Universidad de Cádiz

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The INTERMAE project

Programa de Doctorado en Ciencias de la Salud

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El proyecto INTERMAE

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Dr. D. Jesús Gustavo Ponce González

Profesor Titular de Universidad

Dpto. Didáctica de la Educación Física, Plástica y Musical

Facultad de Ciencias de la Educación

Universidad de Cádiz

JESÚS GUSTAVO PONCE GONZÁLEZ, PROFESOR TITULAR DE LA UNIVERSIDAD DE CÁDIZ

CERTIFICA:

Que la Tesis Doctoral titulada "Diferencias sexuales en las asociaciones de condición física, actividad física, sedentarismo y comportamiento del sueño con el estrés oxidativo en adultos mayores. El proyecto INTERMAE" que presenta D. Juan Corral Pérez al superior juicio del tribunal que designe la Universidad de Cádiz, ha sido realizada bajo mi dirección durante los años 2017-2023, siendo expresión de la capacidad técnica e interpretativa de su autor en condiciones tan aventajadas que le hacen merecedor del Título de Doctor, siempre y cuando así lo considere el citado Tribunal.

Fdo. Jesús Gustavo Ponce González

En Cádiz, 14 de febrero de 2023

Dr. Dª Ana Carbonell Baeza

Profesora Titular de Universidad

Dpto. Didáctica de la Educación Física, Plástica y Musical

Facultad de Ciencias de la Educación

Universidad de Cádiz

ANA CARBONELL BAEZA, PROFESORA TITULAR DE LA UNIVERSIDAD DE CÁDIZ

CERTIFICA:

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Fdo. Ana Carbonell Baeza

En Cádiz, 14 de febrero de 2023

El doctorando D. JUAN CORRAL PÉREZ y los directores de tesis D. JESÚS GUSTAVO PONCE GONZÁLEZ Y Dª ANA CARBONELL BAEZA:

Garantizamos, al firmar esta Tesis Doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección de los directores de tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores al ser citados, cuando se han utilizado sus resultados o publicaciones.

Directores de tesis

Fdo. Jesús Gustavo Ponce González

Fdo. Ana Carbonell Baeza

Doctorando

Fdo. Juan Corral Pérez

En Cádiz, a 14 de febrero de 2023

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RESEARCH PROJECT AND FUNDING

The present International Doctoral Thesis was performed under the framework of the INTERMAE project, which received the following funding:

1) Influence of a physical exercise intervention on markers associated with aging, proteomic profile and fragility. INTERMAE project (INfluencia de una inTervención con EjeRcicio Físico sobre Marcadores Asociados al Envejecimiento, Perfil Proteómico y Fragilidad. Proyecto INTERMAE)
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ABBREVIATIONS

24HR: 24h dietary recalls

4-HNE: Trans-4-hydroxy-2-nonenal

AOPPs: Advanced oxidation protein products.

BMI: Body Mass Index

CRF: Cardiorespiratory fitness

ENMO: Euclidean norm minus one

ES: Effect Size

FFQ: Food Frequency Questionnaire

FL: Functional limitations

FRAP: Ferric-reducing antioxidant power

GQS: Good-quality sleepers

HCY: Homocysteine

ISAK: International Standards for Anthropometric Assessment

LDL: Low-Density Lipoproteins

LPA: Light physical activity

MDA: Malondialdehyde

mG: Miligravities

MPA: Moderate physical activity

MVPA: Moderate to vigorous physical activity

NADPH: Nicotinamide adenine dinucleotide phosphate

NREM: Non-rapid eye movement sleep

O_2^-: Radical superoxide anion

PA: Physical activity

PAC: Physically active

PCs: Proteyn carbonyles,

PI: Physically inactive

PQS: Poor-quality sleepers

PSQI: Pittsburgh Sleep Quality Index

REM: Rapid eye movement sleep

RONS: Reactive oxygen and nitrogen species

SB: Sedentary behaviour

SD: Standard deviation

SOD: Superoxide dismutase

SPPB: Short Physical Performance Battery

TAC: Total Antioxidant Capacity

VO_2peak: Peak oxygen consumption

VPA: Moderate physical activity

WASO: Wake after sleep onset

WFL: Without functional limitations

WHO: World Health Organization

ABSTRACT

Currently, the elderly population is expanding due to an unprecedented increase in longevity. Unfortunately, this trend in the rising ageing population comes with the consequences of the augmentation of physiological decline leading to metabolic diseases or oxidative stress. Oxidative stress has been defined as a pathophysiological state characterised by an imbalance between the excessive production of oxidants (such as homocysteine, HCY) and the inability of the total antioxidant capacity (TAC) of the body to cope with them. When this condition occurs an accumulation of oxidatively damaged macromolecules appears, leading to the loss of function of these macromolecules which contributes to the appearance of no communicable diseases such as cardiovascular diseases or musculoskeletal diseases.

Consequently, it is needed to know how different factors can help to maintain healthy ageing in older adults. Maintaining good physical fitness, adequate levels of physical activity (PA), and healthy sleep behaviour have been shown to reduce the possible mechanisms of ageing such as oxidative stress. However, to our knowledge, it is still unknown whether these factors can differently affect the oxidative stress of older adults depending on sex since most of the studies analysed the data combined.

Therefore, the general aim of this International Doctoral Thesis was to study the sex-specific associations of anthropometry, body composition, physical fitness, PA, sedentarism and sleep behaviour with oxidative stress levels, in a sample of Spanish older adults.

This International Doctoral Thesis includes cross-sectional data from the INTERMAE project. A total of 76 participants (38 women, 68.8 ± 3.0 years old) recruited through the public health care centers of the province of Cádiz were included. Blood samples were obtained from the participants to obtain the plasma levels of TAC and serum levels of HCY. Then participants completed different measurements including body composition, physical fitness through different physical batteries and cardiorespiratory fitness test (CRF), nutritional evaluation, and questionnaires about their PA and sleep manners. In addition to this,

participants wore an accelerometer for at least 7 days to estimate their sedentary (SB), PA and sleep behaviours.

The main findings of this International Doctoral Thesis are i) there are sex differences in basal oxidative stress markers in Spanish older adults, regardless of their nutritional intake., ii) Higher body mass index and thigh perimeter in women and higher fat-free mass in men are associated with higher levels of HCY. iii) In older women a faster gait speed is associated with higher levels of TAC. Better upper body strength, flexibility and gait agility are associated with lower levels of HCY. Higher CRF is associated with lower levels of HCY in both sexes. (**Study 1)**; iv) PA levels seem to be more relevant to older adults' oxidative stress than SB, with moderate to vigorous PA being associated with increasing TAC and light PA with decreasing HCY in both sexes **(Study 2)**; v) Sleep disorders were associated with oxidative stress in older adults, with more time awake during the night associated with a decreased TAC in women and a higher sleep latency being associated with higher levels of HCY in men **(Study 3)**.

The findings of the present International Doctoral Thesis show that there are sex-specific associations of anthropometric, body composition, physical fitness, PA and sleep behaviour with oxidative stress in older adults.

RESUMEN

En la actualidad se está produciendo un aumento en el número de personas mayores debido a un incremento de la longevidad sin precedentes. Por desgracia esta tendencia de envejecimiento viene acompañado de un aumento del deterioro fisiológico derivando en enfermedades metabólicas o estrés oxidativo. El estrés oxidativo se ha definido como un estado pato fisiológico caracterizado por un desequilibrio entre la excesiva producción de oxidantes (como la homocisteína, HCY) sumada a una incapacidad de la capacidad antioxidante total (TAC) de hacerle frente. Cuando esta condición ocurre, se produce una acumulación de macromoléculas con daño oxidativo que contribuyen a la aparición de enfermedades no comunicables como enfermedades cardiovasculares o músculo-esqueléticas.

Por lo tanto, es necesario conocer como diferentes factores pueden ayudar a mantener un envejecimiento saludable en nuestra población. Mantener un buen estado físico, niveles adecuados de actividad física (PA) y unos hábitos de sueño saludable se han determinado como factores protectores ante mecanismos que pueden afectar al envejecimiento como el estrés oxidativo. Sin embargo, bajo nuestro conocimiento, se desconoce si estos factores pueden afectar de forma diferente al estrés oxidativo de las personas mayores dependiendo del sexo debido a que la mayoría de los estudios analizan ambos sexos de forma combinada.

Por lo tanto, el objetivo general de esta Tesis Doctoral Internacional fue el de evaluar las asociaciones de diferentes componentes de antropometría, composición corporal, estado físico, actividad física, sedentarismo y hábitos de sueño sobre el estrés oxidativo en una muestra de adultos mayores españoles y si estas asociaciones tenían diferencias dependiendo del sexo.

Esta Tesis Doctoral Internacional incluye datos transversales del proyecto INTERMAE. Un total de 76 participantes (38 mujeres, 68.8 ± 3.0 años) que fueron reclutados a través centros de salud pública de la provincia de Cádiz fueron incluidos. Se obtuvieron muestras de sangre para analizar la TAC del plasma y los niveles de HCY del suero. Adicionalmente, los participantes completaron diferentes mediciones incluyendo antropometría, composición corporal, estado físico a través

de diferentes baterías y un test de fitness cardiorrespiratorio (CRF), evaluación nutricional, y cuestionarios sobre sus hábitos de actividad física y sueño. Asimismo, los participantes llevaron un acelerómetro durante 7 días para estimar su comportamiento sedentario (SB), de actividad física (PA) y de sueño.

Los principales hallazgos de esta Tesis Doctoral Internacional fueron: i) Existen diferencias sexuales en los niveles basales de estrés oxidativo en nuestros adultos mayores españoles, sin importar su ingesta nutricional. ii) El Índice de Masa Corporal y el perímetro de muslo en mujeres y los niveles de masa libre de grasa en hombres se asocian con niveles incrementados de HCY. En mujeres mayores una velocidad de la marcha alta se asocia con niveles más altos de TAC. Una mayor fuerza y flexibilidad del tren superior, así como una mejor agilidad de la marcha se asocian con menores niveles de HCY en mujeres. Niveles más altos de CRF se asocian con niveles más bajos de HCY en ambos sexos (Estudio 1), iii) Los niveles de PA parecen ser más relevantes para el estrés oxidativo de las personas mayores que el SB, con la PA de moderada a vigorosa estando asociada con una TAC incrementada y los niveles de PA ligera estando asociados con niveles reducidos de HCY (Estudio 2). iv) Los trastornos del sueño se asociaron con el estrés oxidativo de las personas mayores, con un tiempo despierto durante la noche mayor estando asociado con una menor TAC en mujeres y una mayor latencia de sueño estando asociado con mayores niveles de HCY en hombres (Estudio 3).

Los hallazgos de la presente Tesis Doctoral Internacional muestran que existen asociaciones específicas para cada sexo entre variables antropométricas, de composición corporal, de estado físico, actividad física y hábitos de sueño en personas mayores.

INTRODUCTION

INTRODUCTION

General introduction

The ageing process

The advances in modern medicine and improved personal hygiene have led to an increased life expectancy in developed countries and it is estimated that the global population will grow to 3.6 billion people by the end of the century (1). These improvements have obvious consequences for our older population, growing the absolute and relative number of people over 65 years of age (2) and it is estimated that the older population (> 65 years old) will outnumber the young population (<20 years) in 0.67 billion people by 2100 (3). Nonetheless, the population is currently evolving into a more aged society, with data showing that the percentage of the population aged 65 years or older has increased from 16.3% to 21.7% in the European Union and from 16.3% to 21.3% in Spain in the last 20 years (4). This unprecedented increased longevity alongside decreased fertility has developed an aged population which forces the countries to face the challenge of maintaining their health and social systems adapted for this aged population (5,6). Ageing is a multifactorial physiological process that is influenced by several environmental and genetic factors that all living beings experience with time (7,8). Among these environmental factors, we can differentiate between i) behaviours such as smoking (9) or sedentary lifestyles (10), ii) external factors such as chemicals, ultraviolet radiation or x-rays can have harmful effects on biological molecules; iii) internal factors, for instance, excessive production of aftermaths derived from enzymatic reactions such as reactive oxygen and nitrogen species (RONS) modulates the physiological decline that occurs with ageing through continuously damaging cellular structures (11).

