chemokine may contribute to an early influx of monocytes into blood vessels [13]. In the intimal layer of the carotid arteries, chemokines recruit monocytes that trigger the development of foam cells, leading to intimal erosion, which is caused by atherosclerosis and consequently leads to ischemia [12]. The results found in the present study suggest that aquatic physical exercise, especially exercise with aerobic characteristics, helps to prevent the development of cardiovascular diseases by contributing to the reduction in chemokines MCP-1 and MIP-1α.

Exercise in aquatic environments, due to the specific proprieties of water that make the exercise practice safer and more comfortable for the elderly population, reduces the risk of traumatic fractures and joints by giving them less stress and lower impact [44]. The viscosity of the water provides a comfortable resistance that allows the development of muscle strength [22]. The hydrostatic pressure, the force of the viscosity, and the turbulence created by the water during the physical exercise sessions provide a different proprioceptive and sensorial feedback from the land environment and provide periods of instability that must be overcome. These aspects benefit the postural control and balance system [45,46]. The practice of exercise in this environment seems to be a viable strategy, because in addition to its specific characteristics and added benefits, it seems to be equally effective for cardiovascular health when compared to exercise on land.

Study limitations included the fact that a simple randomization method was used, instead of using a blocked randomization method, which would guarantee a balance in the number of participants in each group, reducing sequence unpredictability. Another limitation was the fact that the level of physical activity of the participants in the control group was not evaluated. Although participants were not involved in regular systematic exercise, this assessment would have contributed to a stronger study. The limited number of publications with interventions in an aquatic environment in older populations was also a limitation for the discussion of results.

More intervention exercise studies in aquatic environments are needed in order to reinforce the results found in the present study and better understand the effects and effectiveness of aquatic exercise programs on markers of cardiovascular health.

5. Conclusions

Aquatic physical exercise, regardless of the type of program, seems to lead to benefits in cardiovascular variables, and this type of intervention may be a viable alternative when land-based exercise is not possible and/or desired.

In relation to the intima and average thickness of the carotid arteries, we can say that all the aquatic exercise programs studied (i.e., the continuous aerobic program, the aerobic interval program, and the combined program) can be considered as factors for hemodynamic balance and thus contribute to the reduction of stress on the arterial wall and reduce the risk of cardiovascular diseases. The combined program seems to be slightly more beneficial, compared to the other programs due to the fact that it resulted in significant differences in a higher number of variables.

As for blood pressure, the three exercise groups showed reductions in SBP, DBP, and HR. However, the aerobic and combined exercise programs proved to be more beneficial for blood pressure and present statistically significant differences for both variables (with the same mean variation). Additionally, the combined program also showed a statistically significant reduction for HR.

Regarding the metabolic profile, our results showed that AerG and IntG are equally beneficial in reducing GLU. Regarding TC, HDL, LDL, and TG, the results did not show significant changes; however, they remain within the reference values. We concluded that aquatic physical exercise may contribute to the balance of the lipid profile.

Finally, as for chemokines, the results suggest that aquatic physical exercise, especially that with aerobic characteristics, helps prevent the development of cardiovascular diseases by contributing to the reduction in chemokines MCP-1 and MIP-1α and the subsequent infiltration of monocytes and formation of atherosclerotic plaques.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Faculty of Sport Science and Physical Education, University of Coimbra Ethics Committee (reference: CE/FCDEF-UC/00462019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data are shown in the manuscript.

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