



Universidade da Maia

Departamento de Ciências da Educação Física e Desporto

**DOSE-RESPONSE EFFECTS OF A RECREATIONAL TEAM
HANDBALL-BASED EXERCISE PROGRAMME ON CARDIOVASCULAR,
METABOLIC AND MUSCULOSKELETAL BIOMARKERS OF INACTIVE
OVER 50-YEAR-OLD MEN**

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List of Papers

This thesis was based on the following articles:

Original article I: Dose-response effect of a recreational team handball-based exercise programme on cardiometabolic health and physical fitness in inactive middle-aged-to-elderly males – a randomised controlled trial

Original article II: Bone health, body composition and physical fitness dose–response effects of 16 weeks of recreational team handball for inactive middle-to-older-aged males – A randomised control trial

Original article III: Acute physiological response to different recreational team handball game formats in over 60-year-old inactive men

Original article IV: Mixed-gender small-sided recreational team handball games in middle-aged and elderly are physiologically more demanding for women than men

Original article V: Cardiometabolic effects of recreational team sports for untrained individuals over 60 years of age: a systematic review and meta-analysis (under review)

List of Abbreviations

- AU** – Arbitrary units
- BMC** – Bone mineral content
- BMD** – Bone mineral density
- BMI** – Body mass index
- CG** – Control group
- CTX** – Carboxy-terminal type-1 collagen crosslinks
- CVD** – Cardiovascular disease
- DBP** – Diastolic blood pressure
- BL** – Blood lactate
- BP** – Blood pressure
- GR** – Glutathione reductase
- HbA1c** – Glycated haemoglobin
- HDL** – High-density lipoproteins
- HR** – Heart rate
- HR_{max}** – Maximal heart rate
- LDL** – Low-density lipoproteins
- OC** – Osteocalcin
- P1NP** – Procollagen type-1 amino-terminal propeptide
- PA** – Physical activity
- PL** – Player load
- RPE** – Rating of perceived exertion
- SBP** – Systolic blood pressure
- SSG** – Small-sided games
- T2DM** – Type 2 diabetes mellitus
- TAS** – Total antioxidant status
- TH** – Team handball
- TRG** – Triglycerides
- VO_{2max}** – Maximal oxygen uptake
- VO_{2peak}** – Peak oxygen uptake
- WHO** – World Health Organization

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O andebol de recreação mostrou ser eficaz na melhoria da saúde de diferentes populações. No entanto, os efeitos do andebol de recreação na saúde de homens de meia-idade e idosos ainda não foram estudados. Embora a relação de dose-resposta entre exercício físico (aeróbio e resistido) e as adaptações na saúde já tenha sido abordada, o efeito dose-resposta utilizando andebol de recreação pode diferir devido às suas características multicomponente específicas. Assim, o principal objetivo da presente tese foi analisar o efeito dose-resposta do andebol de recreação na saúde de homens inativos de meia-idade e idosos, após 16 semanas de 1, 2 ou 3 sessões/semana (60 minutos). Pretendemos também analisar as exigências fisiológicas e físicas de diferentes formatos de jogo e formatos mistos vs. mesmo género, e descrever os efeitos cardiometabólicos da prática de desportos coletivos de recreação na população idosa. Os resultados mostraram que o andebol de recreação foi eficaz na melhoria da aptidão cardiorrespiratória quando realizado 3 vezes/semana e da performance aeróbia quando realizado 2–3 vezes/semana. Maiores alterações relativas foram observadas na performance aeróbia no grupo que treinou 3 vezes/semana do que nos grupos que realizaram 1 e 2 sessões/semana e que no grupo controlo. Além disso, 3 sessões semanais de andebol de recreação foram eficazes para induzir benefícios cardiometabólicos, ósseos e de composição corporal. O andebol de recreação apresenta exigências de carga interna semelhantes nos formatos 5x5, 6x6 e 7x7. No entanto, o 5x5 foi o único formato capaz de manter a intensidade cardiovascular ao longo dos três períodos de 15 minutos de jogo. Foi também observada maior frequência de ações de jogo de alta intensidade nos formatos 5x5 e 6x6 vs. 7x7. Os resultados mostraram também que durante formatos de jogos mistos, as exigências cardiovasculares relativas foram maiores para as mulheres de meia-idade e idosas que para os homens. Para os homens, jogar com participantes do mesmo género foi mais exigente do que o formato de jogo misto, enquanto para as mulheres ocorreu o inverso. Embora tenham sido observadas diferenças entre formatos de jogo e género, as exigências fisiológicas e físicas estão dentro dos limites necessários para induzir diversas melhorias na saúde. Por fim, o estudo de revisão mostrou que desportos coletivos de recreação são eficazes na melhoria da aptidão cardiorrespiratória em idosos. Resumindo, o andebol de recreação é uma modalidade de exercício eficaz e segura para melhorar marcadores de saúde e aptidão física em homens de meia-idade a idosos.

PALAVRAS-CHAVE: ENVELHECIMENTO; ANDEBOL; EXERCÍCIO DE ALTA INTENSIDADE; FREQUÊNCIA DE TREINO; SAÚDE CARDIOMETABÓLICA; SAÚDE MUSCULOESQUELÉTICA; COMPOSIÇÃO CORPORAL E APTIDÃO FÍSICA.

Abstract

Recreational team handball (TH) has shown to be effective in improving health in different populations. Nevertheless, the health effects of recreational TH for middle-aged-to-elderly men have not been studied yet. Although, the dose-response relationship between aerobic and resistance training and health adaptations has already been addressed, the dose-response effect of recreational TH may differ due to its specific multicomponent characteristics. Thus, the main aim of the present thesis was to analyse the dose-response health effect of recreational TH in inactive middle-aged-to-elderly males, after 16 weeks of 1, 2, or 3 60-min weekly sessions. Also, we aimed to ascertain the physiological and physical demands in different game and gender formats, and to describe the cardiometabolic effects of recreational team sports practice in older populations. Recreational TH was effective in improving cardiorespiratory fitness when performed 3 times/week and aerobic performance when performed 2–3 times/week. Higher relative changes were observed in aerobic performance for the group that played 3 times/week than the groups that performed 1 and 2 times/week and the control group. Furthermore, 3 weekly TH training sessions were effective in providing cardiometabolic, bone and body composition benefits. Also, recreational TH presents similar internal load demands by playing 5v5 and 6v6 or 7v7 game formats. However, 5v5 was the only game format able to maintain the cardiovascular intensity across the three 15-min match periods. Also, higher frequency of high-intensity game actions was observed in 5v5 and 6v6 compared to 7v7. The results also showed that the relative cardiovascular demands were higher for middle-aged-to-elderly women than for men during mixed-gender matches. Also, in men, the same-gender game format was more demanding than the mixed-gender, while in women the inverse occurred. Although differences observed in game and gender formats, the physiological and physical demands were within the range to improve health. The last study showed that recreational team sports are effective in improving cardiorespiratory fitness for older men and women. To summarise, recreational TH is an effective and safe exercise modality to improve health markers and physical fitness in middle-aged-to-elderly men.

KEYWORDS: AGEING; TEAM HANDBALL; HIGH-INTENSITY EXERCISE; TRAINING FREQUENCY; CARDIOMETABOLIC HEALTH; MUSCULOSKELETAL HEALTH; BODY COMPOSITION AND PHYSICAL FITNESS.

CHAPTER I: Introduction

1. Introduction

1.1. Ageing process

At a biological level, ageing results from the impact of the accumulation of a wide variety of molecular and cellular damage that occurs over time (Steves et al., 2012; Vasto et al., 2010; World Health Organization, 2015). It is a complex biological process associated with a progressive loss of physiological integrity leading to impaired function and increased vulnerability to death (López-Otín et al., 2013). Ageing is associated with an increased risk of multimorbidity (i.e., experiencing more than one chronic condition at the same time) (Marengoni et al., 2011; World Health Organization, 2015), with its prevalence being around 30% among people aged 45–64 years, 65% among people aged 65–84 years and 82% among people aged ≥ 85 years (Barnett et al., 2012). The mechanisms responsible for multimorbidity are complex to understand due to the heterogeneity of patients, however, they can be considered in three broad areas: i) ageing and inflammation; ii) socioeconomic, psychosocial and behavioural determinants of health; and iii) medication-related morbidities (Skou et al., 2022).

The underlying biological mechanisms that influence ageing and inflammation determinants are considered the “hallmarks of ageing”. These have been identified and described by López-Otín and colleagues (2013) as: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication (López-Otín et al., 2013). Secondly, the socioeconomic, psychosocial, and behavioural determinants of health, such as, socioeconomic deprivation and lower education level (Pathirana & Jackson, 2018), as well as several lifestyle factors (i.e., smoking, alcohol intake, physical inactivity and poor diet quality) (Freisling et al., 2020; Katikireddi et al., 2017) influence ageing as they are associated with the development of multimorbidity (Singer et al., 2019). Medication-related determinants may also influence ageing and contribute to the development of multimorbidity as several medications (e.g., anticholinergic and antipsychotic medications) are associated with increased risk of diabetes mellitus, dyslipidaemia, cardiovascular events and cognitive impairment or dementia (Hanlon et al., 2020; Newcomer, 2007). Therefore, preventing the development of multimorbidity is of importance, especially in the socioeconomic, psychosocial, and behavioural area as a healthy lifestyle, that includes regular physical activity (PA), good nutrition and avoids high levels of alcohol consumption

and smoking, has been suggested to increase life expectancy regardless of multimorbidity (Foster et al., 2018).

Although there are several factors influencing health during the ageing process that can be modifiable, there is inter-individual variability due to genetic variations. Thus, we can observe some 70-year-olds ageing independently, while others need help for the basic daily routines much earlier. Nevertheless, all individuals should have the opportunity to live a long and healthy life and thus, acting upon the modifiable factors associated with unhealthy ageing should be addressed (World Health Organization, 2015).

1.1.1. Cardiovascular health

Structural and functional cardiovascular system changes characterise the ageing process. Structural changes occur in the myocardium with the remodelling of the left ventricle, valvular changes, a progressive reduction in the number of pacemakers cells in the sinus node, excitation-contraction coupling and cellular-genetic changes. Also, functional changes in the myocardium can be observed during ageing with alterations in the cardiac rhythm and in systolic and diastolic function. Additionally, the ageing process is also associated to structural alterations in the vessels, with macroscopic and microscopic changes that influence the blood supply of tissues and the cardiac function, and consequently, influence the functional changes in the vessels, i.e., endothelial dysfunction and increase in systolic blood pressure (SBP) and pulse pressure (Karavidas et al., 2010). Furthermore, molecular damage is inevitable throughout life with arteries and heart becoming stiffer and more fibrotic, and with a linear decline in maximal heart rate (HR_{max}) (Fontana, 2018).

Cardiorespiratory fitness is related to functional capacity and human performance and is a strong and independent predictor of all-cause and disease-specific mortality regardless of sex and race (Harber et al., 2017). Moreover, cardiorespiratory fitness is a predictor of dependence (i.e., not being able to live independently and loss of quality of life) (Paterson et al., 2004), which is of relevance considering that the world population is ageing (United Nations Department of Social and Economic Affairs, 2007). Maximal oxygen uptake (VO_{2max}) is the gold standard for cardiorespiratory fitness evaluation. It is the oxygen intake during an exercise intensity at which actual oxygen intake reaches a maximum beyond which no increase in effort can raise it (Hill & Lupton, 1923). VO_{2max} progressively decreases during the ageing process due to a reduction in HR_{max} , cardiac output and arteriovenous oxygen difference (Karavidas et al., 2010; Pimentel et al., 2003). Peak oxygen uptake (VO_{2peak}), the highest value of oxygen

uptake attained on the particular test (incremental or other high-intensity test), also progressively declines over time (Fleg et al., 2005; Jackson et al., 2009b; Letnes et al., 2020), with a more pronounced accelerated decline in cardiorespiratory fitness for men, in advanced age, with the decline progressing from 5% per 10 years in 30-year-olds to >20% in 70-year-old males (Fleg et al., 2005). Nevertheless, accomplishing the recommended guidelines for PA has been associated with a better VO_{2peak} maintenance, and high-intensity PA levels are associated with maintaining a higher VO_{2peak} compared to moderate intensity PA, for both sexes, over the years (Letnes et al., 2020). Furthermore, across the adult life span, having a low body mass index (BMI), being physically active and not smoking have also been positively associated with a higher cardiorespiratory fitness (Jackson et al., 2009b).

An increase in blood pressure (BP) and hypertension prevalence can be observed during the ageing process in both men and women (Singh et al., 2012) and it is known that high SBP and diastolic BP (DBP) is associated to an increased risk of cardiovascular diseases (CVDs) across different age groups (from 30 years to >80 years of age) (Whelton et al., 2018). Hypertension (defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg) (Mancia et al., 2023) is a serious medical condition that significantly increases the risk of CVDs, brain and kidney diseases and other diseases, and it is estimated that 1.4 billion adult people globally are hypertensive (World Health Organization, 2021). Hypertension is the leading modifiable risk factor for CVDs and premature death worldwide. Its prevalence is rising globally due to ageing of the population and increase of unhealthy lifestyle behaviours, including unhealthy diets (i.e. high sodium and low potassium intake) and lack of PA (Mills et al., 2020). Nonetheless, the participation in regular PA is recognized as a key modifiable determinant of hypertension (World Health Organization, 2020).

Resting heart rate (HR) represents the balance between sympathetic and parasympathetic activity and is considered a reliable marker of autonomic nervous system tone (Olshansky et al., 2022). Resting HR values vary from individual to individual, however, the normal values are between 60–100 bpm. Its regulation is a dynamic process that is not only influenced by intrinsic factors but also by extrinsic factors such as exercise, diet or even smoking, and it has a circadian variation that is modulated by sleep, diet and caffeine intake (Olshansky et al., 2022). Elevated resting HR values have shown to be an independent predictor of all-cause mortality and cardiovascular events in older adults (≥ 60 years) (Li et al., 2017). Nevertheless, regular exercise has shown to reduce resting HR values (Huang et al., 2005). In fact, endurance sports and yoga are examples of exercise modalities that have shown to reduce resting HR (Reimers et al., 2018), which is important to reduce the risk of CDVs.

Although the cardiovascular decline is associated with age, the ageing process itself does not cause CVDs. Instead, the combination of unhealthy lifestyle behaviours, such as poor diet and excessive calorie intake, low levels of PA, harmful alcohol consumption, and smoking (World Health Organization, 2013), accelerates cardiovascular functional deterioration and rapidly increases the risk of developing one or more CVDs, i.e., coronary heart disease, stroke, heart failure, aortic aneurysm, peripheral artery disease and vascular dementia (Joseph et al., 2017; Zhang et al., 2021).

1.1.2. Metabolic health and oxidative stress

Excessive energy intake and central adiposity cause insulin resistance, type 2 diabetes mellitus (T2DM), inflammation, dyslipidaemia, elevated BP, oxidative stress, and many other metabolic and hormonal alterations, which trigger several detrimental molecular and cellular adaptations leading to functional and structural CVDs (Lavie et al., 2018). According to the World Health Organization (WHO), overweight (i.e., BMI levels over 25 kg/m²) and obesity (i.e., BMI levels over 30 kg/m²) are described as excessive fat accumulation that represents a risk for health (World Health Organization, 2016). Overweight and obesity are associated with the incidence of cancer, CVDs and T2DM (Guh et al., 2009) and are the main modifiable risk factors for T2DM. Since 80% of people diagnosed with T2DM are overweight (Smyth & Heron, 2006), increased adiposity is considered the main risk factor for the development of this disease (DeFronzo et al., 2015). T2DM is explained by a dysregulation of carbohydrate, lipid, and protein metabolism resulting in impaired insulin secretion, insulin resistance or a combination of both (DeFronzo et al., 2015). Moreover, increased levels of fasting plasma glucose (>110 mg/dL), glucose tolerance (>140 mg/dL) or glycated haemoglobin (HbA1c; >6%) are the main conditions to be prediabetic, and consequently, to develop T2DM (DeFronzo et al., 2015). Furthermore, insulin resistance is the most common metabolic disorder in obesity and it is the main responsible for the development of dyslipidaemia (Klop et al., 2013). Dyslipidaemia is characterised by increased triglycerides (TRG) concentrations and/or plasma low-density lipoproteins (LDL) and also reduced high-density lipoproteins (HDL) cholesterol (Klop et al., 2013). High levels of plasma LDL cholesterol are an important risk factor for the development of CVDs by aggravating plaques, enhancing inflammation, and facilitating foam cell formation. On the other hand, plasma HDL cholesterol has a protective function, by transporting cholesterol from plasma to the liver, leading to reduced plaque formation (Bhargava et al., 2022). During the ageing process the lipid profile deteriorates, with older age being associated

with a higher risk for dyslipidaemia development (Cho et al., 2020). Therefore, low levels of total (<200 mg/dL) and LDL (<100 mg/dL) cholesterol, high levels of HDL cholesterol (≥ 60 mg/dL) and low levels of TRG (<150mg/dL) are recommended to decrease the risk of developing CVDs (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001).

The prevalence of obesity increases with age, and this increase may be explained by a complex interaction between changes in the nutrition, levels of PA, socioeconomic, environmental, and genetic factors (Chooi et al., 2019). Obesity is a risk factor for metabolic diseases (i.e., T2DM), CVDs and some cancers (Nguyen & El-Serag, 2010), and a majority of the older population suffers from one or more chronic conditions (Ward & Schiller, 2013). To worsen the situation, the prevalence of multiple chronic conditions is increasing in all age groups, especially in the older-aged group (Hayek et al., 2017). Nevertheless, metabolic health is not only influenced by genetic factors, but also by lifestyle behaviours, which are a cornerstone in the management of lipid and lipoprotein disorders and obesity (Enkhmaa et al., 2000). For example, regular moderate to vigorous PA and exercise have a pronounced protective effect against metabolic diseases (Thyfault & Bergouignan, 2020) and complying with the recommended PA guidelines can reduce the risk of T2DM by 30% (Tudor-Locke & Schuna, 2012).

One of the starting points for the development of many diseases, that has also huge importance in the development of ageing and chronic and degenerative disorders, is oxidative stress. Oxidative stress results from an imbalance between oxidant and antioxidant substances. Also, biological systems are continuously exposed to oxidants, either from endogenous metabolic reactions or exogenous sources (Liguori et al., 2018). Nevertheless, regular PA lowers peroxidation levels and improves antioxidant defences across all adult populations and by performing regular PA elderly physically active individuals have shown similar antioxidant activity and lipid peroxidation to young inactive individuals (Bouzzid et al., 2018), reinforcing the importance of PA throughout the lifetime. Therefore, at any age, however with particular interest in the older populations, regular moderate and vigorous PA is recommended to counteract and attenuate the negative effects of oxidative stress on health (Simioni et al., 2018).

1.1.3. Body composition and physical fitness

During the ageing process, decreases are shown in lean mass and increases in fat mass, especially in the abdominal area (St-Onge, 2005). The imbalance of energy intake and expenditure, high-calorie foods and an inactive lifestyle increase the obesity levels, that together with the loss of lean mass and increases in fat mass, usually observed during ageing, are the major contributors for the prevalence of T2DM and CVDs (Dominguez & Barbaggio, 2016). Muscular strength also deteriorates with age by ~12–14% per decade in over 50-year-olds (Hurley & Roth, 2000) and lower body strength is commonly the most affected by the ageing process (Candow & Chilibeck, 2005), leading to an increased risk of falls, and consequently, fractures (Deandrea et al., 2010). Arm and leg muscle strength, aerobic endurance, ability of change direction, and dynamic balance have been associated with a lower risk of falls, with individuals that showed better balance and lower risk of falls also presenting better leg muscle strength, aerobic endurance, agility and dynamic balance (Toraman & Yildirim, 2010). Additionally, grip strength is a predictive biomarker of general strength and function, bone mineral density (BMD), fractures and falls, nutritional status and comorbidity, cognition, depression, sleep, hospital-related variables and mortality (Bohannon, 2019). Therefore, although during ageing individuals tend to decrease muscle mass, and consequently, decline their physical fitness, being able to maintain or increase muscle mass and physical fitness is crucial for the ability to perform daily activities (Wang et al., 2020), and consequently, to be independent. Although several factors influence the changes in body composition and physical fitness during the ageing process, regular and adequate PA are recommended to maintain and improve body composition markers and muscular fitness (Izquierdo et al., 2021).

1.1.4. Musculoskeletal health

The musculoskeletal system progressively changes during the ageing process and develops specific adverse structural and morphological characteristics, including loss of muscle mass, strength and bone mass (Frontera, 2017). Muscle and bone mass losses result in a poor quality of life and impaired mobility for older individuals (Padilla Colón et al., 2018). Additionally, the age-related alterations in the musculoskeletal tissues compromise the individual's capacity to perform the daily activities, as well as other more demanding tasks (Frontera, 2017).

Reductions in muscle strength and mass can be observed during ageing, as well as increases in fat and connective tissue that, consequently, cause a decline in the muscle quality. In addition, a reduction in the number of muscular fibers (particularly type II) and changes in

fiber type distribution (i.e., increase in type I and reduction in type II fibers) can also be observed during ageing (Frontera, 2017). These age-related alterations can lead to the development of sarcopenia. Sarcopenia is described as a progressive and generalized loss of skeletal muscle mass and strength, or physical performance (Cruz-Jentoft et al., 2010) and has been associated with metabolic impairment, cardiovascular risk, physical disability, and mortality (Atkins & Wannamethee, 2020). Furthermore, the higher the severity of sarcopenia the higher is the risk of falls and it is progressively related to reduced postural balance in the elderly population (Gadelha et al., 2018). Muscle mass is a source of myokines that can influence bone mass by stimulating bone formation (Hamrick, 2011). Several proteins produced by the skeletal muscle are dependent upon muscle contraction. Thus, lack of PA probably leads to an altered myokine response, which may provide a potential mechanism for the association between sedentary behaviour and many diseases (Pedersen & Febbraio, 2012). Nevertheless, the age-related changes observed in the musculoskeletal system, and consequently, the prevalence of sarcopenia, can be partially modified with an active lifestyle and appropriate exercise training (Frontera, 2017).

Bone is responsible for several mechanical functions such as providing for rigidity and shape, protecting, and supporting the body structures, and locomotion (Datta et al., 2008). In addition, it is a highly dynamic tissue, constantly undergoing remodelling, repairing itself, e.g., after a fracture (Datta et al., 2008). Bone remodelling is a continuous process throughout life, however, with age bone remodelling is reduced leading to a negative bone balance, and consequently, to bone mass loss (Demontiero et al., 2012). Bone mass loss and the prevalence of osteoporosis increases with age (Hernlund et al., 2013). Osteoporosis is a disease described as reduced bone mass and disruption of bone architecture, resulting in increased risk of fragility fractures (World Health Organization Scientific Group on the Prevention Management of Osteoporosis, 2003). It affects millions worldwide, and, therefore, it is considered as a major public health problem. Although osteoporosis has higher incidence in women than men, in the European Union, 6% of the middle-aged-to-elderly males suffer from this disease (Hernlund et al., 2013), being male osteoporosis underestimated and underdiagnosed (Vescini & Chiodini, 2021). The most common osteoporotic fractures are vertebral, hip, forearm and proximal humerus fractures (Johnell & Kanis, 2005). Thus, in order to prevent current or future fractures, it is crucial to optimize bone accumulation during childhood and adolescence (Weaver et al., 2016) and to maintain bone and muscle mass in later life, counteracting the effects of ageing on bone health (Novotny et al., 2015).

BMD is considered as the gold standard for osteoporosis diagnosis (Kanis, 2002) and therefore, is important for determining the risk of potential fractures (Wheater et al., 2013). It represents the amount of bone mass per unit volume (volumetric density, g/cm^3), or per unit area (areal density, g/cm^2), and it is measured by densitometric techniques (Weaver et al., 2016). In addition, recently, significant developments have been made in the identification and characterisation of specific bone turnover markers for use in the therapeutic management of osteoporosis. Bone turnover biomarkers express the metabolic activity and are normally categorised as markers of bone formation or bone resorption. Bone formation (osteoblasts) and bone reabsorption (osteoclasts) cell activity markers are usually measured in the serum or plasma (Wheater et al., 2013). Markers of bone formation [e.g. procollagen type-1 amino-terminal propeptide (P1NP) and osteocalcin (OC)] are products of active osteoblasts expressed during various phases of their development or osteoblastic enzymes, and reflect different aspects of osteoblast function, while markers of bone resorption [e.g. carboxy-terminal type-1 collagen crosslinks (CTX)] are degradation products of type I collagen (Vasikaran et al., 2011; Wheeler et al., 2013). Osteocytes are fully differentiated osteoblasts that can detect changes in bone morphology (i.e., micro-fractures) due to their sensitivity to mechanical forces, and consequently, act as bone mechanoreceptors (Dallas et al., 2013). In addition, osteocytes regulate the function of both osteoclasts and osteoblasts, therefore playing an important role in bone remodelling (Dallas et al., 2013). Sclerostin is a protein secreted by osteocytes that acts as an inhibitor to the Wnt signalling pathway (the pathway that regulates bone homeostasis, repair, and regeneration by influencing stem cell proliferation, differentiation, and maintenance in the bone) (Houschyar et al., 2018). Increased levels of sclerostin block the Wnt effects on osteoblasts, and consequently, decrease bone formation (Li et al., 2005). Thus, sclerostin works as a negative regulator of bone mass.

During the bone remodelling process, osteocytes, osteoblasts and osteoclasts act in a coordinated manner to form or resorb bone as necessary, and their activation is controlled by a range of stimuli including mechanical forces applied to the skeleton, apoptosis of osteocytes, calciotropic and sex hormones, cytokines and local factors (Datta et al., 2008). Bone is a highly adaptative tissue, able to increase or decrease the amount of bone depending in its loading, and it adapts to dynamic loading considerably more than static loading, different strain magnitudes and frequencies (Mellon & Tanner, 2012). PA produces mechanical load (impact and muscle forces) on the bones, activating the mechanosensitive bone cells (i.e., osteocytes), and consequently, signalling molecules to activate osteoblasts and osteoclasts (Weaver et al., 2016). Furthermore, to start an osteogenic response, bone needs to be submitted to a different

strain magnitude, exceeding the habitual strain range in the predominant loading direction (Weaver et al., 2016) and to avoid static and repetitive low magnitude loads (Mellon & Tanner, 2012). Therefore, performing PA and/or exercise that promotes high impact mechanical load is important for bone health, as it results in increases in BMD (World Health Organization Scientific Group on the Prevention Management of Osteoporosis, 2003) reductions in the risk of falls, and possibly decreases in osteoporotic-related fractures (World Health Organization, 2010).

According to the WHO, there are several risk factors that influence bone health and the prevalence of osteoporotic fractures, i.e., trauma, low BMD levels, previous fractures, genetics, poor nutrition, physical inactivity levels, cigarette smoking, alcohol consumption, high BMI, sex hormone deficiency (oestrogen), and prevalence of other diseases and disorders (e.g. endocrine and metabolic). Therefore, modifying behavioural factors such as reducing physical inactivity levels, may have a significant positive impact on muscle and bone mass and consequently, on the incidence of osteoporotic fractures in the future generations (World Health Organization Scientific Group on the Prevention Management of Osteoporosis, 2003).

1.2. Sex-related differences in the ageing process

There is a higher proportion of women than men with advanced age, as women's life expectancy is higher than men's (World Health Organization, 2019). This different sex longevity is influenced and explained by biological and behavioural sex-differences.

The development and progression of CVDs differ between men and women due to intrinsic biological sex-differences and to sex-differences in how age-related alterations in cardiac and vascular anatomy and physiology progress over the time (Merz & Cheng, 2016). Usually, age-related changes in cardiac anatomy can be observed with increases in the left ventricular wall thickness, and consequently, decreases in left ventricular size and concentricity (Merz & Cheng, 2016). These age-related changes are associated with cardiovascular events risk factors (Lieb et al., 2009). A major difference between men and women regarding cardiac anatomy is that throughout life, men have greater left ventricular mass, wall thickness and cavity dimensions than women (St Pierre et al., 2022). Besides cardiac changes, during the ageing process, alterations are also observed in vascular function, such as, increasing endothelial dysfunction and arterial stiffness, combined with increasing SBP and pulse pressure (Lakatta & Levy, 2003). Here, sex-differences can be found with men having greater

endothelial dysfunction and arterial stiffness than women in all age spectrum, until the sixth decade where arterial dysfunction starts progressing at a faster rate in women than in men (Merz & Cheng, 2016). These sex-differences in vascular function contribute for different hypertension prevalence during men's and women's life course. Before 45 years of age, men have higher prevalence of hypertension than women, between 45–64 years men and women show similar levels of hypertension and, after 65 years women overcome men (National Center for Health Statistics, 2015). CVDs remain the most common cause of death in Europe for both men and women, though, interestingly, more women die from CVDs than men. Nevertheless, the rates of morbidity and death are higher in men, in over 70-years-olds (Townsend et al., 2022). It has been reported a higher decline in myocytes and systolic function, a higher rate of death due to ischemic heart disease, and a higher incidence and death rates of stroke, for men than women. On the other hand, women suffer from higher incidence of diabetes in youth, while men experience it in midlife, and women have higher incidence and death rates due to hypertension and heart disease than men (Hägg & Jylhävä, 2021). In summary, biological aspects influence sex-differences in the prevalence and development of CVDs, nevertheless, lifestyle factors such as dietary habits, physical inactivity, smoking, and adiposity, can also influence sex-differences in the prevalence and development of CVDs, as the alteration of the lifestyle factors mentioned before have substantial effects on cardiovascular risk (Mozaffarian et al., 2008).

Regarding metabolic health, ageing is associated with an increase in plasma LDL cholesterol in men and women (Abbott et al., 1983). Nevertheless, menopause is often responsible for an increase of plasma LDL cholesterol, and after 50 years, women regularly have higher plasma total cholesterol than men from the same age group. Men usually decrease plasma HDL cholesterol concentrations during puberty and early adulthood, comparing to childhood, and remain lower than women within the same age group. Furthermore, in men, TRG concentrations increase gradually reaching peak values between 40–50 years and then decrease afterwards, while women show increases in TRG throughout time (Kreisberg & Kasim, 1987). This sex-differences may be related to hormonal differences since, as it has been reported that postmenopausal women show higher plasma LDL and TRG concentrations than premenopausal women, within the same age-group, indicating that oestrogen levels are important for lipid profile (Auro et al., 2014). Older men have higher prevalence of T2DM than women, and the prevalence of T2DM and fasting plasma glucose levels have been associated with the larger amount of visceral fat mass usually found in men (Nordström et al., 2016). The prevalence of obesity is greater in women than men, and increases with age (Chooi et al., 2019).

Also, women have higher total and gynoid fat mass compared to men within the same age group (Nordström et al., 2016). However, one of the explanations for the sex-differences in T2DM prevalence maybe the higher visceral fat mass normally reported for older men than women.

Men show better physical fitness than women, being stronger, faster and showing greater muscle mass, which can be explained by the higher testosterone levels (Hägg & Jylhävä, 2021; Peiffer et al., 2010). Women, start losing bone mass earlier than men and at a faster pace, especially due to menopause, and men normally show a higher hip BMD and lumbar bone mineral content (BMC) than women (Hernlund et al., 2013). In 2010, around 6% of men and 21% of 50–84 year-old women suffered from osteoporosis in the European Union (Hernlund et al., 2013). Women above 50 years show four times higher rate of osteoporosis than men (Alswat, 2017). Moreover, the risk of osteoporosis-related fractures is higher for women than men (Hernlund et al., 2013). Nevertheless, men have higher rates of fracture-related mortality than women (Center et al., 1999). Furthermore, the mortality risk is higher, after the first 5 years of the first fracture in all types of fractures for both men and women, with hip fracture-associated mortality remaining elevated for up to 10 years (Bliuc et al., 2009).

To summarize, cellular and molecular mechanisms of ageing are initially better preserved in women, due to a better cellular maintenance system and protection provided by oestrogens. However, after menopause, women can reach the same ageing levels as men. Furthermore, although women present worse physical function and health in later life, they still outlive men (Hägg & Jylhävä, 2021).

The increased life expectancy shown by women compared to men is explained by several causes, and 33 of the 40 leading causes of death, contribute more to reduced life expectancy in men than in women. This because, ischaemic heart disease, accident road injuries, lung cancer, chronic obstructive pulmonary disease, stroke, cirrhosis of the liver, tuberculosis, prostate cancer and interpersonal violence are the causes of death that most contribute to a lower life expectancy for men than women (Beltrán-Sánchez et al., 2008). For some diseases, the death rates are similar in men and women (when they are exposed to the same risk), however the exposure to risk differs because of gender-related factors (i.e., occupation, access to diagnosis and treatment), which helps to understand the longevity differences between men and women (World Health Organization, 2019). As it has been mentioned before, behaviour and environment factors help to understand the sex-differences during the ageing process. In fact, the death rates sex-differences in many non-communicable diseases are due to the exposure to several modifiable risk factors (i.e., tobacco use, harmful use of alcohol, diet and physical inactivity) that varies between men and women (World Health

Organization, 2019). Historically, men have higher mortality rates from smoking-related diseases and consuming more alcohol than women (McCartney et al., 2011). Additionally, men tend to make less use of health services and to visit less a doctor than women, due to norms of masculinity and other socioeconomic factors (Novak et al., 2019). Also, men usually are harder to engage in exercise interventions than women, when the participation in the program does not align with their concepts of masculinity, as men tend to have a negative view of health promotion programmes and healthcare professionals, and the male gender role does not tend to include seeking care (Anderson et al., 2016). Still, men have lower physical inactivity levels than women (40% vs. 49%, respectively) (European Commission, 2022), as women are often responsible for multiple functions (i.e., childcare, household care-giving and professional duties), consuming women's time and energy (World Health Organization, 2019). Nevertheless, the low levels of regular PA usually presented for both men and women are a concern, as PA and exercise promote benefits in overall health for both sexes (World Health Organization, 2022).

To summarize, in terms of behaviour and environmental factors, men show higher levels of PA than women, which prevents the development of non-communicable diseases. On other hand, men expose themselves to other risk factors (i.e., higher smoking levels and alcohol consumption), and tend to make less use of health services (i.e., visit less a doctor) than women, which leads to higher associated death rates. These facts help to understand the longevity differences between men and women.

1.3. Importance of healthy ageing

The number of people aged 60 years or older is increasing worldwide (United Nations Department of Social and Economic Affairs, 2007). In 2019, 1 billion of the world's population was aged 60 years or more and this number tends to increase with an estimate of 1.4 billion by 2030 and 2.1 billion by 2050. Living a longer life is not just important for older people and their relatives, but also for society, as older people can have a positive contribution in different ways to their families and communities. Nonetheless, this contribution will always depend on a major factor, their health. Healthy ageing is defined by the WHO as the process of developing and maintaining the functional ability that enables well-being at old age (World Health Organization, 2015). Variations in older people's health have been observed, with some of these being due to genetic factors, however, most of them being due to the individuals' physical and

social environments (their homes, neighbourhoods, and communities) and personal characteristics (sex, ethnicity, or socioeconomic status). The environments that surround people as children combined with individuals' personal characteristics have long-term influence on how people age (Commission on Social Determinants of Health, 2008). The physical and social environments where people are inserted in can affect health directly, through barriers or incentives that affect opportunities, decisions, and behaviour. There are several behaviours that contribute for healthy ageing such as a consumption of a balanced diet, performing regular PA, and abstaining from tobacco use, as these behaviours will reduce the risk of non-communicable diseases, improve physical and mental capacity and delay or avoid care dependency (World Health Organization, 2015).

As it been explained before, ageing is associated with the development of several diseases. However, healthy ageing helps to delay and/or counteract the effects of ageing on health. The disease burden at older age is developed due to the prevalence of noncommunicable diseases, therefore, it is important to target the risk factors responsible for these conditions. Furthermore, important strategies such as enabling healthy behaviours and controlling metabolic risk factors, help to reduce the burden of disability and mortality, especially when these strategies start in early life and are maintained throughout the life course (Michel et al., 2008). Therefore, to have the opportunity to live longer and with high quality of life and independence, is a major concern for society (World Health Organization, 2015).

1.4. Broad-spectrum health effects of recreational team sports

As it has been previously described, meeting the recommended guidelines for PA is important for health benefits and may help to reduce and counteract the main ageing effects on health (Bull et al., 2020). Thus, increased levels of PA can reduce the risk of disease development and the use of medication therapy, which consequently, reduces the large global economic burden on the health systems (World Health Organization, 2022). Nevertheless, in the European region, ~30% of males aged 45–69 years and >40% of males with more than 70 years do not meet the recommended guidelines for PA (World Health Organization, 2022). The three main reasons appointed to not exercise or play sport are lack of time (41%), lack of motivation or interest (25%) and fear of having a disability or illness (14%) (European Commission, 2022). This means that finding exercise programmes that are time-effective, motivating, and safe is an important concern to elevate the PA levels.

The PA guidelines for adults and older adults (≥ 65 years) comprise at least 150–300 min/week of moderate-intensity aerobic PA or 75–150 min/week of vigorous-intensity aerobic PA, or an equal combination of both. Additionally, it is recommended in at least 2 days/week muscle-strengthening activities at moderate or greater intensity that involve all major muscle groups. For older adults, adding multicomponent activities for balance and strength development for at least 3 days/week, is also suggested (Bull et al., 2020). Multicomponent exercise modalities are characterised by a combination of resistance, endurance, and balance training, and exercise programmes using a multicomponent approach are considered the best option to improve the rate of falls, gait ability, balance, and strength performance in physically fragile elderly when compared to other exercise programmes that comprise these exercise modalities separately (Cadore et al., 2013).

Recreational team sports, which are an adaptation of the official versions, using modified rules (i.e., less players, smaller areas and other adapted rules), are an example of multicomponent exercise (Castagna et al., 2020a). For more than 15 years, recreational team sports have been studied as exercise intervention programmes aiming at promoting broad health effects, while meeting the recommended PA guidelines (Castagna et al., 2020a; Krstrup et al., 2010; Krstrup et al., 2018a; Krstrup & Krstrup, 2018b; Krstrup et al., 2009; Milanović et al., 2022; Milanović et al., 2015; Milanović et al., 2019). Additionally, recreational team sports are considered as intrinsically motivating through positive social interaction and play (Hornstrup et al., 2018; Nielsen et al., 2014) indicating potential to ensure long-term adherence to exercise. Moreover, studies addressing recreational team sports have reported low injury occurrence (Castagna et al., 2020a).

To this point, recreational football is by far the most studied sport, showing beneficial effects on VO_{2max} for healthy young and middle-aged populations, untrained men and women with mild-to-moderate hypertension, T2DM patients, untrained elderly populations and prostate cancer patients (Milanović et al., 2015). Additionally, improvements in BP, resting HR, body fat mass, LDL cholesterol and countermovement jump performance, have been observed with recreational football practice for different populations and age-groups (Milanović et al., 2019). Furthermore, recreational football exercise programmes lasting 12–64 weeks have showed a large effect on bone turnover markers and a beneficial effect on lower limb BMD across all age groups (Milanović et al., 2022). Recreational football, played as small-sided games (SSGs), has proven to induce several cardiovascular, metabolic, body composition, physical fitness, musculoskeletal benefits for different populations and age groups (Milanović et al., 2022; Milanović et al., 2015; Milanović et al., 2019). Other recreational team sports such as

recreational futsal, basketball, floorball, volleyball, touch rugby and team handball (TH) have also been studied regarding their game-specific demands and used as health-enhancing exercise programmes for young adults, adult/middle-aged and older populations (Castagna et al., 2020a; Pereira et al., 2021; Teixeira et al., 2024).

Recreational futsal played 60 min/week for 12 weeks by adult/middle-aged males (44.5±4.7 years) has showed to induce benefits on cardiovascular health by increasing VO_{2max} and decreasing SBP and mean BP, with no effects on metabolic health (Beato et al., 2017). Other study with adult/middle-aged males with treated hypertension, showed that recreational futsal, played 2–3 60 min sessions/week for 12 weeks, was effective in increasing VO_{2peak} , aerobic performance, left femur BMC and postural balance, in reducing resting HR, HbA1c and fasting blood glucose, although with no effects in BP (Teixeira et al., 2024).

Recreational basketball, played as SSGs (3v3 and occasionally 2v2) 2–3 times/week for 12 weeks for adult/middle-aged untrained male participants (20-42-year-old), in a full and half court, resulted in health benefits (Randers et al., 2018b). Both groups (full vs. half-court) improved their VO_{2max} and the group that played in a full-court showed reductions in SBP and mean arterial BP. However, like recreational futsal, no changes were observed in metabolic profile for both groups compared to the control group (CG). Nevertheless, both groups (full vs. half-court) reduced body and android fat mass and the half-court group showed an increase in lean body mass. The full-court group also showed an increase in whole body BMD (Randers et al., 2018b).

Recreational floorball played 2.2 sessions/week for 12 and 1.7 sessions/week for 26 months by untrained older men (66-78-year-old) showed no improvements in VO_{2max} . However, after 12 weeks of recreational floorball combined with a diet intervention, decreases in body and abdominal fat mass were observed, and after 26 months, a positive reduction in HbA1c and maintenance of muscle mass levels were also reported (Pedersen et al., 2018; Vorup et al., 2017b). Pre and postmenopausal women, aged 48 and 51–52 years, respectively, playing recreational floorball 2 60 min sessions/week for 12 weeks, showed improvements in VO_{2max} (Nyberg et al., 2014; Seidelin et al., 2017) and aerobic performance (Yo-Yo intermittent endurance level 1 test; YYIE1) (Seidelin et al., 2017). After 12 weeks, pre and postmenopausal women showed decreases in DBP, increases in whole-body lean and leg muscle mass and leg BMD, and postmenopausal women showed increases HDL cholesterol (Nyberg et al., 2014; Seidelin et al., 2017). In addition, postmenopausal women also showed an increase in P1NP, while premenopausal women increased OC (Seidelin et al., 2017). Furthermore, after 40 weeks, both groups (pre and postmenopausal women) were able to maintain their previous

achievements and reduce HbA1c, even when reducing the training volume to once a week (Seidelin et al., 2017). Other study combining recreational floorball and cone ball training with protein load supplementation, for 12 weeks with older untrained adults (72 ± 6 years), resulted in a decrease of resting HR for one of the intervention groups (low-protein group), however, no BP effect was observed in this study for any group. Also, an increase in leg muscle mass was observed for the group that consumed a high vs. low-protein supplementation (Vorup et al., 2017a).

Recreational volleyball played 2–3 80-min sessions/week, for 10 weeks, by untrained adult/middle-aged men (33–55 years) with previous experience with the sport resulted in increased aerobic performance and decreased resting HR (Trajković et al., 2020).

Touch rugby, which is an adaptation of rugby, was created to reduce the risk of collision, and consequently, injuries. This modality discourages tackling and is usually played as SSGs (6v6). Recreational touch rugby played 3 sessions/week, for 8 weeks by inactive adult/middle-aged males (49 ± 7 years) showed an improvement in VO_{2max} at $80\%HR_{max}$, reductions in HbA1c and body fat mass, and an increase in body fat free mass (Mendham et al., 2014, 2015). Additionally, recreational touch rugby practice for 90 min/week, for 12 weeks with men and women aged 30–64 years, also resulted in an improvement in VO_{2max} and a reduction in resting HR (Filliau et al., 2015).

Recreational TH has been used as an exercise intervention for different populations and intervention durations, and it is the second most studied recreational team sport (after recreational football). VO_{2max} and aerobic performance have significantly improved, after 12–16 weeks, in adult/middle-age male former TH players (35–55 years), young unexperienced adult men (20–30 years), overweight premenopausal (35–50 years) and postmenopausal women (average age of 67 years) (Hornstrup et al., 2019; Hornstrup et al., 2020; Pereira et al., 2020; Póvoas et al., 2018), while young untrained adult women (20–30 years) only showed improved aerobic performance, after 12 weeks of recreational TH (Hornstrup et al., 2018). Additionally, adult/middle-aged male former TH players exhibited additional cardiometabolic improvements, after 12 weeks of recreational TH, by reducing DBP, mean BP, resting HR, TRG, fasting glucose, and by improving LDL cholesterol (Póvoas et al., 2018). After 16 weeks of recreational TH it was also possible to observe a decrease in fasting insulin in postmenopausal women (Pereira et al., 2020). Recreational TH played as SSGs for 12 weeks also resulted in an increase of total muscle mass for young untrained adult women (Hornstrup et al., 2018) and in a decrease of body fat mass for young unexperienced adult men (Hornstrup et al., 2019). Overweight premenopausal women without experience with TH also showed

decreases in body and android fat mass, after 16 weeks of recreational TH practice (Hornstrup et al., 2020). Playing recreational TH has also shown to be effective in improving bone health, namely, by increasing OC and CTX levels for young untrained adult women and men, respectively, and by showing positive changes in proximal femur BMD (Hornstrup et al., 2019; Hornstrup et al., 2018). Additionally, after 16 weeks, increases in PINP were observed for postmenopausal women (Pereira et al., 2021).

To summarize, different recreational team sports interventions aiming at different age groups, have shown positive health changes impacting multiple organs and systems. Nevertheless, randomised control trials (RCTs) with older populations without experience with team sports are warranted, as this age group population is increasing worldwide, and this type of multicomponent exercise interventions have potential to improve broad-spectrum health and can be easily implemented in the community.

1.5. Specific demands and organizational settings of recreational team sports

Although several health improvements have been shown for exercise interventions using recreational team sports for different populations, choosing the most adequate intervention weekly dose, total volume, session duration, intensity, and organizational settings (i.e., number of players, court/pitch size, type of gender game format, etc) is still a concern.

A dose-response relationship exists between PA and health adaptations with a minimum dose of PA resulting in health benefits, and the benefits increasing with increased dose. Nonetheless, once exceed a certain value, the adverse effects may outweigh the benefits (Lee, 2007). The dose-response effect of PA and conventional exercise modalities such as resistance training has shown that 2 sessions/week (together with 60 s of rest between sets, 2–3 sets/exercise, 7–9 repetitions/set, and 4 s between repetitions) appear to be more effective in improving maximal voluntary strength in healthy older adults than higher weekly doses (Borde et al., 2015). On the other hand, aerobic training has showed a graded dose-response change in cardiorespiratory fitness across levels of exercise training in postmenopausal women (57.3±6.4 years) (Church et al., 2007). Exercise interventions using recreational team sports frequently use 2–3 weekly 60-min sessions (Bangsbo et al., 2015; Castagna et al., 2020a; Krstrup et al., 2018a), with exclusively two studies using lower weekly training dose [recreational football: 2x15-min/week (Connolly et al., 2014) and recreational futsal: 1x60-min/week (Beato et al.,

2017)]. These lower exercise dosages were effective in improving body composition and cardiovascular outcomes, i.e., reducing fat mass and improving aerobic performance (Connolly et al., 2014), and increasing VO_{2max} and lowering BP (Beato et al., 2017). Nevertheless, to a lower extent when compared to other recreational football interventions using higher weekly doses (Milanović et al., 2019). However, the dose-response health effects of recreational team sports have not been systematically studied yet. Therefore, it is important to ascertain if the training-induced effects on overall health and physical fitness are related to exercise dose applied in this type of exercise interventions. From a practical point of view, this analysis is relevant for effective exercise prescription and for determining public health guidelines and policies.

In this regard, recreational team sports training intensity should also be analysed. High-intensity exercise training has been associated with positive health adaptations (Batacan et al., 2017), and it has been suggested that recreational team sports health benefits are related to its high-intensity physical and physiological demands (Castagna et al., 2020a; Milanović et al., 2022; Milanović et al., 2019). In fact, VO_{2max} improvements have been associated with the time spent at high-intensity, namely, with $HRs >90\%HR_{max}$ (Póvoas et al., 2018). Additionally, recreational team sports have been characterised as a high-demanding physical stimulus, as shown by its locomotor movement pattern and specific actions. These high physical demands together with the high-intensity internal load imposed have been proposed as the most likely explanations for the observed health improvements (Castagna et al., 2020a).