In addition to this, genetic factors such as sex can also influence this damaging process to cellular structures. For example, following the excessive production of RONS, it has been shown that women have better protection against the overproduction of these molecules than men (12,13) since women have higher levels of estrogens that could act as a protective factor against these molecular damages (14).

Therefore, the combination of all the aforementioned factors produces numerous molecular and cellular changes which lead to deleterious effects on health that promote ageing (7,8). These deleterious effects are summarized in Figure 1. Among all of these factors, we would like to highlight the mitochondrial dysfunction that leads to altered oxidative stress. Concerning this, the functionality of their mitochondria decreases as people age affecting the efficacy of the respiratory chain and consequently increasing the leaking of electrons and reducing the production of ATP (15). The increased leakage of electrons results in an increased production of RONS producing progressive mitochondria and cellular deteriorations that generates an altered oxidative stress status (8). These changes induce a progressive reduction in organ function and physical capacity that increased the risk of suffering non-communicable diseases such as dementia or sarcopenia and eventually leads to death (16).

This trend in the increasing ageing population augmented the incidence of non-communicable diseases such as musculoskeletal disorders, neurodegenerative diseases, cancer, and cardiovascular diseases, especially ischaemic heart diseases and strokes, which have been the main causes of disability in older adults in recent years (17). Due to the consequences of these diseases, a heavy economic and psychological burden is generated for all of society (18). Additionally, the ageing process has been shown to influence the health of our older adults due to its association with declined physical fitness components (i.e. aerobic capacity, muscle strength, balance or flexibility) which are directly affecting the performance of daily living activities generating dependency (19).

Therefore, if our expanding older population wants to keep maintaining its role in our society, and also to reduce the possible economic and social consequences of these age-related disorders, it is needed to find a strategy to prevent the decline of our older adults such as healthy ageing, which has been proposed since the beginning of the century (20) and has gained notability during recent years until the point that is one of the main aims for this decade (5). Healthy ageing has been defined as the process of not only maintaining but also developing functional ability, and preserving the well-being of our older adults (21). Even though there is a lack of consensus on the term healthy ageing (22), there are several factors that could

contribute individuals to ageing physically and mentally healthy such as physical activity (PA), physical fitness, sleep behaviour, smoking or alcohol intake (23). Maintaining good physical fitness, adequate levels of PA, healthy sleep behaviour and avoiding smoke or alcohol intake have been shown to reduce the possible mechanisms of ageing such as telomere shortening, the presence of senescence genes in the DNA, or oxidative stress (7,8).

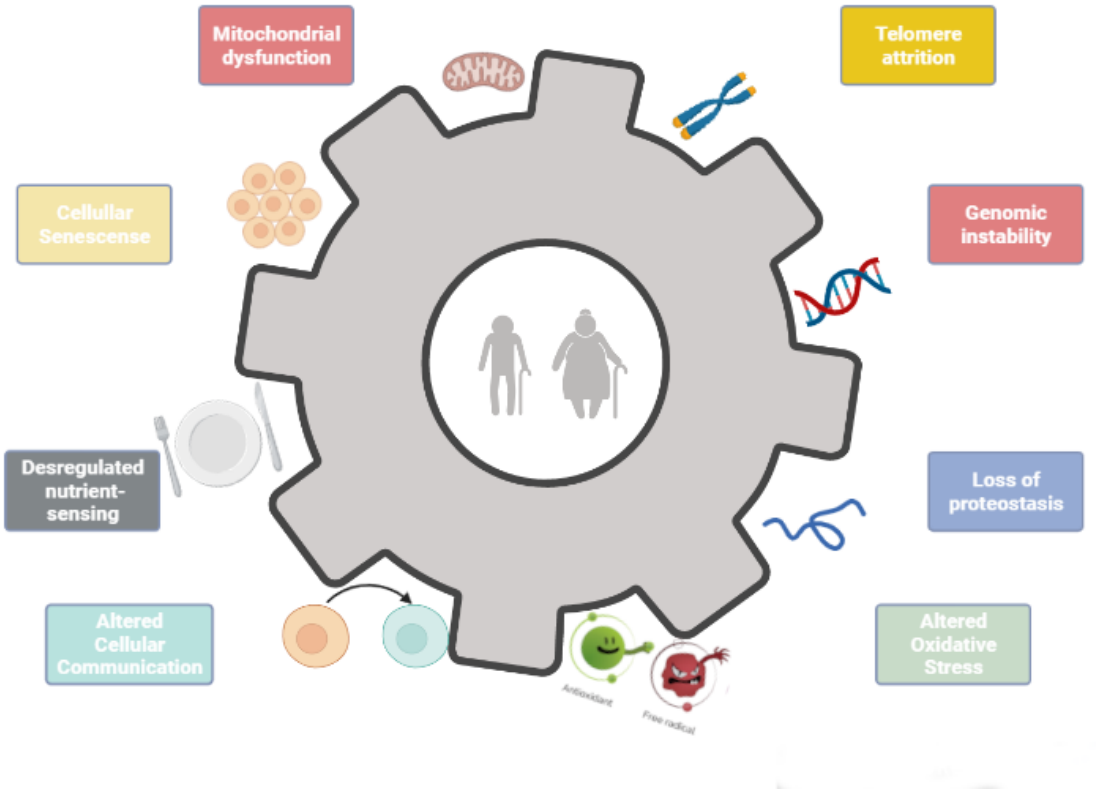

Figure 1. The hallmarks of ageing. Adapted from López-Otín et al (2013) (8)

Oxidative stress and ageing

Oxidative stress has been defined as a pathophysiologic imbalance between pro-oxidants and antioxidants in favour of pro-oxidative with RONS being the most common pro-oxidative (24).

RONS are mostly produced from endogenous and exogenous sources in the cell, primarily by mitochondria, and play an important role in ageing along with age-related diseases (25). These RONS can be produced by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, Homocysteine (HCY), lipoxygenase, and angiotensin II (26). Two of the prevalent sources of RONS are NADPH oxidase, which generates the radical superoxide anion (O2-) during cellular and mitochondrial respiration (27) and HCY which has been associated with an increased level of reactive oxygen species (28). Exogenous sources are pollution, tobacco, alcohol, drugs or radiation among others (27). RONS cause oxidative modifications in the major cellular macromolecular such as the lipids forming oxidative markers such as trans-4-hydroxy-2-nonenal (4-HNE), malondialdehyde (MDA), and isoprostanes (29).

In order to defend the biological system from this oxidative damage, every cell creates molecules to cope with this stress, also known as the antioxidant defence. Similar to RONS, the antioxidant capacity has endogenous and exogenous sources. The endogenous antioxidants include enzymes and non-enzymatic antioxidants. Enzymes include Superoxide Dismutase (which converts O_2^- to H_2O_2), Catalase (which converts H_2O_2 to water and oxygen), and glutathione peroxidase (which converts peroxides and hydroxyl radicals into nontoxic forms by the oxidation of reduced glutathione into glutathione disulfide and then reduced to glutathione by glutathione reductase) (30). In non-enzymatic endogenous antioxidants, we can find bilirubin, α-tocopherol, and β-carotene in blood and albumin and uric acid in plasma (31). Exogenous antioxidants include ascorbic acid, which scavenges hydroxyl and superoxide radical anion, and α-tocopherol (27). Figure 2 summarizes the interactions of the aforementioned pro-oxidants and antioxidants.

When the production of RONS is overproduced or the antioxidant defence is not able to cope with them, oxidative stress occurs. When this condition happens, an accumulation of oxidatively damaged macromolecules (especially DNA, lipids and proteins) appears, leading to the loss of function of these macromolecules. These functional losses are speculated to promote cellular senescence which is defined as a cellular mechanism that inhibits the proliferation of new cells during replication as a reply to the accumulation of damaged macromolecules (27). This cellular senescence is characterized by a pro-inflammatory state as a result of the production of IL-1α (32) as well as an increment in RONS production by inhibition of enzymatic antioxidants such as Superoxide dismutase (33).

However, the exact mechanism of how oxidative stress is inducing ageing is still not clear.

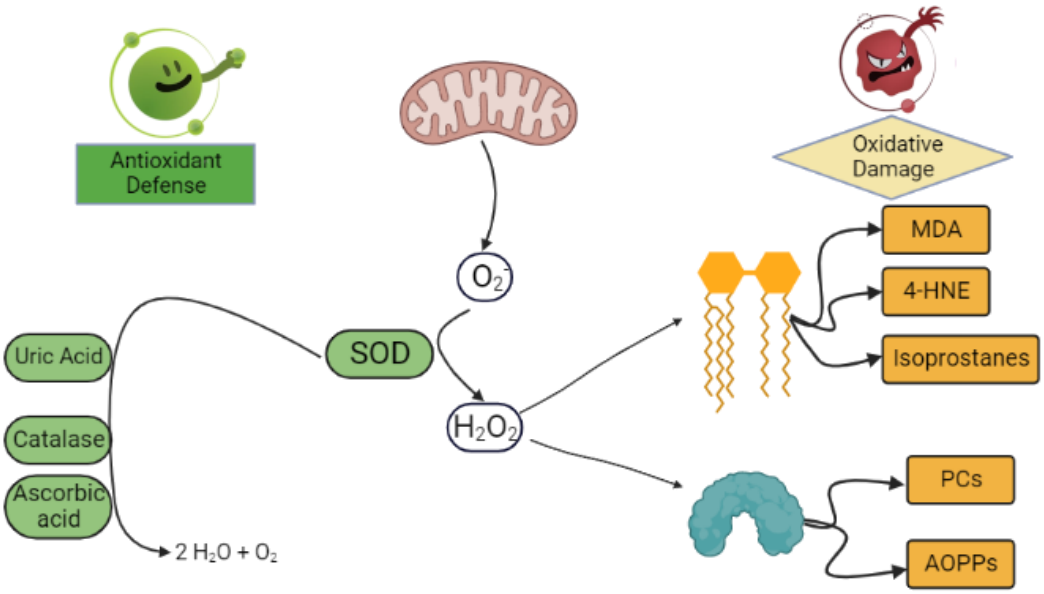

Figure 2. Summary of selected oxidative and anti-oxidative pathways. The antioxidants shown in this figure are in green ovals. The superoxide radical is generated through mitochondrial oxidative phosphorylation, among other cellular processes. Hydrogen peroxide is converted to oxygen and water through reactions with enzymatic (catalase and glutathione peroxidase) or non-enzymatic (primarily uric acid and ascorbic acid) antioxidants. Superoxide and hydrogen peroxide may react with proteins or lipid species to produce tissue damage which we refer to as oxidative stress. 4-HNE = 4-hydroxynonenal, MDA = malondialdehyde, SOD = superoxide dismutase, PCs =Proteyn carbonyles, AOPPs= Advanced oxidation protein products.

Oxidative stress and age-related diseases

As mentioned before, oxidative stress has been associated with several age-related diseases, for instance, cognitive impairment, cardiovascular diseases or sarcopenia (27).

Regarding cognitive diseases, it has been studied if the accumulation of pro-oxidants or the decrease of antioxidants could affect these non-communicable diseases. On one hand, it has been shown that there is an association between higher levels of oxidative stress biomarkers of lipids (MDA) and proteins (protein carbonyl) with lower levels of cognitive performance in older adults (34). On the other hand, other studies found that cognitive impairment was much slower in older adults with higher activity of the antioxidant enzyme glutathione peroxidase (35). Therefore, it seems that maintaining a good oxidative stress balance may help ameliorate the progressive loss of memory that occurs in ageing populations.