Exercises interventions using recreational football usually include 3v3 to 7v7 game formats for 45–60 min training sessions which results in mean and peak HRs of 76–89 and 94–99 $\%HR_{max}$, respectively and 11–21% of total match time $>90\%HR_{max}$. Additionally, blood lactate (BL) concentrations range from 2.6–7.4 mmol·l⁻¹ and rating of perceived exertion (RPE) from 3.9–6.7 arbitrary units (AU; 0–10 scale). Moreover, recreational football is characterised by average distances covered of 3800–5000 m, high-intensity running distances of 400–900 m and a total number of high-demanding activities ranging between 886–951 (including multiple turns, jumps, sprints with accelerations and decelerations) (Fløtum et al., 2016; Krstrup et al., 2018a; Krstrup et al., 2009; Randers et al., 2014; Randers et al., 2010; Randers et al., 2018a). Recreational futsal demands have also been analysed for inactive adult males (44±3 years) and for adult/middle-aged males with treated hypertension (48±8 years), showing mean HRs of 81–85 $\%HR_{max}$ by playing 4v4 and 5v5 matches for 30–52 min sessions (Beato et al., 2016; Teixeira et al., 2024). Recreational futsal, played by adult/middle-aged males with treated hypertension, has also showed peak HRs of 89% HR_{max} , 19% of total match

time $>90\%HR_{max}$ and RPE values of 6.6 AU (0–10 scale) (Teixeira et al., 2024). Distance covered during the matches by inactive adult males was 3412 ± 381 (Beato et al., 2016). Moreover, recreational basketball played as full- and half-court by untrained adult males (20–42 years) has showed mean and peak HRs of 84–85 and 94–95% HR_{max} and ~24–28% of total match time $>90\%HR_{max}$, during 3v3 and occasionally 2v2 game formats for 48 min sessions (Randers et al., 2018b). Recreational floorball, played as 3v3 to 6v6 for 30–40 min sessions, has showed mean HRs between 72–85% HR_{max} and 4–25% of total match time $>90\%HR_{max}$ in pre and postmenopausal women and, older men and women (Nyberg et al., 2014; Pedersen et al., 2018; Seidelin et al., 2017; Vorup et al., 2017a; Vorup et al., 2017b). Also, older males (65–76 years) performing recreational floorball reached peak HRs of $95\pm 1\%HR_{max}$ and covered 2100 ± 200 m during 30 min sessions (Vorup et al., 2017b). Furthermore, untrained older men and women performed an average of 111–201 high-intensity specific floorball game actions during 30–40 min sessions (Pedersen et al., 2018; Vorup et al., 2017a). Recreational volleyball for adult untrained experienced males (35–55 years) results in mean HRs of $72\pm 5\%HR_{max}$ and moderate intensity RPE values [3.4 ± 0.6 AU (0–10 scale)], by playing 3v3 and 4v4 game formats for 80 min sessions. Recreational touch rugby for middle-aged men and women showed mean HRs of 83–86% HR_{max} , BL concentrations of ~ 2.3 mmol·l⁻¹, RPE values between 11–14 AU (6–20 scale) and distances covered from 2696–3173 m, during 40–56 min sessions played as 5v5 and 6v6 game formats (Filliau et al., 2015; Mendham et al., 2012; Mendham et al., 2016; Mendham et al., 2014, 2015). Recreational TH specific demands have been described for adult/middle-age male former TH players (35–55 years), young untrained adult women and men (20–30 years), young untrained college male students (20.8 ± 1.1 years), overweight premenopausal (35–50 years) and postmenopausal women (average of 67 years) with mean HRs ranging from 76–85% HR_{max} (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018; Pereira et al., 2021; Pereira et al., 2020; Pereira, 2024; Póvoas et al., 2018; Póvoas et al., 2017; Stojiljković et al., 2020) and peak HRs 88–95% HR_{max} (Pereira et al., 2021; Pereira et al., 2020; Pereira, 2024; Póvoas et al., 2017; Stojiljković et al., 2020), BL concentrations of 2.4–4.4 mmol·l⁻¹ (Pereira, 2024; Póvoas et al., 2017; Stojiljković et al., 2020) and RPE from 4.8–6.3 AU (0–10 scale) (Pereira et al., 2021; Pereira et al., 2020; Pereira, 2024; Póvoas et al., 2017; Stojiljković et al., 2020). Additionally, during 40–60 min sessions of recreational TH (4v4 to 7v7 game formats), distances covered between 1878 to 6012 m (Póvoas et al., 2017; Stojiljković et al., 2020) and a frequency of 38–89 high-intensity specific game actions (Hornstrup et al., 2020; Hornstrup et al., 2018; Pereira, 2024; Póvoas et al., 2017) have been reported.

To summarize, 30–60 min/sessions, 1–3 sessions/weeks, for at least 8–12 weeks of recreational team sports practice seem enough to induce health improvements. Nevertheless, for greater and additional health benefits (i.e., metabolic profile improvements), a longer session duration (40–60 min/session) and higher weekly dose (2–3 sessions/week), and for a prolonged period (at least 16 weeks) appears to be important. Addressing the adequate exercise intensity is also crucial to induce health benefits, i.e., mean HRs of 72–85%HR_{max} and 15–20% of total match time spent >90%HR_{max} has been suggested to induce cardiometabolic adaptations. Moreover, performing high-demanding actions such as multiple turns, throws, jumps, sprints with accelerations and decelerations and running at different speeds and directions, seem to be important for health improvements in musculoskeletal outcomes (Bangsbo et al., 2015; Castagna et al., 2020a; Krstrup et al., 2010; Krstrup et al., 2018a; Milanović et al., 2022; Milanović et al., 2019).

SSGs have been proven to be a good strategy when implementing recreational team sports, especially for untrained and unexperienced populations (Castagna et al., 2020a). The reasons behind the success of SSGs are probably due to the fact that they use smaller areas, less players, for shorter periods of time, don't allow body contact and use smaller and softer balls than the conventional ones. These adaptations of the official team sports rules have several advantages for untrained and unexperienced populations, i.e., recreational team sports that use smaller pitches/courts avoiding long distance sprints, together with not allowing physical contact, have the potential to reduce the risk of injuries (Malone et al., 2018). The use of less players also allows the participants to perform more game actions, and consequently, to be more actively involved in the training sessions, which increases the motivation and adherence to exercise (Nielsen et al., 2014). Also, the fact that SSGs are normally organised in more than two match periods may help the untrained and unexperienced participants to better recover and manage their effort during the training sessions, keeping the demands high throughout the training session, that usually characterises recreational team sports interventions. Therefore, recreational team sports played as SSGs with adapted rules have become an interesting solution to counteract the low levels of PA, showing high physical and physiological demands, a strong social component, and being easy to implement in the community. Furthermore, because recreational team sports exercise interventions using SSGs are often organised by gender it is important to ascertain the physical and physiological demands imposed in each gender during same and mixed-gender game formats, as it is common to observe mixed-gender exercise classes in the community.

CHAPTER II: Aims

2. Aims

Recreational TH has been previously studied showing several health benefits for different populations. Nevertheless, middle-aged-to-elderly men, that are a population with age-related diseases and one of the most affected age-group by physical inactivity, have not been studied so far regarding the health effects of this specific exercise modality. Also, the dose-response health effects of recreational team sports have not been systematically studied yet. Therefore, the main aims of this doctoral thesis are: 1) to determine the dose-response short-term health effects of recreational TH in middle-aged-to-elderly men without experience with the sport; 2) to describe the physical and physiological demands of recreational TH played as different game formats (5v5, 6v6 and 7v7) for middle-aged-to-elderly men; 3) to analyse the working demands of playing 6v6 same vs. mixed-gender game formats for middle-aged-to-elderly men and women; and 4) to examine the health effects of recreational team sports exercise interventions in older populations.

The general hypothesis of this doctoral thesis is that recreational TH is a multicomponent exercise mode resulting in physical and physiological demands that are within the range shown to induce several health improvements, and that those outcomes are proportional to the training volume and intensity (dose-response effect) in middle-aged-to-elderly men, after 16 weeks of recreational TH. Additionally, it is hypothesised that the TH working demands for middle-aged-to-elderly men would be higher when playing recreational TH same- vs. mixed-game formats, and the opposite would occur for women. Furthermore, it is hypothesised that recreational team sports may be effective in improving cardiometabolic health for the older populations. To challenge the general hypothesis four scientific studies were conducted, which resulted in five original articles.

Original article I

The general aim of this study was to analyse the dose-response effects of a recreational TH-based exercise programme on cardiometabolic health and physical fitness of inactive middle-aged-to-elderly males with no previous experience with the sport. The specific aims were to analyse the dose-response effects of recreational TH on:

- (i) Cardiovascular health (cardiorespiratory fitness, aerobic performance, BP and resting HR);

- (ii) Metabolic health (fasting blood glucose, fasting plasma insulin, HbA1c, total, HDL and LDL cholesterol and TRG) and oxidative stress [total antioxidant status (TAS), glutathione reductase (GR) and glutathione peroxidase];
- (iii) Body composition (body and fat mass).

Original article II

The general aim of this study was to analyse the effects of different weekly exercise volumes on bone health, body composition and physical fitness of inactive middle-to-older-aged males, after 16 weeks of recreational TH. The specific aims were to analyse the dose-response effect of recreational TH on:

- (i) Bone health (BMC, BMD, P1NP, OC, CTX and sclerostin);
- (ii) Body composition markers (limbs total, fat and lean mass);
- (iii) Physical fitness (upper and lower body dynamic strength, postural balance, upper body isometric strength and agility).

Original article III

The general aim of this study was to describe the physical and physiological response of playing 5v5, 6v6 and 7v7 recreational TH game formats in the official court (40x20 m) for over 60-year-old men. The specific aims were to analyse:

- (i) HR, BL and RPE responses;
- (ii) Fun levels;
- (iii) Locomotor profile;
- (iv) High-intensity game actions;
- (v) The time-course intensity of the matches, i.e., exercise intensity throughout the three match periods.

Original article IV

The general aim of this study was to analyse the physiological response, the activity profile and the perceived experience of middle-aged-to-elderly men and women when playing same- vs. mixed-gender recreational TH game formats. The specific aims were to analyse:

- (i) HR, BL and RPE responses;
- (ii) Fun levels;
- (iii) Locomotor profile;
- (iv) High-intensity game actions.

Original article V

The general aim of this study was to perform a systematic review and meta-analysis of the health effects of recreational team sports for older populations. The specific aims were to study:

- (i) Cardiorespiratory fitness;
- (ii) BP and resting HR;
- (iii) Lipid profile (TRG and total, HDL and LDL cholesterol)

CHAPTER III: Experimental Work

3. Experimental Work

3.1. Original Article I

Dose-response effect of a recreational team handball-based exercise programme on cardiometabolic health and physical fitness in inactive middle-aged-to-elderly males – a randomised controlled trial

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Dose-response effect of a recreational team handball-based exercise programme on cardiometabolic health and physical fitness in inactive middle-aged-to-elderly males – a randomised controlled trial

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ABSTRACT

This study aimed at examining the dose-response of a recreational team handball (TH) exercise-based programme on cardiometabolic health and physical fitness in inactive middle-aged-to-elderly males without TH experience. Fifty-four inactive middle-aged-to-elderly men (67.5 ± 4.2 years; stature 168.8 ± 6.2 cm; body mass 78.4 ± 10.7 kg; fat mass 27.1 ± 5.3%; BMI 27.4 ± 2.9 kg/m²; VO_{2peak} 27.3 ± 4.8 mL/min/kg) were randomised into three intervention groups performing 1 (TH1, *n* = 13), 2 (TH2, *n* = 15), or 3 (TH3, *n* = 12) 60-min weekly recreational TH-based training sessions, for 16 weeks, and a control group (CG, *n* = 14). A time × group interaction was observed for VO_{2peak}, aerobic performance, fasting plasma insulin and body and fat mass (*p* ≤ 0.043) with TH3 showing the greatest overall effects. Post-intervention differences were observed in aerobic performance (TH3>CG, TH1 and TH2; TH2>CG), body mass (TH3>CG and TH1), fat mass (TH3>CG), VO_{2peak} (TH3>CG) and plasma insulin (TH3>CG) (*p* ≤ 0.040). In conclusion, recreational TH performed for 60-min thrice and twice per week results in improved aerobic performance for middle-aged-to-elderly men. Moreover, it was observed that three weekly sessions were more effective in providing overall cardiometabolic benefits compared to training with a lower weekly frequency. ClinicalTrials.gov ID: NCT05295511.

Trial registration: ClinicalTrials.gov identifier: NCT05295511.

Highlights:

- We observed high intensities and fun levels during recreational TH, organised as formal and small-sided games, for middle-aged-to-elderly men during a 16-week period, independently of the number of weekly training sessions.
- Marked positive effects on aerobic performance and cardiometabolic health were observed in the intervention group that performed 3 weekly sessions.
- The study results indicate that recreational TH training with low frequency and volume results in some beneficial effects on cardiometabolic fitness and health for middle-aged-to-elderly men, but future studies with more participants or longer intervention periods are warranted to explore this possibility.

KEYWORDS

aerobic fitness; ageing; exercise; health; team sports

1. Introduction

Multicomponent exercise programmes, i.e. a combination of resistance, endurance, and balance training,

have shown to be the best strategy to improve the rate of falls, gait ability, balance, and strength performance in physically frail elderly when compared to

exercise interventions focusing in one of these training modalities alone (Cadore et al., 2013). Increasing population levels of physical activity (PA) needs to be prioritised to contribute to the World Health Organization (WHO) member states 2025 global target, especially because recent data have shown this process to be too slow and not on track (Guthold et al., 2018). Recreational team sports (RTS), an adaptation of the official versions, using less players, smaller areas, and other modified rules, are an example of multicomponent exercise (Castagna et al., 2020; Milanović et al., 2019). Training interventions using RTS, including team handball (TH), have demonstrated to have a high adherence and attendance across different age-groups, and to be efficient in upregulating fitness status, cardiometabolic and musculoskeletal health in inactive men and women, with or without previous experience with the sport (Bangsbo et al., 2015; Castagna et al., 2020; Krstrup et al., 2018). In fact, promoting sport for all is a main recommendation in the WHO Global Action Plan on PA 2018–2030 (World Health Organization, 2018). Nevertheless, the dose-response health effects of recreational TH or any other RTS practice is currently missing, as well as the health effects of recreational TH for middle-aged and elderly populations, that have an elevated risk of developing chronic diseases (Schellevis, 2013).

It is widely accepted that a dose–response relationship exists between PA volume and health adaptations (Lee, 2007). However, in contrast to PA or conventional exercise modalities, such as resistance or endurance training (Borde et al., 2015; Church et al., 2007), the dose-response effect of RTS training may differ due to its specific multicomponent characteristics. The reported beneficial health impact of RTS interventions mostly refers to interventions applying 2–3 weekly 60-min sessions over 12–16 weeks (Bangsbo et al., 2015; Castagna et al., 2020; Krstrup et al., 2018). To our knowledge, exclusively two RTS intervention studies used a lower training volume (2×15 -min/week Connolly et al., 2014 and 1×60 -min/week Beato et al., 2017). These lower exercise dosages were still effective in decreasing fat mass and improving aerobic fitness (Connolly et al., 2014), increasing maximal oxygen uptake (VO_{2max}) and lowering blood pressure (Beato et al., 2017), although to a lower extent compared to the higher volume interventions (Milanović et al., 2019). Understanding whether the training-induced effects on health and physical fitness are proportional to the exercise volume for the most common dosages applied in this training type is important for effective exercise prescription, and for public health guidelines and policies. RTS training intensity should also be described, as high-intensity exercise

training has been associated with positive health adaptations (Batacan et al., 2017).

Therefore, this study aimed at examining the dose-response effect of a recreational TH-based exercise programme on cardiometabolic health and physical fitness of inactive middle-aged-to-elderly males with no previous experience with the sport. We hypothesised a positive effect of training volume on health and physical fitness main outcomes (Borde et al., 2015).

2. Methods

2.1. Trial design

This is an open label four-arm parallel randomised controlled trial (RCT). This study report followed CONSORT 2010 guidelines (Moher et al., 2012). The participants were randomly allocated into a recreational TH training-based intervention group performing 1, 2 or 3 60-min weekly sessions or a control group (CG) (1:1:1:1 ratio). Evaluations were performed at baseline and after the 16-week intervention. The participants were instructed not to perform exhausting physical activities during the 48 h preceding the evaluations and had at least 40 h of recovery between the training sessions. A physical education and sport graduated, and TH certified coach was responsible for the instruction of all training sessions, while the research team supervised and monitored the activity.

The primary outcome was cardiorespiratory fitness evaluated as peak oxygen uptake (VO_{2peak}) relative to body mass and secondary outcomes were aerobic performance, cardiometabolic, oxidative stress-related markers and body composition. Sample size estimation for the main outcome was performed for a sample power of 95%, with an effect size of 0.275, at a significance level of 5%, and for a dropout rate of 20%, resulting in a total of 62 participants to be recruited. A computer-generated list of random numbers was used (random.org) for participants' allocation, and stratified randomisation by VO_{2peak} was performed. The allocation sequence and assignment of the participants to the groups was performed by an independent researcher after baseline evaluations, and thus concealed from the researcher enrolling and assessing the participants.

2.2. Participants

Recruitment was initiated three months prior to baseline evaluations through advertisement in social media, flyers/posters, and face-to-face meetings in local senior institutions, in Porto District, Portugal.

Sixty-three inactive middle-aged-to-elderly males (>50 years old) were found eligible and volunteered to participate. After baseline evaluations, the participants were stratified by VO_{2peak} and randomly allocated into four groups: three recreational TH intervention groups, performing 1 (TH1; $n=16$), 2 (TH2; $n=16$) or 3 (TH3; $n=16$) 60-min sessions/week and a CG ($n=15$). Inclusion criteria were: male participants, inactive (i.e. not complying with the PA guidelines for the last 6 months), aged >50 years and with no medical contraindications to perform moderate-to-vigorous PA or incapacity to run or grip a ball. Baseline and post-intervention evaluations were performed at university facilities (Porto, Portugal). Evaluations during the intervention period were performed in a municipality sports hall (Gaia, Portugal). There were 9 dropouts during the intervention period (Figure 1). Therefore, fifty-four participants completed the intervention and were evaluated at the 16-week follow-up (TH1, $n=13$; TH2, $n=15$, TH3, $n=12$, and CG, $n=14$).

2.3. Interventions

The training sessions consisted of a standardised 15-min warm-up (i.e. running, coordination, strength, flexibility, and balance exercises) as previously described (Carneiro et al., 2022), and 3 × 15-min periods of recreational TH

matches (4v4, 5v5, 6v6 or 7v7) interspersed by 2-min breaks performed in an indoor TH court. The game formats 4v4, 5v5, 6v6, and 7v7 were selected as they are typically used in recreational TH interventions that have shown to have the internal and external load demands previously suggested to result in health improvements (Castagna et al., 2020). No body contact was allowed, and softer and lighter TH balls than the official ones were used, as this population had no experience with this sport, and to avoid potential injuries. Other exceptions to the official TH rules included: no exclusions; no substitutions; no dribbling; the participants rotated positions every 3 min in a random order, including the goalkeeper; and the ball was immediately put back in play by the goalkeeper after a goal. The CG was instructed to maintain their habitual daily PA.

2.3.1. Intervention intensity, perceived experience, and attendance

To describe the intervention intensity (matches), internal [cardiovascular strain: mean and peak heart rate (HR) and time spent in different HR zones; mean and peak blood lactate concentrations; perceived experience: respiratory and global rating of perceived exertion (RPE)], and external load (activity profile characterisation: time motion analysis and accelerometer data) demands and fun levels were analysed. Training attendance, cardiovascular strain, perceived experience, and fun levels

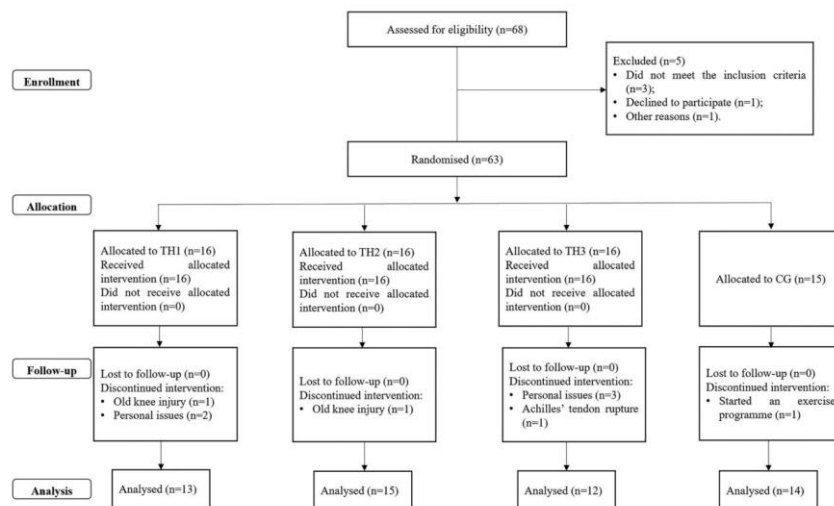


Figure 1. Flowchart for the recruitment process of the fifty-four inactive men.

were registered in all training sessions. The participants were previously familiarised with RPE and fun levels assessment, which was performed at the end of all training sessions. To measure blood lactate concentrations, capillary blood (30 µl) was drawn, and activity profile characterisation (using time-motion analyses and accelerometer data) was performed during 10 training sessions (three matches per participant), according to procedures previously described (Carneiro et al., 2022).

2.4. Outcomes

2.4.1. Cardiorespiratory fitness, aerobic performance, and blood pressure

A laboratory incremental treadmill test until voluntary exhaustion (H/P/Cosmos, Quasar, Germany) (ACSM, 2008) was performed to determine $\dot{V}O_{2peak}$. $\dot{V}O_{2peak}$ and respiratory exchange ratio (RER) were measured by pulmonary gas exchange measurements (Oxycon Pro Metabolic Cart, Jaeger, careFusion, Hochberg, Germany), according to procedures previously described (Carneiro et al., 2022). Time to exhaustion (TTE) was recorded. Aerobic performance (Yo-Yo intermittent endurance level 1 test; YYIE1) was evaluated in an indoor TH court (Bangsbo, 1994) and exercise HR was recorded with short-range telemetry (Firstbeat Technologies Ltd., version 4.5.0.2, Jyväskylä, Finland). Individual maximal HR (HR_{max}) was determined as the highest value reached during the $\dot{V}O_{2max}$ test, the YYIE1 or the matches, according to a multiple testing approach (Póvoas et al., 2019). Systolic and diastolic blood pressure (SBP and DBP) and resting HR were measured in a laboratory by an automatic upper arm blood pressure monitor (multiparameter patient monitor, Omron Z207, Kyoto, Japan) according to standardised procedures (Williams et al., 2018).

2.4.2. Metabolic and oxidative stress-related markers

Before the fasting blood draws, the participants were instructed to follow an 8-h overnight fasting and no further recommendations were given regarding food intake. High- and low-density lipoprotein (HDL and LDL) cholesterol, triglycerides and glucose were collected by fasting blood draws in 8-ml serum separator tubes, by trained nurses, in the morning, in a quiet room and analysed in AU5400[®] (Beckman-Coulter, USA). Fasting blood insulin was determined using a Cobas[®] e411 automated analyser (Roche Diagnostics GmbH, Mannheim) and glycosylated haemoglobin (HbA1c) in Variant II (Bio-Rad[®] Laboratories, Lda., Portugal). Total antioxidant status (TAS), glutathione reductase (GR) and glutathione peroxidase (GPx) were

measured in plasma samples using AU5800 (Beckman-Coulter[®], USA). Pre-to-post-intervention outcome evaluators were blinded to the groups. The intra-assay coefficient of variance was determined using the manufacturer's own controls or sample "pools" according to the EP5-A2 of CLSI (Clinical and Laboratory Standard Institute) protocol in two runs per day in duplicate each one, for 20 days. Triglycerides, HDL and LDL cholesterol, and glucose intra-assay precision was determined using three level of sample "pool" (low, medium, and high) according to the protocol mentioned above. Fasting blood insulin, GR and GPx intra-assay precision was determined using two control levels (low and high) and TAS using three control levels (low, medium, and high) also according to the EP5-A2 (CLSI) protocol.

2.4.3. Anthropometric characteristics and body composition

Body (0.01 kg) and fat mass (%) were measured in a laboratory, by a bioimpedance digital scale (Tanita Inner Scan BC 532, Tokyo, Japan) and stature (cm) was determined by a portable stadiometer (Seca 213, Hamburg, Germany), according to standardised protocols (Norton & Olds, 1996). Body mass index (BMI) was calculated as kg/m^2 .

2.5. Statistical analysis

Results are presented as means \pm standard deviations (SD). To examine the differences between the groups at baseline and after 16 weeks a two-way analysis of variance (ANOVA) for repeated measures with Bonferroni post hoc multiple comparison tests was used. The 95% confidence interval (CI) was considered. Effect size was calculated using partial eta-squared (η^2p) and as Cohen d , and interpreted as small (≥ 0.01), medium (≥ 0.06), or large (≥ 0.14) and as trivial (< 0.2), small (0.2–0.5), medium (0.5–0.8) and large (> 0.8), respectively (Cohen, 1988). One-way ANOVA was used to assess delta values differences between the groups after the 16-week intervention. Statistical Package for the Social Sciences (SPSS Inc., version 23.0) was used. The data were tested for normality using the Shapiro-Wilk test. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Baseline data

Participants' baseline data were 67 ± 4 years, stature 169 ± 6 cm, body mass 78.7 ± 10.9 kg, fat mass $27.5 \pm 5.1\%$, BMI 27.6 ± 3.1 , $\dot{V}O_{2peak}$ 27.3 ± 4.6 mL/min/kg and for those who completed the intervention and were

evaluated at follow-up were 68 ± 4 years, stature 169 ± 6 cm, body mass 78.4 ± 10.7 kg, fat mass $27.1 \pm 5.3\%$, BMI 27.4 ± 2.9 kg/m², VO_{2peak} 27.3 ± 4.8 mL/min/kg; Table 1. There were no significant differences in those variables between the dropouts and the participants that remained in the study (data not shown).

3.2. Intervention intensity, perceived experience, and attendance

Match internal and external load is presented in Table 1. The average weekly attendance was TH1: 0.9 ± 0.3 (14 ± 4 out of 16 sessions), TH2: 1.8 ± 0.3 (28 ± 4 out of 32 sessions) and TH3: 2.6 ± 0.2 (42 ± 3 out of 48 sessions). No significant differences were found between the groups in internal load variables, fun levels and in high-intensity game actions. However, a higher percentage of walking frequency ($p = 0.018$, large) and time spent walking ($p = 0.008$, large) and a lower distance covered in jogging ($p = 0.026$, large) was observed for TH1 than TH3. Also, a lower percentage of time spent in player load zone $>0.1-0.3$ (%) ($p = 0.043$, large) was found for TH1 than TH3.

3.3. Cardiorespiratory fitness, aerobic performance, blood pressure and resting heart rate

A time x group interaction was shown for relative and absolute VO_{2peak} ($p = 0.008$, 0.043 , respectively; Table 2), with TH3 showing higher pre- to post-intervention changes in relative and absolute VO_{2peak} compared to CG ($p = 0.002$, 0.030 , respectively). A significant absolute VO_{2peak} increase (Figure 2) was shown for all intervention groups (TH1: $p \leq 0.001$, 95% CI: 1877–2336, large; TH2: $p \leq 0.001$, 1994–2496, large; TH3: $p \leq 0.001$, 1785–2364, large), while the CG showed no significant pre- to post-changes. Moreover, a significant relative VO_{2peak} increase was observed for all intervention groups (TH1: $p = 0.036$, 6.7–18.6, large; TH2: $p \leq 0.005$, 8.8–21.5, large; TH3: $p \leq 0.001$, 16.6–29.4, large), while the CG showed no significant pre- to post-changes. Moreover, TTE increased for all TH groups (TH1: $p \leq 0.001$, -4.9–47.0, medium; TH2: $p \leq 0.001$, 8.8–29.6, large; TH3: $p \leq 0.001$, 13.6–28.1, large). A time x group interaction was observed for aerobic performance (YYIE1) ($p \leq 0.001$; Table 2). TH3 covered a higher distance than CG, TH1 and TH2 ($p \leq 0.040$), and TH2 covered a higher distance than CG ($p = 0.006$). A significant increase (Figure 2) was observed for the intervention groups in aerobic performance after 16 weeks of recreational TH training (TH1: $p = 0.007$, 20.3–56.0,

large; TH2: $p \leq 0.001$, 31.0–73.6, large; TH3: $p \leq 0.001$, 40.6–120.3, large), while the CG showed no changes.

At post-intervention, SBP decreased for the three intervention groups (TH1: $p = 0.010$, -7.8, -0.5, medium; TH2: $p = 0.042$, -5.7, -0.2, medium); (TH3: $p \leq 0.001$, -9.7, -4.3, large), while was unchanged for CG. DBP decreased for all TH training groups (TH1: $p = 0.002$, -9.2–2.0, large; TH2: $p = 0.022$, -6.9, -0.3, medium; TH3: $p \leq 0.001$, -13.4, -6.1, large), and also for CG ($p = 0.035$, -7.9, -0.7, medium). A significant decrease was also observed for resting HR, though only in TH3 ($p \leq 0.001$, -18.5, -4.1, medium).

3.4. Metabolic and oxidative stress-related markers

A time x group interaction ($p = 0.021$; Table 2) was observed for fasting plasma insulin. After the intervention, a decrease was observed for fasting blood glucose for TH3 ($p = 0.018$, -20.7–5.3, small) and for fasting plasma insulin for the groups that trained 2–3 times a week (TH2: $p = 0.017$; -36.3–11.3, large; TH3: $p = 0.003$, -46.6–34.3, medium). The -5.5 ± 10.3 $\mu\text{mol/L}$ fasting plasma insulin decrease shown in TH3 was different ($p = 0.025$) from the 1.6 ± 2.8 $\mu\text{mol/L}$ increase observed in CG. There were no significant differences in total and LDL cholesterol and triglycerides for any of the groups after the 16 weeks. HDL cholesterol significantly decreased after 16 weeks for CG ($p = 0.004$, -13.9–3.6, large), while the TH groups maintained their baseline values. A significant decrease was observed for HbA1c in TH1 ($p = 0.049$, -5.4–0.3, large), and a significant increase was observed for TAS in TH3 ($p = 0.037$, -1.9, -25.2, medium) and for GR in all groups (TH1: $p \leq 0.001$, 20.6–56.1, large; TH2: $p \leq 0.001$, 19.4–67.1, large; TH3: $p = 0.002$, 5.0–76.9, large; CG: $p \leq 0.005$, 8.3–52.0, medium). Additionally, a significant increase was observed for GPx in CG ($p = 0.036$; 95% 2.6–42.1, medium).

3.5. Body composition

A time x group interaction was observed for body and fat mass ($p \leq 0.032$; Table 2). TH3 showed a higher decrease in body mass than TH1 and CG ($p \leq 0.050$) and in fat mass than CG ($p = 0.032$) ($p \leq 0.035$). After the 16 weeks, a significant body mass decrease was observed in TH2 ($p \leq 0.001$, -3.8, -1.0, large) and TH3 ($p \leq 0.001$, -4.8, -2.1, large) groups and all groups showed a significant decreased in fat mass (TH1: $p \leq 0.001$, -10.2–3.2, large; TH2: $p \leq 0.001$, -14.4, -6.8, large; TH3: $p \leq 0.001$, -18.9–8.1, large; CG: $p = 0.045$, -8.0, -1.6, large).

Table 1. Chronological age, anthropometric characteristics, body composition and cardiorespiratory fitness of the participants in the recreational team handball (TH) groups performing 1 (TH1, $n = 13$), 2 (TH2, $n = 15$) or 3 (TH3, $n = 12$) training sessions per week and the CG (CG, $n = 14$) at baseline, and internal and external load markers of each intervention group during the recreational TH training sessions.

Variables		TH1 ($n = 13$)	TH2 ($n = 15$)	TH3 ($n = 12$)	CG ($n = 14$)
Baseline characteristics	Age (years)	67 ± 5 (60–76)	67 ± 3 (61–72)	68 ± 4 (61–76)	67 ± 5 (59–75)
	Stature (cm)	169 ± 6 (159–177)	168 ± 7 (158–188)	168 ± 6 (156–173)	170 ± 6 (163–180)
	Body mass (kg)	77.1 ± 12.1 (61.8–103.6)	79.2 ± 11.7 (69.1–104.3)	77.6 ± 10.6 (51.3–87.5)	79.3 ± 9.3 (63.9–100.0)
	Fat mass (%)	28.0 ± 6.6 (16.6–41.1)	27.4 ± 3.7 (23.9–35.5)	27.7 ± 6.1 (18.1–36.4)	25.2 ± 4.7 (14.3–31.8)
	BMI (kg/m ²)	26.8 ± 3.1 (23.7–34.2)	27.9 ± 2.8 (24.5–34.7)	27.5 ± 3.3 (21.1–30.9)	27.4 ± 2.8 (20.4–31.1)
	VO _{2peak} (mL/min/kg)	27.0 ± 3.7 (12.3–34.2)	27.5 ± 5.2 (15.8–33.9)	27.1 ± 4.3 (20.9–33.4)	28.8 ± 4.7 (21.0–34.9)
Training sessions	Heart rate				
	Mean HR (b.min ⁻¹)	128 ± 13	127 ± 16	127 ± 11	
	Mean HR (%HR _{max})	80 ± 7	78 ± 10	79 ± 5	
	Peak HR (b.min ⁻¹)	143 ± 13	140 ± 18	141 ± 13	
	Peak HR (%HR _{max})	89 ± 7	86 ± 10	87 ± 8	
RPE and fun	Time above 80%HR _{max} (%)	46 ± 29	40 ± 30	46 ± 16	
	Time above 90%HR _{max} (%)	11 ± 14	7 ± 8	5 ± 7	
	Respiratory RPE (AU, 0–10)	6.0 ± 1.8	6.6 ± 1.3	6.9 ± 1.7	
Blood lactate	Global RPE (AU, 0–10)	6.0 ± 1.9	6.4 ± 1.3	7.0 ± 1.6	
	Fun (AU, 0–10)	8.1 ± 0.6	8.2 ± 1.2	9.0 ± 1.2	
Game actions	Mean blood lactate (mmol·l ⁻¹)	4.9 ± 1.1	3.5 ± 1.5	3.3 ± 1.1	
	Peak blood lactate (mmol·l ⁻¹)	6.9 ± 1.6	4.8 ± 2.0	4.4 ± 1.1	
Locomotor categories	Jumps (n)	7.0 ± 5.6	5.4 ± 2.9	8.1 ± 3.0	
	Throws (n)	5.3 ± 3.0	5.6 ± 2.2	8.4 ± 3.1	
	Stops (n)	9.7 ± 2.2	12.4 ± 4.5	12.4 ± 3.0	
	Changes of direction (n)	9.3 ± 2.3	12.4 ± 4.3	12.0 ± 2.7	
	One-on-one situations (n)	7.0 ± 1.3	7.9 ± 2.1	9.2 ± 1.8	
	Total actions (n)	38.4 ± 13.3	43.6 ± 14.8	50.1 ± 11.3	
	Standing frequency (%)	7 ± 4	4 ± 1	4 ± 2	
	Standing time spent (%)	10 ± 5	7 ± 2	8 ± 3	
	Walking frequency (%)	40 ± 1	38 ± 3	35 ± 4*	
	Walking time spent (%)	56 ± 1	48 ± 2	42 ± 8*	
Accelerometer data	Walking distance (m)	2581 ± 513	2045 ± 434	1858 ± 453	
	Jogging frequency (%)	29 ± 6	32 ± 4	33 ± 3	
	Jogging time spent (%)	22 ± 6	29 ± 9	33 ± 5	
	Jogging distance (m)	1344 ± 339	1698 ± 696	2213 ± 572*	
	Fast running frequency (%)	4 ± 1	6 ± 3	7 ± 2	
	Fast running time spent (%)	1 ± 0	3 ± 2	3 ± 1	
	Fast running distance (m)	128 ± 48	231 ± 163	320 ± 128	
	Sprint frequency (%)	0.1 ± 0.1	0.6 ± 0.8	0.3 ± 0.2	
	Sprint time spent (%)	0.0 ± 0.0	0.2 ± 0.3	0.1 ± 0.1	
	Sprint distance (m)	1.8 ± 1.8	26.0 ± 36.9	32.2 ± 64.8	
	Sideways medium frequency (%)	3 ± 2	3 ± 2	5 ± 3	
	Sideways medium time spent (%)	3 ± 2	3 ± 2	5 ± 3	
	Sideways medium distance (m)	178 ± 124	169 ± 119	274 ± 148	
	Sideways high frequency (%)	0.5 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	
	Sideways high time spent (%)	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	
	Sideways high distance (m)	3.5 ± 7.0	0.1 ± 0.1	0.4 ± 0.5	
	Backward frequency (%)	12 ± 3	14 ± 5	13 ± 4	
Backward time spent (%)	7 ± 2	9 ± 3	9 ± 4		
Backward distance (m)	442 ± 154	507 ± 241	504 ± 254		
HI movement frequency (%)	5 ± 2	7 ± 4	7 ± 2		
HI movement time spent (%)	2 ± 1	3 ± 2	4 ± 1		
HI movement distance (m)	134 ± 53	257 ± 192	352 ± 171		
Total accumulated player load (AU)	Total accumulated player load (AU)	261 ± 74	284 ± 69	326 ± 34	
	Player load zone 0.0–0.1 (%)	19 ± 5	15 ± 5	19 ± 6	
	Player load zone >0.1–0.3 (%)	47 ± 8	42 ± 6	36 ± 5*	
	Player load zone >0.3–0.6 (%)	16 ± 4	18 ± 6	16 ± 3	
	Player load zone >0.6–1.0 (%)	6 ± 2	9 ± 4	9 ± 3	
	Player load zone >1.0–1.5 (%)	7 ± 4	11 ± 5	11 ± 4	
	Player load zone >1.5–2.0 (%)	5 ± 3	3 ± 3	7 ± 2	
	Player load zone > 2.0 (%)	1 ± 2	1 ± 1	2 ± 1	
Total accelerations (n)	28 ± 6	27 ± 13	40 ± 27		
Total deaccelerations (n)	14 ± 3	16 ± 8	17 ± 10		

Data are presented as mean ± SD (range). Abbreviations: AU – Arbitrary units; BMI – Body mass index; CG – Control group; HI – High-intensity; HR – Heart rate; HR_{max} – Maximal heart rate; RPE – Rating of perceived exertion; TH – Team handball; VO_{2peak} – Peak oxygen uptake.

*Significantly different from TH1.

Table 2. Cardiorespiratory fitness, aerobic performance, blood pressure, resting heart rate, metabolic and oxidative stress-related markers, and body composition at 0 weeks (baseline) and after 16 weeks of recreational team handball (TH) training performing 1 (TH1), 2 (TH2) or 3 (TH3) times a week or a continuation of the habitual PA (control group).

Variables	TH1 (n = 13)					TH2 (n = 15)				
	0 weeks	16 weeks	Δ (abs)	Δ (%)	Effect size	0 weeks	16 weeks	Δ (abs)	Δ (%)	Effect size
VO _{2peak} (mL/min)	2106 ± 320	2343 ± 383*	237 ± 188	11.5 ± 9.3	1.327	2200 ± 489	2457 ± 606*	257 ± 214	11.6 ± 9.4	1.415
VO _{2peak} (mL/min/kg)	27.0 ± 3.7	30.5 ± 4.9*	3.5 ± 2.2	12.7 ± 8.4	1.841	27.5 ± 5.2	31.7 ± 6.8*	4.2 ± 3.1	15.2 ± 10.5	1.544
Time to exhaustion (s)	685 ± 160	793 ± 161*	108 ± 136	21 ± 36	0.795	663 ± 155	770 ± 116*	107 ± 83	19 ± 17	1.453
YYIE1 (m)	447 ± 148	629 ± 250*	182 ± 134*	38 ± 27	1.998	531 ± 301	786 ± 425**	254 ± 174*	52 ± 34*	2.061
Systolic blood pressure (mmHg)	134 ± 8	128 ± 9*	-6 ± 8	-4 ± 6	0.708	131 ± 18	127 ± 13*	-4 ± 7	-3 ± 5	0.657
Diastolic blood pressure (mmHg)	77 ± 9	72 ± 8*	-4 ± 5	-6 ± 6	0.978	77 ± 7	74 ± 6*	-3 ± 5	-4 ± 5	0.634
Resting heart rate (bpm)	66 ± 10	65 ± 13	-1 ± 9	-1 ± 13	0.700	68 ± 13	66 ± 10	-2 ± 8	-2 ± 14	0.791
Fasting blood glucose (mg/dl)	102.2 ± 22.8	100.5 ± 18.8	-1.7 ± 13.6	-0.3 ± 14.4	0.127	107 ± 18.1	103.7 ± 13.8	-3.9 ± 11.4	-2.8 ± 9.1	0.368
Fasting plasma insulin (μmol/L)	10.4 ± 4.1	9.1 ± 5.0	-1.3 ± 4.6	-8.4 ± 46.5	0.284	15.8 ± 5.5	11.8 ± 4.5*	-4.0 ± 3.9	-23.8 ± 21.6	1.049
Total cholesterol (mg/dl)	188 ± 46.5	183.0 ± 45.4	-5.4 ± 21.5	-2.5 ± 14.1	0.252	182.4 ± 34.0	173.3 ± 31.6	-9.1 ± 28.9	-3.5 ± 17.5	0.315
HDL cholesterol (mg/dl)	51.9 ± 5.8	52.8 ± 11.3	0.9 ± 8.8	1.5 ± 16.7	0.127	44.9 ± 8.7	44.8 ± 5.7	-0.1 ± 5.4	1.1 ± 11.0	0.031
LDL cholesterol (mg/dl)	122.4 ± 38.2	121.5 ± 31.9	-0.9 ± 14.7	1.6 ± 15.4	0.068	115.1 ± 22.7	108.9 ± 28.8	-6.2 ± 27.1	-1.3 ± 28.5	0.231
Triglycerides (mg/dl)	86.8 ± 32.6	75.8 ± 26.0	-10.9 ± 20.3	-10.7 ± 20.6	0.565	110.3 ± 47.7	97.5 ± 31.9	-12.8 ± 34.6	-5.7 ± 27.4	0.407
HbA1c (%)	5.9 ± 0.8	5.7 ± 0.5*	-0.2 ± 0.3	-2.8 ± 4.0	1.210	5.6 ± 0.5	5.6 ± 0.5	0.0 ± 0.3	-0.1 ± 5.0	0.050
TAS (mmol/L)	1.60 ± 0.10	1.68 ± 0.26	0.08 ± 0.30	5.9 ± 19.7	0.292	1.68 ± 0.17	1.74 ± 0.23	0.06 ± 0.27	4.3 ± 16.5	0.219
GR (U/L)	37.6 ± 9.7	50.8 ± 7.9*	13.1 ± 7.8	38.3 ± 21.2	1.782	34.1 ± 8.1	47.3 ± 5.5*	13.1 ± 7.4	43.2 ± 28.6	1.872
GPx (U/L)	860 ± 121	919 ± 314	58 ± 329	8 ± 39	0.197	734 ± 267	731 ± 219	-3 ± 184	6 ± 33	0.014
Body mass (kg)	77.1 ± 12.1	76.6 ± 2.5	-0.46 ± 1.75	-0.64 ± 2.04	0.284	79.2 ± 11.7	77.3 ± 11.0*	-1.95 ± 2.08	-2.38 ± 2.51	0.984
Fat mass (%)	28.0 ± 6.6	25.8 ± 5.0*	-2.1 ± 2.1	-6.7 ± 5.8	1.561	27.4 ± 3.7	24.4 ± 3.3*	-2.9 ± 2.1	-10.6 ± 6.9	1.458

Table 2. Continued.

0 weeks	TH3 (n = 12)			CG (n = 14)			Two-way ANOVA					
	16 weeks	Δ (abs)	Δ (%)	Effect size	0 weeks	16 weeks	Δ (abs)	Δ (%)	Effect size	Time F (df,error)	Group F (df,error)	Interaction F (df,error)
2074 ± 404	2449 ± 454*	375 ± 206*	18.7 ± 9.4*	1.872	2129 ± 231	2211 ± 278	82 ± 218	4.1 ± 10.3	0.384	52.530 (3,37)	0.262 (3,37)	2.995 (3,37)
27.1 ± 4.3	33.3 ± 6.0*	6.2 ± 2.9*	23.0 ± 9.0*	2.672	28.8 ± 4.7	30.2 ± 5.1	1.4 ± 2.6	5.2 ± 39.4	0.365	<0.001; 0.587	0.853; 0.021	0.043; 0.195
637 ± 78	767 ± 91*	130 ± 60	21 ± 10	2.207	627 ± 147	663 ± 120	36 ± 60	8 ± 14	0.678	75.810 (3,37)	0.139 (3,37)	4.574 (3,37)
429 ± 203	866 ± 471**	436 ± 330**	100 ± 67**	2.079	415 ± 220	320 ± 80	-95 ± 154	-15 ± 22	1.308	<0.001; 0.672	0.936; 0.011	0.908; 0.271
136 ± 9	126 ± 8*	-10 ± 6	-7 ± 4	1.633	134 ± 13	130 ± 13	-4 ± 9	-3 ± 6	0.479	43.506 (3,37)	1.715 (3,37)	1.715 (3,37)
80 ± 8	72 ± 10*	-8 ± 4	-10 ± 6	1.892	77 ± 8	74 ± 8*	-3 ± 6	-4 ± 7	0.506	<0.001; 0.540	0.181; 0.122	0.181; 0.122
72 ± 16	63 ± 11*	-9 ± 11	-11 ± 11	0.737	67 ± 11	63 ± 8	-4 ± 8	-5 ± 12	0.597	35.023(3,40)	2.119(3,40)	9.797(3,40)
113.8 ± 33.0	99.8 ± 15.6*	-14.1 ± 36.6	-7.7 ± 20.4	0.409	102.5 ± 13.3	100.8 ± 11.1	-1.7 ± 7.5	-1.2 ± 7.4	0.236	<0.001; 0.467	0.113; 0.137	<0.001; 0.424
16.2 ± 11.5	10.7 ± 5.7*	-5.5 ± 10.3*	-6.2 ± 63.6	0.603	9.1 ± 3.2	10.6 ± 4.6	1.6 ± 2.8	17.2 ± 29.6	0.625	31.381 (3,49)	0.168 (3,49)	1.452 (3,49)
178.8 ± 31.1	177.2 ± 39.2	-1.7 ± 29.7	-0.6 ± 15.9	0.058	185.1 ± 40.0	172.8 ± 38.4	-12.3 ± 25.1	-5.9 ± 12.8	0.490	<0.001; 0.390	0.918; 0.010	0.239; 0.082
47.7 ± 10.1	47.5 ± 10.4	-0.2 ± 4.1	-0.1 ± 8.6	0.040	54.4 ± 13.6	49.3 ± 12.0*	-5.1 ± 6.1	-8.7 ± 8.9	0.871	44.801 (3,49)	0.118 (3,49)	2.616 (3,49)
107.8 ± 22.8	109.4 ± 31.7	1.7 ± 28.6	2.2 ± 26.5	0.061	107.6 ± 33.8	101.1 ± 30.9	-6.4 ± 19.2	-3.9 ± 16.5	0.338	<0.001; 0.478	0.949; 0.007	0.061; 0.138
117.0 ± 61.7	101.6 ± 47.2	-15.4 ± 38.7	-6.9 ± 37.5	0.425	114.3 ± 39.9	111.6 ± 43.9	-2.7 ± 51.7	-4.8 ± 52.3	0.053	11.402 (3,49)	0.130 (3,49)	1.981 (3,49)
5.9 ± 0.9	5.7 ± 0.5	-0.2 ± 0.5	-2.4 ± 6.7	0.551	5.8 ± 0.4	5.7 ± 0.4	-0.1 ± 0.2	-1.4 ± 3.3	0.423	0.001; 0.189	0.942; 0.008	0.129; 0.108
1.60 ± 0.14	1.77 ± 0.24*	0.17 ± 0.32	11.7 ± 20.2	0.536	1.64 ± 0.10	1.61 ± 0.09	-0.02 ± 0.12	-1.1 ± 7.8	0.188	3.697 (3,48)	0.373 (3,48)	1.075 (3,48)
39.0 ± 7.1	53.6 ± 6.1*	14.6 ± 9.4	41.0 ± 29.0	1.563	38.9 ± 10.5	47.6 ± 4.5*	8.7 ± 12.3	30.2 ± 34.4	0.743	0.060; 0.072	0.773; 0.023	0.369; 0.063
793 ± 278	869 ± 329	76 ± 358	19 ± 47	0.214	829 ± 113	1002 ± 277*	173 ± 281	22 ± 33	0.690	7.655 (3,48)	2.476 (3,48)	3.564 (3,48)
77.6 ± 10.6	75.0 ± 10.2*	-2.68 ± 1.78**	-3.42 ± 2.08**	0.986	79.3 ± 9.3	78.5 ± 9.3	-0.76 ± 1.35	-0.98 ± 1.70	0.571	0.076; 0.138	0.073; 0.134	0.021; 0.182
27.7 ± 6.1	24.0 ± 5.5*	-3.8 ± 2.9*	-13.5 ± 8.5*	1.298	25.2 ± 4.7	24.0 ± 4.4*	-1.2 ± 1.4	-4.8 ± 5.3	0.875	3.706 (3,48)	0.133 (3,48)	0.385 (3,48)
										0.060; 0.072	0.940; 0.008	0.764; 0.024
										1.684 (3,48)	1.868 (3,48)	2.532 (3,48)
										0.201; 0.034	0.148; 0.105	0.068; 0.137
										0.819 (3,48)	0.779 (3,48)	0.377 (3,48)
										0.370; 0.017	0.511; 0.047	0.770; 0.023
										3.805 (3,48)	1.733 (3,48)	0.272 (3,48)
										0.057; 0.073	0.173; 0.098	0.846; 0.017
										6.545 (3,48)	0.219 (3,48)	0.884 (3,48)
										0.014; 0.120	0.882; 0.014	0.456; 0.052
										3.678 (3,45)	1.477 (3,45)	1.092 (3,45)
										0.062; 0.076	0.234; 0.090	0.362; 0.068
										47.554 (3,29)	0.997 (3,29)	0.638 (3,29)
										<0.001; 0.621	0.408; 0.094	0.597; 0.062
										3.385 (3,45)	2.117 (3,45)	0.850 (3,45)
										0.072; 0.070	0.111; 0.124	0.474; 0.054
										36.695 (3,50)	0.165 (3,50)	4.375 (3,50)
										<0.001; 0.423	0.920; 0.010	0.008; 0.208
										71.399 (3,49)	0.487 (3,49); 0.693; 0.029	3.171 (3,49); 0.032; 0.163
										<0.001; 0.593		

Data are presented as mean ± SD. Abbreviations: GR – Glutathione reductase; GPx – Glutathione peroxidase; HbA1c – Glycosylated haemoglobin; HDL – High-density lipoprotein; LDL – Low-density lipoprotein; TAS – Total antioxidant status; TH – Team handball; VO_{2peak} – Peak oxygen uptake; YYIE1 – Yo-Yo intermittent endurance level 1 test.
 *Significantly different from baseline (p ≤ 0.005).
 **Significantly different from control group (p ≤ 0.005).
 †Significantly different from team handball group 1.
 ‡Significantly different from team handball group 2.