Similar to cognitive diseases, the literature has focused on how an imbalance between pro-oxidants and antioxidants could influence the development of cardiovascular diseases, which are one of the leading causes of morbidity and mortality in the elderly (27). Several studies have proven that a reduction of the antioxidant enzymes due to ageing leads to a decrease in heart tolerance to oxidative stress, promoting the appearance and the development of cardiovascular alterations (36). Additionally, it has been demonstrated that people who suffered from cardiovascular disease such as coronary artery disease have depleted levels of total antioxidant capacity (TAC) (37), which consider the synergistic role of both enzymatic and non-enzymatic antioxidants (38). Additionally, pro-oxidants have also been associated with the development of cardiovascular diseases. For instance, due to the reduced antioxidant defences of elderly people, RONS can oxidise Low-Density Lipoproteins (LDL), producing oxidized LDL which can accumulate in the subendothelium, provoking the early stages of atherogenesis (38). Likewise, the concentrations of pro-oxidants such as HCY have been associated with an increased risk of fatal and nonfatal atherosclerotic disease in the coronary, cerebral, and peripheral circulation (39).

Furthermore, oxidative stress has been associated with strength deficits due to a loss in muscle quantity, known as sarcopenia, or loss of muscle strength, known as dynapenia (40,41). The muscle is one of the primary generators of RONS, mainly produced by the mitochondria during normal respiration as a product of oxidative phosphorylation (42). This production of RONS is not indeed bad for the organism due to they play essential roles in redox signalling and cell survival by activating or inhibiting enzymes such as mitogen-activated protein kinase, phosphatases, and gene-dependent cascades (40). However, due to ageing, the antioxidant response is decreased and the RONS production of the mitochondria is augmented as a result of the accumulation of dysfunctional mitochondria (42). This imbalance between pro-oxidant production and antioxidant defences has deleterious effects on the human muscle. For instance, it has been partly attributed to oxidative stress the decline in type II fibres in older adults (43). Additionally, the elevated levels of RONS contribute to sarcopenia by increasing proteolysis and decreasing protein synthesis, which could induce a loss of muscle mass (25).

Thus, our older population needs to maintain a healthy balance between pro-oxidants and antioxidants due to their several implications on age-related diseases. Indeed, several studies have explored the multiple genetic and lifestyle variables that can affect oxidative stress such as age, sex, diet, and smoking status (44), but the influence of other lifestyle factors is not that well studied. Therefore, it is important to evaluate how the status and lifestyle of older adults, such as physical fitness, PA, or sleep behaviour can be associated with oxidative stress, in order to obtain the best approaches to maintaining a proper oxidative stress balance.

Physical fitness, ageing and oxidative stress

Physical fitness is described as a set of attributes (cardiorespiratory capacity, muscle strength, balance, flexibility) that allows the person to perform daily activities (45). Good physical fitness is believed to counteract many of the deleterious effects of ageing, due to the increased resistance to oxidative stress adaptations produced by physical training. However, this relationship is unclear in older adults, considering that the findings of a previous systematic review that has

examined the association between physical fitness and oxidative stress have been equivocal (44) and the investigation is still ongoing (46).

The elderly show an impaired capacity to counteract the adverse health effects of pro-oxidants (47), due to lower levels of TAC in older populations compared with younger populations (48). In the elderly, lower levels of TAC have been associated with higher dependency and cognitive impairment (48,49) by preventing oxidative stress; although, the possible beneficial effect of TAC on ageing is still inconclusive.

In this line, a way to mitigate age-related oxidative stress and promote healthy ageing is improving physical fitness which modulates several factors that participate in both the skeletal muscle and other organs of the human body (50). Increasing physical function and physical fitness have been shown to reduce pro-oxidant levels by increasing the antioxidant defence in middle-aged people (51). Specifically, in the older population, the ability to perform daily activities or functional independence has been related to a lower oxidative stress level (46). Therefore, there is an increasing investigation into different health-related parameters that can be easily modified by physical function to provide new strategies to achieve better and healthier ageing (52) and redox status.

Given the fact that older adults usually present higher levels of pro-oxidants and lower levels of antioxidants, they could benefit more from the advantages of maintaining adequate physical fitness. However, it is still unknown whether these oxidative stress markers are differently affected depending on the sex of the population considering most of the literature evaluates both sexes combined (46,49). In this sense, it has been suggested that younger women had a higher antioxidant capacity and lower levels of oxidant markers compared to men at rest (12,13). Additionally, a deep analysis of different physical fitness components (anthropometry, body composition, static and dynamic balance, flexibility, strength, and cardiorespiratory endurance) is needed to better comprehend its possible influence on oxidative stress.

Physical activity, sedentary behaviour, ageing and oxidative stress.

One of the indispensable parts of a healthy lifestyle is PA which has been defined as any bodily movement produced by the muscles that require energy expenditure. The importance of PA is related to its effects on the prevention of several diseases (53), influencing several mechanisms such as the metabolism or the oxidative stress balance (54).

In this line, acute physical activity increases ROS production, however, structured physical activity induces an increased antioxidant capacity and, in consequence, lower oxidative stress (54). Nevertheless, this beneficial mechanism in response to ROS generation is severely diminished in aged muscles (55) with possible differences between men and women and older adults (56).

Sedentary behaviour (SB) has been described as any movement in a sitting, reclining, or lying position which spends less than 1.5 metabolic equivalents of a task (57). In the past years, a growing body of evidence has shown that SB could be an independent risk factor for several diseases in adults, independently of PA (58).

Contrarily to PA, SB is a lifestyle practice which has been established as a possible enhancer of pro-oxidants as it has been shown that higher levels of SB are related to an imbalance in the redox status and impaired mitochondrial functionality in sedentary older adults (59).

Indeed, low PA and high SB are common in older adults, aggravating age-related oxidative stress and its negative consequences for health (60). The World Health Organization (WHO) guidelines for older adults recommend > 150 min/week of moderate PA or >75 min/week of vigorous PA or an equivalent combination of moderate- and vigorous-intensity PA throughout the week (61). Additionally, functional balance and strength should be trained through varied multicomponent PA three or more days a week (61).

Despite an active lifestyle along with a balanced diet being key to promoting healthy ageing and preventing oxidative stress (62), sex-specific differences in the influence of daily PA, daily SB, and dietary intake on oxidative stress have been poorly studied in older adults. Most of the studies analysed both sexes together, ignoring that older

women could show higher levels of oxidative stress due to they do not have the protective effects of estrogens (63) as well as showing higher levels of SB (64). Only a limited number of studies have evaluated the influence of these behaviours in older adults separately by sex, with a restricted population (56). Therefore, analyses divided by sex should be encouraged.

Moreover, to our knowledge, most of the studies that evaluate the association between PA and SB and oxidative stress have used questionnaires to quantify the amount of time spent on both behaviours (65,66). Thus, studies that quantify both PA and SB with a more objective method such as accelerometry are needed to obtain a better comprehension of the association of both behaviours with oxidative stress markers. Additionally, it is also needed to investigate the possible influence of the duration of these behaviours or the intensity of the PA (light, moderate, vigorous or moderate to vigorous) on the levels of oxidative stress.

Sleep behaviour, ageing and oxidative stress

Sleep is a vital phenomenon occurring in all life forms of the animal kingdom and helps to maintain metabolic homeostasis and consequently and overall optimum health and well-being (67). In humans, sleep is divided into two different phases: rapid eye movement sleep, also known as REM, and non-rapid eye movement sleep, also known as NREM. The REM phase is characterized by low brain cortical activity, rapid eye movement and muscle atonia (68). NREM is divided into three phases: N1, N2 and N3. N1 usually lasts for a few minutes and begins when the heartbeat, eye movements, brain waves and breathing rhythm start to slow down. N2 follows N1 and continues to slow down the aforementioned physiological functions as well as decrease body temperature (68). Usually, a person spends half of the night in this phase. Finally, N3 occurs, also known as deep sleep when heartbeat, muscle and brain activity are at their lowest activity levels. Additionally, the body releases growth hormones during this phase (69) and also is believed that during this phase the body can regulate glucose metabolism, immune system functioning, hormone release, and memory (69).

In this line, one of the proposed roles of sleeping is improving the antioxidant mechanisms. It has been suggested that sleep not only promotes the infusion of antioxidants such as oxidized glutathione in animal models (70) but also has been hypothesized to remove free radicals accumulated during the day (71).

However, an abnormal sleep cycle as it happens in sleep disorders like sleep deprivation, known as the reduction in sleep time below 7 hours for adults (68,72), may affect the well-being of the person. This reduction in total sleep time could be due to different factors, such as a long sleep latency which delays the onset of sleep, the number of awakenings during the night, or the time spent awake after one of these awakenings, among others. Indeed, sleep impairment has been associated with ageing with older adults getting less sleep than the young population (73) as well as physiological disorders related to metabolism including increases in oxidative stress (74).

It has been shown that sleep disorders such as sleep deprivation increase oxidative stress among others by unclear mechanisms (75,76). In addition to this, it has been shown that poorer sleep quality is associated with lowered levels of TAC and elevated values of HCY in older people with mild cognitive impairment (77).

It has been shown that sleep disorders such as sleep deprivation increase oxidative stress among others by unclear mechanisms (68,69). In addition to this, it has been shown that poorer sleep quality is associated with elevated values of HCY and lowered levels of TAC in older people with mild cognitive impairment (70).

However, it is still poorly studied if there are sex-specific differences in how sleep behaviour can influence the oxidative stress of older adults. Older men have shown greater sleep impairments and worse sleep quality than older women, with worse sleep deterioration, greater sleep fragmentation, and higher naptime propensity (73). Regarding oxidative stress, women have been shown to have better protection against oxidative damage in younger populations (78), however, this is not clear in the elderly since most of the studies usually evaluate both sexes combined (46,49). In addition to this, despite the associations between sleep behaviour and oxidative stress that have been studied recently in older people with a risk of dementia, both with self-reported questionnaires (79) and using more precise methods using

overnight polysomnographic recordings (77), it is still poorly studied in healthy older adults.

Therefore, deep analyses including both self-reported and deviced-meausure of sleep habits are needed to understand better how this behaviour can influence the oxidative stress of older adults as well as determine if these relationships are different depending on sex.

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AIMS

AIMS

General aim

The general aim of this International Doctoral Thesis was to study the sex-specific associations of anthropometry, body composition, physical fitness, PA, SB and sleep behaviour with oxidative stress levels, in a sample of older adults.

This general aim was addressed in three studies with the following specific objectives:

Study 1: Sexual differences in the association of physical fitness components with oxidative stress in older adults

1)To investigate whether sex differences in oxidative stress markers are presented in the elderly population.

2) To analyse the possible associations of anthropometry, body composition, and physical fitness components with TAC and HCY in men and women older adults.

Study 2: Sex-specific relationships between physical activity and sedentary behaviour with oxidative stress in older adults

To analyse sex-specific associations between self-reported and accelerometer-measured PA and SB with oxidative stress levels in older adults.

Study 3: Sex-specific associations of sleep parameters with oxidative stress in older adults.

To analyse the sex-specific associations between sleep behaviour and antioxidant and pro-oxidant markers in men and women older adults.

OBJETIVOS

Objetivo general

El objetivo general de esta Tesis Doctoral Internacional fue estudiar las asociaciones específicas del sexo de diferentes componentes de antropometría, composición corporal, estado físico, PA, SB, y hábitos de sueño en el estrés oxidativo, en una muestra de adultos mayores españoles.

El objetivo general se desglosa en tres estudios con los siguientes objetivos específicos:

Estudio 1: Diferencias sexuales en la asociación de múltiples componentes del fitness con estrés oxidativo en adultos mayores.

1) Investigar si las diferencias sexuales en marcadores de estrés oxidativo se encuentran todavía presentes en la población mayor.
2) Analizar las posibles asociaciones de la antropometría, composición corporal, y componentes del estado físico con TAC y HCY en hombres y mujeres adultos mayores.

Estudio 2: Relaciones específicas del sexo entre la actividad física y el comportamiento sedentario con el estrés oxidativo en adultos mayores

Analizar las asociaciones específicas de cada sexo entre la PA y el SB autoreportado y medido con acelerometría con los niveles de estrés oxidativo en adultos mayores.

Estudio 3: Asociaciones específicas del sexo de parámetros del sueño con el estrés oxidativo en personas mayores.

Analizar las asociaciones específicas de cada sexo entre el sueo autoreportado y medido con acelerometría con los niveles de marcadores antioxidantes y pro-oxidantes en adultos mayores.