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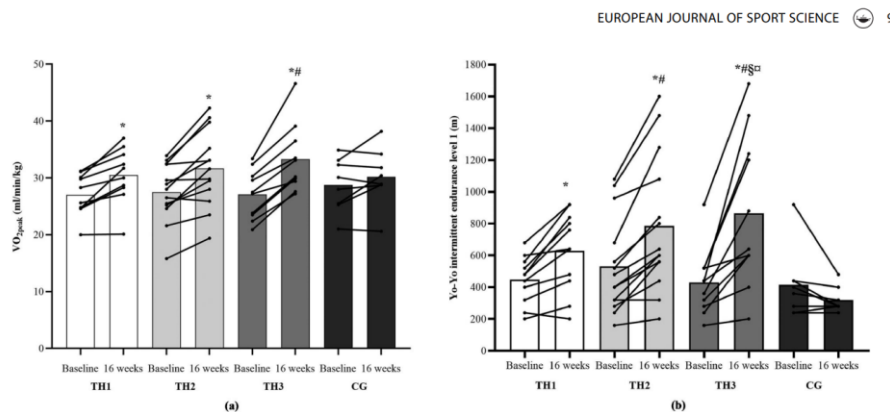


Figure 2. Peak oxygen uptake (mL/min/kg) (a) and yo-yo intermittent endurance level 1 test performance (m) (b) at baseline and after 16 weeks of recreational team handball (TH) training performing 1 (TH1, $n = 13$), 2 (TH2, $n = 15$) or 3 (TH3, $n = 12$) sessions per week (TH1, $n = 10$: white bars; TH2, $n = 13$: light grey bars and TH3, $n = 10$: dark grey bars) or continuation of usual lifestyle (CG, $n = 8$: black bars). *Significantly different from baseline ($p \leq 0.005$); #Significantly different from the CG ($p \leq 0.005$); #Significantly different from the TH1 ($p \leq 0.005$); #Significantly different from the TH2.

4. Discussion

This RCT is the first to provide evidence of a dose-response effect of multicomponent exercise (TH) on cardiometabolic health and aerobic performance in inactive middle-aged-to-elderly males, without previous experience with TH. This study revealed that recreational TH performed for 60-min thrice and twice per week results in improved aerobic performance, and that three weekly sessions were more effective in providing overall cardiometabolic benefits compared to training with a lower weekly frequency. Moreover, recreational TH training was rated as highly joyful, although perceived as very demanding, even though the participants had no previous experience with the sport, which can potentially result in long-term adherence to this exercise mode. In fact, all the participants in the TH training groups continued playing after the intervention ended.

The present study revealed that absolute VO_{2peak} increased by 19% in TH3 and was significantly higher than in CG, and absolute VO_{2peak} also increased in TH2 (12%). These improvements are clinically relevant, as the risk of all-cause and cardiovascular diseases (CVD) mortality decreases by 13% and 15%, respectively, for each 1 MET increase in VO_{2peak} (3.5 mL/min/kg) (Kodama et al., 2009). When expressed in relative terms, 180 min/week of recreational TH practice resulted in remarkable further ~80% improvement in VO_{2peak} in comparison with 60 min/week. Interestingly, the effects of recreational TH practice volume were still evident when expressing VO_{2peak} in absolute terms, despite the significant decrease in the participants' body mass.

Overall, the observed relative VO_{2peak} improvements across all training groups (range: 13-23%) are higher than those reported in previous recreational TH studies (i.e. 7-11%) using 2-3 weekly sessions during 12-16 weeks for inactive populations without previous experience with the sport (Hornstrup et al., 2018; Hornstrup et al., 2019; Hornstrup et al., 2020; Pereira et al., 2021). Surprisingly, actually the within-group changes in VO_{2peak} in TH1 and TH2 were in line with those reported for younger, previously trained populations (i.e. adult/middle-aged former TH players aged 33-55 years; +14%) playing 2-3 times/week, for 12 weeks, with TH3 showing higher pre-to-post changes in relative VO_{2peak} (+23%) (Póvoas et al., 2017). Improvements of 19-21% in TTE (i.e. 1.5-2 min), alongside with the improvement shown in aerobic performance (YYIE1) in all intervention groups (i.e. +38%, +52%, and +100% for TH1, TH2, and TH3, respectively), with no changes in the CG, suggest an improved ability to cope with daily tasks. The YYIE results are in line with previous studies with postmenopausal women (+70%) training recreational TH for 16 weeks, 2-3 times/week (Pereira et al., 2021). Despite population and health status differences, the internal load of the two studies' interventions was similar when considering exercise mean HR (76% vs. 78-89% of HR_{max}, postmenopausal women vs. middle-aged-to-elderly men, respectively) and time spent above 90% of HR_{max} (11 vs. 5-11%, respectively).

Previous recreational TH interventions showed no impact on SBP and DBP after 12-16 weeks (Hornstrup et al., 2018; Hornstrup et al., 2019; Hornstrup et al., 2020; Pereira et al., 2021), except for adult/middle-

aged former players, that reduced DBP by 4% (Póvoas et al., 2017). In this study, a significant decrease was shown in SBP and DBP across the TH groups. The CG showed no significant pre-to-post differences in SBP; however, a significant decrease was observed in DBP. Our participants' baseline SBP and DBP mean values are considered as high blood pressure values (SBP: 130–139 mmHg; DBP: 80–89 mmHg) (Unger et al., 2020). However, after 16 weeks, the participants moved from having high to normal blood pressure (SBP: 120–129 mmHg; DBP <80 mmHg) (Unger et al., 2020). From a clinical and public health perspective, these are relevant results as such reductions may help to reduce CVD probability (Unger et al., 2020). Moreover, TH3 displayed higher blood pressure reductions than the other TH groups, which seems to be related to the higher training volume (Joseph et al., 2019). The magnitude of reduction in arterial pressure in TH3 is comparable to other multicomponent training interventions of similar duration, in participants with the same degree of hypertension (Mohr et al., 2014). Resting HR only decreased significantly in TH3 (–11%). This decrease is in line with what was previously observed in adult/middle-aged former TH players after a 12-week recreational TH-based intervention (–16%) (Póvoas et al., 2017).

In line with prior recreational TH interventions carried out with younger participants (Hornstrup et al., 2018; Hornstrup et al., 2019; Hornstrup et al., 2020), blood lipid profile (LDL, HDL and triglycerides) was unchanged, which can be the consequence of favourable participants' baseline levels. However, in recreational TH interventions performed with postmenopausal women and adult/middle-aged male former players (Pereira et al., 2021; Póvoas et al., 2017), a significant decrease was observed in total cholesterol and LDL (–10 and –2.6%, respectively). These contrasting results may be the consequence of the baseline status of the considered variables and/or the lack of control over the participants' diet during the interventions (Pereira et al., 2021; Póvoas et al., 2017). The significant CG decrease in HDL cholesterol (–5%) promotes the interest of recreational TH in maintaining a healthy blood lipid profile and warrants further studies to understand the mechanisms underpinning the variability of the reported responses.

The serious increase of diabetic patients promotes the interest of novel exercise interventions to counteract this disease (Ghezzi et al., 2017; Powers et al., 2020). Recreational TH interventions with younger populations and postmenopausal women were ineffective in changing fasting blood glucose, HbA1c, and plasma insulin (Hornstrup et al., 2018; Hornstrup et al., 2019; Hornstrup

et al., 2020; Pereira et al., 2021). Nevertheless, in our study, a significant decrease was observed in fasting blood glucose for TH3 (–8%), in plasma insulin for TH2 (–24%) and TH3 (–6%), and in HbA1c (–3%) for TH1. The discrepancy between the studies may be related to the participants under analysis, since the younger participants in the reported studies did not have impaired glucose regulation, while the present study participants can be considered as prediabetic (fasting blood glucose: 100–125 mg/dl) (Colberg et al., 2010). Therefore, reducing these markers was of great health relevance, especially for TH3 that reduced fasting blood glucose to 99.8 ± 15.6 mg/dl, removing them from the prediabetic category.

Although the beneficial effects of moderate and timely-controlled increase in reactive oxygen species production have been extensively defined in the context of physical exercise, such as those linked to the activation of many health-related cellular signalling cascades (Powers et al., 2020), uncontrolled increased of oxidative stress has been associated to the aetiology and development of several pathological conditions (Ghezzi et al., 2017). In fact, PA increases antioxidant defences both in middle-aged and older individuals (Reid, 2001) and long-term adherence to regular PA is recommended to avoid the negative effects of oxidative stress on health (Simioni et al., 2018). In our study, a significant increase was observed for antioxidant stress-related markers TAS in TH3 (+12%) and for GR in TH1 (+13%), TH2 (+13%), TH3 (+15%) and CG (+9%). However, higher changes (%) were observed for TH3 than the other groups. To the best of our knowledge, this is the first study reporting the effects of recreational TH practice on oxidative stress-related markers. This study results are relevant, as antioxidant activity is similar in elderly physically active and young inactive subjects, highlighting the importance of regular PA to attenuate the ageing-associated impairment process (Simioni et al., 2018).

Our study reported a significant decrease in body mass for TH2 (–2%) and TH3 (–3%) and in fat mass for TH1 (–7%), TH2 (–11%), TH3 (–14%) and CG (–5%). The decrease shown in CG fat mass was surprising. However, in PA intervention trials, CG improvements in PA levels are not uncommon (Waters et al., 2012), and even though the participants were asked to keep their habitual PA levels, their lifestyle could have changed throughout the intervention and, consequently, be the cause of the observed CG fat mass loss. It is well-known that with ageing, lean body mass tends to decrease, while body fat mass increases, especially in the abdominal region, even when there are no changes in total body mass (St-Onge & Gallagher,

2010). Increases in abdominal region fat (visceral fat) are strongly associated with several adverse health conditions and older populations' body fat mass is a good predictor of morbidity and mortality (Gambert, 2006). Our results conflict with most of the previous studies addressing recreational TH-based interventions, with different populations and age groups, which did not report body mass improvements by performing an average of 2 weekly sessions for 12–16 weeks (Hornstrup et al., 2018; Hornstrup et al., 2019; Hornstrup et al., 2020; Póvoas et al., 2018), except for postmenopausal women that showed an 11% decrease after 16 weeks of 2–3 60-min sessions/week (Pereira et al., 2020). Nevertheless, postmenopausal women (Pereira et al., 2020) fat mass loss was lower than that shown in our study results (3% vs 7–14%, respectively). Additionally, our participants' post-intervention decrease in fat mass percentage was higher than that reported in younger inexperienced men (−1.7%, 12 weeks) (Hornstrup et al., 2019) and women (−1%, 12 weeks; −4% 16 weeks) (Hornstrup et al., 2018; Hornstrup et al., 2020) playing recreational TH.

In this study, the participants were instructed on how to hold, throw, and catch the ball due to their lack of experience with the sport, and adaptations were performed to the official TH rules, to avoid injuries. Also, match-play was preceded by a standardised warm-up adapted to fulfilled two of the main goals of warming, namely, the progressive physiological preparation for training or match, and the prevention of muscular injuries. During this intervention, two participants experienced knee pain, due to old injuries being instructed by medical indication to stop the intervention, recover, and then restart the exercise programme. These two participants did not finish the intervention and were considered as dropouts. One participant suffered an Achilles tendon rupture during a training session, while running alone during the warm-up, which inhibited the continuation of the exercise programme. The overall injury incidence was 1.3 injuries per 1000 h of exposure, which is in line with the injuries reported in other recreational TH interventions (Hornstrup et al., 2018; Hornstrup et al., 2019; Hornstrup et al., 2020; Pereira et al., 2020; Pereira et al., 2021). There was one injury in each group during the intervention, which resulted in an injury incidence per group of TH1 = 3.9, TH2 = 1.9 and TH3 = 1.3 injuries per 1000 h of exposure.

The major strength of this study is its design (RCT) addressing for the first time the dose-response health and physical fitness effects of RTS (TH) practice on several important parameters. Another strength of this study is the fact that it reports several internal and some external load markers that help to understand

the dose-response results. Nevertheless, a study limitation is that food intake throughout the training intervention was not assessed, although the participants were instructed to keep their regular dietary habits. This may have, at least partially, influenced some health-related variables. Given the impact nature of recreational TH, the analysis of the total external load experienced by the participants during the entire intervention may be helpful in characterising skeletal muscle and bone load. Therefore, future studies monitoring diet and external load are warranted.

In conclusion, recreational TH played as small-sided and formal games is effective for improving aerobic performance when performed thrice or twice per week for 60-min per session, and three weekly TH sessions are more effective in providing overall cardiometabolic benefits for middle-aged-to-elderly men compared to training with a lower weekly frequency. As training intensity and fun levels were similar between the intervention groups, weekly volume seems to be the major contributor for the differential health and physical fitness effects shown for the three training groups after the 16-week recreational TH intervention. Future health-related RCTs using recreational TH are warranted to understand the potential of low frequency TH training for middle-aged-to-elderly men and to explore the effects of low and moderate frequency TH training for untrained women, and for other age groups.

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Disclosure statement

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3.2. Original Article II

Bone health, body composition and physical fitness dose–response effects of 16 weeks of recreational team handball for inactive middle-to-older-aged males – A randomised controlled trial

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Bone health, body composition and physical fitness dose–response effects of 16 weeks of recreational team handball for inactive middle-to-older-aged males – A randomised controlled trial

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ABSTRACT

In this study we aimed at analysing the effects of different weekly exercise volumes (1, 2 or 3 times 60-min) on bone health, body composition and physical fitness of inactive middle-to-older-aged males, after 16 weeks of recreational team handball (RTH). Fifty-four men (68 ± 4 years, stature 169 ± 6 cm; body mass 78.4 ± 10.7 kg; fat mass 27.1 ± 5.3%; BMI 27.4 ± 2.9 kg/m²; VO_{2peak} 27.3 ± 4.8 mL/min/kg) were randomised into three intervention groups (TH1, *n* = 13; TH2, *n* = 15; or TH3, *n* = 12, performing 1, 2 and 3 weekly 60-min training sessions, respectively), and a control group (CG, *n* = 14). The training sessions consisted mainly of RTH matches played as small-sided and formal game formats (4v4, 5v5, 6v6 or 7v7) with adapted rules. Matches' mean and peak heart rate (HR) ranged from 78–80% and 86–89%HR_{max}, respectively, and distance covered from 4676 to 5202 m. A time × group interaction was observed for procollagen type-1 amino-terminal propeptide (P1NP), osteocalcin (OC), carboxy-terminal type-1 collagen crosslinks (CTX), sclerostin, upper and lower body dynamic strength, right arm fat mass, left and right arm, right leg and android total mass (TM; *p* ≤ 0.047) with the greatest effects being shown for TH2 and TH3 groups. Post-intervention group differences were observed in CTX, left arm and right leg TM (TH3 > TH1), P1NP (TH2 > CG), OC, right arm TM (TH3 > CG), upper (CG < TH1, TH2 and TH3) and lower body dynamic strength (CG < TH1 and TH3) (*p* ≤ 0.047). RTH was effective in enhancing bone health, body composition and physical fitness in middle-to-older-aged males, especially for the intervention groups that performed 2–3 weekly training sessions. ClinicalTrials.gov ID: NCT05295511.

KEYWORDS

aging; bone health; musculoskeletal fitness; team sports

Trial registration: ClinicalTrials.gov identifier: NCT05295511.

Highlights

- After 16 weeks of recreational team handball small-sided and formal matches, inactive middle-to-older-aged males improved bone health, body composition and physical fitness, by performing 1, 2 or 3 60-min weekly sessions, however, greater improvements were shown in the groups that performed 2 or 3 weekly training sessions.
- Training intensity was similar across the intervention groups that performed recreational team handball for 1, 2 or 3 60-min weekly sessions, which means that training volume is most likely to be the reason for the different health effects shown.
- The very high fun levels reported by all intervention groups shows that recreational team handball is a social and fun exercise modality for middle-to-older-aged males, with potential to intrinsically motivate the participants and assure long-term adherence to exercise.

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1. Introduction

Aging is associated with an increased risk of multimorbidity, which negatively impacts daily functioning and quality of life, and increases the mortality risk, that consequently increases the rates of health-care use and costs. Therefore, promoting healthy aging is important for maintaining physical and cognitive functions, quality of life and independence (Reginster & Burlet, 2006). Aging is also associated with bone loss and increased prevalence of osteoporosis, which is a musculoskeletal disorder that affects 6% of middle-to-older-aged males in the European Union (Hernlund et al., 2013), with male osteoporosis being underestimated and underdiagnosed (Vescini et al., 2021). Bone mineral density (BMD) is the gold standard for osteoporosis diagnosis (Kanis, 2002), and is thus important for determining the risk of potential fractures (Wheater et al., 2013). The rate of bone turnover is involved in determining bone quality, including bone density and qualitative determinants of bone strength (Datta et al., 2008) as a result of continuous lifelong bone remodelling (Shetty et al., 2016). Bone turnover biomarkers express the metabolic activity and can be used to determine the level of changes in bone turnover. During the remodelling process, osteocytes, osteoclasts and osteoblasts act in a coordinated manner to form or resorb bone as necessary, and their activation is controlled by a range of stimuli including mechanical forces applied to the skeleton, apoptosis of osteocytes, calcitropic and sex hormones, cytokines and local factors (Datta et al., 2008). Biomarkers of bone formation, e.g. procollagen type-1 amino-terminal propeptide (P1NP) and osteocalcin (OC), are products of active osteoblasts, while the majority of bone resorption markers, e.g. carboxy-terminal type-1 collagen crosslinks (CTX), are degradation products of type I collagen, the most abundant collagen in bone (Wheater et al., 2013). Additionally, osteocytes regulate bone turnover both through direct physical contact with other bone cells and by producing various factors which affect bone formation and can be measured in the blood, e.g. sclerostin (Weaver et al., 2016). Sclerostin is a secreted osteocyte marker that acts as inhibitor to the Wnt signalling pathway and hence, blocking the Wnt effects on osteoblasts, and thus decreasing bone formation (Zhang et al., 2004). Also, it is a biomarker that reflects the severity of bone loss and is a candidate biomarker for osteoporosis severity in chronic spinal cord injury (Morse et al., 2013). Exercise is effective in promoting short-term (~2 months) changes in serum bone turnover markers (Banfi et al., 2010; Evans et al., 2008) and, consequently, improvements in BMD. However, bone remodelling

normally takes several months to positively impact BMD, and those changes can only be detected after 6 months or longer (Weaver et al., 2016). Additionally, bone turnover markers' positive response to exercise appears to depend on exercise modality, intensity, age and sex (Smith et al., 2021). Exercise programmes that promote high impact movements with rapid rates of loading are considered as valid tools to attenuate and counteract the rate of bone loss in middle-aged-to-older adults (Smith et al., 2021).

Aging-related decreases in lean mass (LM) and increases in fat mass (FM), namely android FM, which is highly associated with cardiometabolic risk factors (Després et al., 1990), have been shown for men (St-Onge, 2005). Moreover, muscular strength decreases ~12 to 14% per decade in over 50-year-olds (Hurley & Roth, 2000), and lower body strength is commonly the most affected by the aging process (Candow & Chilibeck, 2005), leading to an increased risk of falls and, consequently, fractures (Deandrea et al., 2010). Nonetheless, regular and adequate levels of physical activity (PA) improve bone health, body composition markers and muscular fitness, reducing the risk of falls and femur and lumbar spine fractures (WHO, 2010).

Multicomponent exercise programmes (a combination of resistance, endurance, and balance training) are the best approach to improve the rate of falls, gait ability, balance, and strength performance in physically fragile elderly, when compared to other exercise programmes that prescribe these exercise modalities separately (Cadore et al., 2013). Recreational team handball (RTH), an adapted version of the sport, offers a multicomponent training approach, that positively impacts a range of health and fitness parameters in different populations (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018; Pereira et al., 2021; Pereira et al., 2020; Póvoas et al., 2018). Moreover, it is a social, fun and intrinsically motivating exercise mode (Hornstrup et al., 2018) and therefore, may be a feasible option to improve older males' health and long-term adherence to exercise, which is a major hurdle to overcome in this population (Nielsen et al., 2014). Additionally, it is aligned with the World Health Organization Global Action Plan, as promoting sport for all is a central policy recommendation (WHO, 2020).

It has been proposed that RTH health benefits are related to its high-intensity physical and physiological demands. Indeed, maximal oxygen uptake (VO_{2max}) improvements have been associated with the time spent at high-intensity, namely, with heart rates (HRs) above 90% of maximal heart rate (HR_{max}) (Póvoas et al., 2018). However, bone health, body composition and physical fitness effects appear to depend not only

on exercise type and intensity, but also on exercise volume (Gómez-Cabello et al., 2012; Brown et al., 2017; Kaushal et al., 2019). Yet, to the best of our knowledge, it is still to ascertain the effect of different exercise volumes on bone, body composition and physical fitness adaptations in recreational team sports interventions. Therefore, the present study aims at analysing the effects of different weekly exercise volumes (1, 2 or 3 times 60-min) on bone health, body composition and physical fitness of inactive middle-to-older-aged males, after 16 weeks of RTH. We hypothesized a positive training volume dose-effect on health outcomes.

2. Methods

2.1. Trial design

This is an open label four-arm randomized, parallel controlled trial. Evaluations were performed at baseline and after 16 weeks of the RTH intervention. After baseline testing, the participants were randomly allocated into either a control group (CG) or a RTH training intervention group performing 1, 2 or 3 60-min training sessions/week (1:1:1:1 ratio). During the 48 h preceding the evaluations, the participants were instructed not to perform exhausting physical activities and had at least 40 h of recovery between the training sessions. The RTH training sessions were monitored by the research team, and a physical education and sport graduated, and TH certified coach was responsible for the training instructions.

Sample size estimation for the main outcome was performed for a sample power of 95%, with an effect size of 0.275, at a significance level of 5%, and for a dropout rate of 20%, resulting in a total of 62 participants to be recruited. A computer-generated list of random numbers (random.org) was used to perform a stratified randomization for participants' allocation. The primary outcome was peak oxygen uptake (VO_{2peak}) relative to body mass, however, the results presented in this study are related to the secondary outcomes, namely, bone health [whole-body, proximal femur and lumbar spine bone mineral content (BMC) and BMD, PINP, OC, CTX and sclerostin], body composition [arms and legs LM; arms, legs, gynoid and android FM and total mass (TM)], and physical fitness (upper and lower body dynamic strength, postural balance, upper body isometric strength and agility) markers. The allocation sequence was concealed from an independent researcher enrolling and assessing the participants. The participants were informed about the study purposes, potential risks and benefits and signed a written informed consent according to the Declaration of

Helsinki (World Medical Association, 2013) and ethical approval was provided by the local Institutional Review Board (CEFADE 19 2019). This study was registered at clinicaltrials.gov (identifier: NCT05295511).

2.2. Participants

Three months before baseline evaluations, the recruitment started through advertisement in social media, flyers and in local senior institutions, in Porto District (Portugal). Sixty-three inactive middle-to-older-aged males, that were found eligible and agreed to participate, were stratified by VO_{2peak} and randomly allocated into three RTH intervention groups, performing 1 (TH1; $n = 16$), 2 (TH2; $n = 16$) or 3 (TH3; $n = 16$) 60-min training sessions/week and a CG ($n = 15$). The inclusion criteria were inactive males (i.e. not complying with the PA guidelines for the last 6 months) and over 50 years-old, with no medical contraindications to perform moderate-to-vigorous PA or incapacity to run or grip a ball. University facilities (Porto, Portugal) were used for pre-post-intervention evaluations, while evaluations during the intervention period were performed in a municipality sports hall (Gaia, Portugal). During the intervention period, there were 9 dropouts, (3 in TH1, 1 in TH2, 4 in TH3 and 1 in CG) (Figure 1). Fifty-four participants (59 to 76-year-olds) completed the intervention and were evaluated at follow-up.

2.3. Interventions

All training sessions consisted of a standardized 15-min warm-up, followed by 3×15 -min periods of RTH matches interspersed by 2-min breaks, and were performed on an indoor TH court. The matches were played as small-sided and formal game formats (4v4, 5v5, 6v6 or 7v7). The official TH rules were adapted: no body contact; softer and lighter TH balls (47 cm circumference, GOALCHA, Fredericia, Denmark) than the official ones; no exclusions; no substitutions; no dribbling; the participants rotated positions every 3-min randomly, including the goalkeeper; and the ball was immediately put back in play by the goalkeeper after a goal. The CG was instructed to maintain their habitual daily PA.

2.3.1. Intervention intensity, perceived experience, and attendance

Internal load was assessed as exercise HRs, differential ratings of perceived exertion (RPE) (McLaren et al., 2017) and blood lactate concentrations. The participants wore a HR monitor (Firstbeat Technologies Ltd., version 4.5.0.2, Jyväskylä, Finland) and their differential RPE and fun levels (0–10 AU) were recorded in all training

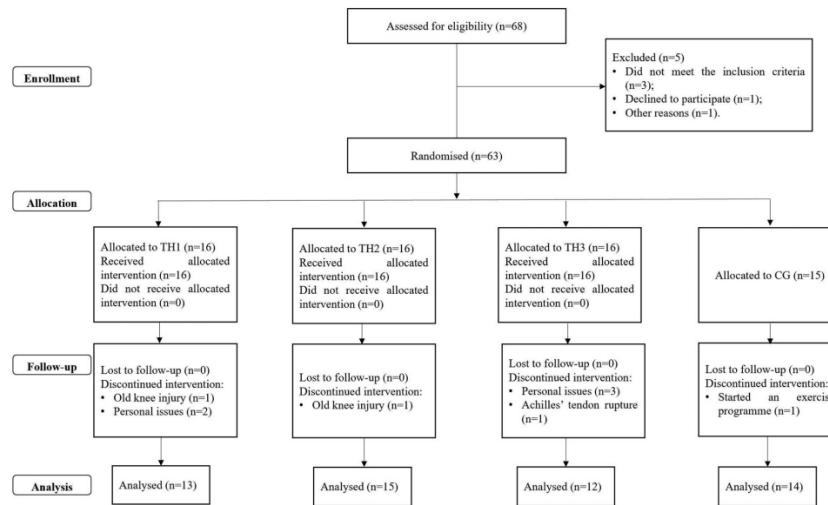


Figure 1. Flowchart for the recruitment process of the fifty-four inactive men.

sessions. Capillary blood (30 μ l) was drawn by a portable electroenzymatic lactate device analyser (Lactate Pro 2 LT-1730, Arkray, Amsterdam, The Netherlands) to determine blood lactate concentrations during 3 sessions per participant, according to procedures previously described (Carneiro et al., 2022). Individual HR_{max} was determined according to a multiple testing approach (Póvoas et al., 2019). External load was described using time-motion analyses of selected locomotor categories (using individualized speed thresholds) and game-specific high-demanding actions (SONY-DCR-SX65E, digital video camera recorder) (Carneiro et al., 2022). Additionally, accelerometer data [player load (PL) and accelerations/decelerations] was measured using Catapult MinimaxX S4 (MinimaxX S4; Catapult Sports, Canberra, Australia) in indoor mode with GPS technology (100 Hz sampling rate) in inactive state and data was analysed using Catapult Sprint Version 5.1.1 (Catapult Innovations, Canberra, Australia). Training attendance was registered for all training sessions.

2.4. Outcomes

Body (0.01 kg) and FM (%) were measured in a laboratory using a bioimpedance digital scale (Tanita Inner Scan BC 532, Tokyo, Japan), and stature (cm) was determined using a portable stadiometer (Seca 213, Hamburg, Germany), according to standardized protocols (Norton & Olds, 1996). Body mass index (BMI) was

calculated as kg/m^2 . To analyse plasma concentrations of the selected biochemical bone turnover markers, a fasting blood sample was drawn by a trained technician. A 6-mL blood sample tube containing ethylenediaminetetraacetic acid (EDTA) was collected and centrifuged, and plasma was pipetted and frozen at $-80^{\circ}C$ for subsequent analysis of P1NP, OC, CTX, and sclerostin. These markers were analysed by a chemiluminescence method using a fully automated immunoassay system [iSYS; Immunodiagnostic System Ltd., Boldon, UK (P1NP, OC, and CTX) and Liaison XL; Diasorin, Salugia, Italy (sclerostin)]. The manufacturer's control specimens were used to verify assay performance. The intermediary precisions expressed as coefficients of variation for P1NP were 5.4% (18.96 μ g/L), 6.5% (48.48 μ g/L) and 6.1% (122.10 μ g/L) for iSYS. For OC, the intermediary precisions were 3.0% (8.73 μ g/L), 3.6% (27.6 μ g/L) and 3.5% (68.7 μ g/L). For CTX were 5.3% (at CTX concentration 213 ng/L), 3.4% (869 ng/L) and 3.5% (2,113 ng/L) for iSYS. For sclerostin, the intraassay precision was 10% at both the 0.2 and 1.9 ng/mL levels. To evaluate potential changes in body composition the participants were submitted to a dual-energy X-ray absorptiometry scan (DXA; Hologic Explorer QDR, Hologic Inc., Belford, MA, USA). Lower and upper body dynamic strength were evaluated by a chair stand and an arm curl test, respectively, according to standardized protocols (Rikli & Jones, 2013), followed by a postural balance test where the number of falls during 1 min was registered

(Deforche et al., 2003). Then, upper body isometric strength was evaluated by a handgrip dynamometer (T.K.K. 5401, Grip-D, Takei, Japan) (Ruiz et al., 2011), and at the end, an agility test (8-foot-up and go) was performed (Rikli & Jones, 2013).

2.5. Statistical analysis

Results are presented as means \pm standard deviations (SD). To examine the differences between the groups at baseline and after 16 weeks a two-way analysis of variance (ANOVA) for repeated measures was used. Bonferroni test was used for post hoc multiple comparisons with 95% confidence interval (CI). Effect size was calculated using partial eta-squared (η_p^2) and as Cohen d , and interpreted as small (≥ 0.01), medium (≥ 0.06), or large (≥ 0.14), and as trivial (< 0.2), small ($0.2 < 0.5$), medium ($0.5 - 0.8$) and large (> 0.8), respectively (Cohen, 1988). One-way ANOVA was used to assess delta values differences between the groups after the 16-week intervention. Statistical Package for the Social Sciences (SPSS Inc., version 23.0) was used for the analyses. Data were tested for normality using the Shapiro-Wilk test. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Baseline data, intervention intensity, perceived experience, and attendance

Participants' baseline data were 67 ± 4 years; stature 169 ± 6 cm; body mass 78.7 ± 10.9 kg; FM $27.5 \pm 5.1\%$; BMI 27.6 ± 3.1 kg/m²; VO_{2peak} 27.3 ± 4.6 mL/min/kg, and for those who completed the intervention and were evaluated at follow-up were 68 ± 4 years; stature 169 ± 6 cm; body mass 78.4 ± 10.7 kg; FM $27.1 \pm 5.3\%$; BMI 27.4 ± 2.9 kg/m²; VO_{2peak} 27.3 ± 4.8 mL/min/kg (Table 1). There were no significant differences between the groups in the assessed variables at baseline (Table 1). Also, there were no significant differences in these variables between the dropouts and those that remained in the study (data not shown).

No significant differences were found between the intervention groups in internal load and fun variables. Mean and peak HR ranged from 78–80 and 86–89% HR_{max}, respectively. Mean and peak blood lactate values ranged from 3.3–4.9 and 4.4–6.9 mmol·l⁻¹, differential RPE and fun levels ranged from 5.8–7.0 and 8.1–9.0 (A.U., 0–10), respectively. Matches' distance covered ranged from 4676 to 5202 m. A higher percentage of frequency and time spent in walking and a lower distance covered in jogging and percentage of time spent in PL

zone 0.1–0.3 was observed for TH1 vs. TH3 ($p \leq 0.043$). Matches' high-demanding actions ranged from 38.4 to 50.1, with no significant differences between the groups. Total PL accumulated ranged from 261 to 326 (AU). Frequency of accelerations and decelerations ranged from 27–40 and 14–17, respectively. The average weekly attendance for TH1 was 0.9 ± 0.3 (14 \pm 4 out of 16 sessions), for TH2 1.8 ± 0.3 (28 \pm 4 out of 32 sessions) and for TH3 2.6 ± 0.2 (42 \pm 3 out of 48 sessions).

3.2. Bone health and bone turnover, body composition and physical fitness markers

A time \times group interaction ($p \leq 0.039$) was shown (Table 2) for P1NP (TH2 > CG), OC (TH3 > CG) and CTX (TH3 > TH1), and for sclerostin ($p = 0.012$). A time effect was shown for whole-body BMC and BMD, lumbar spine BMC and BMD, and proximal femur BMC ($p \leq 0.017$), and for P1NP, OC, CTX, P1NP/CTX and OC/CTX ratios ($p \leq 0.047$). A significant whole-body BMC increase was shown for TH2 and TH3 (TH2: $+1.9 \pm 2.2\%$; $p = 0.004$; 95% CI: 0.64–3.07; $d = 0.823$; TH3: $+1.7 \pm 2.4\%$; $p = 0.037$; 95% CI: 0.12–3.19; $d = 0.664$), and for whole-body BMD (TH2: $+1.5 \pm 2.2\%$; $p = 0.018$; 95% CI: 0.29–2.73; $d = 0.661$; TH3: $+2.3 \pm 2.3\%$; $p = 0.003$; 95% CI: 0.86–3.71; $d = 1.040$). A significant lumbar spine BMC increase was shown for the intervention groups (TH1: $+2.2 \pm 3.6\%$; $p = 0.029$; 95% CI: 0.05–4.43; $d = 0.649$; TH2: $+3.4 \pm 3.5\%$; $p < 0.001$; 95% CI: 1.47–5.29; $d = 1.074$; TH3: $+2.7 \pm 3.5\%$; $p = 0.005$; 95% CI: 0.46–4.88; $d = 1.157$), and in lumbar spine BMD for TH2 and TH3 (TH2: $+1.9 \pm 3.0\%$; $p = 0.006$; 95% CI: 0.26–3.63; $d = 0.655$; TH3: $+1.6 \pm 2.4\%$; $p = 0.026$; 95% CI: 0.07–3.09; $d = 0.912$), while the CG showed no significant pre- to post-changes in lumbar BMC and BMD. However, femur BMC and BMD decreased for CG (femur BMC: $-3.7 \pm 6.7\%$; $p = 0.005$; 95% CI: -7.60–0.56; $d = 0.605$; femur BMD: $-1.5 \pm 2.7\%$; $p = 0.034$; 95% CI: -3.26–0.17; $d = 0.640$), while the intervention TH groups maintained their values. A significant increase (Figure 2) was shown for P1NP in TH2 and TH3 (TH2: $+46.2 \pm 39.9\%$; $p < 0.001$; 95% CI: 23.11–69.20, $d = 1.442$; TH3: $+53.5 \pm 41.3\%$; $p < 0.001$; 95% CI: 27.29–79.79; $d = 1.414$), for OC in all intervention groups (TH1: $+37.8 \pm 70.0\%$; $p = 0.011$; 95% CI: -6.71–82.30; $d = 0.807$; TH2: $+35.2 \pm 28.8\%$; $p < 0.001$; 95% CI: 18.52–51.82; $d = 1.358$; TH3: $+53.6 \pm 40.2\%$; $p < 0.001$; 95% CI: 28.07–79.15; $d = 1.850$) and for CTX in TH3 ($+36.6 \pm 29.2\%$; $p = 0.005$; 95% CI: 18.03–55.15; $d = 1.240$). Moreover, a sclerostin decrease was observed for TH3 ($-7.1 \pm 13.2\%$; $p = 0.032$; 95% CI: -15.48–1.35; $d = 0.678$) while the CG values increased significantly ($+9.7 \pm 14.9\%$; $p = 0.024$; 95% CI: 1.12–18.30; $d = 0.682$). Also, significant increases were shown for TH1 in P1NP/CTX ($+51.7 \pm 74.5\%$; $p =$

Table 1. Baseline chronological age, anthropometric characteristics, body composition and cardiorespiratory fitness of the participants in the recreational team handball (TH) groups performing 1 (TH1, $n = 13$), 2 (TH2, $n = 15$) or 3 (TH3, $n = 12$) training sessions per week and in the control group (CG, $n = 14$).

Variables	TH1 ($n = 13$)	TH2 ($n = 15$)	TH3 ($n = 12$)	CG ($n = 14$)
Age (years)	67 ± 5 (60–76)	67 ± 3 (61–72)	68 ± 4 (61–76)	67 ± 5 (59–75)
Stature (cm)	169 ± 6 (159–177)	168 ± 7 (158–188)	168 ± 6 (156–173)	170 ± 6 (163–180)
Body mass (kg)	77.1 ± 12.1 (61.8–103.6)	79.2 ± 11.7 (69.1–104.3)	77.6 ± 10.6 (51.3–87.5)	79.3 ± 9.3 (63.9–100.0)
Fat mass (%)	28.0 ± 6.6 (16.6–41.1)	27.4 ± 3.7 (23.9–35.5)	27.7 ± 6.1 (18.1–36.4)	25.2 ± 4.7 (14.3–31.8)
BMI (kg/m ²)	26.8 ± 3.1 (23.7–34.2)	27.9 ± 2.8 (24.5–34.7)	27.5 ± 3.3 (21.1–30.9)	27.4 ± 2.8 (20.4–31.1)
VO _{2peak} (mL/min/kg)	27.0 ± 3.7 (12.3–34.2)	27.5 ± 5.2 (15.8–33.9)	27.1 ± 4.3 (20.9–33.4)	28.8 ± 4.7 (21.0–34.9)

Data are presented as mean ± SD (range). Abbreviations: BMI – Body mass index; VO_{2peak} – Peak oxygen uptake.

0.044; 95% CI:4.40–99.02; $d = 0.670$) and in OC/CTX (+64.6 ± 99.3%; $p = 0.012$; 95% CI:1.52–127.69; $d = 0.182$) ratios.

A time × group interaction was observed for right arm FM ($p = 0.047$; Table 2), for right leg and arms TM ($p \leq 0.019$) and for android TM ($p = 0.043$). A time effect was shown for left arm and leg, and gynoid FM ($p \leq 0.029$), left arm LM ($p = 0.006$), for right leg and arms TM ($p \leq 0.041$) and for gynoid and android TM ($p \leq 0.019$). A significant arms FM decrease was observed in TH2 (left arm FM: $-5.1 \pm 11.2\%$; $p = 0.023$; 95% CI: -11.34 – 1.07 ; $d = 0.495$ and right arm FM: $-5.2 \pm 10.6\%$; $p = 0.041$; 95% CI: -11.07 – 0.67 ; $d = 0.553$) and TH3 (left arm FM: $-6.0 \pm 11.3\%$; $p = 0.032$; 95% CI: -13.18 – 1.13 ; $d = 0.553$ and right arm FM: $-6.5 \pm 13.1\%$; $p = 0.020$; 95% CI: -14.79 – 1.81 ; $d = 0.792$) groups. Also, a significant legs FM decrease was shown in TH3 (left leg: $-9.0 \pm 9.6\%$; $p = 0.013$; 95% CI: -15.18 – 2.92 ; $d = 0.909$ and right leg: $-5.4 \pm 9.6\%$; $p = 0.047$; 95% CI: -11.47 – 0.67 ; $d = 1.438$). A significant decrease was also observed for gynoid FM (TH3: $-6.6 \pm 9.1\%$; $p = 0.029$; 95% CI: -12.40 , -0.86 ; $d = 1.063$) and android FM (TH2: $-9.4 \pm 11.6\%$; $p = 0.003$; 95% CI: -15.87 , -3.0 ; $d = 0.328$ and TH3: $-12.5 \pm 9.3\%$; $p < 0.001$; 95% CI: -18.39 , -6.56 ; $d = 0.954$). Additionally, significant decreases were observed in left arm LM for TH3 ($-3.3 \pm 4.1\%$; $p = 0.010$; 95% CI: -5.985 – 0.629 ; $d = 0.883$) and CG ($-2.7 \pm 5.5\%$; $p = 0.026$; 95% CI: -5.861 , -0.683 ; $d = 0.536$), and in right arm LM for TH3 ($-3.5 \pm 5.1\%$; $p = 0.020$; 95% CI: -6.801 , -0.276 ; $d = 0.699$). Moreover, TH3 showed a higher decrease in left arm TM than TH1 ($p = 0.007$) and in right arm TM than CG ($p = 0.002$). A significant decrease was observed for TH2 ($-2.8 \pm 4.4\%$; $p \leq 0.001$; 95% CI: -5.25 , -0.34 ; $d = 0.699$), TH3 ($-4.4 \pm 2.7\%$; $p < 0.001$; 95% CI: -6.09 , -2.67 ; $d = 1.725$) and CG ($-3.0 \pm 2.3\%$; $p = 0.001$; 95% CI: -4.35 , -1.60 ; $d = 1.364$) in left arm TM and for TH2 ($-1.9 \pm 3.6\%$; $p = 0.022$; 95% CI: -3.92 – 0.06 ; $d = 0.688$) and TH3 ($-4.6 \pm 3.5\%$; $p < 0.001$; 95% CI: -6.86 , -2.36 ; $d = 1.292$) in right arm TM. Also, TH3 showed a higher decrease in right leg TM than TH1 ($p = 0.026$) and a significant decrease was observed for TH2 ($-1.5 \pm 3.1\%$; $p = 0.025$; 95% CI: -3.16 – 0.24 ; $d = 0.559$) and TH3 ($-2.3 \pm 2.8\%$; $p = 0.019$;

95% CI: -4.02 , -0.51 ; $d = 0.791$), while in left leg TM, TH1 showed a significant increase ($+15.9 \pm 53.0\%$; $p = 0.025$; 95% CI: -16.14 – 47.90 ; $d = 0.339$). A significant decrease was observed for gynoid (TH2: $-1.6 \pm 3.7\%$; $p = 0.027$; 95% CI: -3.70 – 0.44 ; $d = 0.524$) and android TM (TH2: $-5.7 \pm 4.8\%$; $p < 0.001$; 95% CI: -8.29 , -3.02 ; $d = 1.182$ and TH3: $-5.0 \pm 3.3\%$; $p = 0.043$; 95% CI: -7.07 , -2.88 ; $d = 1.411$), after the intervention period.

A time × group interaction was observed for upper and lower body dynamic strength ($p \leq 0.003$; Table 2) with TH1 showing higher pre- to post-intervention changes compared to CG ($p \leq 0.045$). A time effect was shown for upper and lower body dynamic strength, and agility ($p < 0.001$). An increase was observed for upper and lower body dynamic strength for all intervention groups (upper: TH1: $+33.5 \pm 26.3\%$; $p < 0.001$; 95% CI: 15.78 – 51.12 ; $d = 1.178$; TH2: $+23.3 \pm 20.2\%$; $p < 0.001$; 95% CI: 15.02 – 38.56 ; $d = 1.277$; TH3: $+31.7 \pm 29.9\%$; $p < 0.001$; 95% CI: 12.72 – 50.77 ; $d = 1.529$; lower: TH1: $+49.3 \pm 25.2\%$; $p < 0.001$; 95% CI: 32.39 – 66.25 ; $d = 2.484$; TH2: $+33.6 \pm 38.7\%$; $p < 0.001$; 95% CI: 14.31 – 64.02 ; $d = 0.944$; TH3: $+37.9 \pm 24.2\%$; $p < 0.001$; 95% CI: 22.53 – 53.34 ; $d = 1.771$), while the CG showed no significant pre- to post-changes. TH1, TH2 and TH3 showed higher increases in upper body dynamic strength than CG ($p \leq 0.039$), and TH1 and TH3 in lower body dynamic strength than CG ($p \leq 0.018$). After the intervention, an improvement in postural balance (number of falls: $-16.6 \pm 26.9\%$; $p = 0.022$; 95% CI: -32.76 – 2.29 ; $d = 0.708$) was observed for TH2. Moreover, a decrease in the time to perform the agility test was observed for all groups (TH1: $-13.1 \pm 7.8\%$; $p < 0.001$; 95% CI: -18.36 , -7.87 ; $d = 1.655$; TH2: $-11.7 \pm 9.6\%$; $p < 0.001$; 95% CI: -17.15 , -5.44 ; $d = 1.188$; TH3: $-9.4 \pm 10.9\%$; $p = 0.007$; 95% CI: -16.32 , -2.4 ; $d = 0.894$; CG: $-5.4 \pm 13.1\%$; $p = 0.032$; 95% CI: -13.26 – 4.28 ; $d = 0.508$).

4. Discussion

This study provides, for the first time, evidence of a dose–response effect of recreational team sports (TH) training on bone health, body composition and physical

Table 2. Bone health, body composition and physical fitness markers at baseline and after 16 weeks of recreational team handball (TH) training performed 1 (TH1), 2 (TH2) or 3 (TH3) times a week or a continuation of usual lifestyle (CG).

Variables	TH1 (n = 13)		TH2 (n = 15)		TH3 (n = 12)		CG (n = 14)		Two-way ANOVA		
	Baseline	16 weeks	Baseline	16 weeks	Baseline	16 weeks	Baseline	16 weeks	Time F (df,error) P _t η _p ²	Group F (df,error) P _g η _p ²	Interaction F (df,error) P _i η _p ²
	Whole-body BMC (g)	2323.9 ± 241.2	2355.9 ± 241.3	2470.5 ± 311.2	2515.8 ± 317.7*	2308.5 ± 396.4	2344.9 ± 400.0*	2496.2 ± 482.6	2503.2 ± 470.8	13.845 (3;49) 0.001; 0.220	0.800 (3;49) 0.458; 0.051
Whole-body BMD (g/cm ³)	1.10 ± 0.08	1.11 ± 0.08	1.16 ± 0.09	1.17 ± 0.08*	1.09 ± 0.11	1.11 ± 0.10*	1.16 ± 0.19	1.16 ± 0.18	10.644 (3;49) 0.002; 0.178	1.182 (3;49) 0.326; 0.067	2.106 (3;49) 0.112; 0.114
Lumbar spine BMC (g)	66.7 ± 9.6	68.1 ± 9.3*	73.7 ± 10.9	76.0 ± 10.2*	63.6 ± 12.1	65.6 ± 13.8*	65.5 ± 11.2	66.5 ± 11.6	28.870 (3;48) 0.001; 0.379	2.432 (3;48) 0.076; 0.130	0.846 (3;48) 0.475; 0.049
Lumbar spine BMD (g/cm ³)	0.99 ± 0.16	1.00 ± 0.17	1.08 ± 0.12	1.10 ± 0.10*	1.00 ± 0.14	1.02 ± 0.15*	0.96 ± 0.13	0.97 ± 0.13	15.070 (3;48) 0.001; 0.235	2.180 (3;48) 0.102; 0.118	0.520 (3;48) 0.671; 0.031
Proximal femur BMC (g)	37.4 ± 5.4	36.9 ± 5.2	39.4 ± 7.5	38.9 ± 7.1	39.1 ± 7.4	39.0 ± 7.9	38.6 ± 6.7	37.0 ± 5.4*	6.077 (3;48) 0.017; 0.112	0.298 (3;48) 0.827; 0.018	1.489 (3;48) 0.229; 0.085
Proximal femur BMD (g/cm ³)	0.92 ± 0.09	0.93 ± 0.08	0.99 ± 0.11	0.99 ± 0.11	0.96 ± 0.11	0.96 ± 0.12	0.93 ± 0.12	0.92 ± 0.10*	0.153 (3;48) 0.697; 0.003	1.252 (3;48) 0.301; 0.073	2.234 (3;48) 0.096; 0.123
PINP (µg/L)	46.8 ± 24.1	53.8 ± 19.9	49.1 ± 18.7	71.4 ± 33.4*	46.5 ± 14.8	69.1 ± 23.5*	41.0 ± 14.8	46.6 ± 20.0	42.175 (3;48) 0.001; 0.468	1.826 (3;48) 0.155; 0.102	4.495 (3;48) 0.007; 0.219
OC (µg/L)	13.9 ± 4.8	17.6 ± 6.1*	15.1 ± 5.9	20.5 ± 9.4*	14.4 ± 4.1	22.4 ± 8.8*	13.4 ± 5.1	14.5 ± 4.8	45.031 (3;48) 0.001; 0.484	1.598 (3;48) 0.202; 0.091	4.586 (3;48) 0.007; 0.223
CTX (ng/L)	365.8 ± 151.3	327.2 ± 139.6	361.0 ± 234.1	430.2 ± 301.7	357.8 ± 191.6	470.3 ± 224.8*	302.9 ± 154.5	319.0 ± 182.6	4.719 (3;48) 0.035; 0.090	0.763 (3;48) 0.521; 0.046	3.018 (3;48) 0.039; 0.159
Sclerostin (ng/mL)	0.83 ± 0.24	0.85 ± 0.25	0.97 ± 0.25	0.92 ± 0.26	0.95 ± 0.22	0.87 ± 0.16*	0.90 ± 0.22	0.98 ± 0.25*	0.218 (3;48) 0.643; 0.005	0.589 (3;48) 0.625; 0.035	4.080 (3;48) 0.012; 0.203
PINP/CTX (µg/L)	132.1 ± 65.6	176.0 ± 64.6*	178.9 ± 10.6	205.1 ± 103.6	151.9 ± 61.6	161.6 ± 44.7	158.5 ± 80.2	172.2 ± 75.0	5.215 (3;48) 0.027; 0.098	0.817 (3;48) 0.491; 0.049	0.538 (3;48) 0.659; 0.033
OC/CTX (µg/L)	39.8 ± 13.0	56.6 ± 13.0*	53.3 ± 27.8	56.7 ± 24.1	49.0 ± 23.4	50.8 ± 12.5	51.7 ± 22.7	55.0 ± 22.5	4.147 (3;48) 0.047; 0.080	0.756 (3;48) 0.756; 0.024	1.218 (3;48) 0.313; 0.071
Left arm FM (kg)	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	1.2 ± 0.3*	1.3 ± 0.3	1.2 ± 0.3*	1.3 ± 0.3	1.2 ± 0.3	10.093 (3;49) 0.003; 0.171	0.024 (3;49) 0.995; 0.001	0.588 (3;49) 0.626; 0.035
Right arm FM (kg)	1.3 ± 0.5	1.3 ± 0.4	1.4 ± 0.4	1.3 ± 0.3*	1.4 ± 0.4	1.2 ± 0.3*	1.2 ± 0.3	1.3 ± 0.3	3.579 (3;49) 0.064; 0.068	0.107 (3;49) 0.956; 0.006	2.852 (3;49) 0.047; 0.149
Left leg FM (kg)	3.2 ± 0.8	3.2 ± 0.8	3.0 ± 1.0	2.9 ± 0.9	3.4 ± 0.7	3.1 ± 0.7*	3.1 ± 0.6	3.0 ± 0.7	5.041 (3;49) 0.029; 0.093	0.352 (3;49) 0.788; 0.021	1.476 (3;49) 0.233; 0.083
Right leg FM (kg)	3.2 ± 1.0	3.2 ± 1.0	3.0 ± 1.0	2.9 ± 0.9	3.2 ± 0.9	3.0 ± 0.7*	3.0 ± 0.7	3.0 ± 0.7	2.860 (3;49) 0.097; 0.55	0.229 (3;49) 0.876; 0.014	0.945 (3;49) 0.426; 0.055
Gynoid FM (kg)	3.3 ± 1.0	3.2 ± 0.9	3.1 ± 0.9	3.0 ± 0.7	3.1 ± 0.7	2.9 ± 0.6*	2.9 ± 0.6	3.0 ± 0.9	18.038(3;49) 0.001; 0.269	0.183(3;49) 0.907; 0.011	2.433(3;49) 0.076; 0.130
Android FM (kg)	2.4 ± 1.1	2.4 ± 0.9	2.6 ± 0.7	2.3 ± 0.7*	2.5 ± 0.7	2.1 ± 0.6*	2.3 ± 0.9	2.2 ± 0.8	2.343 (3;49) 0.132; 0.046	0.409 (3;49) 0.747; 0.024	1.636 (3;49) 0.193; 0.091
Left arm LM (kg)	2.6 ± 0.3	2.7 ± 0.3	2.7 ± 0.4	2.7 ± 0.4	2.7 ± 0.5	2.6 ± 0.4*	2.8 ± 0.4	2.7 ± 0.4*	8.414 (3;49) 0.006; 0.147	0.307 (3;49) 0.821; 0.018	2.147 (3;49) 0.106; 0.116
Right arm LM (kg)	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.5	3.0 ± 0.4*	3.2 ± 0.4	3.2 ± 0.4	3.969 (3;49) 0.052; 0.075	0.258 (3;49) 0.855; 0.016	0.974 (3;49) 0.413; 0.056
Left leg LM (kg)	7.8 ± 1.4	7.9 ± 1.3	8.4 ± 1.5	8.3 ± 1.4	8.0 ± 1.1	8.2 ± 1.2	8.1 ± 0.8	8.3 ± 0.8	3.698 (3;49) 0.060; 0.070	0.432 (3;49) 0.731; 0.026	1.331 (3;49) 0.275; 0.075
Right leg LM (kg)	7.7 ± 1.2	7.8 ± 1.2	8.5 ± 1.5	8.4 ± 1.5	8.1 ± 1.1	8.1 ± 1.2	8.4 ± 0.6	8.3 ± 0.7	0.196 (3;49) 0.660; 0.004	0.895 (3;49) 0.451; 0.052	0.329 (3;49) 0.805; 0.020

(Continued)

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Table 2. Continued.

Variables	TH1 (n = 13)		TH2 (n = 15)		TH3 (n = 12)		CG (n = 14)		Two-way ANOVA		
	Baseline	16 weeks	Baseline	16 weeks	Baseline	16 weeks	Baseline	16 weeks	Time F (df,error) P _t η _p ²	Group F (df,error) P _g η _p ²	Interaction F (df,error) P _i η _p ²
	Left arm TM (kg)	4.1 ± 0.6	4.1 ± 0.6	4.2 ± 0.6	4.1 ± 0.6*	4.2 ± 0.7	4.1 ± 0.7*	4.3 ± 0.4	4.2 ± 0.5*	37.625 (3;49) 0.001; 0.434	0.150 (3;49) 0.930; 0.009
Right arm TM (kg)	4.6 ± 0.7	4.6 ± 0.8	4.6 ± 0.6	4.5 ± 0.6*	4.7 ± 0.7	4.5 ± 0.7*	4.6 ± 0.5	4.7 ± 0.5	13.732 (3;49) 0.001; 0.219	0.022 (3;49) 0.995; 0.001	5.211 (3;49) 0.003; 0.242
Left leg TM (kg)	10.8 ± 2.7	11.6 ± 1.9*	11.9 ± 2.4	11.7 ± 2.3	11.9 ± 1.7	11.7 ± 1.8	11.8 ± 1.0	11.8 ± 1.2	0.712 (3;49) 0.403; 0.014	0.369 (3;49) 0.776; 0.022	1.727 (3;49) 0.174; 0.096
Right leg TM (kg)	11.4 ± 1.9	11.5 ± 1.9	11.9 ± 2.5	11.7 ± 2.4*	11.8 ± 1.7	11.5 ± 1.8*	11.8 ± 0.9	11.8 ± 1.1	4.388 (3;49) 0.041; 0.082	0.147 (3;49) 0.931; 0.009	3.647 (3;49) 0.019; 0.183
Gynoid TM (kg)	10.9 ± 1.7	10.9 ± 1.7	11.3 ± 2.0	11.1 ± 1.9*	10.9 ± 1.6	10.7 ± 1.6	10.9 ± 0.9	10.9 ± 1.0	5.871 (3;49) 0.019; 0.107	0.128 (3;49) 0.943; 0.008	1.070 (3;49) 0.371; 0.061
Android TM (kg)	6.5 ± 1.4	6.4 ± 1.4	6.8 ± 1.1	6.4 ± 1.1*	6.4 ± 1.1	6.1 ± 1.0*	6.4 ± 1.1	6.3 ± 1.1	20.196 (3;49) 0.001; 0.292	0.243 (3;49) 0.866; 0.015	2.925 (3;49) 0.043; 0.152
Upper body dynamic strength: 30-s arm curl (n)	17.1 ± 4.5	22.2 ± 4.5*	16.7 ± 1.8	20.5 ± 3.1*	16.8 ± 3.9	21.3 ± 3.5*	17.2 ± 3.6	17.0 ± 2.2	40.096 (3;46) 0.001; 0.466	1.715 (3;46) 0.177; 0.101	5.425 (3;46) 0.003; 0.261
Lower body dynamic strength: 30-s chair stand (n)	17.0 ± 3.3	25.5 ± 6.6*	18.0 ± 3.5	23.4 ± 5.6*	19.1 ± 3.4	26.0 ± 4.6*	17.1 ± 3.6	17.8 ± 2.8	60.764 (3;45) 0.001; 0.575	4.252 (3;45) 0.010; 0.221	5.589 (3;45) 0.002; 0.271
Postural balance: Flamingo test (n)	18.6 ± 10.5	15.2 ± 9.0	20.3 ± 7.4	16.3 ± 6.5*	19.1 ± 6.6	17.5 ± 5.6	19.3 ± 7.5	22.4 ± 6.4	2.632 (3;41) 0.112; 0.060	0.556 (3;41) 0.647; 0.039	2.811 (3;41) 0.051; 0.171
Upper body isometric strength: Handgrip test (kg)	38.8 ± 6.7	40.2 ± 4.0	37.9 ± 6.4	39.2 ± 5.8	38.8 ± 6.8	40.1 ± 7.3	37.7 ± 7.0	35.5 ± 5.4	0.501 (3;45) 0.483; 0.011	0.648 (3;45) 0.588; 0.041	1.847 (3;45) 0.152; 0.110
Agility: 8-foot up and go (s)	4.46 ± 0.61	3.85 ± 0.49*	4.62 ± 0.46	4.06 ± 0.49*	4.26 ± 0.47	3.85 ± 0.51*	4.82 ± 0.58	4.49 ± 0.25*	42.168 (3;45) 0.001; 0.484	4.770 (3;45) 0.006; 0.241	0.745 (3;45) 0.531; 0.047

Data are presented as mean ± SD. Abbreviations: ANOVA – Analysis of variance; BMC – Bone mineral content; BMD – Bone mineral density; CTX – Carboxy-terminal type-1 collagen crosslinks; FM – Fat mass; LM – Lean mass; OC – Osteocalcin; PINP – Procollagen type-1 amino-terminal propeptide; TH – Team handball; TM – Total mass. *Significantly different from baseline (p ≤ 0.005); *Significantly different from control group (p ≤ 0.005).

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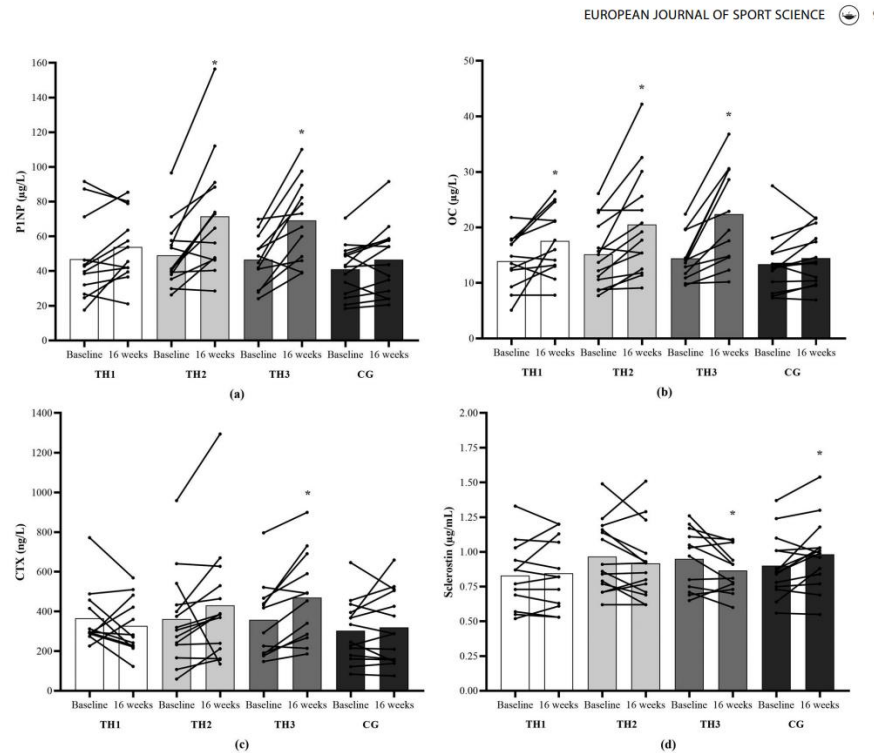


Figure 2. Procollagen type-1 amino-terminal propeptide (P1NP) (a), osteocalcin (OC) (b), carboxy-terminal type-1 collagen crosslinks (CTX) (c) and sclerostin (d) values at baseline and after 16 weeks of team handball training (TH1, $n = 13$; TH2, $n = 15$; TH3, $n = 12$) or a continuation of usual lifestyle (CG, $n = 14$). *Significant differences between baseline and 16 weeks ($p \leq 0.005$).

fitness in middle-to-older-aged males. After 16 weeks, the main findings were that all the intervention groups showed health improvements, however, greater for those who performed 2 and 3 sessions/week. Since the training intensity was similar between groups, it is likely that training volume was the main reason for the differential health effects observed.

A significant increase was shown in whole-body BMC and BMD for TH2 and TH3. Additionally, the intervention groups were able to maintain their femur BMC and BMD values, while a significant decrease was shown in CG. The improvements observed in BMC and BMD after a short-term intervention period (16 weeks) are of interest, since it has been reported that bone remodelling normally takes 3–4 months to change BMD and that those changes are only visible after a training period of approximately 6 months (Weaver et al., 2016). Nevertheless, elderly men with

prostate cancer reported improvements in whole-body and leg BMC (Uth et al., 2016), after only 12 weeks of recreational football practice. This change in leg BMC was positively correlated with the total number of accelerations, decelerations, sum of intense accelerations and decelerations, and total distance covered (Uth et al., 2016). The participants' covered distances (4676–5202 m), accelerations (27–40) and decelerations (14–17) performed by this study participants, may have contributed for the positive results shown in BMC and BMD, especially for TH2 and TH3, after 16 weeks of RTH. Moreover, osteogenesis is upregulated by high-impact forces (Turner & Robling, 2003), and TH may have promoted positive osteogenic activity, since it is characterized by multiple specific high-intensity actions such as stops, jumps, changes of direction and speed, that promote high rates of force application and large ground reaction forces (Póvoas et al., 2012).

Bone turnover biomarkers express the metabolic activity of osteoblasts and osteoclasts (Wheater et al., 2013), and its balance activity results in continuous life-long bone remodelling (Shetty et al., 2016). In our study, a significant increase was observed in bone formation markers P1NP (TH2 and TH3) and OC (TH1, TH2 and TH3), and a significant increase was observed in CTX for TH3. The increase in bone formation markers was higher than the increase shown in bone resorption markers, resulting in a positive net balance. Furthermore, sclerostin concentration decreased significantly in TH3, while an increase was found for CG, which was important since sclerostin reflects the severity of bone loss (Morse et al., 2013). Sclerostin increases with age, and low levels of PA for a prolonged period of time are associated with high sclerostin concentration levels (Smith et al., 2021). Exercise interventions that promote mechanical load, decrease sclerostin levels (Smith et al., 2021), and RTH used as an exercise programme has shown to induce mechanical load throughout different game-specific high-intensity actions such as changes of direction, accelerations, decelerations, jumps, stops and others. This may explain the decrease observed in sclerostin in TH3, while the CG, that was instructed to continue with their normal daily routines, meaning that probably no or little PA was performed, tended to increase sclerostin levels, probably due to low PA levels. This could also be a chance finding as no corresponding effects were shown in OC and P1NP values in the CG. Elderly men that performed recreational football, 2–3 sessions/week, for 16 weeks, showed an increase in P1NP and OC (Helge et al., 2014), which is consistent with our study results. Moreover, studies with younger men and women (RTH, for 12 weeks), also showed similar increases in P1NP, OC and CTX (Hornstrup et al., 2019; Hornstrup et al., 2018), than our TH2 and TH3 groups, meaning that by playing RTH, for 16-weeks, middle-to-older aged males were able to improve bone turnover markers as much as younger populations. The increases shown in BMC and BMD in our study can be explained by the increases observed in bone formation markers P1NP and OC and bone resorption marker CTX and decrease in sclerostin in TH3, which is in line with other studies using recreational team sports (Helge et al., 2014; Hornstrup et al., 2018; Hornstrup et al., 2019; Pereira et al., 2021) that reported positive BMD changes with elevated CTX values. Moreover, TH1 only showed positive changes in OC, however, it was also observed a significant increase in the P1NP/CTX and OC/CTX ratios, which means that even with only 60-min/week of RTH for 16 weeks, TH1 was able to increase the osteogenic activity. The positive

osteogenic response observed in RTH studies using different populations and age groups, can possibly be related to the fact that RTH is a high impact team sport. In fact, similar to ours (Carneiro, et al., 2022), other RTH studies have reported multiple specific high-intense movements and actions, such as stops, jumps, changes of direction, accelerations and decelerations, performed at different speeds and directions, that can possibly stimulate the musculoskeletal system (Hornstrup et al., 2018; Hornstrup et al., 2019; Hornstrup et al., 2020; Póvoas et al., 2017; Póvoas et al., 2018).

Aging is associated with a decline in LM and an increase in FM even with absence of changes in TM (St-Onge, 2005). RTH training resulted in decreases in arms, right leg and android TM in TH2 and TH3, and in gynoid TM in TH2. TH3 decreased arms LM, which was unexpected, however, arms FM decreased almost twice the percentage of LM lost, which helps to explain the decrease in TM in both arms. Furthermore, TH2 and TH3 decreased TM in some regions, which is related to the decrease in FM while maintaining LM in those regions. Android FM is highly associated with cardiometabolic risks factors (Després et al., 1990) and is more common in men (Blaak, 2001). Therefore, it was important to observe positive effects in gynoid and android FM, and especially that higher decreases were observed on android FM for TH2 and TH3. Furthermore, TH3 decreased legs FM, which could possibly explain the decrease in legs TM, and is most likely the result of the this sport physical demands, that include different lower body movement changes, namely in speed and direction (Póvoas et al., 2012). Changes in body composition, especially in the legs, may be expected in several team sports due to the activity pattern (Castagna et al., 2020). However, TH may be responsible for extra benefits in upper body composition (Póvoas et al., 2012). TH movements and actions, including unorthodox movements (Hornstrup et al., 2018; Póvoas et al., 2017), increase the metabolic load and may elevate energy expenditure. This helps to explain the improvements shown in body composition, namely FM decrease, and consequently, legs TM decrease but also in the arms, since TH practice requires higher upper body engagement when compared to other team sports such as football. A dose–response effect was also observed in body composition variables, since only TH2 and TH3 were able to, more markedly, decrease TM and FM.

After 16 weeks of RTH training, positive changes were observed in upper and lower body dynamic strength in all intervention groups. These results were higher than those found for recreational floorball performed 2 × 60-min/weekly during 12 weeks for older men, which

showed increases in upper and lower body dynamic strength of ~2-3 repetitions in the physical fitness tests (Vorup et al., 2017), while our improvements were in the range of 3-9 repetitions. However, there were no changes in upper body isometric strength in our study. In the present study, smaller and softer balls were used during the training sessions in order to prevent injuries and allow a proper ball grip. However, this may have influenced the handgrip strength test results, since these balls do not require a very strong handgrip, unlike what happens with an official TH ball. Nevertheless, this is in line with the results shown by older men playing recreational floorball (Vorup et al., 2017) and by inactive adult/middle-aged men with previous experience in TH, performing 2-3 weekly RTH sessions for 12 weeks (Póvoas et al., 2018). In the present study, a decrease was observed in the number of falls in TH2, indicating improved postural balance. This decrease is lower than the observed in inactive adult/middle-aged males (Shetty et al., 2016), though, relevant compared to the lack of changes in postural balance in older men with prostate cancer that played recreational football for 12 weeks (Uth et al., 2016). Improvements of 9-13% in the agility test were also found in the TH intervention groups. Nonetheless, an improvement, although lower (5%), was also shown for CG. The higher physical fitness improvements shown in the intervention groups, may be related to the high-intensity locomotor movements and game actions elicited by RTH, e.g. changes of speed and direction, and other game-specific actions previously described. This may, potentially, improve dynamic strength, agility, balance and, ultimately, reduce the number of falls in the exercise activity, but also in this population everyday life. Nevertheless, to induce physiological adaptations important for health, a certain volume and intensity of training appears to be necessary.

Total injury incidence was 1.3 injuries per 1000 h of exposure, which is in line with the injuries reported in other RTH intervention studies (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018; Pereira et al., 2021; Pereira et al., 2020). To prevent injuries, a standardized warm-up was performed to progressively prepare the participants to the training sessions and some official TH rules and settings were adapted, since the participants had no experience with the sport. The major strength of this RCT is that addresses for the first time the dose-response effects of recreational team sports on several important health-related markers. Also, this study reports physical and physiological demands that can help to interpret the dose-response results. The fact that food intake was not evaluated throughout the training intervention is a limitation

since it could have provided extra insights on the bone health and body composition results. Moreover, further bone health improvements could have been found if this population was re-evaluated after a longer period (>16 weeks), thus long-term RTH studies are warranted.

In conclusion, since RTH sessions' intensity was similar between the 3 intervention groups, training volume (i.e. 1, 2 or 3 60-min sessions/week) seems to have been the main responsible for the different bone health, body composition and physical fitness improvements found for middle-to-older-aged males, with extra benefits for 2 and 3 sessions/week, indicating a dose-response effect of RTH in this population, and confirming the original work hypothesis.

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3.3. Original Article III

Acute physiological response to different recreational team handball game formats in over 60-year-old inactive men

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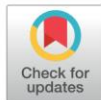
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RESEARCH ARTICLE

Acute physiological response to different recreational team handball game formats in over 60-year-old inactive men

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Abstract

This study described the physical and physiological demands, activity profile and fun levels of recreational team handball (TH) game formats in over 60-year-old men with no previous experience with this sport ($n = 17$, 67.4 ± 3.3 years). The participants performed 5v5, 6v6 and 7v7 matches (3x15-min periods) with fixed pitch size (40x20 m). In all testing sessions, heart rate (HR), differential ratings of perceived exertion and blood lactate were evaluated to measure internal load. Locomotor profile, game actions and accelerometer data were used to access external load. Also, fun levels were registered at the end of all testing sessions. Mean ($76-77\%HR_{max}$) and peak HR ($84-86\%HR_{max}$) decreased from the first to the third match period, in 6v6 and 7v7 ($p \leq 0.034$, $d = 0.730$). Blood lactate increased from baseline to the first period and decreased from the first to the third period in all game formats ($p < 0.001$, $d = 1.646$). The participants covered longer total distances in 6v6 vs 5v5 ($p \leq 0.005$, $d = 0.927$) and spent more time in fast running in 6v6 vs 5v5 and 7v7 ($p < 0.001$, $d = 1.725$) and in 5v5 vs 7v7 ($p = 0.007$, $d = 0.912$). A higher number of throws was performed in 5v5 vs 6v6 and 7v7 ($p < 0.001$, $d = 1.547$), and in 6v6 vs 7v7 ($p = 0.031$, $d = 0.779$). The number of stops and total actions in 7v7 was significantly lower vs 5v5 and 6v6 ($p \leq 0.003$, $d = 1.025$). Recreational TH is a high-intensity and motivating exercise mode for middle-aged and older men, regardless the game format. However, higher high-intensity demands were observed during 5v5 and 6v6 game formats. Therefore, it is suggested a multiple game format (5v5, 6v6 and 7v7) training plan, with more use of 5v5 and 6v6 game formats, with training sessions lasting up to 15-min of warm-up and 3x15-min periods of match-play, when prescribing recreational TH to improve cardiovascular and musculoskeletal health in this population.

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Introduction

As world population' life expectancy is increasing, promoting healthy behaviors and high health-related quality of life at old age, has become a major concern. Physical inactivity is a risk factor for several noncommunicable diseases and a leading cause of death for global mortality [1]. Men have higher incidence and death rates of ischemic heart disease, diabetes mellitus during midlife, and of most cancers (not related to reproduction) than women [2, 3]. On the other hand, the health benefits of physical activity (PA) and exercise for older adults are well established [4, 5]. Those include decreasing the prevalence of common chronic diseases, namely, those previously mentioned [6], and cognitive decline [7], increasing physical function, mental health [8] and, consequently, improving quality of life [9]. Nonetheless, 58% of the European middle-aged and older men (over 55 years old) do not exercise or play any sports, mainly due to lack of time or motivation [10]. Although the benefits of exercising outweigh the risks associated with being physically inactive [11], there is still a concern with the risk of injury [12]. This especially in older, inactive and exercise/sport inexperienced populations. Consequently, finding alternative exercise programs that are effective, safe, and motivating enough to ensure long-term adherence to exercise for this population is essential.

Exercise programs using recreational team sports, an adaptation of the official versions played as different small-sided games (SSGs), have been adopted as a motivating strategy to decrease physical inactivity and promote broad-spectrum health, physical fitness and well-being improvements in different populations [13–17]. Recreational team sports have shown to have a major beneficial impact on cardiorespiratory fitness [13, 15], which has been associated with the time spent with high heart rates (HR) during recreational team handball (TH) matches [18]. Despite the high physical and physiological demands imposed by SSGs, the participants have reported moderate ratings of perceived exertion (RPE) [19].

TH is played by around 30 million of players worldwide [20] being a particularly popular sport in Europe, namely in Portugal. If we add to this, the number of fans and supporters, the social capital emerging from this sport practice can be considered of great interest. Recreational TH has shown to be a high-intensity intermittent exercise mode [15, 21], effective in improving physical fitness and cardiometabolic health (e.g. maximal oxygen uptake (VO_{2max}), blood pressure, aerobic performance, and blood lipid profile) in adult/middle-aged male former TH players [18], premenopausal overweight women [22], postmenopausal women [23] and young adult men [24] with no experience with the sport. In addition, it has proved to induce positive musculoskeletal adaptations (e.g. muscle mass, bone mineral content and density, and on bone metabolism) in young adult men [24] and women [25], and also in postmenopausal women [26].

SSGs are often used in recreational team sports exercise interventions as training tools [14–17, 19]. They are characterized by adapted rules compared to the official ones, such as number of players, size and shape of the court, allowed body contact, coach encouragement, among others, that influence internal and external load markers [27–29]. Recreational TH as exercise mode has been implemented using different game formats, ranging from formal (7v7) to 3v3 formats [15]. Notwithstanding the reported health benefits, no conclusive information exists on what is the most effective recreational TH game format to induce the reported adaptations. This issue is of great practical interest in the daily practice, as different number of participants may attend the training sessions, and also to guide future exercise interventions.

In competitive soccer, the number of players per playing surface has been reported to impact the game demands [30]. On other hand, in recreational soccer, high HRs were observed for different age, sex and social background groups, by playing SSGs, independently of number of players [31]. Additionally, similar physical and physiological demands were reported for

21-year-old college students during recreational TH SSGs (4v4, 5v5 and 6v6) [32]. Nevertheless, the demands of this exercise mode have not yet been described for older populations, and the specific demands of other game formats frequently used in recreational TH-based exercise interventions (i.e., 5v5, 6v6 and 7v7) are still to be ascertained.

In order to induce cardiovascular health improvements, average exercise intensity should range between 60–85%HR_{max} [33], and for optimal improvements it appears to be important to spend a significant amount of time of the training session with HRs above 85%HR_{max} [34]. In fact, in a 12-week recreational TH intervention, post-intervention changes in VO_{2max} were largely correlated with the time spent with HRs >90%HR_{max} [18]. Recreational team sports intensities have shown to be in the range of these intensities in different populations [15]. Nonetheless, this has not yet been described for middle-aged and older men. To optimize training load during recreational TH interventions, it is also important to describe the intensity of the different time periods within a proposed SSG. This to ascertain if a high intensity is maintained throughout the entire training session. Despite the interest of the maintenance of an effective exercise intensity during the matches, recreational TH internal and external load differences between match periods have only been addressed in one study with adult/middle-aged men with previous experience in TH [21]. In that study similar cardiovascular load during the entire match duration (60 min) was reported. However, a decrease was observed in the second half in the frequency and distance covered in some of the locomotor categories, specific game actions and blood lactate (BL) values [21]. Unfortunately, no study is currently available on the effects of game format duration on exercise intensity in over 60-year-old inactive men with no previous experience with recreational TH.

Long-term adherence to the exercise programs is a major concern when planning exercise interventions [35]. Recreational team sports have been considered as a social, fun and intrinsically motivational exercise mode [36], which are important characteristics for long-term adherence to exercise, namely, in the elderly male population [36]. Therefore, it is of relevance to evaluate the self-reported fun levels (which reflect enjoyment) during recreational TH played as different game formats, as it may well be a positive affective response and an intrinsically motivational factor for the participation and adherence of an individual to an exercise program [36].

Given the above reported premises, the aim of this study was to describe the acute physiological response, activity profile and fun levels of 5v5, 6v6 and 7v7 recreational TH game formats in over 60-year-old men when played over the official court (40x20 m). We hypothesized that 5v5 elicits higher cardiovascular (internal load) and activity profile (external load) demands due to the larger playing area, and, consequently, lower player density, and higher fun levels, as a result of higher involvement of the participants in the match.

Materials and methods

Participants

The recruitment process was done through advertisement in social media (Facebook), flyers/posters, and face-to-face meetings in local senior institutions. Seventeen male participants (67.4±3.3 (±SD) years; stature 168.2±5.5 cm; body mass 79.0±11.8 kg; fat mass 29.0±6.2%; body mass index 27.8±3.2 kg·m⁻²; peak oxygen uptake (VO_{2peak}) 27.9±4.1 mL·min⁻¹·kg⁻¹; systolic blood pressure 132±20 mmHg; diastolic blood pressure 78±8 mmHg; resting HR 69±12 b·min⁻¹; Yo-Yo intermittent endurance level 1 test (YYIE1) 480±256 m) with no previous experience with this sport, agreed to participate in this study. Inclusion criteria were: male participants, aged over 60 years, inactive (i.e., not complying with the PA guidelines for the last 6 months). Exclusion criteria were: participants with medical contraindications to perform

moderate-to-vigorous PA or incapacity to run or grip a ball. All the participants were informed about the study purposes, risks and benefits and signed a written informed consent according to the Declaration of Helsinki. Ethical approval was provided by the local Institutional Review Board (CEFADE 19 2019).

Experimental design

All the participants were familiarized with the procedures involved in this study in the week preceding the data collection. Evaluations started with assessment of anthropometric variables, body composition, blood pressure, resting HR and VO_{2max} , in this order. Afterwards, on 2 separate days, the participants were tested for individual locomotor categories speed thresholds by performing each locomotor category at their individual speed, twice, over a 20 m distance, with 90-s recovery in-between, and for aerobic performance (YYIE1). Finally, internal and external load markers were monitored for each participant during 9 testing sessions. These sessions consisted of a standardized warm-up followed by recreational TH matches, 3 of each game format (5v5, 6v6 and 7v7), performed in a random order (Fig 1). These game formats were selected as they are typically used in recreational TH interventions that have shown to result in health improvements [15]. There were 48 hours between each testing session and the participants were asked to refrain from intense PA in the 48h before the testing sessions. The court size was 40x20 m, resulting in ~80, 67 and 57 m² per player for 5v5, 6v6 and 7v7 game formats, respectively, to test the effect of player density.

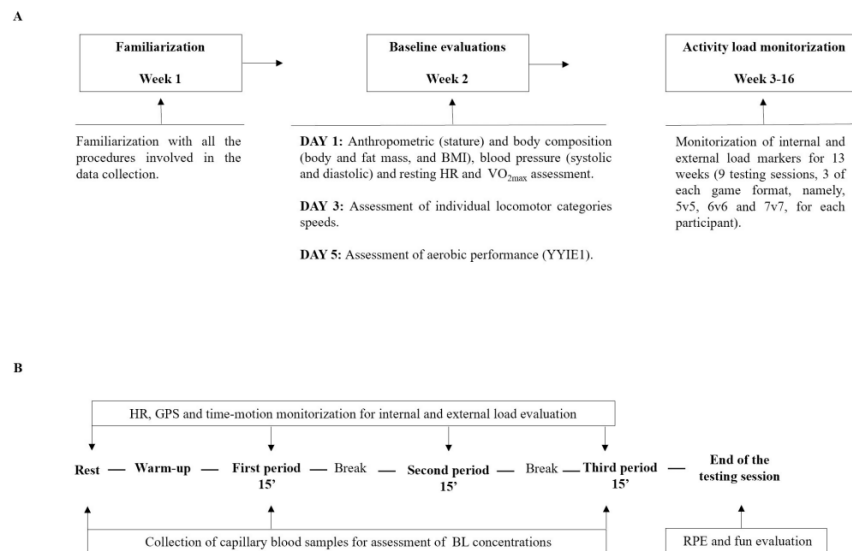


Fig 1. Schematic representation of the study protocol. BMI—Body mass index; HR—Heart rate; VO_{2max} —Maximal oxygen uptake; YYIE1—Yo-Yo intermittent endurance level 1 test; GPS—Global positioning system; BL—Blood lactate; RPE—Rating of perceived exertion.

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Players' internal load was evaluated as exercise HR, BL concentration and differential RPE. Fun levels were also recorded at the end of all testing sessions. TH high-demanding game actions (i.e., jumps, throws, changes of direction, one-on-one situations and stops) and distances covered in selected locomotion categories were considered to profile participants' external load. With the aim to account for inter-individual variability in external load, time-motion analysis was performed according to participants' individual speed categories.

All matches were performed during morning sessions and the participants wore t-shirts and shorts. The participants were hydrated at the beginning of the testing session and were allowed to drink water *ad libitum* to ensure the maintenance of proper hydration throughout the testing sessions. Each testing session comprised a 15-min standardized warm-up, consisting of running, coordination, flexibility, balance, and strength exercises, and three 15-min periods of recreational TH matches played either as 5v5, 6v6 and 7v7, interspersed by 2-min breaks.

The warm-up started with back-and-forth progressive intensity runs in the TH court combined with articular movements for the upper and lower body during approximately 5 min. Then, the other 10 min aimed at flexibility and balance exercises for the upper and lower body and at strength exercises for the main muscles, namely, squats, frontal and side lunges, push-ups, and frontal and side planks. The mean HR during the warm-up of the testing sessions was $69\%HR_{max}$.

After the warm-up, the second part of the testing sessions consisted of three 15-min periods of recreational TH matches. At every 3-min during the matches, the participants were instructed to change their positions assuring even rotation between the participants in the out-field and goalkeeper positions. There were no players' substitutions during the matches and the participants were instructed to follow the basic TH rules. However, the balls used were smaller (47 cm circumference, GOALCHA, Fredericia, Denmark) and made of softer material than the official TH balls, and no body contact was allowed. This, to avoid injuries, since the participants had no experience with this sport. Only data from participants that performed all the three 15-min periods were analyzed.

All testing sessions were instructed by a professional TH coach and physical education teacher and monitored by the research team. All the data collection and analysis were performed by the research team that comprised an experienced group of Sport Science, Physical Exercise and Health and Physical Education Teaching Master and PhD graduates, that had at least 5 years of experience with the testing procedures and analysis.

Experimental procedures

Anthropometric and health outcomes procedures. Body mass (0.01 kg) and fat mass (%) were measured in a bioimpedance digital scale (Tanita Inner Scan BC 532, Tokyo, Japan) and stature (0.1 cm) was determined using a portable stadiometer (Seca 213, Hamburg, Germany), according to standardized protocols [37]. Body mass index was calculated ($\text{kg}\cdot\text{m}^{-2}$). Blood pressure and resting HR measurements were assessed with an automatic upper arm blood pressure monitor (multiparameter patient monitor, Omron Z207, Kyoto, Japan). The participants were required to sit and rest for at least 5 min prior to the first blood pressure measurement. Two measurements were taken after 5 and 10 min of rest from the right arm, with the participants seated, in a relaxed position with their feet resting flat on the ground. The mean of the two measurements was considered for blood pressure analysis. If the two measurements differed by 2 mmHg or more, a third measure was taken. The lowest resting HR value was considered for analysis [38].

To access VO_{2max} , the participants performed an incremental treadmill test until voluntary exhaustion (H/P/Cosmos, Quasar, Germany) [39]. For this purpose, the participants walked on the treadmill for at least 3 min for each stage. The participant's HR was taken every min, and if the participant's HR was not at steady state by the 3rd min, the test continued at that same stage for another min. The first stage was considered the warm-up stage and was performed at 2.7 km·h⁻¹ and 10% inclination, the second stage at a 4 km·h⁻¹ and 12% inclination, the third stage at a 5.4 km·h⁻¹ and 14% inclination, the fourth stage at 6.7 km·h⁻¹ and 16% inclination and the fifth stage at 8 km·h⁻¹ and 18% inclination [39]. The test was performed until voluntary exhaustion. The participants completed at least all the three first stages and the fifth stage was the highest reached. VO_{2max} and respiratory exchange ratio (RER) were determined by pulmonary gas exchange measurements (Oxycon Pro Metabolic Cart, Jaeger, careFusion, Germany) with the participants wearing a HR monitor (Polar Wearlink, Kempele, Finland). VO_{2peak} was considered as the highest 15-s mean value. The test ended at the participants' voluntary exhaustion and the results were considered as VO_{2peak} if two of the following criteria were met: failure of VO_2 to increase with increased exercise intensity; $RER \geq 1.1$; maximal HR (HR_{max}) $\geq 85\%$ of age-predicted HR_{max} [40]. The age-predicted HR_{max} was determined by the formula $208 - (\text{age} \times 0.7)$ [41]. Aerobic performance was evaluated by the YYIE1. The YYIE1 test was performed on the same indoor TH wooden floor court as the matches, after a 10-min warm-up consisting of running at different speeds and changes of direction. The test consists of 2x20 m shuttle runs with increasing speeds interspersed by 5 s of active recovery, with the participants walking around a cone placed 2.5 m behind the starting/finishing line. At set intervals, the running speed increases, starting at 8.0 km·h⁻¹. The total distance (m) covered during the test was recorded as test result for each participant [42].

Internal load outcomes procedures. One hundred and fifty-three HR recordings from 17 participants during the three game formats (9 matches per participant; 3 for each game format) were analyzed. Exercise intensity was assessed using HR monitors (Firstbeat Technologies Ltd., version 4.5.0.2, Jyväskylä, Finland). Selected HR zones were ≤ 60 , 61–70, 71–80, 81–90, 91–100% HR_{max} . In this study, the individual HR_{max} was determined as the highest value reached either during the VO_{2max} test, YYIE1 or matches, according to a multiple testing approach [43]. Capillary blood samples (30 μ l) were drawn from the right earlobe to determine BL concentrations (306 records from 17 players), at baseline (resting conditions) and at the end of the first and third period of the matches. For this analysis, a portable electroenzymatic lactate device analyzer (Lactate Pro 2 LT-1730, Arkray, Amsterdam, The Netherlands) was used. RPE is a practical, reliable and valid tool to estimate internal load and adding differential RPE (i.e., respiratory and muscular), may increase the sensitivity of internal load measurements [44]. Therefore, differential RPE [45] and fun levels (using a visual analogic scale; 0–10 AU) [46] were registered at the end of all game formats. Participants were familiarized with the use of the considered psychometric scales in training sessions performed before this study.

External load outcomes procedures. Video recordings (153 evaluations; 17 participants) (SONY-DCR-SX65E, digital video camera recorder, Weybridge, United Kingdom) were collected for activity profile characterization using time-motion analyses. Players' displacements were divided into eight locomotor categories: 1) standing still, 2) walking, 3) jogging, 4) fast running, 5) sprinting, 6) sideways medium-intensity, 7) sideways high-intensity and 8) backwards movement. High-intensity movements were the result of the sum of fast running, sprinting and sideways high-intensity categories [21]. Individual speed thresholds were determined in order to account for the individual nature of the exercise intensity in each locomotor category [47]. For this purpose, each participant was instructed to perform each locomotor category at their individual speed, twice, over a 20 m distance, with 90 s of recovery in-between.

Telemetric photoelectric cells (Brower Timing System, IRD-T175, Utah, USA) registered the individual speeds. The distance covered in each category was calculated by multiplying each participants' individual speeds by the time spent in each locomotor category. This study participants mean speeds in each locomotor category were 0 km·h⁻¹ for standing, 6 km·h⁻¹ for walking, 9 km·h⁻¹ for jogging, 12 km·h⁻¹ for fast running, 17 km·h⁻¹ for sprinting, 8 km·h⁻¹ for sideways medium-intensity, 10 km·h⁻¹ for sideways high-intensity and 8 km·h⁻¹ for backwards movements. Frequency of the selected high-demanding match actions, i.e., jumps, throws, stops, changes of direction and one-on-one situations, and total number of actions were registered via video-analysis of the matches.

Accelerometer data was collected using Catapult MinimaxX S4 (MinimaxX S4; Catapult Sports, Canberra, Australia) in indoor mode with global positioning system units (GPS) technology in inactive state. Data was downloaded and processed using Catapult sprint Version 5.1.1 (Catapult Innovations, Canberra, Australia). Units were located in a specific vest on players' upper back. The validity and reliability of the accelerometers have been described elsewhere [48]. Player load (PL) (an estimate of physical demand combining the instantaneous rate of change in acceleration in 3 planes [49]) variables were evaluated at a 100 Hz sampling rate. In this study, PL was presented as percentage of time spent in PL zones 0–0.1, >0.1–0.3, >0.3–0.6, >0.6–1.0, >1.0–1.5, >1.5–2.0, >2.0 [49] and total accumulated PL. The matches were held under neutral temperature (20–22°C) and humidity conditions (50–60%).

Statistical analysis

Data was tested for normal distribution using Shapiro-Wilk test. Results are presented as means ± standard deviations (SD). Differences between game formats' internal and external load variables were assessed by repeated measures analysis of variance (ANOVA) with Bonferroni post hoc test for multiple comparisons tests. Power calculations were performed to detect an effect size of 0.25 in a one-way ANOVA of repeated measures (within subjects). Using 3 groups and 3 measurements, with correlation between measures of 0.75, alpha of 5%, and power of 80%, 15 participants were needed. Practical significance was assessed by calculating Cohen *d* and interpreted as trivial (<0.2), small (0.2–0.5), medium (0.5–0.8) and large (>0.8) [50]. IBM Statistical Package for the Social Sciences (SPSS), Statistics for Windows, (Version 25.0, Armonk, New York, USA: IBM Corp.) was used for all analyses. Statistical significance was set at $p \leq 0.05$.

Results

Internal load and fun levels during each game format

Players' internal load and fun variables for each game format (5v5, 6v6 and 7v7) are presented in Table 1 and Fig 2. No significant differences were found between game formats' cardiovascular demands, RPE and BL, except for peak BL, which was significantly higher in 5v5 (5.6±2.1 mmol·l⁻¹) than in 7v7 (4.7±1.7 mmol·l⁻¹; $p = 0.014$, 95% CI: -1.4, -0.3, large).

Players' BL values during 5v5, 6v6 and 7v7 game formats are presented in Fig 3. In all game formats, mean BL values increased significantly from baseline to the first period (5v5: $p < 0.001$, 95% CI: 1.2–2.7, large; 6v6: $p \leq 0.001$, 95% CI: 1.0–2.6, large; 7v7: $p \leq 0.001$, 95% CI: 0.8–2.2, large) and decreased significantly from the first to the third period (5v5: $p \leq 0.001$, 95% CI: -1.5, -0.6, large; 6v6: $p \leq 0.001$, 95% CI: -1.7, -0.6, large; 7v7: $p \leq 0.001$, 95% CI: -1.3, -0.5, large).

Activity profile during each game format

Players' locomotor profile during 5v5, 6v6 and 7v7 game formats is presented in Table 2. During 7v7 game format, the frequency of walking was significantly lower than 5v5 ($p \leq 0.001$, 95%

Table 1. Players' cardiovascular load, perceived experience, and fun levels during 5v5, 6v6 and 7v7 recreational team handball game formats (data are presented as means \pm SD).

Variable	Game formats		
	5v5	6v6	7v7
Heart rate			
Mean HR (b·min ⁻¹)	129 \pm 9	129 \pm 10	128 \pm 11
Mean HR (%HR _{max})	77 \pm 5	77 \pm 4	76 \pm 6
Peak HR (b·min ⁻¹)	145 \pm 10	144 \pm 12	142 \pm 12
Peak HR (%HR _{max})	86 \pm 5	85 \pm 5	84 \pm 6
Time >80%HR _{max} (%)	35 \pm 19	37 \pm 21	32 \pm 21
Time \leq 60%HR _{max} (%)	5 \pm 9	2 \pm 2	2 \pm 4
Time 61–70%HR _{max} (%)	16 \pm 10	17 \pm 12	21 \pm 18
Time 71–80%HR _{max} (%)	44 \pm 15	46 \pm 16	44 \pm 15
Time 81–90%HR _{max} (%)	32 \pm 16	32 \pm 16	28 \pm 17
Time 91–100%HR _{max} (%)	3 \pm 7	4 \pm 8	4 \pm 6
Blood lactate			
Match mean blood lactate (mmol·l ⁻¹)	3.9 \pm 1.5	3.7 \pm 1.3	3.6 \pm 1.4
Match peak blood lactate (mmol·l ⁻¹)	5.6 \pm 2.1	5.0 \pm 1.9	4.7 \pm 1.7
			* <i>p</i> = 0.014; <i>d</i> = 0.843
Perceived experience			
Respiratory RPE (AU, 0–10)	6.6 \pm 2.3	6.4 \pm 2.1	6.4 \pm 2.1
Muscular RPE (AU, 0–10)	6.6 \pm 2.4	6.3 \pm 2.1	6.1 \pm 2.1
Global RPE (AU, 0–10)	6.7 \pm 2.4	6.3 \pm 2.1	6.3 \pm 2.1
Fun (AU, 0–10)	9.0 \pm 1.0	9.0 \pm 0.9	8.6 \pm 1.6

HR—Heart rate; HR_{max}—Maximal heart rate; RPE—Rating of perceived exertion; AU—Arbitrary units.

* Significantly different from 5v5.

<https://doi.org/10.1371/journal.pone.0275483.t001>

CI: 1.3–3.6, large) and 6v6 ($p \leq 0.001$, 95% CI: 1.8–4.7, large). Additionally, 6v6 percentage of time spent jogging ($p \leq 0.001$, 95% CI: -5.1, -1.4, large), and 5v5 and 6v6 total distance covered ($p = 0.004$, 95% CI: -5.9, -1.7, large; $p = 0.034$, 95% CI: -5.7, -0.8, large; respectively; Fig 4) were significantly higher than 7v7. During 5v5 and 6v6, frequency ($p < 0.001$, 95% CI: -9.3, -3.7, large; $p \leq 0.001$, 95% CI: -13.9, -5.4, large; respectively), percentage of time spent ($p = 0.007$, 95% CI: -1.7, -0.4, large; $p < 0.001$, 95% CI: -2.9, -1.3, large; respectively), and total distance covered ($p = 0.011$, 95% CI: -138.0, -31.8, large; $p < 0.001$, 95% CI: -298.5, -111.5, large; respectively) in fast running were significantly higher than during 7v7.

During 7v7 game format, high-intensity movements' frequency was significantly lower than in 5v5 ($p < 0.001$, 95% CI: -10.6, -4.3, large) and 6v6 ($p \leq 0.001$, 95% CI: -14.6, -5.5, large). During 5v5 and 7v7, percentage of time spent ($p = 0.030$, 95% CI: 0.3–1.7, large; $p < 0.001$, 95% CI: -3.0, -1.3, large; respectively), and total distance covered ($p = 0.020$, 95% CI: 36.2–189.5, large; $p < 0.001$, 95% CI: -249.0, -113.3, large; respectively) in high-intensity movements were significantly lower than during 6v6. Moreover, 5v5 percentage of time spent in high-intensity movements was significantly higher than 7v7 ($p = 0.005$, 95% CI: -1.8, -0.5, large). Players' high-intensity actions frequency during the matches is presented in the Table 3. During 5v5 and 6v6, the number of throws ($p < 0.001$, 95% CI: -4.7, -2.1, large; $p = 0.031$, 95% CI: -2.3, -0.4, large; respectively), stops ($p = 0.017$, 95% CI: -4.0, -0.8, large; $p = 0.002$, 95% CI: -3.3, -1.1, large; respectively) and total actions ($p = 0.003$, 95% CI: -13.2, -4.0, large; $p = 0.017$, 95% CI: -9.1, -1.8, large; respectively) was significantly higher than during 7v7 game formats.