MATERIAL AND METHODS

MATERIAL AND METHODS

This International Doctoral Thesis is composed of three studies derived from the INTERMAE project (Influence of a physical exercise intervention on markers associated with aging proteomic profile and fragility, PI-0002-2017, 2018-2022).

In summary, the INTERMAE project is a randomized controlled trial with the aim of analysing the effect of a 20-week supervised physical exercise program on brain structure, cognitive function, proteomics and metabolomics levels in older adults.

The current International Doctoral Thesis is focused on the baseline data of the INTERMAE project using a cross-sectional design. Data collection was carried out during January and February of 2018 (pilot study), 2019 (first wave), and 2020 (second wave). Both studies were evaluated and approved by the Human Ethics and Research Committee of research in Cádiz and the Andalusian Coordinating Committee on Biomedical Research Ethics (code: 04/2018) and conducted following the Declaration of Helsinki (1).

Participants

Participants were recruited from 13 public health care centers from three different cities in the province of Cádiz. In these centers, research team members with the aid of the medical staff were in charge of recruiting potential candidates for the project.

In order to be part of the study, participants needed to comply with the inclusion and exclusion criteria. The compliance with the criteria was divided into two phases. Firstly, the medical staff checked the medical part of the inclusion and exclusion criteria. Then, if the medical criteria were completed, the rest was assessed by the research team members. If participants overcame both phases, they were included in the study.

The inclusion criteria included: i) to be between 65 and 75 years, ii) be able to speak and write properly iii) not to be involved in doing supervised PA greater than 20 minutes per day in more than 3 days per week, iv not be involved in another research project, v) do not suffer from any injury that could avoid them from exercising, vi) to score ≥5 and ≥8 in Lawton and Brody Scale (2) for males and

females respectively, and vii) To want to complete the study if he/she is assigned to the control group.

The exclusion criteria were as follows: i) to suffer from an acute or terminal disease, ii) to suffer from severe depression, iii) to suffer from unstable cardiovascular disease, dementia and/or Alzheimer's disease, iv) to suffer from severe visual problems, v) to have a history of ictus, epilepsy or brain cancer, vi) to have a medical history of head injury with loss of consciousness, and vii) to abuse from alcohol and drugs.

From a total of 280 people interested in participating in the study, 92 participants (41 females) were finally included in the study after completing the entire recruitment process. All participants obtained the information sheet of the entire study and the informed consent with the procedures and potential risks associated with the study before the onset of the study. They returned it signed before doing the first test of the study. However, 76 participants out of 92 (38 women) obtained valid blood samples and only 66 participants (36 women) produced valid accelerometer data (**Figure 3**).

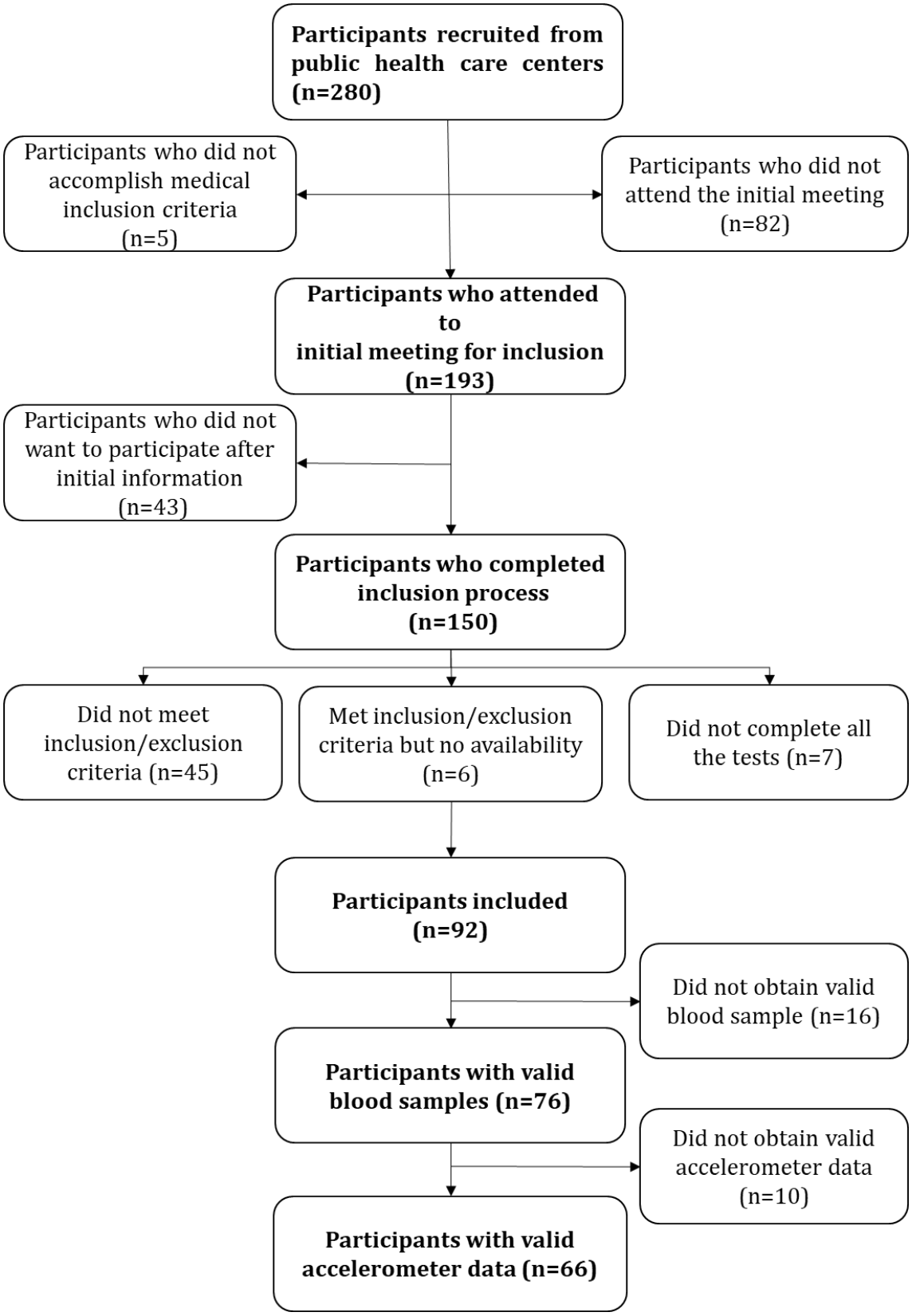

Figure 3. Baseline flow chart of the INTERMAE project.

Measurements

Anthropometry and body composition.

Height was measured in a standing position on the Frankfort plane, after normal expiration with a stature-measuring instrument (SECA 225, Hamburg, Germany).

The waist circumference (cm) was assessed with a metallic non-extensible tape (Lufkin W606PM, Washington, United States) at the level of the thinnest part of the waist between the iliac crest and the last rib. The measurements were taken at the end of normal expiration without compressing the skin.

To assess the hip circumference (cm), participants had to be in a standing position, feet together, with hands on the opposite shoulder and looking forward. On the most prominent gluteal area, the hip circumference was taken horizontally; this region normally coincides with the pubic symphysis and trochanter.

To assess the thigh circumference, participants remained in the same position and the circumference in the midpoint between the femoral trochanter and the tibial condyle was recorded.

All the anthropometric measurements were taken by the International Standards for Anthropometric Assessment (ISAK) guidelines standard techniques by an ISAK level 1 evaluator.

A validated multifrequency bioimpedance (Tanita-MC780MA, Tanita Europe BV, Amsterdam, The Netherlands) was used to assess body composition [weight (kg), body fat (% and kg), and fat-free mass (% and kg)] following the manufacturer's instructions (3,4). Participants were told to be in a fasted state for at least 4 hours and to control their hydration status during the previous week.

Then, the following variables were calculated:

- Body Mass Index (BMI): Weight (kg) / Height $(m)^2$
- Waist-Hip ratio: Waist circumference (cm) / hip circumference (cm)

Physical fitness components.

Physical fitness was evaluated using different fitness batteries. The Senior Fitness Test battery was performed (5), including the following domains:

- CRF was measured using a 6-minute walk test in which participants walk at their own pace, but as quickly as possible during 6 minutes.
- Lower body flexibility using the Chair Sit and Reach Test in which the participant, seated on a chair with an extended leg and knee straight, reaches for the toes of the extended leg.
- Upper body flexibility using the Back Scratch Test in which the participant passes one hand over the shoulder as far as possible trying to connect with the other hand which is placed around the back.
- Lower body strength using the 30 seconds Chair Stand Test in which the participant stands up and sits down as many times as possible in 30 seconds.
- Upper body strength using the Arm Curl Test in which the participant seated on a chair performs a complete flexion and extension of the arm holding a weight as many times as possible in 30 seconds.
- Agility using an 8-foot Up and Go Test in which the participant seated on a chair stands and walks to a cone situated at 8 feet and sits back in a chair as quick as possible.

These evaluations were complemented with the Short Physical Performance Battery (SPPB) (6,7) which includes the following dimensions and tests:

1) Balance

- Side-by-side test in which the participant must stand unassisted with their feet together for ten seconds.
- Semi-tandem test in which the participant must stand unassisted with the heel of one foot touching the big toe of the other foot for ten seconds.
- Tandem test in which the participant must stand unassisted with the heel of one foot touching the toes of the other foot for ten seconds.

2) Gait speed

- 4-meter normal gait speed test in which the participant walks the distance at their usual pace and the time is recorded.

3) Lower body performance

- 5 repetitions Sit to Stand test in which the participant stands up and sits down 5 times as quickly as possible and the time is recorded.

Participants' performance in each dimension was compared to normative data and scored between 0 and 4 points. If the participant could not perform the test, their score was reported as 0. In the end, the SPPB score was calculated as an overall functionality index and participants obtained a score between 0 (highly dependent) to 12 (totally independent). Given the fact that SPPB scores lower than ten are considered to show physical limitations and higher mortality risks (8), participants were divided into two groups, without limitations (with SPPB scores equal to or higher than 10) and with limitations (with SPPB scores lower than 10). Mean lower body velocity and power using the equations proposed by Alcazar et al. (9) were also calculated using the results from the 5-repetition Sit to Stand Test. In addition to both batteries, the following tests were also assessed:

- 6-meter normal gait speed test in which the participant walks the distance at their usual pace and the time spent is recorded.
- 6-meter fast gait speed test in which the participant walks the distance as quickly as possible and the time spent is recorded.
- Handgrip strength (10) in which participants were asked to squeeze a validated dynamometer (TKK 5101 Grip D; Takey, Tokyo, Japan) gradually, by exerting as much pressure as possible with the tested hand, and holding this pressure for approximately two seconds. The test was performed twice with each hand and the mean value from the highest scores attained with each hand was calculated.

Peak oxygen consumption was evaluated by indirect calorimetry with Jaeger MasterScreen CPX® gas analyzer (CareFusion, San Diego, USA) in all participants using a modified Bruce protocol on a treadmill (Lode Valiant, Groningen, The Netherlands) until voluntary exhaustion. The modified Bruce protocol (11) with two-minute steps has been previously used in a similar sample and designed for a geriatric population (12). The participants began the test walking at 2.7 km/h and 0% inclination and every two minutes the speed and/or the inclination were increased. During the first 3 steps, only the inclination was increased by 5% (5% and 10% during the second and third steps) and from that point, the inclination increased by 1% in each step with an increase in the speed of approximately 0.6 km/h. During all the protocols the participant was supervised by at least two researchers and one medical doctor and heart functioning was controlled by a 12-

lead electrocardiogram (Norav 1200W, NORAV Medical, Mainz-Kastel, Germany) to ensure the safety of all participants.

Physical Activity and Sedentary Behaviour.

Self-reported.

The Global Physical Activity Questionnaire was used for the evaluation of both self-reported PA and SB. The Global Physical Activity Questionnaire is a valid measure developed by the WHO (13) to assess PA and SB levels (14). It was administered by trained research personnel and analysed following the WHO procedures (14). Then, participants were divided into meeting the WHO PA guidelines (>150 of moderate to vigorous PA per week) or not meeting them (<150 of moderate to vigorous PA per week) (15).

Accelerometer-measured.

Participants were asked to wear an accelerometer (ActiGraph GT9X, ActiGraphInc, Pensacola, USA) on the hip for at least 7 consecutive days in order to estimate PA and SB, only removing it for water-based activities such as showering. Additionally, during the measurement period, participants were required to make daily notes of their bedtime (time between going to bed and waking) as well as their non-wear time in a diary. ActiGraph is a triaxial monitor that records accelerations in three axes (vertical, anteroposterior, and mediolateral), with a dynamic range of ± 8 g (https://www.actigraphcorp.com/actigraph-link/). Accelerometers were initialized to store accelerations at 100 Hz and raw data files were managed, downloaded as ".gt3x" files, and converted to ".csv" using Actilife v.6.13.3 software (Actigraph) software. Only results from participants with wear time ≥ 16 h/d during at least 4 days (at least 3 weekdays and at least 1 weekend day) were considered valid.

The data were processed with R-package (R Core Team, Vienna, Austria) using the open-source R-package GGIR, version 2.7-0 (https://cran.r-project.org/web/packages/GGIR/index.html). This open sources code has been validated with self-calibrated functions (16). Previously published methods were used to minimize the sensor calibration error (auto-calibration of the data based on local gravity) (16) and accelerations determined by calculating the Euclidean norm

minus one (ENMO). Non-wear time periods (based on the raw acceleration of two of the three axes was <13 mG during the surrounding 60-minute moving window or if the value range for two of the three axes was <50 miliGravities [mG]), and sustained abnormally high accelerations (higher than 5.5 G during at least 15 minutes, related to device malfunctioning) were taken into account (17). Lastly, non-wear time waking hours were identified with an automatized algorithm guided by the participants' diary reports (18).

Time in PA and SB intensities were classified using previously proposed SB thresholds for ENMO in the hip for older adults (a) SB: ≤15 mG, (b) Light PA (LPA): >15 mG and <69 mG, (c) Moderate PA (MPA): ≥69 mG and <190 mG, (d) Vigorous PA (VPA): ≥190 mG (19,20). Bouts in each category were considered when 80% of the minimum required time met the threshold criteria. The following PA variables were obtained: total time in LPA, time spent in LPA in bouts between 1 and 5 minutes, between 5 and 10 minutes, and longer than 10 minutes, the number of bouts of LPA, total time in MPA, total time in VPA, total time in moderate to vigorous PA (MVPA), time spent in MVPA in bouts between 1 and 5 minutes, between 5 and 10 minutes, and longer than 10 minutes, and the number of bouts of MVPA. Depending on their MVPA levels, participants were allocated to meeting the WHO recommendations and those who did not (15).

Regarding SB variables were obtained: total time spent in SB, time spent in SB in bouts of 10 and 20 minutes, between 20 and 30 minutes, between 30 and 45 minutes, between 45 and 60 minutes, and longer than 60 minutes as well as the number of bouts of these SBs.

Finally, fragmentation metrics were calculated. One of the metrics analysed the probability of transitioning from a SB bout to a PA bout or vice-versa. Transition probability (TP) from SB to PA (SB2PA), from PA, LPA, and MVPA to SB (PA2SB, LPA2SB, MVPA2SB) were calculated using the GGIR code. The transition probability from SB to LPA and MVPA was calculated as 1 divided by the mean fragment (considered as a sequence of bout that belongs to the same behaviour, PA or SB) duration.

Additionally, the Gini coefficient was calculated for PA and SB levels using the R package "reldist" (21). The Gini coefficient assesses the dispersion of the behaviour and it ranges from 0 to 1, with smaller values indicating that the behaviour was accumulated more evenly across the day (22) and it was only calculated if there were at least 10 fragments of valid accelerometer data, for instance, 5 SB and 5 PA fragments.

Sleep behaviour.

Self-reported.

The Pittsburgh Sleep Quality Index (PSQI) was obtained from all the participants (23), which through 19 items measures 7 different aspects of sleep:

- Subjective sleep quality: Based on how the participants rate their sleep quality.
- Sleep latency: Based on how much time the participant reported that they need to fall sleep.
- Sleep duration: Calculated by the reported sleep time by the participants.
- Habitual sleep efficiency: Calculated by the reported sleep onset and the reported wake-up time.
- Sleep disturbances: Based on how often the participant had trouble sleeping.
- The use of sleeping medication: Based on the used of medication to facilitate sleep reported by the participant.
- Daytime dysfunction: Based on troubles stating awake during the day reported by the participant.

Participants answered about their sleep behaviour during the past month. Each aspect was valued from 0 to 3, with the final score ranging from 0 to 21 (23). Additionally, to detect if there were any differences in oxidative stress parameters between PSQI classifications, participants were divided into 2 groups, poor-quality sleepers (if they obtained scores higher than 5) and good-quality sleepers (if they obtained scores lower than 5).

Accelerometer-measured.

Participants were asked to wear an accelerometer (ActiGraph GT9X, ActiGraphInc, Pensacola, USA) on the wrist for at least 7 consecutive days in order to estimate their

sleep behaviour. Accelerometers were initialized to store accelerations at 100 Hz and raw data files were managed, downloaded as ".gt3x" files, and converted to ".csv" using Actilife v.6.13.3 software (Actigraph) software. The data were processed with R-package (R Core Team, Vienna, Austria) using the open-source R-package GGIR, version 2.7-0 with the same procedures previously explained for PA and SB.

During the measurement period, subjects were required to make daily notes of their bedtime (time between going to bed and waking) in a diary. The identification of waking and sleeping hours were determined with an automatized algorithm guided by the participants' diary reports (18). Then, inactivity periods with low variability in the z angle (<5º over 5 minutes) during sleeping hours were categorized as sleep time by the algorithm with the aid of the participant's diary. Due to the change in accelerometer position, the threshold for inactivity was ≤57 mG (19).

Sleep behaviour variables of interest were estimated daily and consisted of the following: bedtime, sleep time, wake after sleep onset (WASO), sustained inactivity time, awakenings, sleep onset, wake-up time, sleep regularity Index, and sleep efficiency. Bedtime was defined as the duration between participants who registered they went to bed and got out of bed. Sleep time was estimated with the difference between sleep onset and wake-up time. WASO was defined as the sum of the time a person was awake between sleep onset and sleep termination (i.e., during the sleep duration period). Sustained inactivity time was measured as any behaviour that could be detected as sleep time during the day (<5º over 5 minutes), behaviours such as naps. Awakenings were defined as the number of times a person was awake >5 minutes during sleep time. The sleep regularity index calculates the probability of the individual being in the same state (asleep vs. awake) during the study and it is scaled so that an individual who sleeps and wakes at the same time each day scores 100 (24). Lastly, Sleep efficiency was defined as the proportion of time spent sleeping from onset to wake-up time:

- Sleep Efficiency: (sleep time–WASO) / sleep time.

Sleep efficiency ranges from 0 to 100, where a score of 100 means the individual did not wake between sleep onset and termination.

Blood sampling.

Blood samples were extracted from the antecubital vein in the morning and under fasting conditions for at least 8 hours. To obtain blood plasma, blood samples were centrifuged (1280 g) at 4 °C for 20 minutes. For blood serum, after 30 minutes to obtain complete coagulation, blood samples were centrifuged (1990 g) for 15 minutes at 20 ºC. Both samples were stored at −80 °C, and thawed immediately before assay.

Total antioxidant capacity.

Plasma TAC was measured using the ferric-reducing antioxidant power (FRAP) method (25) with some modifications (26). The FRAP reagent was freshly prepared by mixing 10 mM of 2,4,6-Tris (2-pyridyl)-1,3,5-triazine in 40 mM of aqueous hydrochloric acid, 20 mM of ferric chloride in deionized water, and 300 mM of acetate buffer (pH 3.6) in the ratio of 1:1:10. Plasma samples were thawed in dark on ice for 45 min. The standard calibration curve was prepared using a deionized aqueous solution of ferrous sulfate (1 mM) at different concentrations (100, 250, 400, 500, 600, 700, 800, 900, and 1000 µM). 20 µL of ferrous sulfate standard or plasma were mixed with 150 µL of FRAP reagent (pre-warmed at 37ºC for 30 min) and incubated for 4 min. Absorbance was read at 595 nm on a Victor X3 multilabel plate reader (PerkinElmer, Waltham, MA, USA). FRAP values were expressed as ferrous equivalents in µmol/L.

Homocysteine.

Serum HCY level (µmol/L) was measured in each participant using standard enzymatic methods (A15 Random Access Analyzer, Biosystems, Spain).

Dietary assessment.

Dietary intake assessment was performed by trained surveyors in a personal interview using a Food Frequency Questionnaire (FFQ) and three 24h dietary recalls (24HR). The FFQ has been previously validated in an elderly Mediterranean population of Spain (27) and consisted of 137 items plus vitamin/mineral supplements and alcohol consumption patterns. A semi-quantitative analysis of total energy, macro and micronutrients was performed by converting the results into intake per day and multiplying by the standard serving size indicated in the

FFQ; if not, the amount of food was estimated according to the Spanish reference tables used in the DIAL® software for Windows, version 3.7.1.0 (Table S1) (28,29). Quantification of dietary intake was completed with three 24HR in non-consecutive days including one weekend day. In the three unannounced 24HR, participants were asked to detail food and beverage descriptions including ingredients, methods of food preparation, and portion sizes with the help of a trained interviewer. The 24HR results were analyzed through the DIAL® software (28), estimating the average total energy, and macro and micro nutrients for each participant.

Due to the fact that diet can influence the levels of plasma TAC, being the main external contributor to oxidative damage (30) an analysis to detect if there were differences in TAC levels depending on the diet was performed. Therefore, participants were stratified into two groups, participants who met their specific nutritional recommendations and those who did not in each specific macro and micro nutrient (**ANNEX I, Table S1**)(29).

Statistical analyses.

All statistical analyses were performed by STATA 13.0 (StataCorp LLC, Texas, United States). The level of significance was set at $p<0.05$.

All data are expressed as mean ± standard deviation (SD). Descriptive statistics were used to analyse the characteristics of the participants after verifying the normal distribution of the data (Shapiro-Wilk test).

Study 1

Statistical differences between sexes and groups based on SPPB scores were calculated by using Student's t-test and the standardized effect sizes using Cohen's d coefficients.

Single and multiple linear regressions were applied including confounders in the adjusted models that met scientists and statistical criteria (changes of >10% on the unadjusted regression coefficient). The following potential confounders based on scientific criteria were analysed (Age, smoking status, Vitamin C, Vitamin D, Vitamin B6 and Vitamin K), however, only age met the statistical criteria to be included as a

cofounder. Preliminary analyses showed a significant interaction of sex in most of the variables, therefore, the analyses were performed separately by sex.

Study 2

Statistical differences between sexes and compliance with WHO PA guidelines were calculated by using Student's t-test and the standardized effect sizes using Cohen's d coefficients.

Single and multiple linear regressions were applied including confounders in the adjusted models that met scientists and statistical criteria (changes of >10% on the unadjusted regression coefficient). Of all possible confounders such as age, smoking status, body mass index, weight or macro and micronutrients (Vitamin C, Vitamin D, Vitamin B6 and Vitamin K), age was the only potential confounder that met the scientists and statistical criteria. Preliminary analyses showed a significant interaction of sex in most of the variables, therefore, the analyses were performed separately by sex.

Study 3

Statistical differences between sex for oxidative stress, sleep parameters and diet assessment were calculated by using Student's t-test and the standardized effect sizes using Cohen's d coefficients. Additionally, the same test was used for evaluating the differences between poor-quality sleepers and good-quality sleepers in oxidative stress markers. The significant differences in PSQI aspects of sleep were analysed using the chi-squared test.

Single and multiple linear regressions were applied including confounders in the adjusted models that met scientist and statistical criteria (changes of >10 % on the unadjusted regression coefficient The following potential confounders were analysed (Age, smoking status, Vitamin C, Vitamin D, Vitamin B6 and Vitamin K), however, only age met the statistical criteria to be included as a cofounder. Preliminary analyses showed a significant interaction of sex in most of the variables, therefore, the analyses were performed separately by sex.