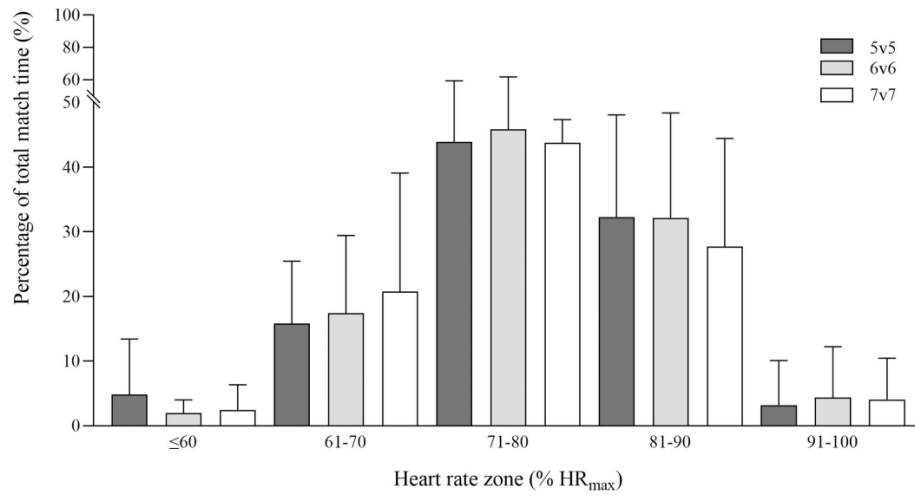


Fig 2. Percentage of total match time spent in each intensity zone expressed as percentage of players' maximal heart rate (HR_{max}) during 5v5 (dark grey bars), 6v6 (light grey bars) and 7v7 (white bars) recreational team handball game formats (data are presented as means ± SD).

<https://doi.org/10.1371/journal.pone.0275483.g002>

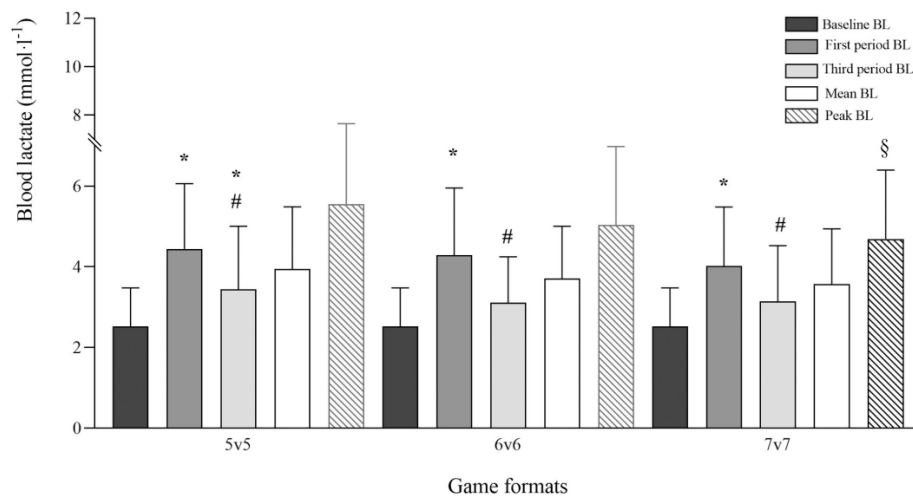


Fig 3. Baseline (dark grey bars), first period (medium grey bars), third period (light grey bars) mean blood lactate and match mean (white bars) and peak (listed bars) blood lactate levels during 5v5, 6v6 and 7v7 recreational team handball game formats (data are presented as means ± SD). BL—Blood lactate. * Significantly different from baseline; # significantly different from the first period and § significantly different from 5v5 ($p < 0.05$).

<https://doi.org/10.1371/journal.pone.0275483.g003>

Table 2. Players' locomotor profile during recreational team handball game formats (5v5, 6v6 and 7v7) (data are presented as means ± SD).

	Locomotor categories									Total
	Standing	Walking	Jogging	Fast running	Sprinting	Side Med	Side High	Back	High-intensity	
Freq (n)										
5v5	16±6	89±12	78±16	17±8	1.4±1.9	14±9	0.1±0.1	30±11	18±9	245±35
6v6	8±5 <i>p</i> <0.001; <i>d</i> = 1.880	84±15	76±16	20±10	0.8±1.0	18±7 <i>p</i> = 0.038; <i>d</i> = 0.707	0.1±0.2	30±13	21±11	237±45
7v7	9±5 <i>p</i> <0.001; <i>d</i> = 1.303	92±10	72±14	10±6 <i>p</i> <0.001; <i>d</i> = 1.236 <i>p</i> ≤0.001; <i>d</i> = 1.325	0.4±0.9 <i>p</i> = 0.021; <i>d</i> = 1.199	19±11 <i>p</i> = 0.002; <i>d</i> = 1.066	0.0±0.1	33±11	11±7 <i>p</i> <0.001; <i>d</i> = 1.332 <i>p</i> ≤0.001; <i>d</i> = 1.301	236±28
Freq (%)										
5v5	7±3	37±4	32±4	7±3	0.5±0.7	6±4	0.0±0.0	12±4	7±3	
6v6	3±2 <i>p</i> <0.001; <i>d</i> = 1.627	36±5	32±4	8±4 <i>p</i> = 0.035; <i>d</i> = 0.782	0.3±0.4	8±3 <i>p</i> = 0.004; <i>d</i> = 0.994	0.0±0.1	13±4	8±4	
7v7	4±2 <i>p</i> <0.001; <i>d</i> = 1.683	39±3 <i>p</i> <0.001; <i>d</i> = 1.121 <i>p</i> ≤0.001; <i>d</i> = 1.287	31±4	4±2 <i>p</i> <0.001; <i>d</i> = 1.273 <i>p</i> <0.001; <i>d</i> = 1.524	0.2±0.4 <i>p</i> = 0.018; <i>d</i> = 1.227	8±4 <i>p</i> <0.001; <i>d</i> = 1.466	0.0±0.1	14±4 <i>p</i> = 0.043; <i>d</i> = 0.669	4±3 <i>p</i> <0.001; <i>d</i> = 1.406 <i>p</i> <0.001; <i>d</i> = 1.457	
Mean duration (s)										
5v5	17±6	13±3	9±2	4±1	2±2	4±1	0.3±0.7	6±2	6±2	
6v6	21±6	15±2	11±2 <i>p</i> = 0.035; <i>d</i> = 0.691	5±1	1±1	6±1 <i>p</i> <0.001; <i>d</i> = 1.628	0.0±0.1	7±1	6±2	
7v7	21±5 <i>p</i> = 0.045; <i>d</i> = 0.662	14±2	9±1 <i>p</i> = 0.004; <i>d</i> = 1.088	4±1 <i>p</i> = 0.002; <i>d</i> = 1.058	1±1	5±1 <i>p</i> = 0.002; <i>d</i> = 1.052	0.2±0.7	7±2	5±2 <i>p</i> = 0.045; <i>d</i> = 0.661 <i>p</i> = 0.004; <i>d</i> = 0.962	
Total duration (s)										
5v5	249±88	1182±236	734±213	69±37	4±7	68±57	0.2±0.4	192±73	73±42	2498±107
6v6	181±126 <i>p</i> = 0.040; <i>d</i> = 0.716	1226±237	808±232	103±64 <i>p</i> = 0.005; <i>d</i> = 1.292	3±3	113±56 <i>p</i> <0.001; <i>d</i> = 1.622	0.1±0.2	220±100	106±67 <i>p</i> = 0.011; <i>d</i> = 1.019	2654±298
7v7	202±90 <i>p</i> = 0.037; <i>d</i> = 0.685	1256±248	683±183 <i>p</i> = 0.032; <i>d</i> = 0.768 <i>p</i> = 0.015; <i>d</i> = 0.823	44±30 <i>p</i> = 0.009; <i>d</i> = 0.866 <i>p</i> ≤0.001; <i>d</i> = 1.439	1±3	110±75 <i>p</i> <0.001; <i>d</i> = 1.739	0.0±0.1	229±94 <i>p</i> = 0.020; <i>d</i> = 0.824	45±32 <i>p</i> = 0.007; <i>d</i> = 0.921 <i>p</i> ≤0.001; <i>d</i> = 1.420	2526±234
Total duration (%)										
5v5	10±3	47±9	30±9	3±1	0.2±0.3	3±2	0.0±0.0	8±3	3±2	
6v6	7±4 <i>p</i> = 0.009; <i>d</i> = 0.884	46±9	16±3 <i>p</i> <0.001; <i>d</i> = 0.213	4±2 <i>p</i> = 0.011; <i>d</i> = 0.985	0.1±0.1	4±2 <i>p</i> <0.001; <i>d</i> = 1.199	0.0±0.0	8±4	4±2 <i>p</i> = 0.030; <i>d</i> = 0.781	

(Continued)

Table 2. (Continued)

	Locomotor categories									Total
	Standing	Walking	Jogging	Fast running	Sprinting	Side Med	Side High	Back	High-intensity	
7v7	8±4 * <i>p</i> = 0.021; <i>d</i> = 0.755	50±8	27±7 * <i>p</i> < 0.001; <i>d</i> = 0.950	2±1 * <i>p</i> = 0.007; <i>d</i> = 0.912 * <i>p</i> < 0.001; <i>d</i> = 1.725	0.1±0.1	4±3 * <i>p</i> < 0.001; <i>d</i> = 1.634	0.0 ±0.0	9±4 * <i>p</i> = 0.037; <i>d</i> = 0.756	2±1 * <i>p</i> = 0.005; <i>d</i> = 0.969 * <i>p</i> < 0.001; <i>d</i> = 1.699	
Mean distance (m)										
5v5		22±5	24±6	15±4	7±8	10±3	0.6 ±1.5	15±5	22±9	
6v6		25±6	27±7 * <i>p</i> = 0.037; <i>d</i> = 0.692	18±5	5±6	14±4 * <i>p</i> < 0.001; <i>d</i> = 1.613	0.1 ±0.2	16±5	23±8	
7v7		24±7	23±4 * <i>p</i> = 0.006; <i>d</i> = 1.171	14±4 * <i>p</i> = 0.002; <i>d</i> = 1.046	3±5	12±4 * <i>p</i> = 0.005; <i>d</i> = 0.963	0.6 ±1.7	16±6	17±9 * <i>p</i> = 0.002; <i>d</i> = 1.009	
Total distance (m)										
5v5		1962±525	1816±619	237±131	20±36	154±127	0.4 ±1.0	443±207	257±156	4632±617
6v6		2088±557	2041±666	357±231 * <i>p</i> = 0.007; <i>d</i> = 1.224	13±18	258±125 * <i>p</i> < 0.001; <i>d</i> = 1.592	0.2 ±0.7	504±260	370±243 * <i>p</i> = 0.020; <i>d</i> = 0.906	5260±768 * <i>p</i> = 0.005; <i>d</i> = 0.927
7v7		2198±683	1713±524 * <i>p</i> = 0.010; <i>d</i> = 0.571	152±109 * <i>p</i> = 0.011; <i>d</i> = 0.838 * <i>p</i> < 0.001; <i>d</i> = 1.414	37±123	258±170 * <i>p</i> < 0.001; <i>d</i> = 1.524	0.1 ±0.4	524±240 * <i>p</i> = 0.033; <i>d</i> = 0.725	189±163 * <i>p</i> < 0.001; <i>d</i> = 1.688	4883±795
Total distance (%)										
5v5		43±12	39±12	5±3	0.4±0.8	3±3	0.0 ±0.0	9±4	5±3	
6v6		40±11	38±10	6±4 * <i>p</i> = 0.032; <i>d</i> = 0.777	0.2±0.3	5±2 * <i>p</i> < 0.001; <i>d</i> = 1.067	0.0 ±0.0	10±5	7±4	
7v7		45±11 * <i>p</i> = 0.007; <i>d</i> = 0.880	35±9 * <i>p</i> = 0.004; <i>d</i> = 1.178 * <i>p</i> = 0.034; <i>d</i> = 0.717	3±2 * <i>p</i> = 0.003; <i>d</i> = 1.017 * <i>p</i> < 0.001; <i>d</i> = 1.694	0.9±2.9	5±3 * <i>p</i> < 0.001; <i>d</i> = 1.368	0.0 ±0.0	11±5	4±4 * <i>p</i> = 0.034; <i>d</i> = 0.699 * <i>p</i> < 0.001; <i>d</i> = 1.100	

Freq—Frequency; Side Med—sideways medium-intensity movements; Side High—sideways high-intensity movements; Back—backwards movements; High-intensity—sum of fast running, sprinting and sideways high-intensity movements.

* Significantly different from 5v5 and * significantly different from 6v6.

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During 5v5, 6v6 and 7v7 game formats, players' percentage of time spent in 0.0–0.1, >0.1–0.3, >0.3–0.6, >0.6–1.0, >1.0–1.5, >1.5–2.0 and above 2.0 PL zones were 17–19%, 40–41%, 16–17%, 8%, 10–11%, 5–6% and 1%, respectively, and the total PL accumulated during the matches ranged between 288 to 310. The number of low, medium, high, and total accelerations during the game formats, ranged between 13–17, 7–9, 9–11 and 29–36 and the number of low, medium, high, and total decelerations ranged between 8–10, 4–5, 2–4 and 14–18, respectively.

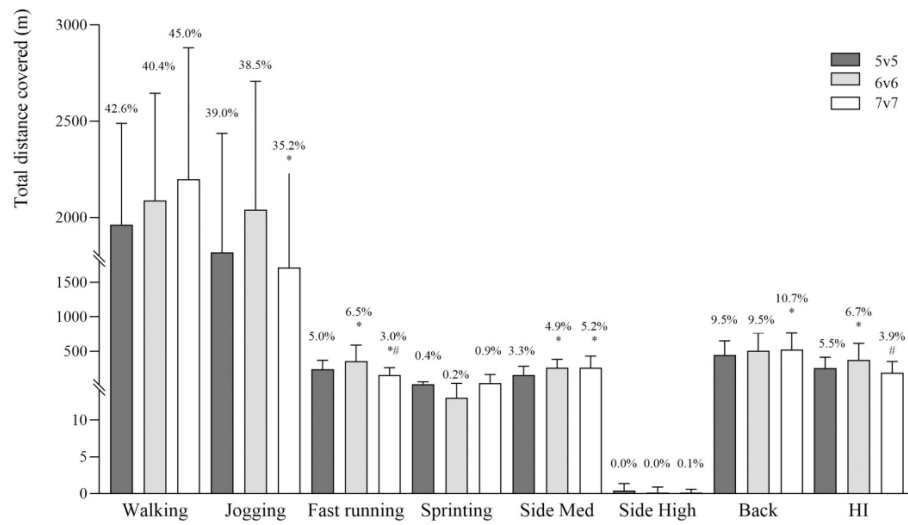


Fig 4. Total absolute and relative distance covered in the selected locomotor categories during 5v5 (dark grey bars), 6v6 (light grey bars) and 7v7 (white bars) recreational team handball game formats (data are presented as means \pm SD). Side Med—sideways medium-intensity movements; Side High—sideways high-intensity movements; Back—backwards movements; High-intensity—sum of fast running, sprinting and sideways high-intensity movements. * Significantly different from 5v5 and # significantly different from 6v6 ($p \leq 0.05$).

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No significant differences were found between the game formats in PL zones and in low, medium, high, and total accelerations and decelerations variables.

Differences between match periods

During 5v5, absolute and relative mean HR increased from the first to the second periods ($p = 0.003$, 95% CI: 1.5–4.9, large; $p = 0.003$, 95% CI: 0.9–3.0, large; respectively), remaining

Table 3. Players' high-intensity game actions (data are presented as means \pm SD) during 5v5, 6v6 and 7v7 recreational team handball game formats.

Actions	5v5	6v6	7v7
Jumps (n)	7.6 \pm 4.5	7.2 \pm 3.6	6.3 \pm 3.6
Throws (n)	8.7 \pm 3.8	6.6 \pm 3.4	5.3 \pm 2.6
		* $p = 0.003$; $d = 1.001$	* $p < 0.001$; $d = 1.547$ # $p = 0.031$; $d = 0.779$
Stops (n)	12.6 \pm 4.0	12.4 \pm 4.0	10.2 \pm 3.2
			* $p = 0.017$; $d = 0.793$ # $p = 0.002$; $d = 1.102$
Changes of direction (n)	11.7 \pm 3.2	12.0 \pm 4.1	10.7 \pm 3.5
One-on-one situations (n)	8.8 \pm 2.9	8.0 \pm 2.2	8.2 \pm 1.8
Total actions (n)	49.3 \pm 15.1	46.2 \pm 14.4	40.7 \pm 11.9
			* $p = 0.003$; $d = 1.025$ # $p = 0.017$; $d = 0.824$

* Significantly different from 5v5 and

significantly different from 6v6.

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Table 4. Players' mean, peak and percentage of total match time spent in the different heart rate zones during the three match periods in 5v5, 6v6 and 7v7 recreational team handball game formats (data are presented as means \pm SD).

Variables	Game periods								
	5v5			6v6			7v7		
	1 st period	2 nd period	3 rd period	1 st period	2 nd period	3 rd period	1 st period	2 nd period	3 rd period
Mean HR (b·min ⁻¹)	127±8	130±9 * <i>p</i> = 0.003; <i>d</i> = 1.017	129±9	131±10	130±12	127±11 * <i>p</i> = 0.035; <i>d</i> = 0.692	131±9	127±13	125±11 * <i>p</i> = 0.010; <i>d</i> = 0.855
Mean HR (% HR _{max})	76±4	77±5 * <i>p</i> = 0.003; <i>d</i> = 1.124	77±5	77±4	77±5	75±5 * <i>p</i> = 0.034; <i>d</i> = 0.730	78±5	76±6	74±6 * <i>p</i> = 0.008; <i>d</i> = 0.872
Peak HR (b·min ⁻¹)	145±10	146±10	144±10	147±12	145±12	141±14 * <i>p</i> ≤ 0.001; <i>d</i> = 1.142 * <i>p</i> = 0.017; <i>d</i> = 0.819	145±11	142±14	139±13 * <i>p</i> = 0.026; <i>d</i> = 0.736
Peak HR (%HR _{max})	86±6	87±6	85±5	87±5	86±5	84±6 * <i>p</i> ≤ 0.001; <i>d</i> = 1.247 * <i>p</i> = 0.014; <i>d</i> = 0.833	86±6	84±7	83±7 * <i>p</i> = 0.020; <i>d</i> = 0.772
Time >80%HR _{max} (%)	33±18	40±23	34±23	39±22	41±23	30±26	42±26	29±24 * <i>p</i> = 0.027; <i>d</i> = 0.717	24±21 * <i>p</i> = 0.005; <i>d</i> = 0.950 * <i>p</i> = 0.027; <i>d</i> = 0.368
Time ≤60%HR _{max} (%)	7±7	4±10	3±10 * <i>p</i> = 0.045; <i>d</i> = 0.734	3±3	1±2 * <i>p</i> = 0.008; <i>d</i> = 0.892	2±2	3±5	2±3	2±5
Time 61–70% HR _{max} (%)	18±12	14±10	16±11	13±9	14±11	25±23	17±16	21±21	24±20 * <i>p</i> = 0.023; <i>d</i> = 0.799
Time 71–80% HR _{max} (%)	42±15	42±18	48±18	46±19	42±19	49±20	40±17	45±20	46±16
Time 81–90% HR _{max} (%)	31±15	35±20	30±19	35±17	36±21	26±18 * <i>p</i> = 0.015; <i>d</i> = 0.799	37±22	24±17	22±18
Time 91–100% HR _{max} (%)	2±4	4±9	3±9	5±7	5±9	4±11	5±7	5±10	2±5

HR—Heart rate; HR_{max}—Maximal heart rate.

* Significantly different from the first match period; † significantly different from the second match period.

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unaltered in the third period (Table 4). Absolute and relative mean HR, in 6v6 ($p = 0.035$, 95% CI: -6.2, -0.9, medium; $p = 0.034$, 95% CI: -3.6, -0.5, medium; respectively) and 7v7 ($p = 0.010$, 95% CI: -9.2, -2.2, large; $p = 0.008$, 95% CI: -5.4, -1.4, large; respectively), decreased as the match time progressed, showing significant differences between the first and third periods (Table 4). During 7v7 game format time spent above 80%HR_{max} significantly decreased from the first to the second ($p = 0.027$, 95% CI: -22.3, -3.7, medium) and third ($p = 0.005$, 95% CI: -27.3, -7.9, large) period, and from the second to the third period ($p = 0.027$, 95% CI: -11.4–2.0, medium).

Total number of high-intensity game actions decreased from the first to the second period for 6v6 ($p = 0.016$, 95% CI: -6.6, -1.4, large) and 7v7 ($p = 0.020$, 95% CI: -4.1, -0.8, large), and from the first to the third period for 6v6 ($p < 0.001$, 95% CI: -8.1, -3.8 large). Distance covered

in fast running ($p < 0.001$, 95% CI: -4.7, -2.5, large) and sprinting ($p = 0.032$, 95% CI: -0.5, -0.1, large) decreased from the first to the third period for 6v6.

Discussion

The aim of this study was to describe the acute physiological response, activity profile and fun levels of 5v5, 6v6 and 7v7 recreational TH game formats in over 60-year-old men. This to provide physical exercise and sport professionals, evidence on the internal and external load characteristics of the game formats analyzed, that will allow them to make informed decisions according to the defined purposes for the training sessions. The main findings of this study were that game format had no significant impact on match internal load, although a tendency was observed for higher demands in 5v5 and 6v6 than in 7v7. Significant differences were evident in the external load variables, with 5v5 and 6v6, showing a higher number of high-intensity movements and total high-intensity game actions when compared to 7v7.

Internal load and fun levels during the game formats

During recreational TH training sessions using SSGs, mean HR is typically reported to be within 76–85%HR_{max} [18, 21–26, 32]. In the present study, mean HR values for the three game formats were lower (76–77%HR_{max}) than values observed in young adult men and women, premenopausal women and adult/middle-aged men (81–85%HR_{max}) [18, 24, 25], but equal to or higher than those reported for postmenopausal women (76%HR_{max}) enrolled in a TH intervention study that resulted in cardiovascular improvements [23]. In fact, these values are in the range of the vigorous exercise intensity threshold (60–85%HR_{max}) proposed to promote cardiovascular improvements [33].

Peak HR values were lower than those reported in studies using recreational soccer SSGs with elderly males (84–86 vs 99%HR_{max}, respectively) and, consequently, the percentage of time spent above 90% HR_{max} was also lower (4 vs 48% of total match time) [31]. However, studies using recreational floorball [51, 52] and recreational TH [23] with similar age groups showed results in line with our study. Additionally, it is worth noting that in the present study we assessed the participants' HR_{max} making use of a multiple approach in order to have an as accurate as possible value [43]. Having an accurate HR_{max} is of great practical interest as time spent above 90%HR_{max} was reported to be related to improvements in cardiorespiratory fitness in recreational TH [18]. Given that, the above differences in exercise HR may be the result of unsuitable HR_{max} assessment [15].

No significant differences were found in exercise HR between the three game formats. This is in accordance with a recent study comparing 4v4, 5v5 and 6v6 game formats using the same pitch size (40x20 m) for young (20.8±1.1 years) active college students with no competitive experience in TH [32]. However, our study reported a significant decrease in 6v6 and 7v7 game formats' mean and peak HR in the third comparing to the first match period, while mean HR in 5v5 significantly increased from the first to the second period and was then maintained during the last 15-min period of the matches. This decrease in intensity was also shown in the activity profile. The main relevance of these results is that 5v5 game format seems to be more efficient in maintaining the cardiovascular load throughout 45-min matches, perhaps due to the greater involvement in the game imposed by the lower number of players. Nonetheless, exercise intensity during all match duration in the three game formats was within the range (60–85%HR_{max}) proposed for cardiovascular improvements [33].

The practical implication of this study HR results is that they were in line with the results from other studies that used recreational team sports, especially TH, that reported

cardiovascular improvements after 12–16-week interventions. Thus, future studies should address the role of training volume and intensity on practitioners' health and fitness.

In this study, peak BL was significantly lower in 7v7 than in 5v5, meaning that in 5v5, the participants, may achieve higher anaerobic intensities. Mean and peak BL concentrations (3.6 ± 1.4 and 4.7 ± 1.7 $\text{mmol}\cdot\text{l}^{-1}$) were in line with the values reported in former TH players, during 7v7 matches (3.6 ± 1.3 and 4.2 ± 1.2 $\text{mmol}\cdot\text{l}^{-1}$) [21]. Significantly higher values were found in all game formats in the first period comparing to baseline conditions and in 5v5, in the third period comparing to baseline. This is in line with a study involving former TH players playing recreational matches [21]. Additionally, a decrease in BL values was found from the first to the third period in all game formats, which again, is in line with what has been shown for recreational TH players with previous experience with the sport [21], evidencing an intensity decrease from the beginning to the end of the recreational TH matches. This decrease is in accordance with the mean and peak HR decrease shown from the first to the third match period, as well as with the decrease in the number of high-intensity game actions during the match in 6v6 and 7v7 game formats, although not observed for 5v5.

In our study, no differences were found in RPE between game formats, with the intensity being perceived as strong-to-very-strong (6.1–6.7 AU). These values were lower than those reported for former TH players playing the recreational version of this sport [18]. However, higher than observed for postmenopausal women (4.8, AU, playing 5v5 and 6v6 matches) [23] and for male college students (3.9 AU, playing 6v6 matches) [32] performing recreational TH. The absence of differences in RPE when playing 5v5, 6v6 or 7v7 may be explained by participants' lack of experience with this sport and by a spontaneous adjustment to game format demands as shown by exercise HR consistency across the proposed game formats. It is important to highlight the very high fun levels reported by the participants (9 out of 10) while playing recreational TH, since enjoyment is a key factor to increase motivation and assure long-term adherence to an exercise program [53]. Also, the perceived high rate of fun in this population may mask the high metabolic, musculoskeletal, and cardiorespiratory strain during training interventions.

Activity profile during the game formats

Significant differences were found between the game formats' external load, namely high-intensity locomotor activity variables. Standing frequency was significantly higher in 5v5 than in 6v6 and 7v7, which may be related to more stops being needed to recover since there are less players involved in the match. The walking frequency was significantly higher in 7v7 than in 5v5 and 6v6 and the jogging frequency showed no significant differences between the game formats. These variables were the ones in which the participants spent more time during the matches (76–77% of total match time) in all game formats. Furthermore, the frequency of fast running movements was significantly higher in 6v6 than in 5v5 and 7v7. Frequency of sprints, resulted higher in 5v5 than in 7v7. Moreover, the frequency of high-intensity movements was significantly higher in 5v5 and 6v6 than in 7v7. These results show that 5v5 and 6v6 may induce higher load on muscles and bones than 7v7.

Standing time was significantly higher in 5v5 than in 6v6 and 7v7. When comparing to former TH players playing the recreational version of the sport, our participants' standing time was higher (8–10 vs 4%), which may be related to their lower physical fitness (27.9 ± 4.1 vs 40.2 ± 7.0 $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$). The 7v7 game format revealed to promote significantly more jogging and less high-intensity distance covered than the other game formats. Interestingly, match time spent with high-intensity movements (2–4%) was similar to that reported in elite (4%) and recreational TH players with previous experience in the sport (6%) [21]. Time spent at

high-intensity seems to be a good indicator when the purpose is to achieve cardiovascular adaptations [13]. However, caution should be taken when comparing these results with those studies, due to differences in speed thresholds. The 5v5 game format showed a significantly higher number of throws and stops than 6v6 and 7v7, suggesting a greater individual game involvement due to the reduced number of players. The practical implication of these results is that 5v5 and 6v6 may be better options than 7v7, when the aim is to induce musculoskeletal improvements in this population. Moreover, when playing 5v5 and 6v6, the participants performed higher number of specific TH game actions, which leads to a higher participation in the training session, and consequently, may result in higher motivation.

We had hypothesized that 5v5 game format would elicit higher cardiovascular (internal load) and activity profile (external load) demands due to the larger playing area, and, consequently, lower player density, and higher fun levels as a result of higher participant involvement in the match. However, changing the number of players (5v5, 6v6 and 7v7) in the same pitch size (40x20 m) did not result in significant differences in majority of these variables. The practical application of these results is that all the three game formats elicited high loading in over 60-year-old inactive men and therefore, all can be recommended as options for organizing recreational TH.

In summary, this study results may guide physical exercise professionals and/or TH coaches, on the best practices when using recreational TH as exercise mode to promote physical fitness and health of older populations.

Although in this study we analyzed the game formats typically used in recreational TH-based exercise interventions (i.e., 5v5, 6v6 and 7v7), a study limitation is the fact that we did not study other game formats' demands, namely 4v4 or 3v3, as there could be differences in internal and external load in comparison to other populations. Additionally, other contextual variables should be addressed in the future, namely court dimensions and comparing indoor vs outdoor pitches, as this type of exercise program may be implemented in different environments. Future studies with more participants may be used to elucidate whether there are some minor advantages in relation to intensity and fun scores by using 5v5 and 6v6 in comparison to 7v7, in a 40x20 m TH court, for over 60-year-old inactive men. Future research should also test the training effects of the proposed different recreational TH formats on participants' health and physical fitness.

Conclusions

Recreational TH internal load demands are similar either played as small-sided (5v5, 6v6) or formal game formats (7v7), in the same pitch size (40x20 m), and are within the range to induce cardiovascular adaptations. This, across match time periods (i.e., 3x15-min). Higher frequency of high-intensity game actions was found in 5v5 and 6v6. Accordingly, these game formats may be better options when the purpose is to induce musculoskeletal improvements in this population.

The higher number of total actions and throws found in 5v5 and 6v6 may also reveal to be of practical importance as a greater involvement may lead to a higher level of motivation and therefore, to higher fun levels and long-term adherence to the exercise program. Nonetheless, recreational TH practice is a highly motivational activity, whatever the chosen game format.

From a practical point of view, this study results suggest that recreational TH can be a valid exercise option to promote health improvements in over 60-year-old men.

A multiple game format approach may be used in recreational TH interventions to provide training variety and training sessions should last up to 60 min. Additionally, considering the very high fun levels reported during recreational TH matches and that lack of motivation to

exercise is a major hurdle, future intervention studies using this exercise mode for this population are warranted.

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3.4. Original Article IV

Mixed-gender small-sided recreational team handball games in middle-aged and elderly are physiologically more demanding for women than men

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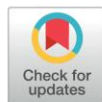
RESEARCH ARTICLE

Mixed-gender small-sided recreational team handball games in middle-aged and elderly are physiologically more demanding for women than men

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Abstract

This study examined the physical and physiological demands and perceived experience of a multicomponent exercise mode, recreational team handball (TH), for middle-aged/elderly men and women, played as same- vs. mixed-gender 6v6 game formats. Matches' heart rate (HR), blood lactate (BL), perceived experience, activity profile, player load and accelerometer variables were assessed. Forty-one participants, with at least 12 weeks of experience with recreational TH (22 men; 69±4 years, 19 women; 66±6 years), performed 2 same- and 2 mixed-gender matches on an indoor 40x20 m TH court. A game format-by-gender interaction was observed for mean HR (%HR_{max}), time spent >80 and >90%HR_{max}, respiratory rating of perceived exertion and for several of the external load variables ($p \leq 0.05$). During mixed-gender matches, time spent >80 and >90%HR_{max} was higher for women vs. men ($p \leq 0.017$). During same- and mixed-gender matches, BL was lower for women than men ($p \leq 0.015$). Time spent >90%HR_{max} was lower for women ($p = 0.036$), whereas time spent >80%HR_{max} was higher for men during same- vs. mixed-gender matches ($p = 0.034$). The frequency, %total match time and distance covered with high-demanding movements were higher for men during same-gender than during mixed-gender matches ($p \leq 0.036$), and higher for men vs. women in same- and mixed-gender matches ($p \leq 0.046$). The frequency of high-intensity actions, accelerations, time spent in the higher player load zones and total accumulated player load, were higher for men vs. women during same- and mixed-gender matches ($p \leq 0.044$). Fun levels were very high (9.1–9.3 AU, 0–10). Mixed-gender small-sided recreational TH games are physiologically more demanding for middle-aged/elderly women compared to men. Men showed higher cardiovascular and activity profile demands when playing same-gender matches, which was opposite to women. Nevertheless, TH is a

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high-intensity and motivating exercise mode for both genders, regardless the gender game format, meaning that exercise interventions may use same- and mixed-gender matches to promote participants' health.

Introduction

Multicomponent exercise interventions (i.e. a combination of resistance, endurance, and balance training) have shown to be the best strategy to improve the overall health status of frail elderly individuals [1]. Recreational team handball (TH) is a multicomponent exercise, as it requires high levels of maximal strength and muscle power to sustain the forceful muscle contractions requested during TH specific movements, a high aerobic and anaerobic turnover to cope with the intermittent high-intensity nature of the game and specific agility and balance to respond to frequent changes of direction, speed and actions [2,3].

Played as formal (i.e., 7v7) or small-sided games (3v3 to 6v6), TH has shown to improve cardiometabolic health and physical fitness of different populations with different levels of fitness, and with or without experience with the sport [4–7]. Additionally, it has proved to induce positive musculoskeletal adaptations in young adult men [5] and women [8] and in postmenopausal women [9].

Recreational TH activity profile has only been described in detail for adult/middle-aged formerly trained men [3], male college students, [10] and unexperienced older men [11]. For these participant groups, the average distance covered was 3–5 km during 40–60 min matches and 40–54 specific TH high-intensity game actions were performed, which may provide a combined positive impact on cardiovascular, metabolic, and musculoskeletal health. These physical demands imposed by recreational TH practice alongside with the high physiological stress [mean heart rates (HR): 77–85% of maximal HR (HR_{max}), time spent $>90\%HR_{max}$: 4–22% of total match time and average blood lactate (BL): 3.6–4.4 $mmol\cdot l^{-1}$] have been suggested as the main reasons for the broad-spectrum health benefits observed in the studied populations [3,10,11].

The studies reporting the health and physical fitness effects of recreational TH interventions have only been organized as gender-specific exercise programs. The possibility of having mixed-gender groups in recreational TH interventions, which mainly use match-playing as training tool, is of practical advantage in a community setting where classes frequently include participants of both genders.

Although time-course aging-related changes differ in men and women, it is known that the cellular and molecular mechanisms of aging are initially better maintained in women. However, after menopause, women seem to catch up and, in several parameters, reach the same levels of aging as men [12]. Nevertheless, elderly men are still stronger and faster than women, which is probably related to higher testosterone levels resulting in higher muscle mass in men [12]. Moreover, in young adult men and women, sex differences in fatigue and ability to recover during high-intensity training are shown even when matching for exercise parameters such as maximal oxygen uptake (VO_{2max}) [13,14]. Despite male superior absolute performance due to genetic differences [15,16], questioning the valence of proposing mixed-gender game formats in recreational team sports-based exercise interventions, similar cardiovascular, metabolic and bone health improvements were shown both for men and women, after 16 weeks of mixed-gender recreational football training [17]. Nonetheless, to the best of our knowledge, the physiological demands and activity profile of same- vs. mixed-gender large and small-

sided games have only been analysed for children and adolescents during football practice and in a school setting [18,19]. HR response and perceived experience (namely the perceived effort and fun levels) showed a significantly lower mean value for girls when playing mixed with boys than when playing same-gender matches. No differences were reported in the game demands for boys when playing mixed- or same-gender matches [18]. However, boys perceived less fun when playing with girls than when playing within the same gender [18]. Interestingly, no significant differences were found in HR response between the genders for 8- to 9-year-old schoolchildren during small-sided team sports [19].

Therefore, understanding whether the physical and physiological demands of middle-aged and elderly men and women playing same- vs. mixed-gender recreational TH game formats differs is of utmost importance. Moreover, since motivation and enjoyment are key factors for long-term adherence to this type of exercise programmes [20], it is also important to ascertain the perceived experience during these different game formats.

Thus, the aim of this study was to analyse the physiological response, the activity profile and the perceived experience of middle-aged and elderly men and women playing same- vs. mixed-gender recreational TH game formats. We hypothesized that the demands for men would be higher when playing same- vs. mixed-game formats, while the opposite would occur for women.

Materials and methods

Participants

Forty-one participants (22 men and 19 women) were invited to participate in this study. Descriptive characteristics of the participants are presented in Table 1. Men's stature, body mass, and distance covered in the Yo-Yo intermittent endurance level 1 test (YYIE1) were higher than women's ($p \leq 0.038$), while body mass index (BMI), fat mass values and time experience with recreational TH, were higher for women than men ($p < 0.001$). No differences were shown between the genders for chronological age. Inclusion criteria were: male and female participants aged over 50 years, that were at the moment involved in a recreational TH-based training programme for at least the last 12 weeks, with medical clearance to perform this type of exercise program.

All the participants were informed about the study purposes, risks and benefits and signed a written informed consent according to the Declaration of Helsinki. Ethical approval was provided by the local Institutional Review Board (CEFADE 19 2019).

Table 1. Chronological age, stature, body composition, aerobic performance and recreational team handball experience (data are presented as mean \pm SD (range)) of the men (n = 22) and women (n = 19) that played the recreational team handball matches.

Variable	Men (n = 22)	Women (n = 19)
Age (years)	69 \pm 4 (63–76)	66 \pm 6 (56–77)
Stature (cm)	168 \pm 5 (159–177)	153 \pm 4 (148–161) [*]
Body mass (kg)	74.8 \pm 8.6 (51–85)	64.7 \pm 9.0 (52–79) [*]
BMI	26.3 \pm 2.6 (20–30)	27.6 \pm 3.8 (21–34) [*]
Fat mass (%)	25.3 \pm 4.8 (15–36)	37.3 \pm 4.7 (31–44) [*]
YYIE1 (m)	754 \pm 439 (320–1560)	395 \pm 158 (200–600) [*]
Recreational TH experience (months)	17 \pm 7 (4–24)	28 \pm 12 (3–36) [*]

BMI—Body mass index; TH—Team handball; YYIE1—Yo-Yo intermittent endurance level 1 test.

^{*}Significantly different from men ($p \leq 0.038$).

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Experimental design

The participants were evaluated for anthropometric variables, body composition and YYIE1 performance, in the week before the data collection. All the participants were familiarized with the procedures involved in the evaluations. Additionally, match internal and external load variables were monitored for each participant during 4 testing sessions. Each participant performed 4 recreational 6v6 (66.6 m² per player) TH matches, 2 with only same-gender players (i.e., same-gender game format) and 2 with participants of both genders (i.e., mixed-gender game format: 3 men and 3 women), resulting in a total of 20 matches to be analysed. The same- and mixed-gender matches were performed in an indoor TH court (40x20 m). There were 48 hours between each testing session and the participants were asked to refrain from intense physical activity in the 48h before the testing sessions. All testing sessions were performed in the morning. To ensure the maintenance of proper hydration throughout the testing sessions, all participants were instructed to be hydrated and to drink water *ad libitum*.

Each testing session started with a standardized 15-min warm-up (consisting of running, coordination, strength, flexibility, and balance exercises), followed by 3x15-min periods of recreational TH playing, interspersed by 2-min breaks. Some adaptations to the TH rules were made, such as, no body contact was allowed, and softer and lighter TH balls (47 cm circumference, GOALCHA, Fredericia, Denmark) than the official ones were used during the matches. There were some exceptions to the official TH rules, namely, no exclusions, no substitutions, no dribbling. Furthermore, the participants rotated positions every 2 min in a random order, including the goalkeeper, and the ball was immediately put back in play by the goalkeeper after a goal. All training sessions were instructed by a professional TH coach and physical education teacher and monitored by the research team. All the data collection and analysis were performed by the research team that comprised an experienced group of Sport Science, Physical Exercise and Health and Physical Education Teaching Master and PhD graduates.

Participants' internal load was evaluated as exercise HR, BL concentrations and differential rating of perceived exertion (RPE). Fun levels were also registered at the end of all testing sessions. External load was evaluated as frequency (n), percentage of total match duration (%) and, absolute (m) and relative (%) match distance covered in selected locomotor arbitrary categories [3], considering this study participants' individual speed thresholds, in order to account for inter-individual variability in external load, as well, as total distance covered.

Experimental procedures

Body (kg) and fat mass (%) were measured in a bioimpedance digital scale (Tanita Inner Scan BC 532, Tokyo, Japan) and stature (cm) was determined using a portable stadiometer (Seca 213, Hamburg, Germany), according to standardized protocols [11]. BMI was calculated as kg/m². Aerobic performance was evaluated by the YYIE1. The YYIE1 test was performed in the same indoor TH wooden floor court as the matches, according to a protocol already described [11].

The cardiovascular load was monitored in the four testing sessions for each participant. For this purpose, the participants wore a HR monitor (Firstbeat Technologies Ltd., version 4.5.0.2, Jyväskylä, Finland) and their differential RPE (i.e., respiratory, muscular, and global) [21] and fun levels [22] were recorded immediately after the end of each testing session [23]. The participants were familiarised with the considered psychometric scales in training sessions performed before this study. Individual HR_{max} was determined as the highest value reached either during a VO_{2max} test, the YYIE1 test or the matches, according to a multiple testing approach [24]. Moreover, capillary blood samples (30 µl) were drawn from the right earlobe at rest and during the last 5 min of the first and third match periods by a portable electroenzymatic lactate device analyser (Lactate Pro 2 LT-1730, Arkray, Amsterdam, The Netherlands), for

measurements of mean and peak BL concentrations. Each of the 41 participants were evaluated 4 times during the study (2 during same- and 2 during mixed-gender matches).

External load was evaluated as match activities variables using time-motion analysis performed by video recordings (SONY-DCR-SX65E, digital video camera recorder) and accelerometer variables using Catapult MinimaxX S4 units (MinimaxX S4; Catapult Sports, Canberra, Australia). Frequency of the selected high-intensity match actions, i.e., jumps, throws, stops, changes of direction and one-on-one situations, and total number of actions were registered via video-analysis of the matches during 20 sessions (4 sessions per participant). Players' displacements were divided into eight locomotor categories: 1) standing still, 2) walking, 3) jogging, 4) fast running, 5) sprinting, 6) sideways medium-intensity, 7) sideways high-intensity, and 8) backwards movement [3]. High-intensity movements were the result of the sum of fast running, sprinting and sideways high-intensity categories. Individual speed thresholds were considered to determine the individual nature of the exercise intensity in each locomotor category [25] and were registered according to the protocol already described [11]. A test-retest analysis was performed for the selected time-motion variables in 8 matches (4 same-gender and 4 mixed-gender; randomly selected) and the analysis was initiated when intraclass correlation coefficient was >0.80 . Table 2 shows the average speed thresholds calculated for each gender in the studied population. Accelerometer data was collected using Catapult MinimaxX S4 units in indoor mode with global positioning system technology in inactive mode. Data was downloaded and processed using Catapult Sprint Version 5.1.1 (Catapult Innovations, Canberra, Australia). The units were located in a specific vest on players' upper back. The validity and reliability of the accelerometers have been described elsewhere [26]. Player load (an estimate of the physical demand combining the instantaneous rate of change in acceleration in 3 planes [27]) variables were evaluated at a 100 Hz sampling rate. In this study, time spent in each player load zone, i.e., $0-0.1$, $>0.1-0.3$, $>0.3-0.6$, $>0.6-1.0$, $>1.0-1.5$, $>1.5-2.0$, >2.0 [27] was presented as percentage of total match time, whereas total accumulated player load was presented as arbitrary units [26]. Frequency of accelerations and decelerations, categorized as low (1.50 to 2.14 $\text{m}\cdot\text{s}^{-1}$), medium (2.14 to 2.78 $\text{m}\cdot\text{s}^{-1}$) and high-intensity (>2.78 $\text{m}\cdot\text{s}^{-1}$), according to manufacture settings (Catapult Sprint Version 5.1.1 software manual, Catapult Innovations, Canberra, Australia), was determined.

Statistical analyses

Results are presented as means \pm standard deviations (SD) and 95% of confidence interval (CI). Students' unpaired t-test was used to assess differences between genders in chronological age, stature, body composition, BMI, aerobic performance and time experience with recreational TH. To examine differences between the genders during the same- and mixed-gender

Table 2. Player's locomotor speed categories according to gender (average values for this population).

Locomotor category	Men	Women
Standing	0 km h^{-1}	0 km h^{-1}
Walking	6 km h^{-1}	6 km h^{-1}
Jogging	9 km h^{-1}	8 km h^{-1}
Fast running	12 km h^{-1}	11 km h^{-1}
Sprinting	17 km h^{-1}	13 km h^{-1}
Sideways medium-intensity movements	8 km h^{-1}	6 km h^{-1}
Sideways high-intensity movements	10 km h^{-1}	7 km h^{-1}
Backwards movements	8 km h^{-1}	5 km h^{-1}

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matches a two-way analysis of variance (ANOVA) for repeated measures with Bonferroni post hoc multiple comparison tests was used. Power calculations were performed to detect an effect size of 0.18 in repeated measures ANOVA (within factors only). With 2 measurements, alpha of 5%, and power of 80%, 39 participants were needed. Effect size was calculated using Cohen d and interpreted as trivial (<0.2), small (0.2–0.5), medium (0.5–0.8) and large (>0.8) [28]. Relative reliability of key HR variables was reported as Intraclass Correlation Coefficients ($ICC_{3,1}$). Magnitude of the ICC values was rated as excellent, good, and poor for 0.75–1.00, 0.41–0.74, and 0.00–0.40 scores, respectively [29,30]. Statistical Package for the Social Sciences (SPSS Inc., version 23.0) was used for the analyses. The data were tested for normality using the Shapiro-Wilk test. Statistical significance was set at $p \leq 0.05$.

Results

Internal load and perceived experience

A game format \times gender interaction was observed for relative mean HR ($p = 0.043$; Table 3 and Fig 1), for the percentage of time spent $>80\%HR_{max}$ ($p = 0.051$) and $>90\%HR_{max}$ ($p = 0.011$), and for respiratory RPE ($p = 0.032$). Percentages of time spent $>80\%HR_{max}$ ($p = 0.008$; 95% CI: -39.96, -6.25; $d = 0.360$) and $>90\%HR_{max}$ ($p = 0.017$; 95% CI: -19.41, -2.06; $d = 0.451$) were higher for women than for men, while playing mixed-gender matches. Men's percentage of time spent $>80\%HR_{max}$ was higher during same- vs. mixed-gender matches ($p = 0.034$; 95% CI: -0.53–23.29; $d = 0.141$; Fig 2), while women's percentage of time spent $>90\%HR_{max}$ was lower during same- vs. mixed-gender matches ($p = 0.036$; 95% CI: -12.86–0.44; $d = 0.459$). Women's BL values were lower than men's during same- (mean BL: $p = 0.002$; 95% CI: 0.49–1.92; $d = 0.980$; peak BL: $p = 0.011$; 95% CI: 0.28–2.02; $d = 0.999$; first period BL: $p \leq 0.001$; 95% CI: 0.61–2.08; $d = 1.179$; third period BL: $p = 0.008$; 95% CI: 0.29–1.85; $d = 0.980$) and mixed-gender matches (mean BL: $p = 0.005$; 95% CI: 0.37–1.97; $d = 0.972$; peak BL: $p = 0.015$; 95% CI: 0.29–2.48; $d = 0.887$; first period BL: $p = 0.009$; 95% CI: 0.34–2.19; $d = 1.094$; third period BL: $p = 0.010$; 95% CI: 0.27–1.88; $d = 0.743$) (Figs 3 and 4). No significant differences were observed for muscular and global RPE and fun levels between the gender game formats. In men, the ICC across the same- and mixed-gender game formats for mean HR, time $>80\%HR_{max}$ and time $>90\%HR_{max}$ were 0.61 (0.18–0.84, good), 0.51 (0.12–0.76, good), and 0.35 (0.18–0.84, poor), respectively. For mean HR, time $>80\%HR_{max}$ and time $>90\%HR_{max}$, the ICC values for the different gender game formats in the female participants were 0.77 (0.50–0.91, excellent), 0.67 (0.31–0.86, good), and 0.72 (0.40–0.88, good), respectively.