A summary of the methodologies of the included studies is shown in **Table 1.**

Table 1. General overview of the methodology followed in the studies included in this International Doctoral Thesis

Study	Design	Participants	Independent (exposure) variables	Dependent (outcome) variables
Study 1: Sexual differences in the association of physical fitness components with oxidative stress in older adults	Cross-sectional	76 older adults (38 females)	Anthropometry variables Body composition variables Senior Fitness test Battery Short Physical Performance Battery 6-meter normal gait speed 6-meter fast gait speed Handgrip Test CRF	TAC HCY
Study 2: Sex-specific relationship between physical activity and sedentary behaviour and oxidative stress in older adults	Cross-sectional	66 older adults (36 females)	Accelerometer-measured PA Accelerometer-measured SB Self-reported PA Self-reported SB	TAC HCY
Study 3: Sex-specific associations of sleep parameters with oxidative stress in older adults.	Cross-sectional	66 older adults (36 females)	Accelerometer-measured sleep behaviour Self-reported sleep behaviour	TAC HCY

CRF: Cardiorespiratory fitness; HCY: Homocysteine; PA: Physical activity; SB: Sedentary Behaviour; TAC: Total Antioxidant Capacity

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RESULTS

RESULTS

The results of the individual studies included in this International Doctoral Thesis are presented underneath.

Study 1: Sexual differences in the association of physical fitness components with oxidative stress in older adults

The sociodemographic characteristics of the participants are presented in **Table 2.**

The basic characteristics of the participants are given in **Table 3**. Regarding anthropometry and body composition, men showed significantly higher values of weight and absolute and relative fat-free mass whereas women showed higher values of absolute and relative fat mass. Despite this, no differences were found for BMI, showing overweight in both sexes, close to obesity.

Concerning physical fitness components, our men reported being fitter than their women counterparts, showing better values for all gait speed tests (4-meter normal gait speed, 6-meter normal gait speed and 6-meter fast gait speed), agility (8-foot Up and Go test), upper body strength (handgrip and arm curl tests), lower body strength (sit to stand tests) and a higher SPPB score (all $p<0.05$). Both sexes showed fair VO_{2peak} (28.2±4.7 and 22.4±4.4 for men and women, respectively) with significant differences in both absolute and relative values ($p<0.001$).

Respecting oxidative stress, men showed higher values for anti-oxidative (+169.18 ± 13.5 μmol/l) and pro-oxidative values (+2.74 ± 0.2 μmol/l), with both values being highly significant ($p<0.001$). These differences in antioxidant capacity and pro-oxidative values remained after adjusting by fat mass percentage ($p=0.005$ and $p<0.001$, respectively) or relative VO_{2peak} ($p=0.001$ and $p<0.001$, respectively).

When the oxidative stress values were compared by SPPB groups, no significant differences were found neither for the total sample nor for men and women for TAC and HCY (**Figure 4**).

Table 2. Sociodemographic characteristics of the total sample and by sex.

	Total (n=76)	Men (n=38)	Women (n=38)	p
	Marital Status			
Single (%)	4 (5.26)	0 (0)	4 (10.53)	
Married or with a couple (%)	58 (76.32)	31 (81.58)	27 (71.05)	0.265
Widowed (%)	8 (10.53)	3 (7.89)	5 (13.16)	
Legally separated or divorced (%)	6 (7.89)	4 (10.53)	2 (5.26)	
	Level of education completed			
Without studies (%)	9 (11.84)	2 (5.26)	7 (18.42)	
Primary (%)	28 (36.84)	14 (36.84)	14 (36.84)	0.293
Secondary/Job Training (%)	24 (31.58)	13 (34.21)	11 (28.95)	
University Studies (%)	15 (19.74)	15 (39.47)	6 (15.79)	
	Contributory pension			
Own quotation (%)	52 (68.42)	38 (100)	13 (34.21)	
Quotation from another person (%)	2 (2.63)	0 (0)	2 (5.26)	**0.001**
Both own and from another person (%)	1 (1.32)	0 (0)	1 (2.63)	
No quotation (%)	22 (28.95)	0 (0)	22 (57.89)	
	Smoking status			
Current smoker (%)	5 (6.58)	5 (13.16)	0 (0)	0.054
Non-smoker (%)	71 (93.42)	33 (86.84)	38 (100)	
	Alcohol status			
Regular drinker (%)	20 (26.3)	15 (39.5)	5 (13.2)	
Occasional drinker (%)	34 (44.7)	16 (42.1)	18 (47.4)	**0.018**
Abstemious (%)	22 (29)	7 (18.4)	15 (29)	

Table 3. Participant characteristics of the total sample and by sex.

	Total (n=76)	Men (n=38)	Women (n=38)	p	ES
Age (years)	68.84 ± 3.02	68.8 ± 3.08	68.88 ± 3.01	0.903	0.0
Anthropometry and body composition					
Height (cm)	159.71 ± 9.11	166.03 ± 6.76	153.38 ± 6.37	**<0.001**	1.9
Weight (kg)	74.35 ± 14.46	81.61 ± 14.52	67.09 ± 10.27	**<0.001**	1.2
BMI (kg/m^2)	29.08 ± 4.59	29.54 ± 4.45	28.61 ± 4.74	0.306	-0.2
Waist circumference (cm)	99.24 ± 12.23	104.69 ± 11.41	94.31 ± 11.09	**<0.001**	0.9
Hip circumference (cm)	105.15 ± 9.57	104.22 ± 9.54	106.08 ± 9.63	0.345	-0.2
Waist/Hip Index	0.94 ± 0.08	1.00 ± 0.06	0.89 ± 0.06	**<0.001**	1.8
Thigh circumference (cm)	51.54 ± 4.84	51.72 ± 4.33	51.36 ± 5.36	0.685	0.1
Fat-Free Mass (kg)	50.45 ± 10.54	59.26 ± 7.30	41.63 ± 3.50	**<0.001**	-3.2
Fat-Free Mass (%)	68.05 ± 7.70	73.33 ± 5.56	62.76 ± 5.63	**<0.001**	1.9
Fat Mass (kg)	23.91 ± 8.10	22.35 ± 1.36	25.43 ± 7.61	0.065	-0.4
Fat Mass (%)	31.94 ± 5.64	26.68 ± 5.56	37.26 ± 5.64	**<0.001**	-1.9
Senior Fitness test Battery					
6-minute walk Test (m)	555.19 ± 87.29	595.02 ± 92.27	516.19 ± 87.29	**<0.001**	1.0
Sit and Reach Test (cm)	-7.49 ± 11.86	-9.03 ± 11.78	-5.99 ± 11.90	0.264	-0.3
Back Scratch Test (cm)	-11.05 ± 9.61	-12.14 ± 8.86	-10.00 ± 10.30	0.332	-0.2
30 seconds Chair Stand Test (repetitions)	11.32 ± 2.22	12.34 ± 2.22	10.25 ± 1.95	**<0.001**	1.0
Arm Curl Test (repetitions)	15.76 ± 3.49	16.32 ± 3.11	15.21 ± 3.79	0.237	0.3
8-foot Up and Go Test (s)	6.41 ± 1.53	5.78 ± 1.17	7.03 ± 1.60	**<0.001**	1.0
Short Physical Performance Battery					
Side-by-side Test (s)	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	1.000	0.0
Semi-tandem Test (s)	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	1.000	0.0
Tandem Test (s)	9.38 ± 1.77	8.93 ± 2.36	9.76 ± 0.95	0.146	-0.2
4-meter normal gait speed Test (m/s)	1.31 ± 0.26	1.41 ± 0.25	1.23 ± 0.24	**0.005**	0.7
5 repetition Sit to Stand Test (s)	12.97 ± 2.74	11.66 ± 1.75	14.17 ± 2.94	**<0.001**	1.1
SPPB score	10.52 1.26	11.06 1.05	10.05 1.25	**<0.001**	0.9
Additional physical evaluations					
6-meter normal gait speed (m/s)	1.30 ± 0.22	1.38 ± 0.21	1.22 ± 0.20	**0.001**	0.8
6-meter fast gait speed (m/s)	1.84 ± 0.32	1.97 ± 0.28	1.70 ± 0.31	**<0.001**	0.9
Handgrip Test (kg)	28.75 ± 8.97	36.26 ± 5.89	21.24 ± 0.57	**<0.001**	-3.0
Sit to Stand Test mean velocity (m/s)	0.79 ± 0.05	0.83 ± 0.04	0.76 ± 0.03	**<0.001**	2.0
Sit to Stand Test relative power (w/kg)	2.49 ± 0.5	2.71 ± 0.53	2.29 ± 0.39	**0.003**	0.9
Cardiorespiratory fitness					
VO_2peak (ml/min)	1874.92 ± 497.54	2260.79 ± 346.53	1489.05 ± 275.45	**<0.001**	2.5
VO_2peak (ml/kg/min)	25.3 ± 5.37	28.17 ± 4.73	22.42 ± 4.37	**<0.001**	1.3

Oxidative Stress

TAC (µmol/l)	977.62 ± 184.9	1063.31 ± 158.22	894.13 ± 171.76	**<0.001**	1.0
HCY (µmol/l)	13.77 ± 3.12	15.18 ± 2.92	12.44 ± 2.71	**<0.001**	1.0

Values are expressed as mean ± SD. BMI, Body Mass Index; ES, Effect Size (Cohen's D); HCY, Homocysteine; TAC, Total Antioxidant Capacity; VO_2peak, peak oxygen consumption.

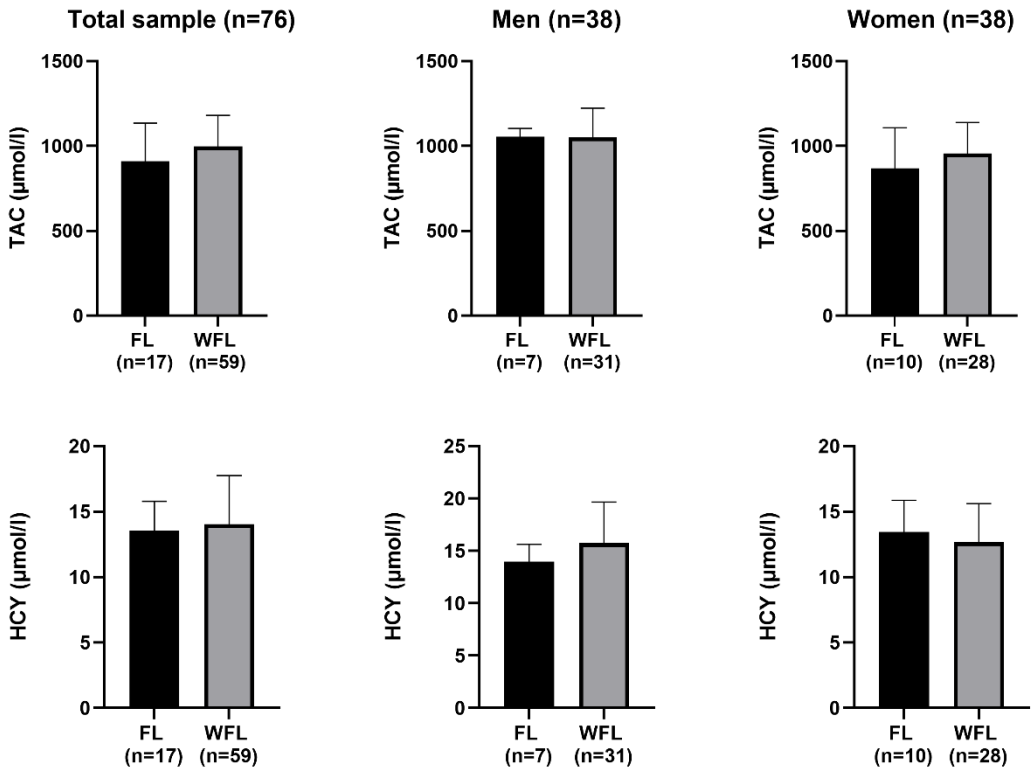

Figure 4. Differences in TAC and HCY levels between participants with functional limitations (FL) and participants without limitations (WFL) based on the SPPB score in the total sample and separately by sex. TAC: Total Antioxidant Capacity; HCY: Homocysteine; SPPB: Short Physical Performance Battery.

Associations of anthropometry and body composition with pro- and anti-oxidative markers

No significant associations between any of the anthropometrics body composition variables and TAC divided by sex were reported. (**Table 4**).

There were associations between fat-free mass and HCY in men and BMI and thigh circumference with HCY in women (both β=0.1, $p<0.05$). Moreover, a trend for a positive association between waist circumference and HYC in women was identified ($p<0.06$) (**Table 5**).

Associations of physical fitness with pro- and anti-oxidative markers

No significant associations were found for any of the physical fitness variables and TAC in men (**Table 6A** and **Table 6B**). Women showed a significant association between 6-meter normal gait speed and TAC after adjusting by age (β=0.3, $p<0.05$) (**Table 6B**).

Concerning pro-oxidative markers, an inverse association between the 6-minute test and HCY was found in both sexes (**Table 7A**). For men, only the relative VO_{2peak} maintained a significant association with pro-oxidant markers (β=-0.4, $p<0.02$) **(Table 7B)**. For women, upper limb flexibility and strength were inversely associated with HCY (β=-0.5 and β=-0.3, both $p<0.05$) and the time completing the 8-foot Up and Go test (β=0.4, $p<0.01$) was also directly associated with HCY.

Dietary assessment

Regarding the dietary assessment, no differences were found between macro and micro intakes in both sexes, except for Vitamin C and D (both $p<0.020$) (**Table 8**). When the TAC and HCY levels were compared between meeting or not the dietary recommendations groups, only Vitamin B6 and K showed significant differences in both parameters (**Table 9**). These four variables were introduced in simple linear regressions to find any associations with TAC, but no significant associations were found, supporting that those statistically significant results found were not due to dietary imbalances.

Table 4. Associations of anthropometry and body composition with TAC (μmol/l) separately by sex.

	TAC (μmol/l)							
	Men (n=38)				Women (n=38)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
BMI (kg/m^2)								
Model 1	-4.016	-0.113	-15.964 ; 7.932	0.500	4.259	0.121	-7.503 ; 16.022	0.467
Model 2	-3.967	-0.115	-16.01 ; 8.07	0.508	0.861	0.025	-10.353 ; 12.074	0.877
Waist circumference (cm)								
Model 1	-2.905	-0.209	-7.49 ; 1.679	0.207	1.308	0.087	-3.743 ; 6.359	0.603
Model 2	-2.793	-0.201	-7.434 ; 1.848	0.230	-0.238	-0.016	-5.048 ; 5.572	0.921
Hip circumference (cm)								
Model 1	-1.383	-0.083	-6.969 ; 4.203	0.619	1.869	0.108	-3.933 ; 7.671	0.518
Model 2	-1.349	-0.081	-6.98 ; 4.28	0.630	0.443	0.026	-5.034 ; 5.922	0.870
Waist/Hip Index								
Model 1	-759.831	-2.272	-1667.822 ; 148.161	0.098	47.125	0.018	-821.842 ; 916.092	0.913
Model 2	-729.992	-0.261	-1654.575 ; 16.940	0.118	-96.367	-0.037	-902.653 ; 709.918	0.810
Thigh circumference (cm)								
Model 1	-3.153	-0.086	-15.462 ; 9.158	0.607	1.097	0.035	-9.393 ; 11.587	0.833
Model 2	-4.281	-0.117	-16.94 ; 8.378	0.497	0.089	0.003	-9.602 ; 9.78	0.985
Fat-Free Mass (kg)								
Model 1	-1.643	-0.758	-8.944 ; 5.659	0.651	7.081	0.149	-8.779 ; 22.954	0.372
Model 2	-3.024	-0.140	-10.902 ; 4.853	0.441	4.420	0.093	-10.193 ; 19.26	0.549

Fat-Free Mass (%)								
Model 1	6.470	0.227	-2.892 ; 15.831	0.170	-4.165	-0.141	-14.045 ; 5.715	0.398
Model 2	6.109	0.215	-2.892 ; 15.831	0.203	-0.602	-0.020	-14.045 ; 5.715	0.899
Fat Mass (kg)								
Model 1	-3.895	-0.209	-10.152 ; 2.362	0.215	4.110	0.188	-3.141 ; 11.361	0.258
Model 2	-3.972	-0.210	-10.273 ; 2.329	0.209	1.355	0.062	-5.787 ; 8.497	0.703
Fat Mass (%)								
Model 1	-6.527	-0.229	-15.885 ; 2.831	0.166	4.178	0.142	-5.688 ; 14.043	0.396
Model 2	-6.165	-0.217	-15.722 ; 3.392	0.199	0.580	0.020	-9.014 ; 10.174	0.903

B, regression coefficient; β means standardized coefficient; BMI, Body Mass Index; CI, confidence interval; TAC, Total Antioxidant Capacity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 5. Associations of anthropometry and body composition with HCY (µmol/l) separately by sex.

	HCY (µmol/l)							
	Men (n=38)				Women (n=38)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
BMI (kg/m^2)								
Model 1	0.109	0.160	-0.114 ; 0.331	0.329	0.189	0.343	0.014 ; 0.363	**0.035**
Model 2	0.104	0.159	-0.117 ; 0.326	0.345	0.175	0.319	-0.006 ; 0.356	**0.050**
Waist circumference (cm)								
Model 1	0.069	0.256	-0.019 ; 0.151	0.126	0.072	0.308	-0.003 ; 0.148	0.060
Model 2	0.061	0.238	-0.024 ; 0.147	0.156	0.066	0.281	-0.012 ; 0.145	0.096
Hip circumference (cm)								
Model 1	0.072	0.237	-0.029 ; 0.172	0.157	0.074	0.275	-0.014 ; 0.162	0.095
Model 2	0.070	0.233	-0.030 ; 0.170	0.162	0.067	0.249	-0.023 ; 0.158	0.139
Waist/Hip Index								
Model 1	6.365	0.119	-11.892 ; 24.623	0.484	6.657	0.165	-6.775 ; 20.080	0.322
Model 2	4.657	0.087	-13.801 ; 23.116	0.611	5.810	0.144	-7.764 ; 19.384	0.391
Thigh circumference (cm)								
Model 1	0.128	0.190	-0.098 ; 0.354	0.259	0.159	0.326	0.003 ; 0.314	**0.046**
Model 2	0.162	0.242	-0.065 ; 0.389	0.155	0.153	0.314	-0.003 ; 0.309	**0.050**
Fat-Free Mass (kg)								
Model 1	0.133	0.332	0.128 ; 0.187	**0.045**	0.176	0.237	0.056 ; 0.317	0.152
Model 2	0.186	0.467	0.086 ; 0.196	**0.007**	0.161	0.216	-0.087 ; 0.409	0.195

Fat-Free Mass (%)								
Model 1	-0.034	-0.065	-0.214 ; 0.145	0.702	-0.120	-0.260	-0.271 ; 0.030	0.115
Model 2	-0.017	-0.033	-0.198 ; 0.164	0.849	-0.011	-0.227	-0.264 ; 0.054	0.189
Fat Mass (kg)								
Model 1	0.055	0.158	-0.063 ; 0.174	0.350	0.100	0.292	-0.011 ; 0.210	0.076
Model 2	0.056	0.161	-0.061 ; 0.174	0.339	0.089	0.261	-0.028 ; 0.207	0.133
Fat Mass (%)								
Model 1	0.036	0.068	-0.144 ; 0.215	0.687	0.121	0.262	-0.03 ; 0.272	0.112
Model 2	0.019	0.036	-0.162 ; 0.200	0.835	0.106	0.229	-0.053 ; 0.265	0.186

B, regression coefficient; β means standardized coefficient; BMI, Body Mass Index; CI, confidence interval; HCY, Homocysteine; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 6A. Associations of physical fitness components with TAC (μmol/l) separately by sex

	TAC (μmol/l)							
	Men (n=38)				Women (n=38)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
	Senior Fitness test Battery							
6-minute walk Test (m)								
Model 1	-0.031	-0.018	-0.611 ; 0.549	0.914	0.455	0.164	-0.456 ; 1.360	0.320
Model 2	-0.139	-0.080	-0.777 ; 0.499	0.662	0.433	0.156	-0.422 ; 1.280	0.311
Sit and Reach Test (cm)								
Model 1	0.376	0.028	-4.164 ; 4.916	0.868	1.191	0.082	-3.602 ; 5.984	0.618
Model 2	0.096	0.007	-4.552 ; 4.744	0.967	0.219	0.015	-4.36 ; 4.799	0.923
Back Scratch Test (cm)								
Model 1	0.762	0.040	-5.266 ; 6.792	0.799	2.571	0.154	-2.918 ; 8.059	0.349
Model 2	0.400	0.022	-5.772 ; 6.575	0.896	1.548	0.092	-3.706 ; 6.804	0.554
30 seconds Chair Stand Test (repetitions)								
Model 1	14.010	0.197	-9.597 ; 37.61	0.237	6.386	0.073	-24.002 ; 36.774	0.672
Model 2	12.670	0.177	-11.955 ; 37.306	0.303	-1.848	-0.021	-30.753 ; 27.058	0.897
Arm Curl Test (repetitions)								
Model 1	3.696	0.152	-4.534 ; 11.927	0.368	1.409	0.062	-6.328 ; 9.146	0.714
Model 2	3.429	0.141	-5.632 ; 12.491	0.447	-0.356	-0.016	-7.751 ; 7.038	0.923
8-foot Up and Go Test (s)								
Model 1	-26.590	-0.197	-71.221 ; 18.04	0.235	-10.608	-0.090	-46.142 ; 24.924	0.549
Model 2	-23.900	-0.081	-71.113 ; 23.31	0.311	-10.286	0.026	-43.577 ; 23.000	0.535

			Short Physical Performance Battery					
Tandem Test (s)								
Model 1	12.627	0.199	-10.872 ; 36.126	0.281	-10.222	-0.057	-71.339 ; 50.896	0.736
Model 2	11.550	0.180	-12.472 ; 35.586	0.230	-12.490	-0.070	-68.136 ; 43.140	0.651
4-meter normal gait speed (m/s)								
Model 1	172.524	0.294	-40.109 ; 385.156	0.108	242.268	0.335	8.833 ; 475.703	**0.042**
Model 2	162.883	0.278	-68.325 ; 394.091	0.160	172.260	0.239	-53.413 ; 397.933	0.130
5-repetition Chair Stand Test (s)								
Model 1	3.919	0.080	-18.142 ; 25.98	0.716	0.831	0.011	-30.050 ; 31.710	0.956
Model 2	5.090	0.104	-17.67 ; 27.848	0.646	-3.670	-0.049	-33.059 ; 25.710	0.799
SPPB score								
Model 1	15.610	0.100	-42.442 ; 73.679	0.587	5.554	0.040	-41.311 ; 52.42	0.811
Model 2	2.992	0.196	-25.764 ; 81.749	0.295	-12.226	-0.089	-56.533 ; 32.081	0.579

B, regression coefficient; β means standardized coefficient; CI, confidence interval; TAC, Total Antioxidant Capacity; SPPB, Short Physical Performance Battery; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 6B. Associations of physical fitness components with TAC (µmol/l) separately by sex.