External load

Locomotor activity profile. Participants' locomotor activity profile is presented in Table 4 and Fig 5. A game format \times gender interaction was found for frequency of standing, walking, sprinting, and backwards movements ($p \leq 0.048$), for the percentage of total match duration spent standing, walking, jogging and in backwards movements ($p \leq 0.020$), for the absolute and percentage of total match distance covered jogging and in backwards movements ($p \leq 0.037$) and for the percentage of total match distance covered walking ($p = 0.010$). A game format effect was observed for frequency of standing, fast running, sideways medium-intensity, backwards and high-intensity movements ($p \leq 0.050$; Table 4), for percentage of total match duration in sideways medium-intensity and backwards movements ($p \leq 0.031$) and for absolute and percentage of total match distance covered fast running, and in sideways medium-intensity, backwards and high-intensity movements ($p \leq 0.022$).

Men's frequency of high-demanding movements was higher during same- vs. mixed-gender matches (sprinting: $p = 0.015$; 95% CI: -1.02–0.02; $d = 0.707$; high-intensity: $p = 0.036$; 95%

Table 3. Men and women's internal load during 6v6 same- and mixed-gender recreational team handball game formats (data are presented as mean \pm SD).

	Men		Women		Game format	Gender	Interaction
	Same-gender	Mixed-gender	Same-gender	Mixed-gender			
	(n = 22)		(n = 19)				
Cardiovascular demands							
Mean HR (%HR _{max})	78 \pm 5	76 \pm 5	76 \pm 8	79 \pm 6	0.617	0.427	0.043
Peak HR (%HR _{max})	86 \pm 6	85 \pm 6	88 \pm 6	88 \pm 6	0.498	0.340	0.150
Time >80% HR _{max} (%)	36 \pm 28	32 \pm 28 ^b	44 \pm 32	47 \pm 33 ^a	0.332	0.043	0.051
Time >90% HR _{max} (%)	7 \pm 16	3 \pm 5	5 \pm 5	9 \pm 18 ^a	0.614	0.136	0.011
Time \leq 60% HR _{max} (%)	1 \pm 2	2 \pm 3	9 \pm 13	1 \pm 2	0.923	0.565	0.104
Time 61–70% HR _{max} (%)	17 \pm 15	19 \pm 12	21 \pm 17	14 \pm 15	0.728	0.101	0.299
Time 71–80% HR _{max} (%)	47 \pm 21	48 \pm 18	27 \pm 7	38 \pm 18 ^a	0.261	0.083	0.231
Time 81–90% HR _{max} (%)	29 \pm 17	29 \pm 25	39 \pm 28	38 \pm 24	0.201	0.059	0.503
BL concentrations							
First period mean BL (mmol \cdot l ⁻¹)	4.0 \pm 1.4	4.1 \pm 1.8	2.5 \pm 0.5 ^b	2.5 \pm 0.7 ^a	0.344	0.001	0.820
Third period mean BL (mmol \cdot l ⁻¹)	3.0 \pm 1.5	3.1 \pm 1.6	1.7 \pm 0.5 ^b	1.9 \pm 0.8 ^a	0.426	0.004	0.989
Match mean BL (mmol \cdot l ⁻¹)	3.5 \pm 1.7	3.6 \pm 1.6	2.1 \pm 0.5 ^b	2.2 \pm 0.7 ^a	0.284	0.001	0.895
Match peak BL (mmol \cdot l ⁻¹)	4.2 \pm 1.5	4.5 \pm 2.0	2.8 \pm 0.7 ^b	2.9 \pm 0.9 ^a	0.103	0.008	0.529
RPE							
Respiratory RPE (AU, 0–10)	6.8 \pm 1.8	5.9 \pm 2.2	5.9 \pm 1.8	6.1 \pm 2.9	0.918	0.948	0.032
Muscular RPE (AU, 0–10)	6.8 \pm 1.7	6.0 \pm 2.0	5.6 \pm 2.1	5.3 \pm 2.5	0.709	0.543	0.206
Global RPE (AU, 0–10)	6.6 \pm 1.8	5.6 \pm 2.5	5.9 \pm 1.7	5.9 \pm 2.6	0.659	0.991	0.069
Fun (AU, 0–10)	9.3 \pm 0.9	9.1 \pm 1.1	9.3 \pm 1.0	9.1 \pm 1.5	0.811	0.849	0.335

AU—Arbitrary units; BL—Blood lactate; HR—Heart rate; HR_{max}—Maximal heart rate; RPE—Rating of perceived exertion.

^aSignificantly different from Men Mixed-Gender

^bSignificantly different from Women Same-Gender

^cSignificantly different from Men Same-Gender.

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CI: -5.88–0.43; $d = 0.558$). During same-gender game formats, men's frequency of fast running ($p = 0.005$; 95% CI: 1.89–9.66; $d = 0.960$) and high-intensity movements was also higher than during mixed-gender matches ($p = 0.006$; 95% CI: 2.23–10.72; $d = 1.011$). During mixed-gender matches the frequency of fast running ($p = 0.005$; 95% CI: 1.50–7.80; $d = 1.038$), sprinting ($p = 0.019$; 95% CI: -0.18–0.37; $d = 0.599$) and in high-intensity movements ($p = 0.004$; 95% CI: 1.45–8.05; $d = 1.013$) was higher for men than in women.

Men's percentage of total match duration spent standing ($p = 0.007$; 95% CI: 0.78–7.06; $d = 0.663$) and walking ($p = 0.008$; 95% CI: 0.80–7.84; $d = 0.740$) was higher when playing mixed- vs. same-gender matches, however, in jogging ($p = 0.018$; 95% CI: -6.31–0.09; $d = 0.576$), sprinting ($p = 0.026$; 95% CI: -0.12–0.01; $d = 0.958$) and backwards movements ($p < 0.001$; 95% CI: -7.49, -2.83; $d = 1.455$) was lower during mixed- vs. same-gender matches. During mixed-gender game formats, men's percentage of total match duration spent fast running ($p = 0.002$; 95% CI: -0.65–1.27; $d = 0.632$), sprinting ($p = 0.042$; 95% CI: -0.02–0.04; $d = 0.00$), in backwards ($p < 0.001$; 95% CI: -1.67–1.35; $d = 0.005$) and high-intensity movements ($p = 0.002$; 95% CI: -0.64–1.29; $d = 0.004$) was higher than for women. For women, the percentage of total match duration spent walking ($p = 0.044$; 95% CI: -6.50, -0.41; $d = 0.769$) was higher, and jogging was lower ($p = 0.038$; 95% CI: 1.05–4.77; $d = 0.928$) during same- vs. mixed-gender matches.

During mixed-gender matches, men's absolute match distance covered fast running ($p = 0.004$; 95% CI: 11.71–121.83; $d = 1.022$), sprinting ($p = 0.046$; 95% CI: -2.46–4.63; $d = 0.627$), and in backwards ($p < 0.001$; 95% CI: -39.95–111.51; $d = 0.438$) and high-intensity movements

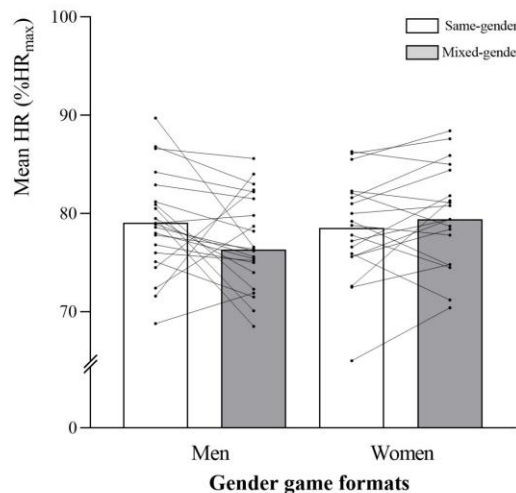


Fig 1. Mean heart rate (mean HR) for men and women in each gender game format (same- and mixed-gender). Data are presented as mean \pm SD.

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($p = 0.003$; 95% CI: 10.98–124.73; $d = 1.023$) as well as total distance ($p = 0.003$; 95% CI: -425.52–826.37; $d = 0.098$) were higher than women's. Men's absolute and percentage of total match distance covered with high-intensity movements, namely, fast running (absolute: $p = 0.003$; 95% CI: -111.60, -18.06; $d = 0.888$; percentage: $p = 0.012$; 95% CI: -2.11, -0.15; $d = 0.970$) and sprinting (absolute: $p = 0.018$; 95% CI: -16.36–0.74; $d = 0.778$; percentage: $p = 0.019$; 95% CI: -0.33–0.02; $d = 1.125$) was higher when playing same- vs. mixed-gender matches. For women, percentage of total match distance covered walking was higher ($p = 0.037$; 95% CI: -7.10, -0.99; $d = 1.106$), and jogging was lower ($p = 0.049$; 95% CI: 0.13–6.26; $d = 1.016$) during same- vs. mixed-gender matches. During mixed-gender matches, the percentage of total match distance covered walking ($p = 0.003$; 95% CI: -5.57–6.32; $d = 0.019$), fast running ($p = 0.008$; 95% CI: 0.23–2.46; $d = 1.096$), and in backwards ($p < 0.001$; 95% CI: -0.89–2.13; $d = 0.006$), and high-intensity movements ($p = 0.006$; 95% CI: 0.23–2.52; $d = 1.102$) was higher for men than women. During same-gender matches, the absolute and percentage of total match distance covered fast running (absolute: $p = 0.019$; 95% CI: 41.95–198.25; $d = 1.063$; percentage: $p = 0.019$; 95% CI: 0.59–3.62; $d = 1.067$) and in high-intensity movements (absolute: $p = 0.021$; 95% CI: 48.11–212.51; $d = 1.141$; percentage: $p = 0.021$; 95% CI: 0.71–3.91; $d = 1.086$) was higher for men than for women.

High-intensity game actions. A game format \times gender interaction was observed for the frequency of throws ($p = 0.045$; Table 5). During mixed-gender matches, frequency of jumps ($p = 0.009$; 95% CI: 1.07–5.05; $d = 1.501$; Table 5), stops ($p = 0.008$; 95% CI: 0.06–4.45; $d = 0.598$), changes of direction ($p = 0.004$; 95% CI: -0.07–3.16; $d = 1.020$) and total high-intensity game actions ($p = 0.014$; 95% CI: 4.44–18.16; $d = 0.712$) was higher for men than for women. During same-gender matches, men showed higher frequency of jumps ($p = 0.004$; 95% CI: 0.77–4.96; $d = 0.622$), throws ($p = 0.005$; 95% CI: -2.21–2.84; $d = 0.537$), stops ($p = 0.044$; 95%

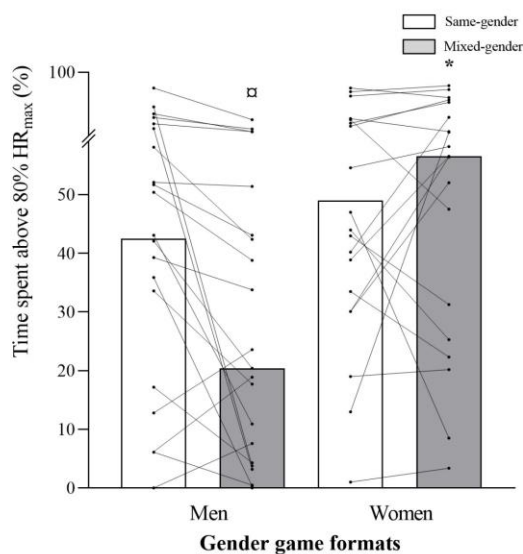


Fig 2. Percentage of total match time spent with heart rates above 80% of individual maximal HR (%HR_{max}) for men and women in each gender game format (same- and mixed-gender). Data are presented as mean±SD.

*Significantly different from Men Mixed-Gender; *Significantly different from Men Same-Gender.

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CI: 0.89–5.51; $d = 2.104$), one-on-one situations ($p = 0.028$; 95% CI: -0.53–2.59; $d = 0.546$) and total high-intensity game actions ($p = 0.002$; 95% CI: 2.30–19.00; $d = 0.727$) than women.

Player load and accelerometer data. A game format x gender interaction was observed for time spent in >0.3–0.6% player load zone ($p = 0.042$; Table 6). During same- and mixed-gender matches, time spent in 0.0–0.1% player load zone was lower for men than for women (same-gender: $p = 0.004$; 95% CI: -13.66, -5.05; $d = 2.048$; mixed-gender: $p < 0.001$; 95% CI: -9.28, -1.91; $d = 1.232$, respectively; Table 6). However, time spent in >0.1–0.3 (same-gender: $p = 0.022$; 95% CI: 0.43–8.22; $d = 0.898$; mixed-gender: $p = 0.031$; 95% CI: 0.74–8.99; $d = 1.323$), >1.5–2.0 (same-gender: $p < 0.001$; 95% CI: 1.97–5.61; $d = 8.276$; mixed-gender: $p < 0.001$; 95% CI: 1.39–5.34; $d = 5.183$) and >2.0% (same-gender: $p = 0.002$; 95% CI: 0.62–3.05; $d = 5.484$; mixed: $p = 0.004$; 95% CI: 0.95–3.47; $d = 6.458$) player load zones and total accumulated player load (same-gender: $p = 0.034$; 95% CI: 9.77–78.75; $d = 1.494$; mixed-gender: $p = 0.013$; 95% CI: 3.13–73.34; $d = 2.589$) was higher for men than for women during same- and mixed-gender matches. Men's low-intensity ($p = 0.012$; 95% CI: -4.70–22.49; $d = 0.981$) and total accelerations ($p = 0.040$; 95% CI: -0.97–37.80; $d = 1.735$) were higher than women's during mixed-gender matches. During same-gender matches, medium ($p = 0.014$; 95% CI: -0.87–5.87; $d = 2.196$) and high-intensity ($p = 0.005$; 95% CI: -1.29–6.71; $d = 3.541$) accelerations were higher for men than for women. No significant differences were found for decelerations.

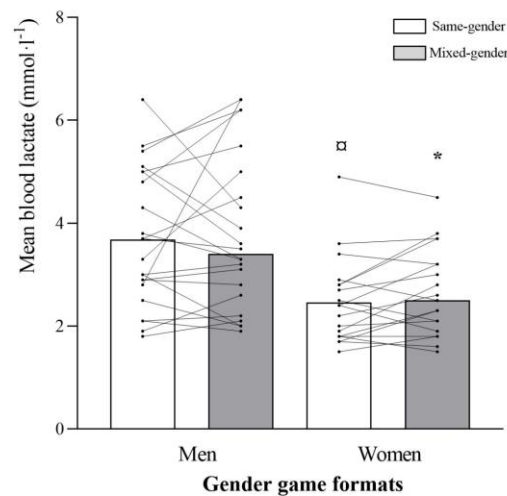


Fig 3. Mean blood lactate (mmol·l⁻¹) values for men and women in each gender game format (same- and mixed-gender). Data are presented as means±SD. [□]Significantly different from Men Mixed-Gender; *Significantly different from Men Same-Gender.

<https://doi.org/10.1371/journal.pone.0286008.g003>

Discussion

This is the first study analysing the internal and external load of same- vs. mixed-gender recreational TH game formats for middle-aged and elderly men and women. Game format-by-gender interactions were found for relative mean HR, time spent >80%HR_{max} and >90%HR_{max} and respiratory RPE, and also for several of the external load variables considered. The main findings were that during mixed-gender matches, time spent >80%HR_{max} and >90%HR_{max} was higher for women than men, while mean and peak BL values were lower. Furthermore, in same-gender matches, men's time spent with HR >80%HR_{max}, as well as several activity profile variables, such as high-intensity locomotor movements were higher than in mixed-gender matches.

Studies using recreational TH as an exercise intervention have shown that this exercise mode is effective in inducing several health improvements in different age groups and in both genders [4–9]. It has also been shown that the time spent in high HRs zones (i.e., HR >90%HR_{max}) is positively associated ($r = 0.61$) with VO_{2max} improvement [7]. Moreover, studies using recreational football have also suggested that time spent with HR >90%HR_{max} (~20% of total match time) was the possible cause of the reported improvements in VO_{2max} [31]. In the present study, the participants spent less time with HR >90%HR_{max} (3–9% of total match time) than the studies reported above. Nevertheless, improvements in VO_{2max} were observed in postmenopausal women by spending around 11% of total match time in HR >90%HR_{max} [4], meaning that for this age population, spending less than 20% of total match time in HR >90%HR_{max} is still able to induce improvements in cardiorespiratory fitness.

In the present study, during mixed-gender matches, time spent >80% and >90%HR_{max} was higher for women than for men. This may indicate that during mixed-gender matches,

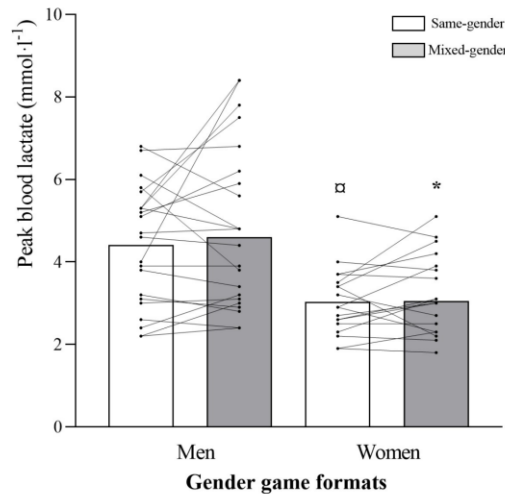


Fig 4. Peak blood lactate (mmol·l⁻¹) values for men and women in each gender game format (same- and mixed-gender). Data are presented as means±SD. Significantly different from Men Mixed-Gender; *Significantly different from Men Same-Gender.

<https://doi.org/10.1371/journal.pone.0286008.g004>

women increase their physical demands in order to keep up with the pace imposed by men. In fact, the ability to perform high-intensity intermittent activity was higher in the male vs. female participants (YYIE1: 754±439 vs. 395±158 m, men and women, respectively). Yo-Yo tests' performance has been positively associated with the amount of high-intensity running during football matches [32]. Men's performance superiority was observed in the matches' external demands. In fact, during same- and mixed-gender matches, the frequency of high-intensity movements and specific high-intensity game actions, the time spent in the highest player load zones, the total accumulated player load and frequency of total accelerations were higher for men than for women, which may be the reason why women showed lower mean and peak BL values. Nevertheless, during mixed-gender matches, women showed higher cardiovascular load than men, while performing less external load (e.g., less time spent and lower distance covered in high-intensity movements and lower total distance covered), which can be attributed to their lower aerobic performance level compared to men [32]. During same-gender matches, men's time spent >80%HR_{max} was higher than in mixed-gender matches, which is in line with the locomotor activity profile. Men performed higher intensity locomotor movements during same- than during mixed-gender matches, which may explain the higher HRs shown during same-gender matches.

Both during same- and mixed-gender matches, men's and women's relative mean and peak HR values were slightly lower than those reported for younger populations [3,5–8]. However, they were in line with those reported for postmenopausal women [4,9], who showed cardiovascular and musculoskeletal health improvements after 16 weeks of recreational TH training with those intensities. Relative reliability is a viable strategy to assess the interindividual consistency from evaluation-to-evaluation, providing information over the underpinning variability

Table 4. Men and women's locomotor activity profile during 6v6 same- and mixed-gender recreational team handball game formats (data are presented as mean ± SD).

Locomotor categories	Men		Women		Game format <i>p</i>	Gender <i>p</i>	Interaction <i>p</i>
	Same-Gender (n = 22)	Mixed-Gender	Same-Gender	Mixed-Gender (n = 19)			
Freq (n)							
Standing	12±8	21±5 ^o	17±6 ^o	16±7 [*]	0.038	0.913	<0.001
Walking	94±17	101±10	93±8	92±35	0.645	0.459	0.048
Jogging	74±18	74±14	73±13	75±30	0.965	0.747	0.526
Fast running	13±8	10±6	6±4 ^o	5±2 [*]	0.050	0.002	0.500
Sprinting	0.8±1.4	0.1±0.3 ^o	0.0±0.0	0.0±0.0 [*]	0.180	0.025	0.043
Side Med	15±9	18±8 ^o	12±6	15±7	0.009	0.548	0.276
Side High	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0			
Back	30±17	10±6 ^o	10±8	9±6 [*]	<0.001	0.008	<0.001
High-intensity	14±9	10±6 ^o	6±4 ^o	5±2 [*]	0.050	0.001	0.355
Total	240±42	234±30	211±23	212±75	0.744	0.084	0.371
Total match duration (%)							
Standing	9±7	14±5 ^o	17±5	15±2 [*]	0.158	<0.001	0.020
Walking	50±10	55±6 ^o	54±6 ^o	50±4 [*]	0.706	0.366	0.001
Jogging	28±8	24±6 ^o	25±6	28±3 ^o	0.915	0.904	0.002
Fast running	2.2±1.4	1.6±1.1	1.0±0.7	1.5±2.6 [*]	0.739	0.037	0.092
Sprinting	0.1±0.2	0.0±0.0 ^o	0.0±0.0	0.0±0.0 [*]	0.209	0.048	0.070
Side Med	3.1±2.4	3.4±2.0	2.2±1.3	3.3±1.5	0.031	0.703	0.586
Side High	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0			
Back	7.7±5	2.0±1.5 ^o	2.0±1.8	1.9±1.6 [*]	<0.001	0.006	<0.001
High-intensity	2.3±1.5	1.6±1.1	1.0±0.7	1.5±2.6 [*]	0.675	0.032	0.076
Total match distance (m)							
Walking	2203±443	2534±501 ^o	2433±579	2456±1047	0.107	0.875	0.080
Jogging	1804±697	1614±494	1319±375	1724±762 [*]	0.499	0.374	0.010
Fast running	231±165	147±102 ^o	93±74 ^o	56±30 [*]	0.016	0.003	0.088
Sprinting	12.8±24.1	2.0±4.8 ^o	0.0±0.0	0.0±0.0 [*]	0.169	0.059	0.057
Side Med	183±143	203±116	89±48	152±66	0.020	0.251	0.669
Side High	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0			
Back	442±339	101±82 ^o	64±60	70±53 [*]	<0.001	<0.001	<0.001
High-intensity	244±175	149±105 ^o	93±74 ^o	56±30 [*]	0.014	0.003	0.061
Total	4875±713	4601±629	3998±671	4457±1729 [*]	0.674	0.063	0.084
Total match distance (%)							
Walking	46±13	55±9 ^o	61±8	55±8 ^{o*}	0.438	0.478	0.010
Jogging	36±10	35±9	33±7	39±7 ^o	0.421	0.835	0.037
Fast running	5±3	3±2 ^o	2±2 ^o	1±1 [*]	0.022	0.005	0.232
Sprinting	0.3±0.5	0.0±0.1 ^o	0.0±0.0	0.0±0.0	0.162	0.062	0.065
Side Med	4±3	4±3 ^o	2±1	4±2	0.009	0.491	0.973
Side High	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0			
Back	9±6	2±2 ^o	2±2	2±1 [*]	<0.001	0.001	<0.001
High-intensity	5±3	3±2 ^o	2±2 ^o	1±1 [*]	0.018	0.004	0.163

Back-backwards movements; Freq-Frequency; High-intensity-sum of fast running, sprinting and sideways high-intensity movements; Side High-sideways high-intensity movements; Side Med-sideways medium-intensity movements.

^oSignificantly different from Men Mixed-Gender

^{*}Significantly different from Women Same-Gender

^oSignificantly different from Men Same-Gender.

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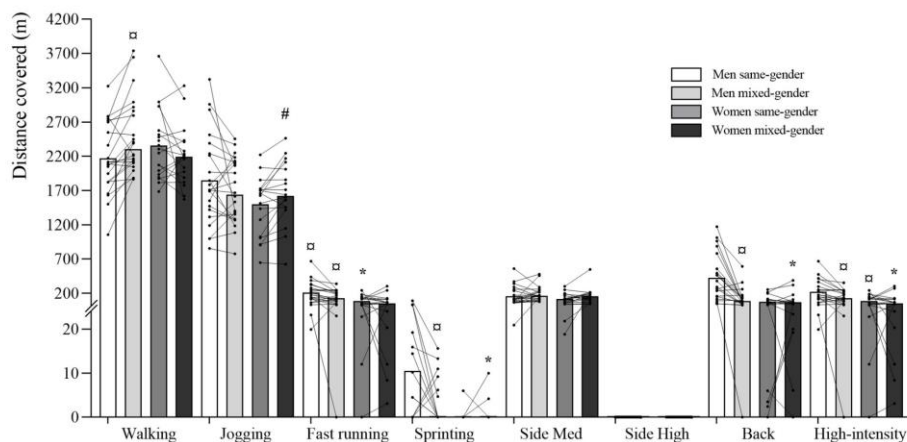


Fig 5. Distance covered (m) for the locomotor activity categories in each gender game format (same- and mixed-gender). Data are presented as means \pm SD. Back-backwards movements; High-intensity-sum of fast running, sprinting and sideways high-intensity movements; Side High-sideways high-intensity movements; Side Med-sideways medium-intensity movements. *Significantly different from Men Mixed-Gender; #Significantly different from Women Same-Gender; ^aSignificantly different from Men Same-Gender.

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within and between the evaluations. In this study, the ICC value of the key HR variables was studied across the gender game formats (i.e., same- vs mixed-games). The results showed poor-to-good match intensity (HR) consistency for the men and good-to-excellent scores for the women. This data suggests individual monitoring of exercise intensity across the gender game formats when men play with their female counterparts. Interestingly, women's mean and peak BL values were lower than the men's (3.5–3.6 and 4.2–4.5 $\text{mmol}\cdot\text{l}^{-1}$, respectively), which could be related with the higher frequency, percentage of total match time and distance covered in high-intensity movements by men compared to women, during both gender game formats. Nevertheless, men's mean and peak BL values were similar to those reported for

Table 5. Men and women's high-intensity game actions during 6v6 same- and mixed-gender recreational team handball game formats (data are presented as means \pm SD).

Game actions/gender game format	Men		Women		Game format	Gender	Interaction
	Same-Gender	Mixed-Gender	Same-Gender	Mixed-Gender			
	(n = 22)		(n = 19)				
Jumps (n)	9 \pm 0	7 \pm 2	6 \pm 7 ^a	2 \pm 3 ^a	0.402	0.002	0.829
Throws (n)	11 \pm 1	9 \pm 1	8 \pm 7 ^a	6 \pm 3	0.947	0.121	0.045
Stops (n)	16 \pm 2	15 \pm 8	8 \pm 5 ^a	11 \pm 4 ^a	0.161	0.007	0.414
Changes of direction (n)	12 \pm 4	12 \pm 5	8 \pm 4	9 \pm 2 ^a	0.332	0.007	0.063
One-on-one situations (n)	11 \pm 0	12 \pm 5	8 \pm 8 ^a	7 \pm 4	0.182	0.029	0.399
Total high-intensity actions (n)	43 \pm 15	44 \pm 11	32 \pm 11 ^a	33 \pm 11 ^a	0.363	0.003	0.841

^aSignificantly different from Men Mixed-Gender;

^bSignificantly different from Men Same-Gender.

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Table 6. Men and women's player load and accelerometer data during 6v6 same- and mixed-gender recreational team handball game formats (data are presented as mean \pm SD).

Accelerometer variables/gender game formats	Men		Women		Game format	Gender	Interaction
	Same-Gender	Mixed-Gender	Same-Gender	Mixed-Gender			
	(n = 22)		(n = 19)				
Player load zones							
Time 0.0–0.1 (%)	12 \pm 6	17 \pm 3	25 \pm 4 ^o	22 \pm 2 [*]	0.511	<0.001	0.082
Time >0.1–0.3 (%)	37 \pm 4	40 \pm 0	34 \pm 1 ^o	36 \pm 5 [*]	0.577	0.018	0.694
Time >0.3–0.6 (%)	21 \pm 1	19 \pm 2	20 \pm 4	23 \pm 6 ^e	0.129	0.773	0.042
Time >0.6–1.0 (%)	9 \pm 2	8 \pm 2	8 \pm 4	7 \pm 3	0.206	0.414	0.448
Time >1.0–1.5 (%)	10 \pm 2	11 \pm 1	11 \pm 5 ^o	10 \pm 6	0.611	0.131	0.169
Time >1.5–2.0 (%)	8 \pm 0	5 \pm 0	2 \pm 0 ^o	2 \pm 1 [*]	0.275	<0.001	0.554
Time >2.0 (%)	2 \pm 1	1 \pm 0	0 \pm 0 ^o	0 \pm 0 [*]	0.404	0.001	0.478
Total accumulated (AU)	353 \pm 22	317 \pm 4	278 \pm 51 ^o	263 \pm 29 [*]	0.533	0.008	0.740
Accelerations							
Low-intensity (n)	24 \pm 6	14 \pm 7	10 \pm 6	10 \pm 0 [*]	0.829	0.042	0.721
Medium-intensity (n)	14 \pm 5	12 \pm 3	6 \pm 1 ^o	7 \pm 0	0.115	0.016	0.708
High-intensity (n)	18 \pm 6	17 \pm 8	6 \pm 0 ^o	5 \pm 1	0.673	0.006	0.182
Total (n)	55 \pm 17	43 \pm 18	21 \pm 6	22 \pm 1 [*]	0.753	0.011	0.531
Decelerations							
Low-intensity (n)	13 \pm 0	14 \pm 5	11 \pm 9	7 \pm 8	0.982	0.734	0.962
Medium-intensity (n)	6 \pm 0	7 \pm 5	6 \pm 6	5 \pm 4	0.431	0.479	0.737
High-intensity (n)	6 \pm 5	8 \pm 5	21 \pm 28	2 \pm 1	0.094	0.627	0.841
Total (n)	24 \pm 6	29 \pm 14	38 \pm 43	13 \pm 13	0.275	0.598	0.951

AU—arbitrary units.

^oSignificantly different from Men Mixed-Gender

^eSignificantly different from Women Same-Gender

^{*}Significantly different from Men Same-Gender.

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middle-aged former TH players (3.6 and 4.2 mmol \cdot l $^{-1}$, respectively) [3]. Based on this study results, playing same- or mixed-gender matches results in internal load values in the range to induce cardiovascular adaptations seen in intervention studies with other age-groups using recreational TH as exercise mode.

During same-gender matches, the lower BL values observed for women when compared to men, could be related with the higher frequency of TH high-demanding game-specific actions such as jumps, throws, stops, one-on-one situations and total high-intensity actions, more time spent in higher player load zones (>1.5–2.0 and >2.0), higher total accumulated player load, and higher frequency of medium and high-intensity accelerations found in men. The BL values were also lower for women in mixed-gender matches, which may have been the result of men also showing higher frequency of TH high-intensity game-specific actions such as jumps, stops, changes of direction and total high-intensity actions, spending more time in higher player load zones (>1.5–2.0 and >2.0), showing higher total accumulated player load and performing more low-intensity and total accelerations than women, indicating that men were more involved in the matches than women and, consequently, had a higher level of participation in the matches. This is important to maintain the participants' motivation to keep playing [33]. Accordingly, women could have felt less involved and motivated in the game. Nevertheless, this was not the case, since differential RPE were similar for men and women as

well as fun levels, which were very high (9.1–9.3 AU on a 0–10 scale) in both gender game formats. Therefore, for this population, both gender game formats could be recommended for both genders, contrary to children, with boys reporting more fun when playing football in same- vs. mixed-gender matches [18].

Our study showed that in recreational TH, men and women spent around 50–55% of the total match time walking and 24–28% jogging. The intensities alternate during a TH game due to its intermittent nature, with the locomotor activity pattern changing from standing and walking, jogging and moderate running, fast running and sprinting, sideways and backwards movements [34,35], requiring a high level of endurance to keep up with the game demands. Nevertheless, TH performance is also highly influenced by cognitive, tactical, and social factors [2]. This may explain the higher amount of time spent jogging (24–28% vs. 16%, respectively) and the less time spent in high-intensity movements (1–2% vs. 15%, respectively), by this study participants, that have a clear lack of knowledge and experience with the sport, when compared to former TH players [3], whose technical-tactical sport background may have allowed them to better manage and control the game than the unexperienced older participants.

From a physiological point of view, in order to achieve the highest intensities, recreational TH interventions should be preferentially organized as same-gender game formats for men and as mixed-gender for women. Nevertheless, men's and women's HRs in both same- and mixed-gender TH matches, were in line with studies showing cardiovascular health improvements, which from a practical perspective, means that both gender game formats may be used to induce cardiovascular adaptations for this population. Perhaps, it would be of interest to organize different recreational TH training sessions by changing the gender game formats, allowing men and women to alternate and benefit from different match demands given by the different gender game formats.

Strengths and limitations

The main strength of this study is that it is the first to analyse the differences in internal and external load variables in both men and women playing same- vs. mixed-gender recreational TH games. This is important since this multicomponent exercise programme is usually implemented in community settings, aiming at men and women, and therefore understanding the physical and physiological demands and the perceived experience for each gender when playing same vs. mixed-gender games is crucial to plan and organize the best training settings for this population to achieve broad health improvements. Nevertheless, one study limitation is the fact that during this study the participants were only evaluated during 4 TH training sessions (2 same- and 2 mixed-gender matches).

Given the practical value of organising mixed-gender games-based exercise interventions, future randomized controlled studies aiming at assessing participants' health impact are needed.

Conclusion

Cardiovascular demands were higher for middle-aged and elderly women than for age-matched men during mixed-gender matches. In men, same-gender game formats were more demanding than mixed-gender game formats, while in women the inverse occurred. Interestingly, fun levels were reported as being very high for both genders, independently of the gender game format, which can possibly lead to greater long-term adherence to this exercise program.

It could be concluded that recreational TH is an intermittent high-intensity and motivating exercise mode with potential to induce several health improvements for middle-aged and elderly men as well as for women, regardless the gender game format. From a practical point of view, same- and mixed-gender matches may be organised with the aim to promote health and physical fitness for this population. Since women are more physically and physiologically challenged when playing with men, a lead-in period with same-gender formats may be recommended for women, when implementing mixed-gender recreational TH-based exercise interventions.

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CHAPTER IV: Review Work

4. Review Work

4.1. Original article V

Cardiometabolic effects of recreational team sports for untrained individuals over 60 years of age: a systematic review and meta-analysis

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ABSTRACT

The aim was to evaluate the effects of recreational team sports (RTS) on cardiometabolic markers for individuals over 60 years of age. Eligibility criteria were randomized controlled trials that used RTS with a non-exercise control group as a comparator; participants >60 years; studies that analysed at least one of the selected outcomes [maximal oxygen uptake (VO_{2max}), systolic and diastolic blood pressure (SBP; DBP), resting heart rate (HR), triglycerides and total, high- and low-density lipoproteins (HDL; LDL) cholesterol]. At post-intervention, RTS training groups showed a superior VO_{2max} (3.28 mL/min/kg; 95% CI: 0.86; 5.70), while no significant differences were observed in SBP (-3.35 mmHg, 95% CI: -10.52; 3.82), DBP (-1.84 mmHg, 95% CI: -5.14; 1.46), resting HR (0.07 bpm, 95% CI: -2.44; 2.57), triglycerides (-0.08 mmol/L, 95% CI: -0.27; 0.11), total (0.19 mmol/L, 95% CI: -0.10; 0.48), LDL (0.02 mmol/L, 95% CI: -0.18; 0.22) and HDL (0.03 mmol/L, 95% CI: -0.08; 0.14) cholesterol, when comparing to non-exercise control groups. GRADE assessment showed very low and low certainty of evidence for cardiovascular and metabolic outcomes, respectively. RTS for over 60-year-old individuals may effectively enhance cardiorespiratory fitness, nevertheless, it is imperative to conduct additional high-quality studies within this population to corroborate these findings. CRD42023418690.

Keywords: Blood pressure, cholesterol, maximal oxygen uptake, resting heart rate, triglycerides.

1. INTRODUCTION

In today's world, older individuals are expected to enjoy significantly longer lifespans compared to the past. Life expectancy has risen from 67 years in 2000 to 73 years in 2019 (World Health Organization, 2023). Ideally, increased longevity should be accompanied by an extended period of good health (World Health Organization, 2015). Indeed, older individuals

can continue to actively contribute to society in different ways, namely, by helping their offspring and the community, which is contingent on their health status (World Health Organization, 2015). Numerous deleterious physiological changes are anticipated as part of the aging process. However, the notable decline in cardiorespiratory fitness (namely, maximal oxygen uptake, VO_{2max}) holds great significance. This, because the ability of older individuals to maintain independence functioning within the community largely depend on their capacity to preserve adequate levels of aerobic fitness necessary for daily activities (Fleg et al., 2005). Low cardiorespiratory fitness has been associated with the prevalence of coronary heart disease, cardiovascular diseases (CVD), cancer, and all-cause mortality (Lee et al., 2010). Therefore, cardiorespiratory fitness has been recognized as an independent health marker that has become a priority in health promotion for the elderly (Lee et al., 2010).

CVD are the major cause of death in the elderly population (Liu & Li, 2015), with hypertension being the leading modifiable risk factor for these diseases (Mills et al., 2020). Hypertension has higher prevalence in older than in the younger adult populations (Oliveros et al., 2020). Nevertheless, its development can be prevented and controlled by the reduction of some risks factors, such as high sodium and low potassium intake, obesity, alcohol consumption and physical inactivity (Mills et al., 2020). Moreover, dyslipidaemia, which is also a well-established risk factor for CVD in the elderly, often comprising metabolic abnormalities such as high levels of total and low-density lipoproteins (LDL) cholesterol, and lower levels of high-density lipoproteins (HDL) cholesterol (Liu & Li, 2015), can be counteracted by physical activity (PA) and exercise (Sun et al., 2013; World Health Organization, 2015). In fact, the broad-spectrum health benefits of PA and exercise for older individuals are well described (Sun et al., 2013; Taylor et al., 2004). Nevertheless, PA levels tend to decrease as age progresses (Gomes et al., 2016) and one of the main reasons is lack of motivation (European Commission, 2022). Thus, exercise programmes that are motivating enough to keep long-term adherence to exercise in this population are crucial.

Over the last two decades, recreational team sports (RTS) have emerged as an alternative exercise approach to conventional exercise modalities. Various benefits, including improvements in cardiometabolic health, bone health, body composition, physical fitness, and overall well-being have been shown across diverse populations and age groups, including elderly. These positive effects have the potential to mitigate and counteract some of the primary detrimental effects of aging (Bangsbo et al., 2015; Castagna et al., 2020; Milanović et al., 2022; Milanović et al., 2015; Milanović et al., 2019). RTS programs are characterised as multicomponent exercise regimens that encompass resistance, endurance and balance training

(Castagna et al., 2020; Krstrup et al., 2009). This comprehensive approach has demonstrated superiority in reducing the incidence of falls and enhancing gait ability, balance, and strength performance among physically frail older adults when compared to interventions focusing solely on one of these training modalities (Cadore et al., 2013). Additionally, RTS programs are a social and fun activity (Carneiro et al. (2023a); Carneiro et al., 2022; Carneiro et al., 2023b; Carneiro et al., 2023c; Pereira et al., 2023), consequently showing potential to boost exercise motivation and thereby to foster sustained long-term adherence to exercise (Nielsen et al., 2014). Until now, no consistent conclusions about the cardiovascular and metabolic health effects of RTS in untrained individuals over 60 years of age have been reported. Therefore, the aim of this systematic review and meta-analysis is to evaluate the health effects of RTS for over 60-years-olds, with focus on (i) cardiovascular and (ii) metabolic markers.

2. METHODS

This study was registered in PROSPERO: CRD42023418690. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement guided the reporting of this systematic review and meta-analysis (Page et al., 2021).

2.1. Eligibility criteria

The eligibility criteria used for this systematic review and meta-analysis was based on PICO questions. The inclusion criteria were: randomized controlled trials (RCTs) published in English in a peer-reviewed journal that used any RTS as an exercise programme; male and female participants >60 years on average; interventions with a duration of at least 12 weeks; studies with a non-exercise control group as the comparator group; studies that analysed one of any of the following health markers pre-to-post-intervention: VO_{2max} , systolic and diastolic blood pressure (SBP and DBP), resting heart rate (HR), triglycerides, total, LDL and HDL cholesterol. The exclusion criteria were RCTs that combined exercise with other interventions (e.g., diet).

2.2. Information sources and search strategy

The electronic databases used were MEDLINE (PubMed), Scopus and Web of Science. All available records since January 2008 to June 2023 were considered once the first RCT exercise intervention using RTS was published in 2009. An electronic search was performed using the following key terms: randomized controlled trial, random, randomly, random allocation, recreational football/soccer, recreational team handball, floorball, team sports,

rugby, handball, basketball, recreational basketball, hockey, recreational volleyball, volleyball, baseball, cricket sport, cricket. Adaptations were made for each database, and the search strategies are reported in Table S1.

2.3. Selection process

Duplicated papers were excluded with Endnote. Non-RCTs were excluded. Then studies with inadequate titles were excluded, and the remaining studies were checked for inclusion criteria adequacy in their abstracts. The studies that met the inclusion criteria were used for consideration of eligibility criteria. Two reviewers (IC and RO) independently reviewed the titles and abstracts. Each full text was reviewed by the same two reviewers. Any discrepancy identified after the comparison between the two reviewers was solved by consensus and with a third reviewer (JT) available as an adjudicator. Additionally, manual searches of the reference lists of RTS narrative or systematic reviews was conducted to potentially identify additional studies.

2.4. Data collection process

Two independent reviewers (IC and RO) were responsible for the data extraction in duplicate. The data extraction was performed following a standardized protocol. First, the study characteristics, such as authors, title, year of publication, country, funding, and protocol were extracted. Then, the baseline participants' characteristics (age, sex, and health status), intervention detailed information (RTS modality, intervention and training sessions duration, weekly frequency of the training sessions, intensity and game format type used in the intervention) and the study outcomes, namely [VO_{2max} , SBP, DBP, resting HR, triglycerides, total, LDL and HDL cholesterol] at post-intervention, were registered. The reviewers were not blinded to authors, institutions, or manuscript journals. Some authors provided additional data and/or clarifications after being contacted (Andersen et al., 2014; Carneiro et al., 2023a; Pereira et al., 2023; Pereira et al., 2020; Skoradal et al., 2018; Uth et al., 2014). A graph digitiser software (Web Plot Digitizer 4.6, Pacifica, California, United States of America) was used to obtain data values in the studies that the required data were published as plots (Andersen et al., 2014; Skoradal et al., 2018).

2.5. Data synthesis and analysis

A random-effects model was fitted to the data, using REML estimator. Mean differences (MD) and corresponding 95% confidence intervals (95% CI) were calculated using mean scores and standard deviations at post-intervention for all outcome measures. In the case of those studies where two time points were reported at post-intervention, the time point that most closely resembled those used in the remaining studies included in our analysis was selected. This approach aimed to reduce heterogeneity and improve comparability among the studies. To ensure the robustness of our findings, we further conducted a sensitivity analysis using the other time point. VO_{2max} sensitivity analysis was calculated with standardized mean difference (SMD), as one study (Schmidt et al., 2014) reported this outcome in different units than the other studies. Q statistic, prediction intervals (PI), t_2 , I², and forest plots were reported to assess the degree of heterogeneity. Subgroup analyses, meta-regression, funnel plots and Egger's regression were not conducted due to the small number of studies included. Analyses were performed with R Statistical Software, with “meta” package.

2.6. Study risk of bias assessment

To evaluate the quality assessment of the included studies, the Cochrane Risk of Bias 2 (Flemyng et al., 2023) tool was used by two independent reviewers (IC and JT). This included evaluating the studies in five domains respective to the bias arising from the randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome and bias in selection of the reported.

2.7. Certainty assessment

The certainty of the evidence was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach (Guyatt et al., 2011). The target of the certainty rating was the null (that is, the true effect is a non-null effect).

3. RESULTS

3.1. Study selection and characteristics

The study selection process is described in Figure 1, according to PRISMA flow diagram. This resulted in eight studies and 245 participants (n=146 in RTS and n=99 in control groups) included in this systematic review and meta-analysis. Five studies used recreational football (Andersen et al., 2014; Andersen et al., 2016; Schmidt et al., 2014; Skoradal et al., 2018; Uth et al., 2014) and three studies used recreational team handball (Carneiro et al., 2023a; Pereira et al., 2023; Pereira et al., 2020), as RTS. Three out of the eight studies included in the

analysis were the result of the publication of multiple manuscripts with different outcomes or different follow-ups emerging from the same original study. The studies characteristics are presented in Table 1. Seven studies have registered their protocols (Andersen et al., 2014; Andersen et al., 2016; Carneiro et al., 2023a; Pereira et al., 2023; Pereira et al., 2020; Schmidt et al., 2014; Uth et al., 2014). All the selected studies reported VO₂max values and the respective assessment protocols (Andersen et al., 2014; Andersen et al., 2016; Pereira et al., 2023; Pereira et al., 2020; Schmidt et al., 2014; Skoradal et al., 2018; Uth et al., 2014). Sample size ranged from 9 to 41 participants (Andersen et al., 2014; Pereira et al., 2020). Studies' duration ranged from 12 to 52 weeks [median=16 weeks; interquartile range (IQR)=16-26]. The participants' characteristics are presented in Table 2. Mean age ranged between 60-70 years (median=67 years; IQR=67-68) and mean VO₂max values ranged from 20.2 to 30.8 mL/min/kg (median=26.8 mL/min/kg; IQR=25.1-27.4).

****Figure 1****

****Table 1 and 2****

3.2. Adverse events

Eight adverse events were reported in the selected studies, namely, 2 Achilles tendon ruptures, 2 knee injuries, 2 finger subluxations, 1 shoulder injury and 1 muscle strain, that led to drop-out of the studies (Andersen et al., 2014; Carneiro et al., 2023a; Pereira et al., 2023; Pereira et al., 2020; Schmidt et al., 2014; Uth et al., 2014).

3.3. Effect of RTS on cardiovascular and metabolic markers

RTS elicited a superior increase in VO₂max (3.28 mL/min/kg; 95% CI: 0.86; 5.70; I²=69%; PI: -4.79; 11.35; p<0.01) compared to the non-exercise control group (Figure 2). No significant between-group differences were observed for SBP, DBP, resting HR, nor for the metabolic markers assessed (i.e., triglycerides, total, LDL and HDL cholesterol; Table 3 and Figures S1-S7). The certainty of the evidence was very low for VO₂max, SBP, DBP and resting HR and low for triglycerides, total, LDL and HDL cholesterol. The details of GRADE assessment are presented in Table S2.

****Figure 2****

****Table 3****

3.4. Risk of bias

All studies were rated to be at high risk of bias regarding VO_{2max} , SBP, DBP and resting HR. The majority of the studies were rated as having some concerns regarding triglycerides, total, LDL and HDL cholesterol (Andersen et al., 2016; Carneiro et al., 2023a; Skoradal et al., 2018), except one with high risk of bias (Pereira et al., 2023) – see Table S3.

3.5. Sensitivity analysis

Sensitivity analyses were conducted and showed no statistically significant differences for VO_{2max} (0.41 mL/min/kg; 95% CI: -0.33; 1.15), SBP (-4.05 mmHg, 95% CI: -9.58; 1.47), resting HR (-0.44 bpm, 95% CI: -3.26; 2.38), triglycerides (-0.14 mmol/L; 95% CI: -0.33; 0.04), total cholesterol (0.02 mmol/L; 95% CI: -0.33; 0.37), LDL cholesterol (-0.99 mmol/L; 95% CI: -0.24; 0.07) and HDL cholesterol (0.06 mmol/L; 95% CI: -0.07; 0.18), however DBP was significantly lower (-3.05 mmHg; 95% CI: -5.72; -0.38) when comparing the effects of RTS in older populations to non-exercise control groups – Table S4 and Figures S8-S15.

4. DISCUSSION

The main finding of the present systematic review and meta-analysis was that RTS (football and team handball) training is effective in improving VO_{2max} for over 60-year-olds when compared to non-exercise control groups. The VO_{2max} showed very low certainty of evidence in GRADE assessment. In contrast, to the results found for VO_{2max} , no significant effects were observed for the secondary cardiometabolic outcomes (SBP, DBP, resting HR, triglycerides, total, LDL and HDL cholesterol). The secondary cardiovascular and metabolic outcomes showed very low and low certainty of evidence, correspondently, in GRADE assessment. Nevertheless, the key strength of this systematic review and meta-analysis is that it focused on RCT's for individuals over 60 years of age.

Regular and adequate levels of PA and exercise are important to increase and maintain cardiorespiratory fitness levels in over 60-year-old men and women (Letnes et al., 2020). In fact, the World Health Organization PA guidelines for adults and older adults recommend at least 150-300 min/week of moderate-intensity aerobic PA or 75–150 min/week of vigorous-intensity aerobic PA or an equal combination of both, muscle-strengthening activities at moderate or greater intensity that involve all major muscle groups on 2 or more days a week, and in the case of older adults, adding varied multicomponent activities for balance and strength development, for at least 3 days/week. These PA guidelines are important to promote health benefits and to prevent and help to manage CVDs, to reduce the risk of developing several

cancers and symptoms of depression and anxiety, to improve brain health (cognitive function and academic performance), to strengthen muscles and bones and, consequently, to help to prevent falls among older adults (World Health Organization, 2022). Therefore, multicomponent exercise programmes (combining endurance, resistance, and balance exercises) are important for the overall health of older populations.

In the present study, RTS elicited a superior increase in VO_{2max} when compared to the non-exercise control group. This is of importance as cardiorespiratory fitness is related to functional capacity and human performance, and it is a strong and independent predictor of all-cause and disease-specific mortality regardless of sex and race (Harber et al., 2017). RTS fall within this scope, being characterised by high physical and physiological demands (Castagna et al., 2020; Milanović et al., 2022; Milanović et al., 2019), which may be the underlying cause of the observed improvements in VO_{2max} in the over 60-year-old population. In fact, in a study using a 12-week recreational team handball intervention for adult/middle-aged former team handball male players, the time spent $>90\%HR_{max}$ (21% of total match time) was associated with improvements in VO_{2max} (Póvoas et al., 2018). Additionally, time spent $>90\%HR_{max}$ (~15% of total match time) has also been suggested to potentially induce physiological adaptations, in studies using recreational football with different age-populations (Randers et al., 2010). In the studies analysed in this work, older men and prostate patients playing recreational football have spent 16-18% and 27%, respectively, of total match time above $>90\%HR_{max}$ (Andersen et al., 2014; Uth et al., 2014). Slightly lower values of 5-11% and 9-11% of total match time above $>90\%HR_{max}$, were observed for middle-aged-to-elderly men and postmenopausal women (Carneiro et al., 2023a; Pereira et al., 2023; Pereira et al., 2020), by performing recreational team handball. Nonetheless, all the studies used in this systematic review and meta-analysis reported high HRs which is most likely the main responsible for the increase in VO_{2max} observed after the interventions (Andersen et al., 2014; Carneiro et al., 2023a; Pereira et al., 2020; Skoradal et al., 2018; Uth et al., 2014). Based on our comprehensive meta-analysis, RTS could significantly enhance and sustain cardiorespiratory fitness for this population. Moreover, these positive outcomes have the potential to facilitate greater independence, ultimately leading to an enhanced quality of life (Paterson et al., 2004), thereby implying far-reaching and enduring repercussions over the long-term well-being of older individuals. Even though an effect of RTS was observed in VO_{2max} in the primary analysis of the present study, the results from the sensitivity analysis accounting for longer intervention durations showed no significant differences in VO_{2max} , which shows lack of robustness in the

data analysed. Therefore, future analysis should be performed to confirm the effect of RTS in VO_{2max} .

High BP levels have been associated with an increased risk of developing CVDs across different age groups (Whelton et al., 2018). Also, elevated resting HR has shown to be an independent predictor of all-cause mortality and cardiovascular events in over 60-year-olds (Li et al., 2017). Therefore, it is important to reduce the elevated BP and resting HR values found in individuals over 60 years of age, in order to lower the CVD risk and mortality. Still, in the present systematic review and meta-analysis, no effects were shown on these cardiovascular variables of RTS practice when compared to non-exercise control groups. It is worth noting that none of the participants in the studies included in the present systematic review and meta-analysis were classified as hypertensive patients, with only two studies (Carneiro et al., 2023a; Skoradal et al., 2018) reporting high BP levels at baseline (SBP: 130-139 mmHg and DBP: 80-89 mmHg) (Unger, 2020). This may explain the absence of significant effects on these variables. However, a systematic review and meta-analysis addressing the effect of recreational football on various age groups revealed that engaging in recreational football interventions for 12-16 weeks (2-3 per week, each lasting for 45-60 min) was most likely beneficial for SBP and DBP, when compared with non-exercise control groups. Nevertheless, a greater significant BP reduction was observed among participants with hypertension compared to normotensive individuals (Milanović et al., 2019). This finding is in line with our results and is aligned with the literature (Pedersen & Saltin, 2006). In the sensitivity analysis using longer follow-ups, a significant decrease for DBP was detected. This suggests that longer interventions might be required to observe changes in BP. Nonetheless, no significant differences were observed for SBP and resting HR outcomes in the sensitivity analysis.

In the present study, RTS showed no effect in the lipid profile markers. A narrative review on the effects of recreational football on global health of untrained men has reported mainly non-significant changes in total and LDL cholesterol (Bangsbo et al., 2015). Additionally, a more recent systematic review and meta-analysis examining the effect of recreational football on overall health of individuals of all age groups showed similar results, with possibly small beneficial decrease in LDL cholesterol and most likely and likely trivial decreases in HDL cholesterol and triglycerides, and total cholesterol (Milanović et al., 2019). Notably, the lack of changes in lipid profile has also been reported in studies using RTS with younger populations for the same intervention duration (12-16 weeks; recreational team handball) (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018). It is well-known that elevated triglycerides concentrations and/or LDL cholesterol, as well as decreased

HDL cholesterol, are indicators of dyslipidaemia (Klop et al., 2013), and the prevalence of dyslipidaemia worsens with age (Cho et al., 2020). Therefore, levels of <150 mg/dL for triglycerides, <200 mg/dL for total cholesterol, <100 mg/dL for LDL cholesterol and \geq 60 mg/dL for HDL cholesterol are recommended in order to decrease the risk of developing dyslipidaemia, and consequently, CVDs (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001). The four studies used in the present systematic review and meta-analysis, which assessed lipid profile, had a duration of 16 weeks, which may have also influenced these results. There is a limited number of studies with longer intervention durations that have evaluated lipid profile during RTS interventions for older populations (Andersen et al., 2014; Carneiro et al., 2023a; Pereira et al., 2020; Skoradal et al., 2018). Consequently, further research on the short and long-term effects of RTS is warranted, as other exercise modalities such as aerobic and resistance exercise, as well as a combination of both, are efficient in reducing high lipid profile values over short- and long-term interventions (Mann et al., 2014). The study included in the current systematic review and meta-analysis that implemented a diet intervention for both the football and the control groups, showed the overall greatest improvement in lipid profile (Skoradal et al., 2018) compared to the other studies considered. This indicates that combining RTS with dietary interventions in short-term exercise programmes may be important when the aim is to improve the lipid profile in individuals over 60 years of age. It is noteworthy that the other studies included in this analysis did not control for food intake, which is a limitation as such information could have contributed to a better understanding of lipid profile behaviour over time.

The present systematic review and meta-analysis has some limitations. The fact that not all the eight studies assessed BP, resting HR and lipid profile markers is a main limitation. If more studies had addressed these important health markers for this population, the results from the present study might have been different. Additionally, the limited availability of RCTs for individuals over 60 years of age, with only recreational football and team handball being included, restricted the number of studies used in this work. Another limitation identified is that some of the studies used were follow-up studies from the primary RCTs, resulting in even smaller sample sizes for certain outcomes. Nevertheless, the results of the studies analysed were consistent with the findings of the present systematic review and meta-analysis, since it is suggested that engaging in RST exercise interventions is associated with better cardiovascular health. Further RCTs using different RTS for older populations that address cardiovascular and metabolic outcomes over intervention durations exceeding 16 weeks are warranted.

The practical application of this systematic review and meta-analysis is that RTS, such as recreational football and team handball training programmes, hold the potential to enhance cardiorespiratory fitness in both men and women over the age of 60. Furthermore, despite the lack of significant changes in BP, resting HR, triglycerides, total, LDL and HDL cholesterol with RTS, this type of intervention might be capable of maintaining normal cardiometabolic markers values, that typically tend to deteriorate with age.

5. CONCLUSIONS

This systematic review and meta-analysis revealed that RTS, when played as small-sided and formal games (3v3 to 7v7) for 30-60 min/session, 1-3 times/week for at least 12 weeks, improves VO_{2max} for men and women over 60 years of age. However, no significant improvements were detected on BP and resting HR, as well as on lipid profile markers (triglycerides, total, LDL and HDL cholesterol). Moreover, the certainty of the evidence for cardiovascular and lipid profile outcomes was of very low and low quality, respectively. Therefore, further high-quality studies involving this population are required to confirm the present findings.

STATEMENTS AND DECLARATIONS

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Declaration of interest

The authors report there are no competing interests to declare.

Data availability statement

The data generated and/or analysed during this study are included in this published article (and its supplementary information files). The remaining data generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Registration and protocol

This study was registered in PROSPERO: CRD42023418690.

Author contributions

IC designed the study, conducted all stages of the review, and drafted the manuscript. IC and RO performed the screening and extracted the data. IC and JT analysed the data and rated the quality of the studies. JT designed the study and performed GRADE assessment. PK, CC, RR and SP contributed to the study design and manuscript draft and critically revised the work. All authors read and approved the final manuscript.

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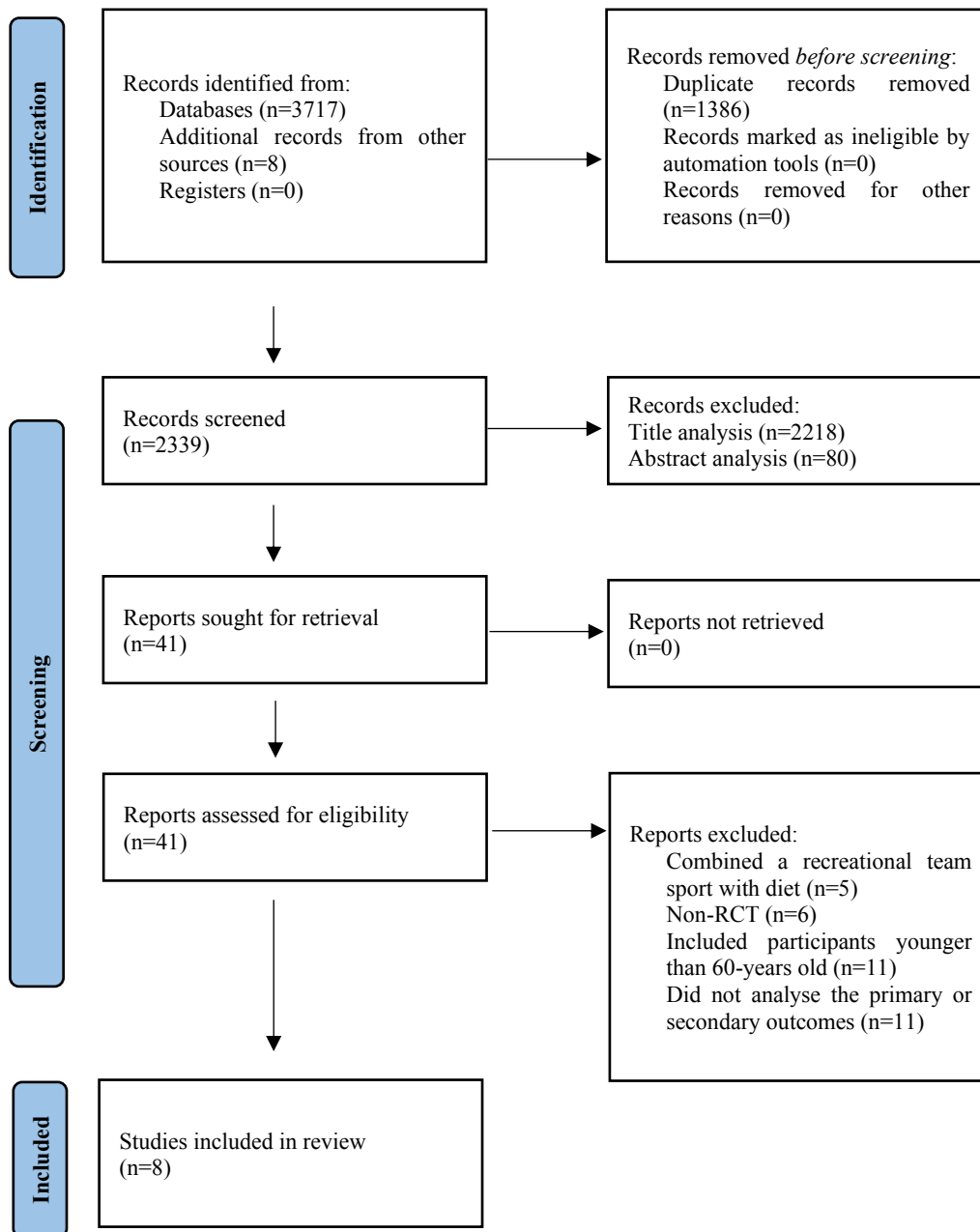


Figure 1. PRISMA flow diagram of the process of study selection.

Table 1. Main characteristics of the included studies.

Study	Reference	Origin	VO _{2max} evaluation	Intervention	Control group	Interventions characteristics				
						Type of format	Session	Intensity	Frequency	Weekly attendance
NCT01530035*	Andersen, et al., 2014	Denmark	Standardized cycling protocol performed on an electronically braked ergometer bike	Recreational football for 16 weeks	Instructed to continue with their daily routines and not to change lifestyle during the intervention period	4v4; 5v5; 6v6	0-12 weeks: 3x15 min periods	<u>Mean HR:</u> Week 1: 138±3 bpm, 81±2%HR _{max} Week 16: 143±3 bpm, 84±1%HR _{max}	2 sessions/week	1.6±0.1, 77.1±2.4%
	Andersen, et al., 2016	Denmark	-				60min/session	<u>Peak HR:</u> Week 1: 92±2%HR _{max} Week 16: 93±1%HR _{max}	0-16 weeks: 2 sessions/week	
	Schmidt, et al., 2014	Denmark	Standardized cycling protocol performed on an electronically braked ergometer bike	Recreational football for 52 weeks		3v3; 4v4; 5v5		-	17-52 weeks: 3 sessions/week	1.7±0.3, 66±4%
NCT05295511	Carneiro, et al., 2023	Portugal	Standardized incremental treadmill protocol test until voluntary exhaustion	Recreational team handball for 16 weeks	Advised to maintain habitual PA	4v4; 5v5; 6v6; 7v7	3x15 min periods 60min/session	<u>Mean HR:</u> 127.4±13.7 bpm, 78.9±7.4% HR _{max} <u>Peak HR:</u> 141.2±15.3 bpm, 87.4±7.5% HR _{max}	1-3 sessions/week	1.7±0.7
NCT05292261*	Pereira, et al., 2020	Portugal	Standardized incremental treadmill protocol test until voluntary exhaustion	Recreational team handball for 16 weeks	Advised to maintain habitual PA	5v5; 6v6	3x15 min periods 60min/session	<u>Mean HR:</u> 76±6%HR _{max} <u>Peak HR:</u>	2-3 sessions/week	1.9±0.4

* Note that some studies resulted in multiple publications. Abbreviations: BP- Blood pressure; CG- Control group; DBP- Diastolic blood pressure; HR- Heart rate; HR_{max}- maximal heart rate; RTS- Recreational team sport; SBP- Systolic blood pressure; VO_{2max} – Maximal oxygen uptake.

Table 2. Participants' characteristics of the included studies.

Reference	Main characteristics	RTS					CG				
		Sample (n); Sex (M/F)	Age	Baseline VO _{2max}	Baseline BP (SBP;DBP)	BMI	Sample (n); Sex (M/F)	Age	Baseline VO _{2max}	Baseline BP (SBP;DBP)	BMI
Andersen, et al., 2014 Andersen, et al., 2016 Schmidt, et al., 2014	Inactive healthy males	9 (M)	68±4	27.5±5.4	125±13; 74±7	26.1±3.9	8 (M)	67±3	30.8±3.3	130±13; 77±6	27.9±4.6
Carneiro, et al., 2023	Inactive healthy males	40 (M)*	68±4*	27.1±4.9*	133±13; 78±8*	27.4±4.0*	14 (M)	67±5	28.8±4.7	134±13; 77±8	27.4±2.8
Pereira, et al., 2020	Inactive	41 (F)	67±7	25.8±3.3	126±14; 74±7*	27.7±4.0*	26 (F)	70±5	24.5±3.6	133±16; 76±7*	26.2±3.8*
Pereira, et al., 2023	postmenopausal	31 (F)	64±7	25.8±3.9	126±13; 74±7*	27.3±4.0*	14 (F)	67±5	25.2±2.5	134±15; 79±7*	26.0±3.9*
Skoradal, et al., 2018	Inactive prediabetes males and females	27 (M/F) #	60±6	24.9±5.3	138±16; 84±11	28.6±4.1	23 (M/F) #	62±6	20.2±4.8	142±18; 87±11	30.9±4.9
Uth, et al., 2014	Inactive prostate cancer patients	29 (M)	67±7	27.2±4.6	-	26.6±3.2	28 (M)	67±5	26.4±3.5	-	27.6±2.8

* Additional information given by the authors; # Sex ratio was not reported. Abbreviations: BMI- Body mass index; BP- Blood pressure; CG- Control group; DBP- Diastolic blood pressure; RTS- Recreational team sport; SBP- Systolic blood pressure; VO_{2max}- Maximal oxygen uptake.

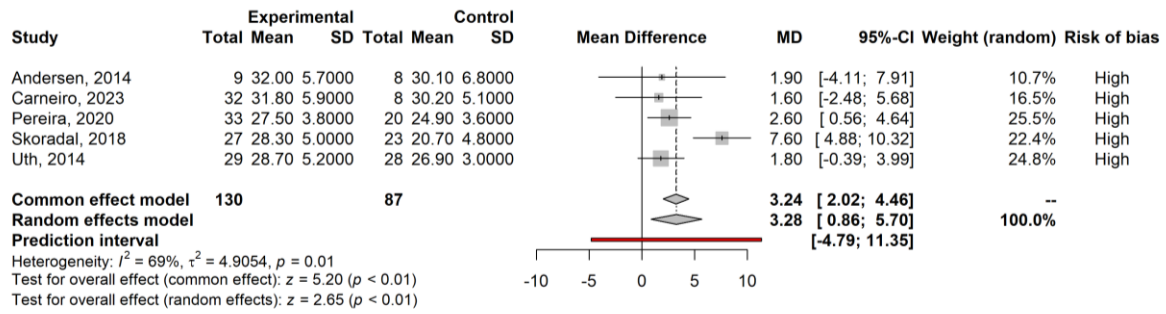


Figure 2. Forest plot of the effects of recreational team sports on maximal oxygen uptake.

Table 3. Effects of recreational team sports on the secondary cardiometabolic outcomes.

Outcomes	k	Random-effects model			Heterogeneity		
		Mean Difference (95% CI)	p	PI	I ²	t ²	Q
SBP (mmHg)	3	-3.35 (-10.52; 3.82)	0.36	-78.66; 71.96	54%;	21.75	0.11
DBP (mmHg)	3	-1.84 (-5.14; 1.46)	0.27	-29.81; 26.13	29%	2.02	0.25
Resting HR (bpm)	3	0.07 (-2.44; 2.57)	0.96	-5.43; 5.56	0%	<0.01	0.61
Triglycerides (mmol/L)	4	-0.08 (-0.27; 0.11)	0.42	-0.75; 0.59	31%	≤0.01	0.23
Total cholesterol (mmol/L)	4	0.19 (-0.10; 0.48)	0.20	-0.44; 0.82	0%	<0.01	0.86
LDL cholesterol (mmol/L)	4	0.02 (-0.18; 0.22)	0.86	-0.61; 0.64	0%	≤0.01	0.42
HDL cholesterol (mmol/L)	4	0.03 (-0.08; 0.14)	0.58	-0.22; 0.28	0%	<0.01	0.66

Abbreviations: 95% CI- 95% confidence interval; DBP- diastolic blood pressure; HDL- high-density lipoproteins; HR- heart rate; k- number of trials; LDL- low-density lipoproteins; PI- Prediction intervals; SBP- systolic blood pressure.

SUPPLEMENTARY INFORMATION

Table S1. Search strategy used in databases.

Data base	Search strategy
MEDLINE (PUBMED)	"Randomized Controlled Trial"[Publication Type] OR random[Title/Abstract] OR random*[Title/Abstract] OR randomly[Title/Abstract] OR "random allocation"[MeSH Terms] AND ("recreational football"[Title/Abstract] OR "recreational soccer"[Title/Abstract] OR "recreational team handball"[Title/Abstract] OR floorball[Title/Abstract] OR "team sports"[Title/Abstract] OR rugby[Title/Abstract] OR handball[Title/Abstract] OR "basketball"[MeSH Terms] OR "recreational basketball"[Title/Abstract] OR hockey[Title/Abstract] OR "hockey"[MeSH Terms] OR "recreational volleyball"[Title/Abstract] OR "volleyball"[MeSH Terms] OR "baseball"[MeSH Terms] OR baseball[Title/Abstract] OR "cricket sport"[MeSH Terms] OR cricket[Title/Abstract])
Web of science	((Randomized Controlled Trial OR random OR randomly OR random allocation) AND (recreational football OR recreational soccer OR recreational team handball OR football OR

	team sports OR rugby OR handball OR basketball OR recreational basketball OR hockey OR recreational volleyball OR volleyball OR baseball OR cricket sport OR cricket))
Scopus	TITLE-ABS-KEY (("Randomized Controlled Trial") OR ("random allocation") AND ("recreational football") OR ("recreational soccer") OR ("recreational team handball") OR ("floorball") OR ("team sports") OR ("rugby") OR ("handball") OR ("basketball") OR ("recreational basketball") OR ("hockey") OR ("recreational volleyball") OR ("volleyball") OR ("baseball") OR ("cricket sport") OR ("cricket")) AND (LIMIT-TO (DOCTYPE , "ar")) AND (EXCLUDE (PUBYEAR , 2000) OR EXCLUDE (PUBYEAR , 1999) OR EXCLUDE (PUBYEAR , 1998) OR EXCLUDE (PUBYEAR , 1997) OR EXCLUDE (PUBYEAR , 1996) OR EXCLUDE (PUBYEAR , 1995) OR EXCLUDE (PUBYEAR , 1994) OR EXCLUDE (PUBYEAR , 1993) OR EXCLUDE (PUBYEAR , 1992) OR EXCLUDE (PUBYEAR , 1991) OR EXCLUDE (PUBYEAR , 1990) OR EXCLUDE (PUBYEAR , 1989) OR EXCLUDE (PUBYEAR , 1986) OR EXCLUDE (PUBYEAR , 1984) OR EXCLUDE (PUBYEAR , 1983) OR EXCLUDE (PUBYEAR , 1977) OR EXCLUDE (PUBYEAR , 1975) OR EXCLUDE (PUBYEAR , 1974) OR EXCLUDE (PUBYEAR , 2008) OR EXCLUDE (PUBYEAR , 2007) OR EXCLUDE (PUBYEAR , 2006) OR EXCLUDE (PUBYEAR , 2005) OR EXCLUDE (PUBYEAR , 2004) OR EXCLUDE (PUBYEAR , 2003) OR EXCLUDE (PUBYEAR , 2002) OR EXCLUDE (PUBYEAR , 2001))

Table S2. Results of the GRADE assessment.

Certainty assessment							
N° of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Certainty
VO_{2max}							
5	randomized trials	very serious ^a	not serious	not serious	not serious	yes ^b	⊕○○○Very low
SBP							
3	randomized trials	very serious ^a	serious ^c	not serious	serious ^d	none	⊕○○○Very low
DBP							
3	randomized trials	very serious ^a	serious ^c	not serious	serious ^d	none	⊕○○○Very low
Resting HR							
4	randomized trials	very serious ^a	serious ^c	not serious	not serious	none	⊕○○○Very low
Triglycerides							

4	randomized trials	not serious	serious ^c	not serious	serious ^d	none	⊕⊕○○Low
Total cholesterol							
4	randomized trials	not serious	serious ^c	not serious	serious ^d	none	⊕⊕○○Low
LDL cholesterol							
4	randomized trials	not serious	serious ^c	not serious	serious ^d	none	⊕⊕○○Low
HDL cholesterol							
4	randomized trials	not serious	serious ^c	not serious	serious ^d	none	⊕⊕○○Low

Abbreviations: DBP- diastolic blood pressure; HDL- high-density lipoproteins; HR- heart rate; LDL-Low-density lipoproteins; SBP- systolic blood pressure; VO_{2max}- Maximal oxygen uptake.

Explanations:

- a. Frequent high risk of bias in several domains (downgraded by two levels).
- b. Downgraded by one level by the accumulation of several reasons. First, Skoradal (2018) inflated the results due to large baseline imbalances. Second, in the sensitivity analysis, the results were no longer statistically significant. Finally, although, Uth's protocol pre-registered a long-term assessment of several health outcomes, including VO_{2max}, the published follow-up RCT did not report the long-term VO_{2max} results.
- c. Variability in point estimates and confidence intervals.
- d. Very wide confidence interval.

Table S3. Results of the risk of bias assessment (RoB2).

Study	Domain 1	Domain 2	Domain 3	Domain 4	Domain 5	Overall
VO_{2max}						
Andersen, et al., 2014	Some concerns	Low risk	Low risk	High risk	Some concerns	High risk
Carneiro, et al., 2023	Low risk	High risk	High risk	Some concerns	Some concerns	High risk
Pereira, et al., 2020	Low risk	Low risk	Low risk	High risk	Low risk	High risk
Pereira, et al., 2023	Low risk	High risk	High risk	High risk	Low risk	High risk
Schmidt, et al., 2014	Some concerns	Low risk	Low risk	High risk	Some concerns	High risk
Skoradal, et al., 2018	Some concerns	Some concerns	Low risk	High risk	Some concerns	High risk
Uth, et al., 2014	Low risk	Some concerns	Low risk	High risk	Some concerns	High risk
SBP						
Carneiro, et al., 2023	Low risk	Some concerns	Low risk	High risk	Some concerns	High risk
Pereira, et al., 2020	Low risk	Low risk	Low risk	High risk	Low risk	High risk
Pereira, et al., 2023	Low risk	High risk	High risk	High risk	Low risk	High risk
Schmidt, et al., 2014	Some concerns	Some concerns	Low risk	High risk	Low risk	High risk

Abbreviations: DBP- diastolic blood pressure; HDL- high-density lipoproteins; HR- heart rate; LDL-Low-density lipoproteins; SBP- systolic blood pressure; VO_{2max}- Maximal oxygen uptake.

Table S4. Sensitivity analysis conducted for the primary and secondary outcomes.

Outcomes	k	Random-effects model			Heterogeneity			
		Mean Difference (95% CI)	p	PI	I ²	t ²	Q	
VO _{2max} (mL/min/kg)	5	0.41* (-0.33; 1.15)	0.27	-2.26; 3.09	78%	0.57	<0.01	
SBP (mmHg)	3	-4.05 (-9.58; 1.47)	0.15	-51.02; 42.92	30%	5.72	0.24	
DBP (mmHg)	3	-3.05 (-5.72; -0.38)	0.03	-20.35; 15.26	0%	<0.01	0.44	
Resting HR (bpm)	4	-0.44 (-3.26; 2.38)	0.76	-6.62; 5.75	0%	<0.01	0.40	
Triglycerides (mmol/L)	4	-0.14 (-0.33; 0.04)	0.13	-0.58; 0.30	0%	<0.01	0.40	
Total cholesterol (mmol/L)	4	0.02 (-0.33; 0.37)	0.91	-0.75; 0.79	0%	<0.01	0.95	
LDL cholesterol (mmol/L)	4	-0.09 (-0.24; 0.07)	0.27	-0.42; 0.25	0%	<0.01	0.77	
HDL cholesterol (mmol/L)	4	0.06 (-0.07; 0.18)	0.37	-0.21; 0.33	0%	<0.01	0.47	

* Reported as standardized mean difference. Abbreviations: 95% CI- 95% confidence interval; DBP- diastolic blood pressure; HDL- high-density lipoproteins; HR- heart rate; k, number of trials; LDL-Low-density lipoproteins; PI- Prediction intervals; SBP- systolic blood pressure; VO_{2max}- Maximal oxygen uptake .

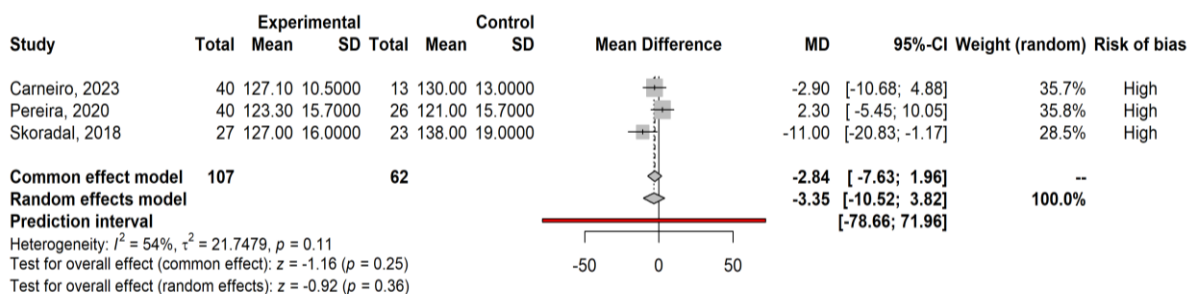


Figure S1 Forest plot of the effects of recreational team sports on systolic blood pressure.

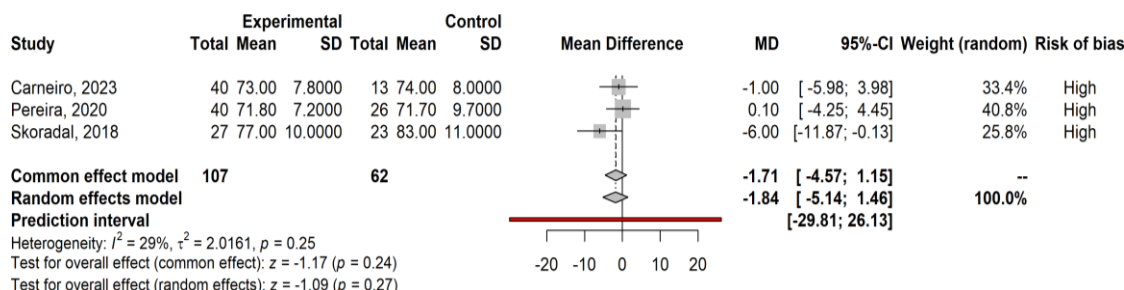


Figure S2. Forest plot of the effects of recreational team sports on diastolic blood pressure.

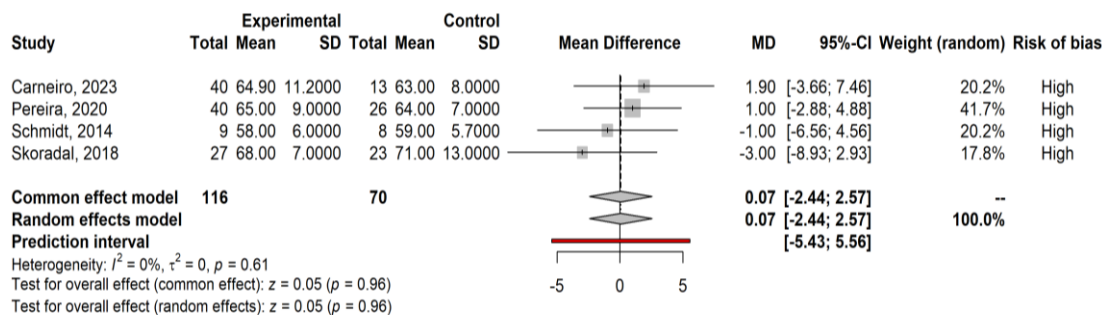


Figure S3. Forest plot of the effects of recreational team sports on resting heart rate.

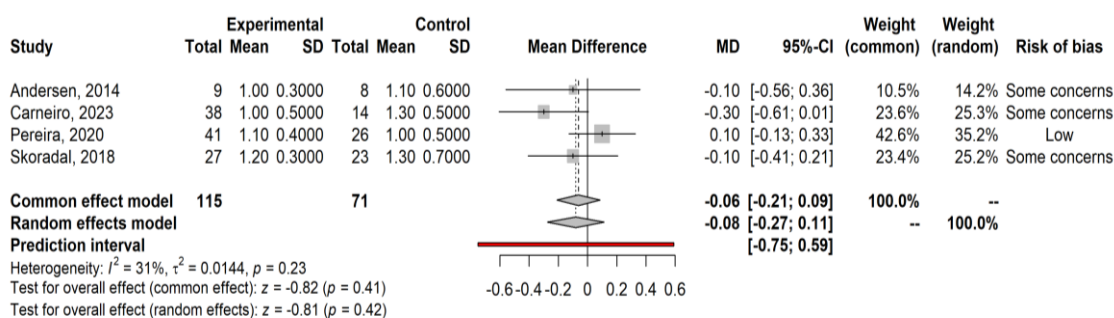


Figure S4. Forest plot of the effects of recreational team sports on triglycerides.

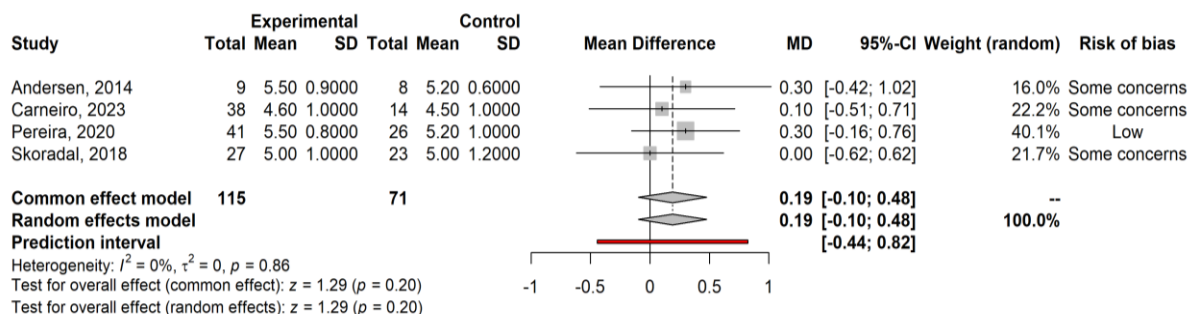


Figure S5. Forest plot of the effects of recreational team sports on total cholesterol.

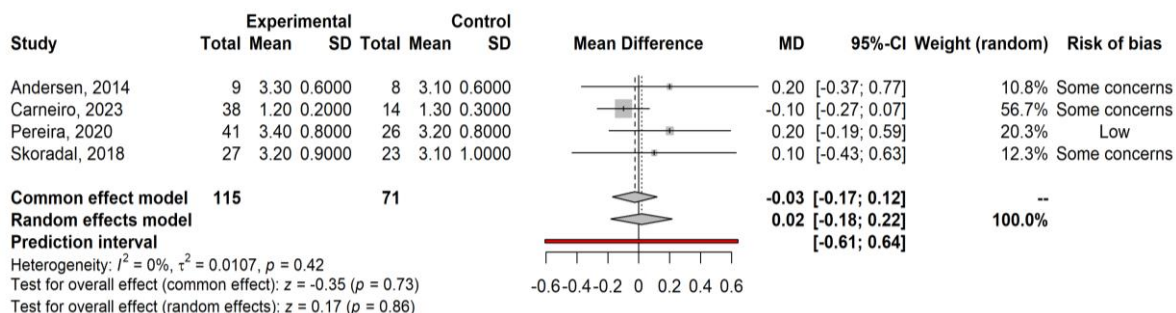


Figure S6. Forest plot of the effects of recreational team sports on low-density lipoproteins cholesterol.

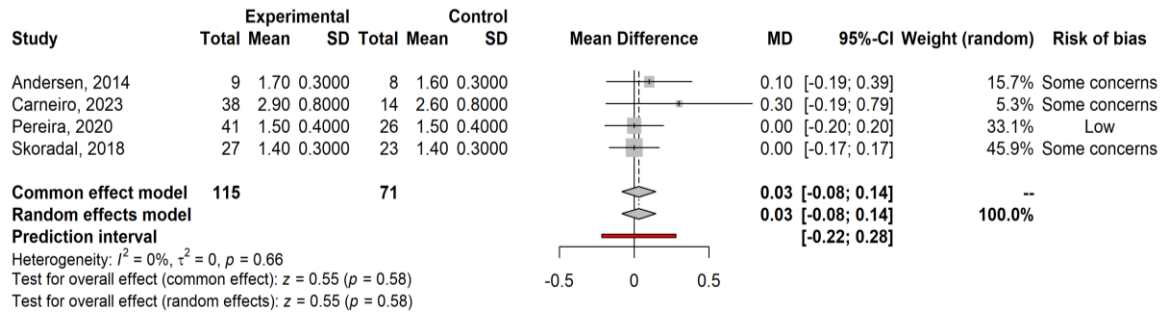


Figure S7. Forest plot of the effects of recreational team sports on high-density lipoproteins cholesterol.

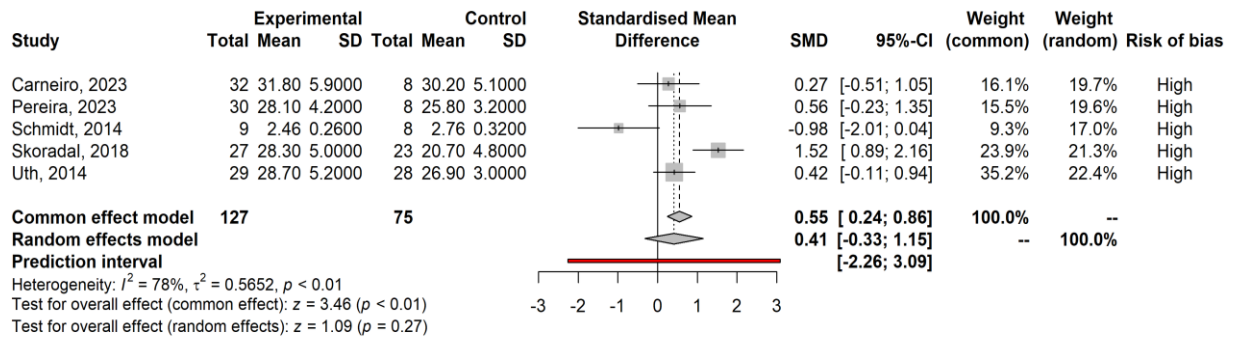


Figure S8. Forest plot of the sensitivity analysis conducted for maximal oxygen uptake.

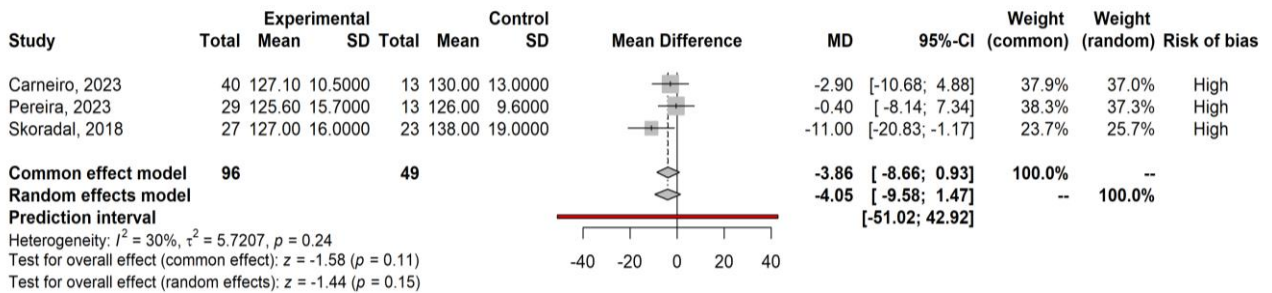


Figure S9. Forest plot of the sensitivity analysis conducted for systolic blood pressure.

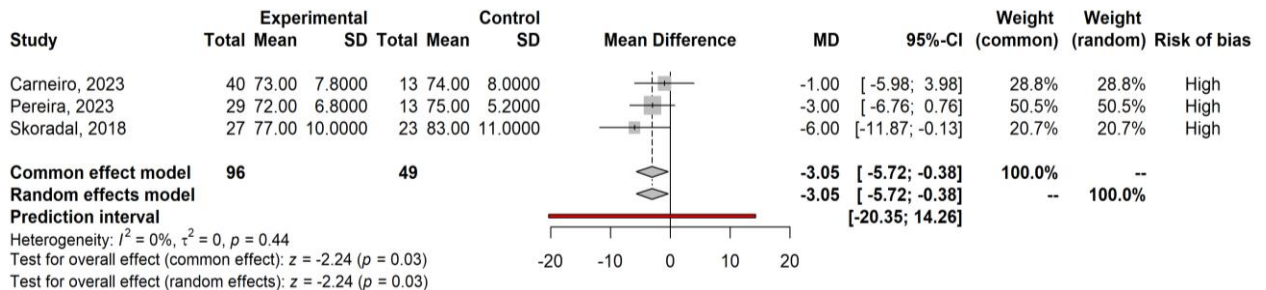


Figure S10. Forest plot of the sensitivity analysis conducted for diastolic blood pressure.

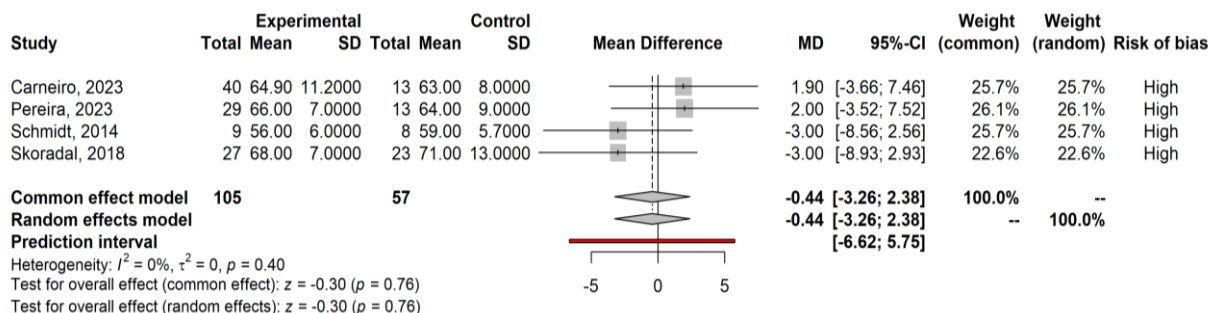


Figure S11. Forest plot of the sensitivity analysis conducted for resting heart rate.

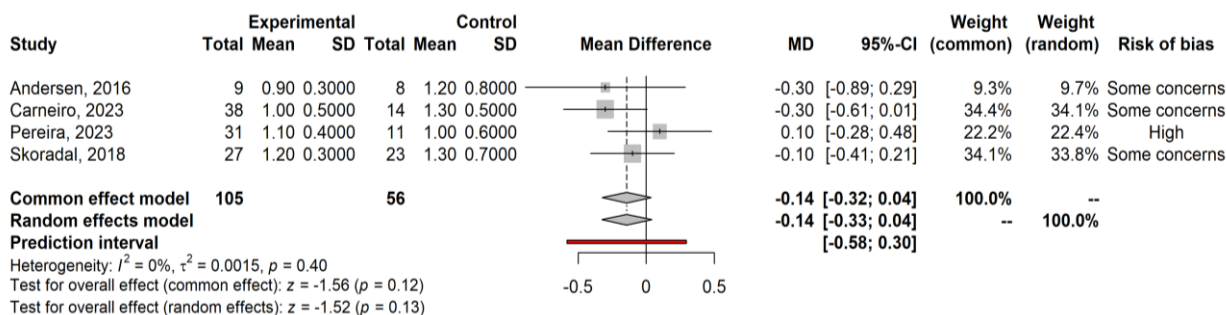


Figure S12. Forest plot of the sensitivity analysis conducted for triglycerides.

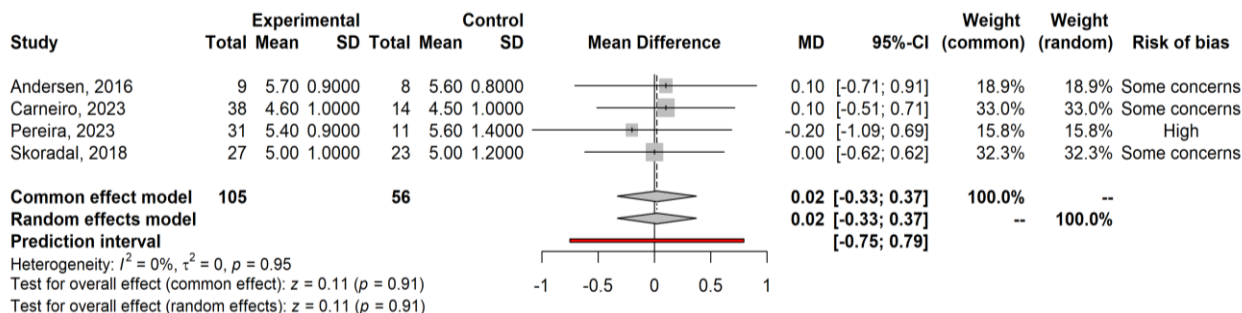


Figure S13. Forest plot of the sensitivity analysis conducted for total cholesterol.

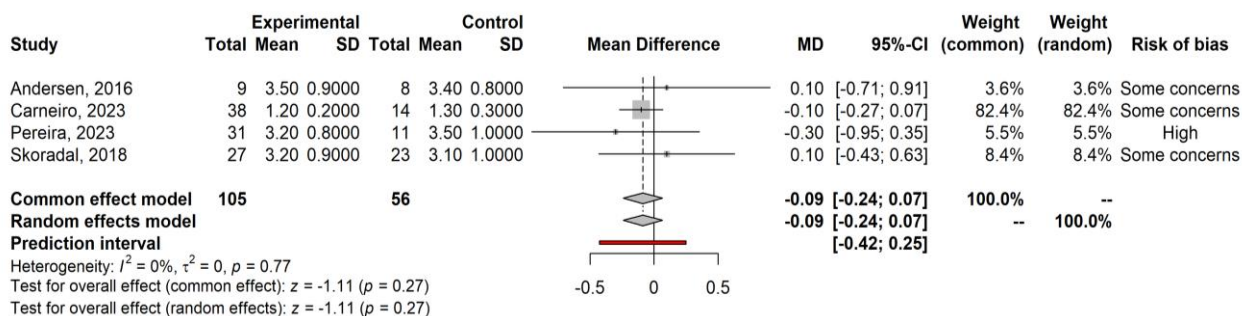


Figure S14. Forest plot of the sensitivity analysis conducted for low-density lipoproteins cholesterol.

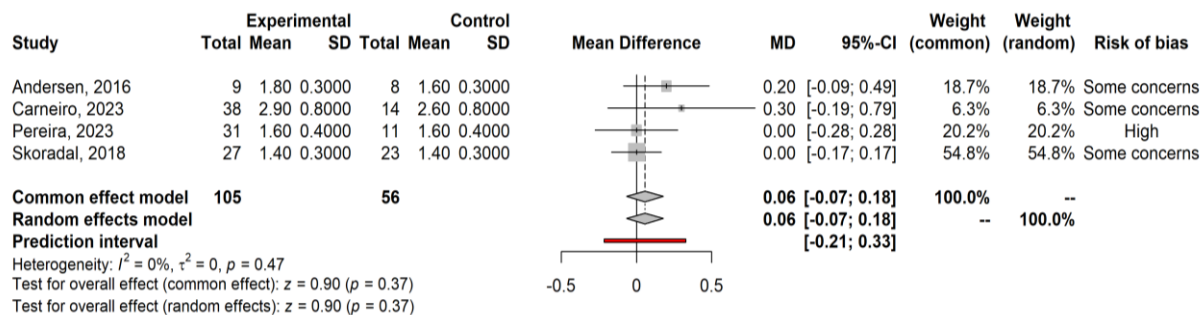


Figure S15. Forest plot of the sensitivity analysis conducted for high-density lipoproteins cholesterol.

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CHAPTER V: Discussion

5. Discussion

The present work is the first RCT to provide evidence of a dose-response effect of a multicomponent exercise modality, namely, recreational TH, on cardiometabolic health, aerobic performance, bone health, body composition and physical fitness in inactive middle-aged-to-elderly males, without previous experience with the sport. The main findings were that after 16 weeks, health improvements were observed in all the intervention groups, with greater benefits shown for those who performed 2 and 3 sessions per/week. Because the training intensity was similar between groups, it is likely that the frequency of training, and therefore, also the training volume, was the main reason for the differential health effects observed. This is also the first work to describe the physical and physiological demands of recreational TH for middle-aged-to-elderly males when playing 5v5, 6v6 and 7v7, as well as the differences in the demands when playing same- vs. mixed-gender game formats for men and women in this age group. The conclusions from these two studies are important to provide physical exercise and sport professionals evidence on the internal and external load characteristics of the game and gender formats analysed, which will allow them to make informed decisions according to the defined purposes for the training sessions. The main findings of these studies were that game format had no significant impact on the internal load, although a tendency was observed for higher cardiovascular demands in 5v5 and 6v6 vs. 7v7. Significant differences were found in the analysed external load variables, with 5v5 and 6v6, showing a higher number of high-intensity movements and total high-intensity game actions when compared to 7v7. Moreover, when comparing the demands of men and women playing same- vs. mixed-gender games formats, higher time spent $>80\%HR_{max}$ and $>90\%HR_{max}$ was found for women than men during mixed-gender matches. Additionally, during mixed-gender matches, mean and peak BL values were lower for women than men. During same-gender matches, men's time spent $>80\%HR_{max}$, as well as some activity profile variables, such as high-intensity locomotor movements, were higher than in mixed-gender matches. In all studies that compose this thesis, the fun levels were analysed and reported as very high (~ 9 on a 0-10 AU scale), which is an important finding since according to the latest Eurobarometer, having fun is one of the main reasons for people to exercise (European Commission, 2022). Furthermore, this thesis also includes a systematic review and meta-analysis addressing the cardiometabolic health effects of recreational team sports for older populations. The main findings of this study were that recreational team sports were effective in improving cardiorespiratory fitness (VO_{2max}) compared to non-exercise CG,

while no effect was observed for the secondary cardiometabolic outcomes (SBP, DBP, resting HR, TRG, total, LDL and HDL cholesterol).

5.1. Dose-response health effects of recreational team handball

5.1.1. Cardiorespiratory fitness, aerobic performance, blood pressure and resting heart rate

The training protocol used in the RCT designed to ascertain the dose-response health effect of recreational TH in middle-aged-to-elderly males comprised a 15 min warm-up and 3x15-min periods of recreational TH, performed 1, 2 or 3 times 60 min/week (60, 120 and 180 min/week; TH1, TH2 and TH3, respectively). After the exercise intervention, a change of +19% in absolute and +23% in relative VO_{2peak} was shown for TH3, which was higher than the CG. On the other hand, no significant differences were shown in TH1 and TH2 compared to CG, or between the intervention groups, in absolute and relative VO_{2peak} . However, after 16 weeks, the absolute VO_{2peak} values were significantly higher by 12% for both TH1 and TH2, and changes of 3.5 and 4.2 mL/min/kg were shown in relative VO_{2peak} for TH1 and TH2, respectively. These results are clinically relevant since the risk of all-cause and CVDs mortality reduces by 13–15%, respectively, for each 1 MET increase, corresponding to a 3.5 mL/min/kg increase in VO_{2peak} (Glass et al., 2007; Kodama et al., 2009). Moreover, body mass was reduced after 16 weeks, however, changes in absolute VO_{2peak} of 12–19% for the intervention groups were still observed, which means that the decrease in body mass did not influence the results found in VO_{2peak} . This highlights the important role that recreational TH may have on the improvement of cardiorespiratory fitness for this population. Moreover, improvements of 19–21% corresponding to 1.5–2 min, in time to exhaustion, during VO_{2max} test, were observed for all TH training groups, indicating an improvement in the VO_{2max} test performance, which is in accordance with the increases found for absolute and relative VO_{2peak} values.

The improvements found in relative VO_{2peak} after 16 weeks for all interventions groups (TH1=13%, TH2=15% and TH3=23%) were higher than the 7–11% increases reported for untrained adult men and women, premenopausal overweight women, and postmenopausal women performing recreational TH 2–3 sessions/week for 12–16 weeks (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018; Pereira et al., 2020). Moreover, the within-group changes in relative VO_{2peak} in TH1 and TH2 were in line with those reported for adult/middle-

aged male former TH players (+14%) that played recreational TH 2–3 times/week, for 12 weeks (Póvoas et al., 2018). Nevertheless, TH3 showed higher pre-to-post changes in relative VO_{2peak} (+23%) than the adult/middle-aged male former TH players. The differences in the results from the studies may be explained by the baseline characteristics of the population in each study and the exercise intervention protocols, as it has been shown that VO_{2max} response to training may vary depending on age, body mass and training status and implemented exercise protocol design (i.e., intensity, duration, weekly frequency) (Wen et al., 2019). As an example, the time spent $>90\%HR_{max}$ has been previously associated with improvements in VO_{2max} (Póvoas et al., 2018), with adult/middle-aged male former TH players spending 21% of total match time $>90\%HR_{max}$. In the present work, the participants spent 5–11% of the total match time $>90\%HR_{max}$, corresponding to half of the time of adult/middle-aged male former TH players. In this case, even though adult/middle-aged male former TH players spent more time $>90\%HR_{max}$ than our participants, the improvements in cardiorespiratory fitness were similar for both studies, with TH3 even showing higher changes than the adult/middle-aged male former TH players (Póvoas et al., 2018). The volume of recreational TH training sessions (2.2 ± 0.7 times/week for 12 weeks) to which the adult/middle-aged male former TH players were exposed, compared to TH3 from our study (2.6 ± 0.2 times/week for 16 weeks) may have influenced the cardiorespiratory fitness results. Additionally, untrained older men, within the same age group as our participants, playing recreational football 1.6 ± 0.1 times/week for 16 weeks, showed a 16% increase in absolute VO_{2max} (Andersen et al., 2014), in line with our recreational TH groups (12–19%). Nonetheless, after 12 months and by increasing the weekly volume to 3 sessions an even higher improvement (18%) in absolute VO_{2max} was shown compared to the baseline values (Schmidt et al., 2014), which are consistent to our TH3 increase in absolute VO_{2peak} after 16 weeks of recreational TH (19%). These comparisons suggest, again, that volume is important when the aim is to improve cardiorespiratory fitness, which is expectable as a dose-response relationship exists between PA volume and VO_{2peak} adaptations (Church et al., 2007; Lee, 2007). Moreover, a study using recreational floorball (which has higher utilization of the upper limb muscles than recreational football or futsal) for untrained older males (1.9 sessions/week for 12 weeks), did not show improvements in VO_{2max} (Vorup et al., 2017b). The same group was not able to even maintain their VO_{2max} values (Pedersen et al., 2018) by performing recreational floorball twice a week (40 min/sessions) for 26 months. Nevertheless, the reduction observed in VO_{2max} , for the floorball group, was lower than the reduction found for the age-matched CG. This suggests that by performing recreational floorball for 12 weeks and 26 months, older untrained men may not benefit from cardiorespiratory fitness improvements,

however, this type of exercise modality may help to decelerate the ageing-related decline in cardiorespiratory fitness. However, the sessions' duration was shorter than the usual recreational TH sessions' duration, which can be a plausible explanation for the different results shown in these two recreational team sports interventions. Since the cardiovascular load demands were similar between recreational TH and floorball, with mean HRs ranging from 78–80%HR_{max} vs. 77–80% HR_{max}, correspondently, it is plausible that the floorball interventions did not have the adequate session volume to induce cardiorespiratory fitness benefits. On the other hand, these studies addressing older untrained men playing recreational floorball (Pedersen et al., 2018; Vorup et al., 2017b) that reported similar cardiovascular training load did not perform a multiple approach to assess HR_{max} as we did. Instead, an estimated HR_{max} was used, which can also explain the high cardiovascular training load (which may be overestimated), but lack of cardiorespiratory fitness benefits, as it can be a result of unsuitable HR_{max} assessment.

Recreational TH was effective in improving aerobic performance (YYIE1) for TH2 and TH3 compared to the CG, and for TH3 compared to TH1 and TH2, showing a dose-response effect in this outcome. Additionally, improvements after 16 weeks were shown in YYIE1 in TH1 (+38%), TH2 (+52%) and TH3 (+100%), with no changes in the CG. Aerobic performance, measured by the YYIE1 test, is important for estimating the capacity of an individual to perform repeated exercise (Grgic et al., 2019). The observed improvements in YYIE1 suggest the potential to achieve a higher amount of time at high-intensity during the training sessions [which has been associated with VO_{2max} changes (Póvoas et al., 2018)], as it has been shown that a higher performance in the Yo-Yo intermittent tests is associated with a higher amount of high-intensity exercise during a football match (Bangsbo et al., 2008). From a practical point of view, a higher aerobic performance will allow these participants a higher ability to cope with daily tasks and to prolong independence (Shephard, 2009). The YYIE1 results found in this study are in line with a recent study using recreational TH, for 16 weeks, 2–3 sessions/week, for postmenopausal women (+70%) (Pereira et al., 2020). The internal load shown by the postmenopausal women (mean HR of 76%HR_{max} and time spent >90% of HR_{max} of 11% of total match time) is similar to that experienced by the present study middle-aged-to-elderly males (mean HR of 78–80%HR_{max} and time spent >90% of HR_{max} of 5–11% of total match time), which may explain the similar results found for YYIE1 performance. Studies with younger populations (untrained adult men and women) performing TH have showed improvements in YYIE1 of 32–35%, after 12 weeks (Hornstrup et al., 2019; Hornstrup et al., 2018), which are much lower than the improvements showed for older populations such as ours

and postmenopausal women (Pereira et al., 2020). Nevertheless, the distance covered in YYIE1 by the younger participants was approximately twice the value at baseline and at post-intervention than the older ones, which can explain the lower percentages of change reported compared to middle-aged-to-elderly men and postmenopausal women. This because participants with lower baseline aerobic capacity, when submitted to exercise interventions, show greater training-related improvements in VO_{2peak} (Tang et al., 2013) and thus, higher aerobic performance improvements than participants with a higher baseline value, as aerobic fitness is associated with aerobic performance (Castagna, 2020b).

At baseline, the participants from the present work had high BP values (SBP: 130–139 mmHg; DBP: 85–89 mmHg) (Mancia et al., 2023). Although time x group differences were not found for BP, a significant reduction in SBP and DBP was found for all intervention groups after 16 weeks, which from a clinical and public health point of view is relevant since the participants were able to change their BP from high to normal reference values [i.e., SBP: 120–129 mmHg; DBP: 80–84 mmHg (Mancia et al., 2023)]. These reductions are important as normal BP values in this population may help to reduce CVDs risk, especially, in TH3 that experienced the higher reduction among the TH groups, which may suggest that the dimension of the reduction can be related to the higher training volume (Joseph, 2019). Furthermore, the TH3 reduction observed for SBP of 10 mmHg, after 16 weeks, of recreational TH is important to highlight as it has been shown that a 10 mmHg reduction in SBP reduces the risk of major CVD events by 20%, coronary heart disease by 17%, stroke by 27%, heart failure by 28% and of all-cause mortality by 13% (Ettehad et al., 2016). Previous studies using recreational TH as exercise interventions for younger populations showed no impact on BP after 12–16 weeks of training (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018), while adult/middle-aged male former TH players reduced DBP (-4%) after 12 weeks. Moreover, recreational football for older untrained male participants practiced for 4 and 12 months, as well as recreational floorball for untrained older men for 12 weeks, did not show BP changes either (Schmidt et al., 2014; Vorup et al., 2017b). Nevertheless, the participants from the studies mentioned before showed normotensive baseline values, which can possibly explain the lack of or small reductions in BP observed for those populations, contrary to the participants from our study that had high BP levels at baseline. On the other hand, a study combining recreational football and a diet intervention for 55-70-year-old males and females, for 16 weeks, showed decrements in SBP and DBP values compared to the respective CG that had a diet intervention (Skoradal et al., 2018). The recreational football exercise programme combined with a diet showed decreases in SBP from 138 ± 16 to 127 ± 15 mmHg and in DBP from 84 ± 11 to 77 ± 10

mmHg, which are higher BP reductions than the observed in our participants. Nevertheless, the recreational football intervention combined with a diet intervention showed even better results than our study. This because it is known that different types of exercise modalities, as well as, a lifestyle modification including a low calorie diet have positive effects on BP reduction (Fu et al., 2020).

A postintervention reduction in resting HR was observed for TH3 (-11%). Resting HR predicts various outcomes, such as, mortality, sudden cardiac death, and cardiovascular mortality, and although the ideal resting HR value may vary in each individual, presenting a resting HR of 70 bpm or higher may be a concern, specially, when an underlying disease (i.e., hypertension, heart failure and cancer) is present (Olshansky et al., 2022). In the present study, TH3 showed a reduction from 72 ± 16 to 63 ± 11 bpm, after 16 weeks. The decrease presented by TH3 was in line with the -16% reduction found for adult/middle-aged male former TH players, after 12 weeks of recreational TH (Póvoas et al., 2018), as well as the reductions found for untrained older men (recreational football) of -6 and -8 bpm, after 4 and 12 months of training, respectively (Schmidt et al., 2014). These results suggest that recreational TH and football may have the potential to reduce resting HR in males, and consequently, reduce the risk of CVD (Olshansky et al., 2022).

5.1.2. Metabolic health and oxidative stress-related markers

After 16 weeks of the recreational TH intervention, no changes in blood lipid profile were observed for the intervention groups while a significant decrease (-5%) was observed in plasma HDL cholesterol for the CG. Since lipid profile tends to suffer negative alterations during the ageing process and older age has been associated with a higher risk for dyslipidaemia development (Cho et al., 2020), being able to maintain plasma HDL cholesterol, while the CG decreased their values, is relevant and promotes the interest of recreational TH in maintaining a healthy blood lipid profile. The results found in the present intervention are in line with prior recreational TH implementations with younger participants, where blood lipid profile (LDL, HDL and TRG) was unchanged, which could be the consequence of favourable participants' baseline levels (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018). On the contrary, significant decreases were observed in total and LDL cholesterol for postmenopausal women (-2.6 and -2.3%, respectively) and for adult/middle-aged male former players (-10 and -14%, respectively) after 12–16 weeks of recreational TH interventions. However, these contrasting results may have been the consequence of the high baseline status of the considered

variables and/or the lack of control over the participants' diet during the interventions (Pereira et al., 2020; Póvoas et al., 2018). Moreover, untrained older men playing recreational football for 4 and 12 months did not show any change in lipid profile (Andersen et al., 2016), which is also in line with our study results. Conflicting results have been observed for recreational floorball interventions with untrained older men after 12 weeks, showing decreases in LDL (-11%) and in TRG (-8%), however, after 26 months the same group did not show extra changes in lipid profile (Pedersen et al., 2018; Vorup et al., 2017b). Additionally, in the same study, decreases were shown in body and especially in visceral fat mass for untrained older men (Vorup et al., 2017b). These results may be associated with the decreases showed in LDL and TRG, as improvements in lipid profile are related to reductions in body and visceral fat mass (Sarin et al., 2019).

Impaired fasting plasma glucose levels, reduced glucose tolerance or increased HbA1c levels are the main criteria to be determined as prediabetic, and consequently, to develop T2DM (DeFronzo et al., 2015). The serious increase of diabetic patients encourages the awareness of new exercise interventions to tackle this disease (Ghezzi et al., 2017; Powers et al., 2020). In our study, a higher absolute change in fasting plasma insulin was observed for TH3 (-5.5 $\mu\text{mol/L}$) compared to CG (+1.6 $\mu\text{mol/L}$), showing that recreational TH was effective in reducing fasting plasma insulin. Nonetheless, no significant interactions were shown for other markers regarding glycaemic profile. On the other hand, at baseline, the participants from all groups could be considered as prediabetic as fasting blood glucose levels were between 100–125 mg/dl (Colberg et al., 2010). However, a significant decrease was observed in fasting blood glucose for TH3 (-8%) after the intervention, which is of great health relevance as this group was excluded from the prediabetic category by reducing fasting blood glucose to 99.8 ± 15.6 mg/dl (Colberg et al., 2010). Studies addressing recreational TH with younger populations and postmenopausal women, as well as recreational football and floorball with untrained older men were ineffective in changing fasting blood glucose, HbA1c, and plasma insulin. However, these participants did not have impaired glucose regulation like the participants from the present thesis, which may explain the discrepancy with our results (Andersen et al., 2016; Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018; Pereira et al., 2020; Vorup et al., 2017b). Conversely, adult/middle-aged male former TH players have shown to reduce fasting blood glucose (-7%), after only 12 weeks of recreational TH (Póvoas et al., 2018) which is in line with our study results. This means that both adult/middle-aged male former TH players and the participants from TH3 of our study, reduced their risk of develop T2DM, by maintaining

normal and reducing impaired fasting blood glucose, respectively. However, caution should be taken regarding the cause of these results as diet was not controlled in both interventions.

Antioxidant defences in middle-aged-to-elderly individuals have shown to increase with PA (Reid, 2001), and long-term adherence to regular PA is recommended to prevent the negative effects of oxidative stress on health (Simioni et al., 2018). In the present thesis, after 16 weeks, a significant increase was observed for antioxidant stress-related markers, such as TAS in TH3 (+12%) and GR in TH1 (+13%), TH2 (+13%), TH3 (+15%) and CG (+9%). The results found in the present thesis are relevant as uncontrolled increased of oxidative stress has been associated to the aetiology and development of several diseases (e.g., renal failure, ischaemia, heart failure, diabetes, cancer, and others). Additionally, antioxidant activity is similar in elderly physically active and young inactive subjects, therefore, highlighting the importance of regular PA to attenuate the ageing-associated impairment process (Ghezzi et al., 2017). To the best of our knowledge, this is the first study reporting the effects of a recreational team sport practice (namely, TH) on oxidative stress-related markers.

5.1.3. Body composition

During the ageing process lean body mass tends to decrease, while body fat mass increases, especially in the abdominal region, even when there are no changes in total body mass (St-Onge & Gallagher, 2010). These alterations in body composition occur due to an increase in adiposity and an age-related fat redistribution, namely, the lower body subcutaneous fat (considered as beneficial adipose tissue for metabolism) to the visceral region (considered as harmful adipose tissue for metabolism) (Kuk et al., 2009). Increases in abdominal region fat (visceral fat) are strongly associated with several adverse health conditions, and older populations' body fat mass is a good predictor of morbidity and mortality (Gambert & Pinkstaff, 2006). Additionally, android fat mass is more common in men than women (Blaak, 2001) and is highly associated with cardiometabolic risks factors (Després et al., 1990). In the present study, a dose-response effect was observed in body composition variables, since TH3 was able to more markedly, decrease body and fat mass. Higher absolute and relative changes in body mass were shown for TH3 compared to CG and TH1 and in fat mass for TH3 compared to CG. In addition, after 16 weeks, within group alterations were observed with a significant decrease in body mass for TH2 (-2%) and TH3 (-3%) and in fat mass for TH1 (-7%), TH2 (-11%), TH3 (-14%) and CG (-5%). Moreover, decreases in arms, right leg and android total mass in TH2 and TH3, and in gynoid total mass in TH2 were observed. Unexpectedly, TH3 decreased arms'

lean mass, however, arms' fat mass decreased almost twice the percentage of lean mass lost, which helps to explain the decrease in total mass in both arms. Furthermore, TH2 and TH3 decreased total mass in some regions, which was related to the decrease in fat mass since lean mass in those regions was maintained.

The body mass results found in the present work conflict with most of the previous studies addressing recreational TH-based interventions with different populations and age groups, that did not report body mass improvements by performing an average of 2 weekly sessions for 12–16 weeks (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018; Póvoas et al., 2018). However, a decrease was found in body mass for postmenopausal women, after 16 weeks of 2–3 60-min sessions/week (Pereira et al., 2021). Additionally, the decrease in fat mass percentage shown in our participants' post-intervention was higher than that reported for younger untrained men (-1.7%, 12 weeks) (Hornstrup et al., 2019) and women (-1%, 12 weeks; -4% 16 weeks) (Hornstrup et al., 2020; Hornstrup et al., 2018), and for postmenopausal women (-2.5%, 16 weeks) (Pereira et al., 2021) by playing recreational TH. The conflicting results found in body composition patterns, between our study participants and younger populations (playing TH), may be justified by the age and sex-differences. This because, men's peak body and fat mass are typically achieved between 64–81 years of age (Kuk et al., 2009). Also, women show different responses, namely, lower improvements in body composition than men, after exercise interventions (Williams et al., 2015), which can partially explain the differences between our participants' and postmenopausal women's results. On the other hand, changes in body and visceral fat mass (-5 and -14%, respectively) were observed for untrained older men by playing floorball for 12 weeks, which is in line with our results. Nevertheless, a combination of floorball training with diet was used for that intervention, as it was explained before, and this may have influenced the results. Interestingly, no changes in lean mass were observed in this floorball training intervention (Vorup et al., 2017b). Also, untrained older men, that played recreational football, showed a significant decrease in leg lean mass (-0.6 kg) from 16 to 52 weeks, while the total and upper body lean mass remained unchanged (Andersen et al., 2016). Changes in body composition, mainly in the legs, may be expected in recreational team sports, especially in recreational football, due to its demands (Krustrup et al., 2010; Krustrup et al., 2018a). However, TH is also considered a high-intensity intermittent exercise (Póvoas et al., 2012), which can help to explain the decrease found in the present study for TH3 in legs' fat mass (that could possibly explain the decrease in legs total mass), as high-intensity intermittent exercise is known to influence body composition by reducing fat mass (Boutcher, 2011). However, in the present study TH may have induced extra

benefits in the upper body composition such as decreases in arms' fat mass, probably due to the higher upper body engagement required when compared to other team sports such as football.

5.1.4. Physical fitness

Individuals' risk of falls has been associated to arm and leg muscle strength, dynamic balance and agility (Toraman & Yildirim, 2010). During the ageing process, individuals decrease their muscle mass and decline their physical fitness, namely, cardiorespiratory fitness and muscle strength (Jackson et al., 2009a; Keller & Engelhardt, 2013). Therefore, increasing, maintaining or at least, minimizing the decrease of muscle mass and physical fitness during the ageing process is fundamental. In the present work, TH1, TH2 and TH3 showed higher absolute changes in upper and lower body dynamic strength than CG. These results are relevant as the TH groups improved their physical fitness, which is important for the ability to perform daily activities (Wang et al., 2020), and consequently, to be independent. The results found for lower body dynamic strength (sit-to-stand test: TH1, +49%; TH2, +34%; TH3, +38%) are higher and in line with those found after 16 weeks and 1 year, respectively, of recreational football for untrained older men (+29 and +32%, respectively) (Andersen et al., 2014; Sundstrup et al., 2016). Additionally, the results found in the present study for upper (arm curl-up test: TH1, +5.1 repetitions; TH2, +3.8 repetitions; TH3, +4.5 repetitions) and lower (sit-to-stand test: TH1, +8.5 repetitions; TH2, +5.4 repetitions; TH3, +6.9 repetitions) body dynamic strength were higher than those reported for recreational floorball training, for 12 weeks, with untrained older men (~2–3 repetitions) (Vorup et al., 2017b). These results are important to highlight as a high body dynamic strength is important to reduce the risk of falls among older adults (Smee et al., 2012).

In the present work, there were no changes in upper body isometric strength. The lack of changes observed for upper body isometric strength may be related to the fact that smaller and softer balls were used during the recreational TH training sessions. This, to prevent injuries, to allow a proper ball grip and higher players' collaboration, and consequently, engagement in the activity. Nevertheless, it may have influenced the handgrip strength test results, since these balls do not require a strong handgrip, unlike what happens with an official TH ball. The fact that in TH proximally half of the playing time is spent in defensive actions (Póvoas et al., 2012) may also help to explain the lack of changes in handgrip strength, as the participants are likely to have spent less than half of match time with ball possession. The results from the present study are in line with those shown for adult/middle-aged male former TH players, performing

recreational TH, for 12 weeks (Póvoas et al., 2018), and untrained older men playing recreational floorball for 12 weeks (Vorup et al., 2017b). Additionally, postmenopausal women playing recreational TH did not report changes in upper body isometric strength (handgrip test) after 16 weeks, which is also in line with our results. Nevertheless, after 36 weeks, an improvement of 12% was observed for postmenopausal women, suggesting that 16 weeks may not be sufficient to induce upper body isometric strength positive changes for these specific populations (Pereira et al., 2023).

A decrease in the number of falls was observed in TH2 (-17%) indicating an improvement in postural balance, which is important as this group may have reduced the risk of fractures resulting from falls (Papalia et al., 2020). The decrease found in the number of falls that represents an increase in postural balance for TH2, after 16 weeks, is within the range of that observed in adult/middle-aged male former TH players (-27%) (Póvoas et al., 2018) and in postmenopausal women after playing recreational TH for 16 and 36 weeks (-7 and -12%, respectively).

Moreover, improvements of 9–13% in the agility test were found in the TH intervention groups from the present thesis, although an improvement was also showed for CG, though significantly lower (-5%) than compared to the pre-to-post intervention increases observed for TH1 (-13%) and TH3 (-9%). The fact that the participants from the present work have decreased their time performing the agility test is important to highlight, since older adults that present reduced functional fitness capacity, especially in agility, have higher risk of falling than older adults with better functional fitness capacity (Zhao & Chung, 2016).

To summarize, significant positive alterations were observed in physical fitness in middle-aged-to-elderly participants. The main reason to explain the higher physical fitness improvements shown in the TH intervention groups, may be the fact that these groups were exposed to high-intensity locomotor movements and game actions elicited by recreational TH, e.g., changes of speed and direction, and other game-specific actions previously described (Póvoas et al., 2017). From a practical point of view, recreational TH improves dynamic strength and agility, and consequently, has the potential to reduce the number of falls during the exercise program and in this population everyday life.

5.1.5. Bone health

Bone remodelling usually takes 3–4 months to change BMD and those changes are normally visible after a training period of approximately 6 months (Weaver et al., 2016). For

this reason, the short-term (16 weeks) improvements in BMC and BMD shown in our participants are of interest. This includes the significant increase in whole-body BMC and BMD for TH2 and TH3 and the maintenance of femur BMC and BMD values, while a significant decrease was shown in CG. Exercise interventions using recreational TH for 12–16 weeks have showed significant improvements in proximal femur BMD for young untrained women (+0.8%) and men (+2%), in femur BMC for postmenopausal women (+2.2%), and also in whole-body BMC for young untrained men (+53 g) (Hornstrup et al., 2019; Hornstrup et al., 2018; Pereira et al., 2021). Additionally, men with prostate cancer reported improvements in whole-body and leg BMC, after only 12 weeks of recreational football practice, and a positive correlation was found between the change in leg BMC and the total number of accelerations, decelerations, sum of intense accelerations and decelerations, and total distance covered (Uth et al., 2016a). In our study, the participants' covered higher distances (4676–5202 m) and performed a higher number of accelerations (27–40) and decelerations (14–17) than the men with prostate cancer, which may have contributed for the positive results shown in BMC and BMD, especially for TH2 and TH3. After 26 months of recreational floorball training, older males have shown to increase leg BMD (Pedersen et al., 2018). Moreover, after 32 weeks of recreational football practice, prostate cancer patients, showed additional improvements compared to 12 weeks, namely, increases in hip, femoral neck and lumbar spine BMD (Uth et al., 2016b). Also, untrained older men playing recreational football showed improvements in proximal femur and femoral neck BMD after 4 and 12 months (Helge et al., 2014). These three recreational football and floorball interventions with older male populations suggest that longer periods of time are important for additional bone health improvements for this population (Helge et al., 2014; Pedersen et al., 2018; Uth et al., 2016b).

Bone turnover biomarkers express the metabolic activity of osteoblasts and osteoclasts (Wheater et al., 2013) and its balance activity results in continuous life-long bone remodelling (Shetty et al., 2016). In the present study, recreational TH was effective in inducing alterations in bone turnover markers in TH2 and TH3, by showing a higher absolute change in P1NP for TH2 (+22 µg/L) compared to the CG (+5.6 µg/L), and in OC for TH3 (+8.0 µg/L) compared to CG (+1.1 µg/L). Also, a higher absolute change in CTX was observed for TH3 (+113 ng/L) compared to TH1 (-37 ng/L). In addition, a significant increase after 16 weeks, was observed in bone formation markers P1NP (TH2 and TH3) and OC (TH1, TH2 and TH3) and CTX (TH3). These results indicate that there was a positive net balance as the increase in bone formation markers was higher than the increase observed in bone resorption markers. Moreover, in the present work, higher absolute and relative changes in sclerostin concentrations

were observed in TH3 (-0.08 ng/mL and -7.1%, respectively) compared to CG (+0.08 ng/mL and +9.7%, respectively). In addition, a significant decrease was found after 16 weeks, in sclerostin concentrations in TH3 and an increase in CG. Sclerostin reflects the severity of bone loss as it is a secreted Wnt pathway antagonist and therefore, inhibits bone formation (Morse et al., 2013), and it increases with age (Smith et al., 2021). High sclerostin concentration levels are associated with low levels of PA for a prolonged period of time and, exercise interventions that promote mechanical load decrease sclerostin levels (Smith et al., 2021). Therefore, the significant decrease found in sclerostin concentrations for TH3, and the increase shown by CG, are important as it indicates bone gain for the exercise group. Recreational TH induces mechanical load throughout different game-specific high-intensity actions such as changes of directions, accelerations, decelerations, jumps, stops and others, which may explain the decrease observed in sclerostin in TH3, while the CG increased sclerostin levels, probably due to low PA levels. Nevertheless, this could also be a chance finding as no matching effects were shown in OC and P1NP values in the CG. Studies using recreational TH with younger men and women (12 weeks) showed similar increases in P1NP, OC and CTX (Hornstrup et al., 2019; Hornstrup et al., 2018) than our TH2 and TH3 groups. These results indicate that by playing recreational TH for 16 weeks, our participants were able to improve bone turnover markers as much as younger populations using recreational TH for 12 weeks. Additionally, the present study results in bone turnover markers for TH2 and TH3 were slightly higher in P1NP, however similar for OC and CTX, when compared to postmenopausal women results after playing recreational TH for 16 weeks (Pereira et al., 2021). Moreover, untrained older men playing recreational football showed similar changes, as our study, in P1NP and OC after 16 weeks (Helge et al., 2014; Uth et al., 2016a), and after 12 months were able to maintained those changes (Helge et al., 2014). Recreational team sports studies (Helge et al., 2014; Hornstrup et al., 2019; Hornstrup et al., 2018; Pereira et al., 2021) have reported positive BMD changes and elevated CTX values, which is in line and explains the results from the present work, such as the improvements found in BMC and BMD. Furthermore, in TH1 a positive change was observed in OC, P1NP/CTX and OC/CTX ratios, which means that even with only 60 min/week of recreational TH for 16 weeks, there is osteogenic activity.

The positive osteogenic response observed in the present work and in studies using recreational TH with different genders and age groups, can possibly be related to the fact that TH is a high impact team sport with several studies reporting multiple specific high-intense movements and actions performed with different speeds and in different directions (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018; Pereira, 2024; Póvoas et al., 2018;

Póvoas et al., 2017). This type of exercise interventions may have important load properties to stimulate the musculoskeletal system [i.e., dynamic, load cycles (4–5 jumps), high magnitude and rate, and unusual distribution of strain] (Russo, 2009), which consequently, induce positive osteogenic activity responses.

5.2. Harms

Due to the lack of experience with TH, all participants were instructed on how to hold, throw, and catch the ball. Also, some adaptations were made to the official TH rules, such as the balls used were smaller and softer than the official TH balls, and no body contact was allowed, to avoid potential injuries. Match-play was preceded by a standardised warm-up with the aim to fulfil two of the main goals of warming, namely, the progressive physiological preparation for training or match, and the prevention of muscular injuries. During the 16 weeks intervention, two participants experienced knee pain, due to old injuries and were instructed by medical indication to stop the intervention, recover, and then restart the exercise programme. However, both did not complete the intervention and were considered as dropouts. Also, one participant suffered an Achilles tendon rupture during a training session, while running alone during the warm-up, which inhibited the continuation of the exercise programme. Therefore, there was one injury in each group during the exercise intervention, which is in line with the frequency of injuries reported in similar intervention (Hornstrup et al., 2019; Hornstrup et al., 2018; Pereira et al., 2021; Pereira et al., 2020), and resulted in an injury incidence per group of TH1=3.9, TH2=1.9, and TH3=1.3 injuries per 1000 h of exposure.

5.3. Working demands of different TH game and gender formats

5.3.1. Internal and external load demands

For the present thesis, two studies were conducted to examine the internal and external load demands of recreational TH practice. One of the studies examined the recreational TH demands for middle-aged-to-elderly males while playing three game formats (5v5, 6v6 and 7v7). This because in the community setting the number of participants may vary in each exercise session and therefore, understanding the demands of different game format is of importance for exercise prescription, namely in this population. The other study aimed at

analysing the demands of recreational TH for middle-aged-to-elderly men and women when playing same vs. mixed-gender 6v6 game formats, as it is common to have both men and women participating in the same class in community exercise programmes. Therefore, the internal and external load demands, as well as the perceived experience (fun and RPE levels) during different game (5v5, 6v6 and 7v7) and gender formats (same- and mixed-gender) were analysed.

Recreational TH training sessions using SSGs, have reported mean HRs within 76–85%HR_{max} (Hornstrup et al., 2019; Hornstrup et al., 2020; Hornstrup et al., 2018; Pereira et al., 2021; Pereira et al., 2020; Pereira, 2024; Póvoas et al., 2018; Póvoas et al., 2017; Stojiljković et al., 2020). In our study addressing recreational TH demands for middle-aged-to-elderly men, mean HR values for 5v5, 6v6 and 7v7 game formats were 76–77%HR_{max}. Also, when comparing same- vs. mixed-gender formats (6v6), the HRs ranged from 76–79%HR_{max} for middle-aged-to-elderly men and women. These intensities are lower than those reported for young adult populations without experience with the sport and adult/middle-aged male former TH players (82–85 HR_{max}) (Hornstrup et al., 2019; Hornstrup et al., 2018; Póvoas et al., 2018). Nevertheless, they are in line with those observed for postmenopausal women (76–79% HR_{max}) (Pereira et al., 2021; Pereira et al., 2020; Pereira, 2024), namely with the intensities shown to result in cardiovascular improvements (76% HR_{max}) (Pereira et al., 2020). Also, the mean HRs reported in both of our studies were within the range of the vigorous exercise intensity threshold (60–85% HR_{max}) proposed to promote cardiovascular improvements (Williams, 2013). The time spent >90%HR_{max} in both of our studies was lower than the reported for younger populations playing recreational TH, although in line with those reported for postmenopausal women that improved cardiorespiratory fitness after 16 weeks (Pereira et al., 2020; Pereira, 2024). The results from the studies using recreational TH with other populations suggest that the HRs and time spent >90%HR_{max} achieved by our participants in 5v5, 6v6 and 7v7 game formats, as well as, in same vs. mixed-gender game formats, are within the range to induce cardiovascular adaptations.

In our first study, addressing the recreational TH demands, a comparison was performed between the three game formats showing no significant differences between 5v5, 6v6 and 7v7, which is in line with a study comparing 4v4, 5v5 and 6v6 game formats using the same court size (40x20 m) for young active college students (20.8±1.1 years) with no competitive experience in TH (Stojiljković et al., 2020). Nonetheless, in the study addressing the recreational TH demands of playing 6v6 same vs. mixed-gender game formats, significant differences were found between the gender game formats. In fact, during mixed-gender

matches, time spent $>80\%HR_{max}$ and $>90\%HR_{max}$ was higher for women than men, which may indicate that during mixed-gender matches, women increased their physical demands to keep up with the pace imposed by men. Also, during same-gender matches, men's time spent $>80\%HR_{max}$ was higher than in mixed-gender matches, which may be related to the higher amount of high-intensity' locomotor movements (e.g., higher frequency of sprinting and high-intensity movements; higher percentage of time spent in sprinting movements; higher absolute and relative distance covered in fast running, sprinting, backwards and high-intensity movements) shown during same- vs. mixed-gender matches, providing evidence that the external load is higher.

In both our studies (i.e., analysing the game and the gender game formats), the mean and peak BL concentrations were similar to those reported for adult/middle-aged male former TH players during 7v7 matches, young active college students during 4v4, 5v5 and 6v6 matches, and postmenopausal women during 4v4, 5v5 and 6v6 recreational TH matches (Pereira, 2024; Póvoas et al., 2017; Stojiljković et al., 2020). The results mentioned before are interesting as peak BL concentrations decrease with age (Korhonen et al., 2005; Peserico et al., 2018), however, the participants from our two studies (middle-aged-to-elderly men and women) were able to achieve similar BL concentrations as the younger populations when playing recreational TH.

When comparing the demands of different game formats (5v5, 6v6 and 7v7) for middle-aged-to-elderly males, lower peak BL concentrations were found for 7v7 compared to 5v5, indicating that in 5v5 the participants may have achieved higher anaerobic intensities. This is in accordance with the results found in the external load, namely in high-intensity locomotor activity, shown in the different game formats, such as, higher frequency of fast running movements found in 6v6 than 5v5 and 7v7, higher frequency of sprints observed for 5v5 than in 7v7, and higher frequency of high-intensity movements observed for 5v5 and 6v6 than in 7v7. Also, a higher frequency of throws, stops and total actions was found for 5v5 and 6v6 than 7v7. The overall results indicate a higher load on muscles and bones in 5v5 and 6v6 than in 7v7.

In our study comparing the gender game formats, men's mean, and peak BL values were higher than women's, in both same- and mixed-gender formats. These results can be the consequence of the higher frequency, percentage of total match time and distance covered at high-intensity movements by men compared to women, during both gender game formats. In addition to the locomotor movements, during same-gender matches, men performed higher frequency of TH high-demanding game-specific actions (jumps, throws, stops, one-on-one

situations and total high-intensity actions), spent more time in higher player load (PL) zones (>1.5–2.0 and >2.0), showed higher total accumulated PL, and higher frequency of medium and high-intensity accelerations compared to women. Furthermore, during mixed-gender matches, men also showed higher frequency of TH high-intensity game-specific actions (jumps, stops, changes of direction and total high-intensity actions), spent more time in higher PL zones (>1.5–2.0 and >2.0), showed higher total accumulated PL and performed more low-intensity and total accelerations than women. This means that in both same- and mixed-gender matches men showed higher external load demands than women, which can explain the higher BL concentrations shown by men compared to women. Also, men performed higher high-intensity locomotor movements during same- than during mixed-gender matches, which can justify the higher HRs shown during same- vs. mixed-gender matches observed in men.

In the study addressing the recreational TH demands of the different game formats analysed (5v5, 6v6 and 7v7), match intensity was perceived as strong-to-very-strong (6.1–6.7, 0-10 AU), with no differences shown between the game formats. The values reported in our study are lower than those reported for adult/middle-aged male former TH players, but slightly higher than those reported for postmenopausal women and male active college students (Pereira et al., 2020; Pereira, 2024; Póvoas et al., 2017; Stojiljković et al., 2020). In our study, as well as in postmenopausal women and male active college students studies, the participants had no experience with the sport. The lack of experience with TH may justify the lower RPE results compared to adult/middle-aged male former TH players, as several factors, e.g., the exercise modality expertise level, may influence RPE (Haddad et al., 2017). No differences were shown in RPE between the game formats analysed in our study, and the results were consistent with the absence of differences in HR between 5v5, 6v6 and 7v7. Additionally, no differences were found in respiratory, muscular, or global RPE, when comparing same- vs. mixed-gender matches.

The activity profile results of the study addressing the recreational TH demands in three game formats showed higher intensity in 5v5 and 6v6 than 7v7 game formats. Furthermore, the activity profile results of the study addressing the same vs. mixed-gender 6v6 game formats showed higher intensity for men than women by playing both same- and mixed-gender matches. Therefore, based on the demands observed, in the study addressing recreational TH demands in three game formats, the participants were more involved in the matches when they played 5v5 and 6v6 than 7v7 game formats. While in the study addressing the same vs. mixed-gender 6v6 game formats, men were more involved in the matches in both same- and mixed-gender game formats than women. These results could have influenced the fun levels as the higher

level of participation in the matches may be important to maintain the participants' motivation to keep playing (Nielsen et al., 2014). Nevertheless, no significant differences were found in fun levels for men playing 5v5, 6v6 and 7v7, and for men and women, when playing same- and mixed-gender game formats. In both of our studies, fun levels were reported as very high, which can be a good indicator of long-term adherence to exercise in this population (Lakicevic et al., 2020).

The practical application of the results emerging from the game formats study is that all three (5v5, 6v6 and 7v7) may be used for middle-aged-to-elderly men when the aim is to induce broad health adaptations. Higher external load demands were shown for 5v5 and 6v6 when compared to 7v7, allowing the participants to be highly involved in the training sessions. However, RPE and fun levels were not different between game formats showing that these participants perceived similar effort and levels of fun during the three game formats. Furthermore, the results from the study addressing same and mixed-gender game formats showed that both may be implemented for middle-aged-to-elderly men and women. This because, the internal and external load results showed that it was more demanding for men to play same-gender matches, while for women was more demanding to play mixed-gender matches. Nevertheless, both gender game formats elicited high-intensity demands in the range to induce positive health adaptations. Additionally, in both gender formats, men and women reported similar fun levels, showing that the participants enjoyed playing same- and mixed-gender matches, which is important for adherence to exercise in this population (Collado-Mateo et al., 2021).

The information provided by these two studies may help to prescribe and design future community exercise programmes for this population, as playing different game formats such as 5v5, 6v6 and 7v7 may induce broad-spectrum positive health adaptations in middle-aged-to-elderly men. Therefore, this exercise modality played in different game formats can be implemented in community groups that usually vary in the number of participants. Furthermore, middle-aged-to-elderly men and women may integrate both same- and mixed-gender matches during recreational TH training sessions. However, given the different match demands experienced by men and women, it can be interesting to often change the gender game formats.

5.3.2. Training sessions' intensity in the different match periods

The physical and physiological demands of the 3x15 min periods were analysed during the three game formats (5v5, 6v6 and 7v7) with the aim to ascertain if this game formats would

be able to elicit a high-intensity throughout all match duration. Differences between the 3x15 min periods, in the three game formats studied, were observed in some internal and external demands variables. Nevertheless, the exercise intensity during all match duration in 5v5, 6v6 and 7v7, was within the range proposed for cardiovascular improvements (Williams, 2013), and the participants performed different locomotor movements at different speeds and in different directions, as well as a relevant frequency of TH-specific game actions with potential to induce musculoskeletal adaptations, throughout all match duration.

In 6v6 and 7v7 game formats, a decrease was shown in mean and peak HR from the first to the third periods. However, in 5v5, mean HR increased from the first to the second period and was maintained from the second to the third period. In 7v7 game format, a decrease in time spent $>80\%HR_{max}$ was observed from the first to the second and third match periods, and from the second to the third period, indicating a decrease in intensity in this format over the match duration, which was not observed for 5v5 and 6v6.

BL concentrations were significantly increased from baseline comparing to the first period in all game formats and in 5v5 from baseline to the third period. Also, for all game formats, a decrease in BL values was found from the first to the third period, indicating an intensity decrease from the beginning to the end of the recreational TH matches. These results are in line with the HRs' decrease observed from the first to the third match period, for 6v6 and 7v7 game formats, although not observed in 5v5. Furthermore, in 6v6 a decrease was observed from the first to the third period in the distance covered in fast running and sprinting movements. Moreover, in 6v6 and 7v7, a decrease was observed from the first to the second period and in 6v6 from the first to the third period in the total number of high-intensity game actions.

From a practical point of view, the results found suggested that 5v5 game format may be more efficient in maintaining cardiovascular and musculoskeletal load throughout the 3x15 min recreational TH matches.

5.4. Recreational team sports and cardiometabolic health for over 60-years-olds

The systematic review and meta-analysis included in this thesis was the last study to be performed as there were only a few RCTs addressing recreational team sports with older populations at the beginning of this PhD project. For this reason, one of our studies addressing

the dose-response health effects of recreational TH was part of the list of studies used for the systematic review and meta-analysis.

The main finding of the systematic review and meta-analysis performed in this thesis is that recreational team sports, more specifically, recreational football and TH training are effective in improving VO_{2max} for over 60-year-old men and women, when compared to non-exercise CGs. VO_{2max} showed very low certainty of evidence in GRADE assessment. No effect was observed for SBP, DBP, resting HR, TRG, total, LDL and HDL cholesterol. The secondary cardiovascular and metabolic outcomes showed very low and low certainty of evidence, correspondently, in GRADE assessment.

From a practical point of view, the results from the systematic review and meta-analysis indicated that recreational team sports for over 60-year-old men and women have the potential to help reducing the risk of CVDs and the prevalence of other comorbidities, since high cardiorespiratory fitness is associated with improved survival and decreased incidence of CVDs, hypertension, diabetes, heart failure, and atrial fibrillation (Al-Mallah et al., 2018).

To summarize, the effects of recreational team sports interventions on cardiorespiratory fitness after 12–16 weeks (30–60 min/sessions) are of importance as they showed that this type of exercise programmes may help to reduce and counteract the ageing effects in VO_{2max} , on over 60-years-old. Nevertheless, high-quality studies with this population are warranted to corroborate the current findings.

5.5. Strengths and limitations

The major strength of the present thesis is its design (RCT) addressing for the first time the dose-response effects of recreational team sport practice, namely TH, on cardiometabolic, musculoskeletal, and body composition health, and physical fitness. Also, it examines several internal and external load markers that helped to understand the dose-response results. Other strengths of this thesis are the fact that it addressed the physical and physiological demands of different game formats frequently used in TH interventions and analysed the differences between match periods in internal and external load markers. Moreover, this thesis analysed for the first time the recreational TH demands in same- and mixed-gender matches for men and women, within the same age group, which is important for future exercise prescription, as it is common to have mixed-gender groups in a community setting. Additionally, the effects of recreational team sports for over 60-year-old men and women analysed in a systematic review

and meta-analysis are important as little is known about the effects of recreational team sports on health for this specific population, as this is the population with the higher risk of development and prevalence of CVDs (Rodgers et al., 2019). Nevertheless, this thesis has limitations. Although the participants were instructed to keep their regular dietary habits, food intake throughout the training intervention was not assessed. This may have, at least partially, influenced some health-related variables, especially the metabolic and body composition markers. Moreover, further bone health improvements could have been found if this population was re-evaluated after a longer period (>16 weeks). Furthermore, although the most typically used game formats in recreational TH-based exercise interventions were analysed, the working demands of other game formats such as 3v3 or 4v4 were not studied. Finally, in the systematic review and meta-analysis, the fact that only eight studies were eligible to analyse the effect of recreational team sports on cardiometabolic markers, and some of those studies did not evaluate all the defined cardiometabolic markers is a study limitation.

CHAPTER VI: Conclusions and perspectives

6. Conclusions and perspectives

Recreational TH played as SSGs and formal games is effective in improving cardiorespiratory fitness when performed 3 times/week and aerobic performance when performed 2–3 times for 60 min per training session. Additionally, aerobic performance showed higher relative changes in the group that performed 180 min/week compared to the groups that performed 120 and 60 min/week and CG. Nonetheless, 3 weekly TH training sessions are more effective in providing overall cardiometabolic, skeletal and body composition benefits for middle-aged-to-elderly men compared to training with a lower weekly frequency. As the training intensity was similar between training TH performed one, two or three times per week, the weekly volume seems to be the major contributor for the differential health and physical fitness benefits shown after the 16-week recreational TH intervention.

Recreational TH internal load demands were similar either played as small-sided (5v5, 6v6) or formal game formats (7v7), when using the same court dimensions (40x20 m) and were within the range to induce cardiovascular adaptations. Nevertheless, 5v5 was the game format that was able to maintain the cardiovascular intensity across the three 15-min match periods. Additionally, higher frequency of high-intensity game actions was found in 5v5 and 6v6 compared to 7v7. Therefore, 5v5 and 6v6 may be better options when the purpose is to induce musculoskeletal improvements in middle-aged-to-elderly men. Indeed, the higher number of total actions and throws found in 5v5 and 6v6 revealed a greater involvement in the training sessions by the participants, which may lead to higher motivation, and consequently, long-term adherence to the exercise program. In fact, fun levels were reported as very high in all game formats, indicating that 5v5, 6v6 and 7v7 are highly motivational game formats. In conclusion, a multiple game format approach may be used in recreational TH interventions to provide training variety by using different game formats.

When comparing the recreational TH demands of playing same- vs. mixed-gender game formats, the relative cardiovascular demands were higher for middle-aged-to-elderly women than for men during mixed-gender matches. Additionally, in men, same-gender game formats were more demanding than mixed-gender game formats, while in women the inverse occurred. Interestingly, independently of the gender game format, the fun levels reported were very high for both genders. To summarize, recreational TH can be considered as an intermittent high-intensity and motivating exercise modality with potential to induce several health improvements for middle-aged and elderly men as well as for women, regardless the gender

game format. From a practical point of view, same- and mixed-gender matches can be organised with the aim to promote health and physical fitness for this population. Because women are more physically and physiologically challenged when playing with men, a lead-in period with same-gender formats may be suggested for women, when implementing mixed-gender recreational TH-based exercise interventions.

Furthermore, based on the results from our systematic review and meta-analysis on the cardiometabolic effects of recreational team sports in over 60-year-old men and women, we concluded that recreational team sports (especially football and TH) may be effective in improving cardiorespiratory fitness.

Future RCTs using recreational TH for long-term periods in middle-aged-to-elderly men are warranted to assess the long-term dose-response health effects, as 16 weeks may be too short to induce benefits in some health parameters in this population. Additionally, studies addressing other recreational TH SSGs, such as 3v3 and 4v4, as well as different court dimensions and comparing indoor vs outdoor pitches are warranted to provide information regarding the best settings to implement this exercise modality. This is because, the number of participants, court dimensions and types of pitches frequently vary in the community setting. Also, it would be of interest to run RCTs using mixed-gender games-based exercise interventions, aiming at assessing its impact on participants' health, as mixed-gender groups are common in exercise programmes implemented in the community. Further systematic reviews and meta-analysis using more studies with high quality, various team sports and other important health outcomes are required to analyse the health effects of recreational team sports on older populations.

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