	TAC (µmol/l)							
	Men (n=38)				Women (n=38)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Other physical evaluations								
6-meter normal gait speed (m/s)								
Model 1	196.845	0.264	-46.169 ; 439.860	0.109	286.654	0.340	22.560 ; 550.748	**0.034**
Model 2	190.916	0.256	-77.029 ; 458.862	0.157	253.512	0.300	2.832 ; 504.192	**0.048**
6-meter fast gait speed (m/s)								
Model 1	100.708	0.181	-84.492 ; 285.908	0.277	119.870	0.214	-62.738 ; 302.478	0.192
Model 2	92.980	0.167	-96.110 ; 282.070	0.325	123.153	0.219	-47.066 ; 293.370	0.151
Handgrip Test (kg)								
Model 1	4.917	0.183	-4.005 ; 13.840	0.271	0.537	0.010	-16.127 ; 17.200	0.948
Model 2	4.344	0.162	-5.807 ; 14.494	0.391	1.038	0.021	-14.635 ; 16.711	0.894
Sit to Stand Test mean velocity (m/s)								
Model 1	-251.621	-0.063	-2052.807 ; 1549.564	0.774	1283.139	0.207	-1263.006 ; 3829.283	0.309
Model 2	-1001.550	-0.252	-3374.817 ; 1371.171	0.389	920.961	0.149	-1458.697 ; 3300.619	0.432
Sit to Stand Test relative power (w/kg)								
Model 1	-62.530	-0.223	-187.547 ; 62.479	0.310	35.709	0.083	-143.601 ; 215.020	0.685
Model 2	-79.091	-0.280	-210.302 ; 52.12	0.223	79.471	0.186	-86.248 ; 244.742	0.332

				Cardiorespiratory fitness				
VO_2peak (ml/min)								
Model 1	0.021	0.046	-0.132 ; 0.175	0.782	-0.048	-0.079	0.252 ; 0.155	0.635
Model 2	0.002	0.005	-0.163 ; 0.168	0.975	-0.103	-0.171	-0.292 ; 0.084	0.273
VO_2peak (ml/kg/min)								
Model 1	6.189	0.185	-4.931 ; 17.309	0.266	-6.617	-0.175	-19.337 ; 0.599	0.292
Model 2	5.880	0.176	-5.388 ; 17.149	0.297	-7.500	-0.197	-19.070 ; 4.070	0.197

B, regression coefficient; β means standardized coefficient; CI, confidence interval; TAC, Total Antioxidant Capacity; VO_2peak, peak oxygen consumption; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 7A. Associations of physical fitness components with HCY (µmol/l) separately by sex.

	HCY (µmol/l)							
	Men (n=38)				Women (n=38)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Senior Fitness test Battery								
6-minute walk Test (m)								
Model 1	-0.013	-0.397	-0.023 ; 0.002	**0.015**	-0.017	-0.392	0.030 ; 0.003	**0.014**
Model 2	-0.012	-0.383	-0.024 ; 0.001	**0.037**	-0.017	-0.390	-0.031 ; 0.004	**0.012**
Sit and Reach Test (cm)								
Model 1	-0.043	-0.171	-0.129 ; 0.420	0.312	-0.062	-0.271	-0.135 ; 0.011	0.095
Model 2	-0.034	-0.133	-0.121 ; 0.053	0.439	-0.072	-0.314	-0.144 ; 0.001	0.054
Back Scratch Test (cm)								
Model 1	-0.060	-0.176	-0.177 ; 0.055	0.298	-0.111	-0.422	-0.190 ; -0.031	**0.007**
Model 2	-0.047	-0.136	-0.166 ; 0.072	0.430	-0.123	-0.467	-0.200 ; -0.045	**0.003**
30 seconds Chair Stand Test (repetitions)								
Model 1	-0.182	-0.136	-0.637 ; 0.273	0.422	-0.337	-0.250	-0.793 ; 0.118	0.141
Model 2	-0.113	-0.084	-0.588 ; 0.361	0.630	-0.423	-0.313	-0.878 ; 0.032	0.068
Arm Curl Test (repetitions)								
Model 1	-0.038	0.000	-0.213 ; 0.019	0.643	-0.097	-0.276	-0.213 ; 0.019	0.098
Model 2	0.007	0.014	-0.175 ; 0.184	0.940	-0.117	-0.332	-0.232 ; -0.002	**0.047**
8-foot Up and Go Test (s)								
Model 1	0.624	0.201	-0.415 ; 1.664	0.231	0.740	0.439	0.235 ; 1.247	**0.005**
Model 2	0.421	0.136	-0.754 ; 1.596	0.472	0.744	0.440	0.243 ; 1.244	**0.005**

	Short Physical Performance Battery							
Tandem Test (s)								
Model 1	0.171	0.119	-0.382 ; 0.724	0.532	-0.499	-0.174	-1.470 ; 0.471	0.303
Model 2	0.237	0.165	-0.330 ; 0.804	0.399	-0.513	-0.179	-1.485 ; 0.458	0.290
4-meter normal gait speed (m/s)								
Model 1	2.460	0.204	-2.117 ; 7.037	0.280	-2.093	-0.180	-6.016 ; 1.829	0.286
Model 2	3.808	0.315	-1.058 ; 8.673	0.120	-2.755	-0.237	-6.774 ; 1.265	0.173
5 repetition Chair Stand Test (s)								
Model 1	0.133	0.012	-17.67 ; 27.848	0.646	0.070	0.060	-33.059 ; 25.715	0.799
Model 2	-0.018	-0.017	-0.540 ; 0.502	0.941	0.069	0.058	-0.429 ; 0.566	0.778
SPPB score								
Model 1	10.282	0.217	-5.330 ; 25.893	0.190	-3.309	-0.052	-24.419 ; 17.801	0.753
Model 2	1.153	0.020	-18.270 ; 20.575	0.905	-8.236	-0.130	-28.227 ; 11.759	0.409

B, regression coefficient; β means standardized coefficient; CI, confidence interval; HCY, Homocysteine; SPPB, Short Physical Performance Battery; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 7B. Associations of physical fitness components with HCY (µmol/l) separately by sex.

	HCY (µmol/l)							
	Men (n=38)				Women (n=38)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Other physical evaluations								
6-meter normal gait speed (m/s)								
Model 1	1.520	0.111	-3.158 ; 6.197	0.514	-3.369	-0.254	-7.650 ; 0.911	0.119
Model 2	3.251	0.237	-1.760 ; 8.264	0.196	-3.700	-0.279	-7.957 ; 0.555	0.086
6-meter fast gait speed (m/s)								
Model 1	0.036	0.068	-0.144 ; 0.215	0.687	0.121	0.262	-0.030 ; 0.272	0.112
Model 2	0.019	0.036	-0.162 ; 0.200	0.835	0.106	0.229	-0.053 ; 0.265	0.186
Handgrip Test (kg)								
Model 1	-0.020	-0.041	-0.188 ; 0.148	0.810	0.018	0.024	-0.241 ; 0.277	0.888
Model 2	0.035	0.071	-0.154 ; 0.224	0.707	0.022	0.020	-0.235 ; 0.280	0.859
Sit to Stand Test mean velocity (m/s)								
Model 1	16.760	0.189	-23.887 ; 57.400	0.400	-16.090	-0.169	-55.640 ; 23.460	0.409
Model 2	46.951	0.529	-3.429 ; 97.330	0.066	-16.236	-0.170	-57.129 ; 24.658	0.420
Sit to Stand Test relative power (w/kg)								
Model 1	0.227	0.036	-2.709 ; 3.164	0.873	-1.310	-0.200	-4.036 ; 1.400	0.327
Model 2	0.596	0.094	-2.495 ; 3.687	0.691	-1.399	-0.212	-4.240 ; 1.444	0.320

	Cardiorespiratory fitness							
VO_2peak (ml/min)								
Model 1	-0.001	-0.148	-0.004 ; 0.002	0.381	0.000	0.009	-0.003 ; 0.003	0.953
Model 2	-0.001	-0.083	-0.004 ; 0.003	0.645	-0.002	-0.027	-0.004 ; 0.003	0.336
VO_2peak (ml/kg/min)								
Model 1	-0.263	-0.413	-0.478 ; -0.064	**0.011**	-0.115	-0.187	-0.309 ; 0.086	0.261
Model 2	-0.251	-0.394	-0.467 ; -0.050	**0.016**	-0.117	-0.197	-0.315 ; 0.082	0.236

B, regression coefficient; β means standardized coefficient; CI, confidence interval; HCY, Homocysteine; VO_2peak, peak oxygen consumption; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 8. Nutritional Intake by total sample and by sex.

	Total (n=76)	Men (n=38)	Women (n=38)	p
Energy (kcal)	1780.85 ± 392.22	1762.10 ± 392.22	1796.57 ± 351.59	0.704
Water (ml)	1242.54 ± 401.66	1196.90 ± 407.54	1280.78 ± 398.19	0.190
Protein (g)	77.77 ± 20.49	78.29 ± 23.34	77.33 ± 18.08	0.850
Carbohydrates (g)	162.54 ± 38.49	157.70 ± 42.88	166.59 ± 34.46	0.346
Lipids (g)	82.33 ± 21.54	81.08 ± 20.29	83.36 ± 22.75	0.707
Cholesterol (mg)	288.87 ± 112.08	294.22 ± 124.08	284.38 ± 102.49	0.605
Vitamin B1 (mg)	1.18 ± 0.32	1.17 ± 0.33	1.19 ± 0.31	0.578
Vitamin B2 (mg)	1.44 ± 0.34	1.40 ± 0.34	1.47 ± 0.33	0.353
Vitamin B6 (mg)	1.96 ± 0.65	1.93 ± 0.73	1.99 ± 0.57	0.218
Vitamin B12 (mg)	5.34 ± 4.21	4.85 ± 2.48	5.76 ± 5.24	0.542
Vitamin C (mg)	136.21 ± 66.01	116.06 ± 59.59	153.08 ± 67.14	**0.020**
Vitamin A (µg)	875.03 ± 531.45	830.06 ± 626.62	912.70 ± 441.86	0.527
Retinol (µg)	388.26 ± 304.99	395.71 ± 317.70	382.03 ± 298.19	0.707
Carotens (µg)	2622.90 ± 2634.46	2348.71 ± 3139.85	2852.62 ± 2140.90	0.064
Vitamin D (µg)	4.73 ± 9.93	2.90 ± 3.70	6.26 ± 12.92	**0.020**
Vitamin E (µg)	8.00 ± 3.05	7.71 ± 2.98	8.25 ± 3.13	0.399
Vitamin K (µg)	139.77 ± 65.86	145.81 ± 78.17	134.71 ± 54.07	0.902

Values are expressed as mean ± SD. Significant differences appeared in bold.

Table 9. TAC and HCY levels by meeting sex-specific nutritional recommendations.

	n	Meet the recommendations	n	Not meeting the recommendations	p
TAC in Water recommendations (umol/l)	4	951.04 ± 187.23	72	1116.13 ± 1117.78	0.088
TAC in Protein recommendations (umol/l)	64	948.9 ± 187.29	12	1022.26 ± 184.05	0.237
TAC in Carbohydrates recommendations (umol/l)	4	941.82 ± 378.9	72	961.94 ± 174.6	0.837
TAC in Lipids recommendations (umol/l)	8	990.39 ± 245.96	68	957.36 ± 181.79	0.662
TAC in Cholesterol recommendations (umol/l)	44	945.45 ± 199.31	32	981.36 ± 171.32	0.439
TAC in Vitamin B6 recommendations (umol/l)	58	931.4 ± 190.02	18	1056.21 ± 146.19	**0.019**
TAC in Vitamin B12 recommendations (umol/l)	75	958.75 ± 188.06	1	1095.53 ± N.A	N.A
TAC in Vitamin C recommendations (umol/l)	63	959.56 ± 180.29	13	966.38 ± 226.53	0.910
TAC in Vitamin A recommendations (umol/l)	49	931.52 ± 177.41	27	1011.06 ± 196.93	0.092
TAC in Vitamin D recommendations (umol/l)	8	1053.92 ± 227.79	68	951.74 ± 182.7	0.205
TAC in Vitamin E recommendations (umol/l)	1	1015.55 ± N.A	75	959.95 ± 188.68	N.A
TAC in Vitamin K recommendations (umol/l)	53	928.03 ± 171.68	23	103.03 ± 204.16	**0.030**
HCY in Water recommendations (umol/l)	4	12.48 ± 2.31	72	13.89 ± 3.62	0.445
HCY in Protein recommendations (umol/l)	64	13.60 ± 3.77	12	14.31 ± 2.66	0.511
HCY in Carbohydrates recommendations (umol/l)	4	13.83 ± 3.09	72	13.80 ± 3.60	0.900
HCY in Lipids recommendations (umol/l)	8	12.67 ± 3.42	68	13.94 ± 3.56	0.378
HCY in Cholesterol recommendations (umol/l)	44	13.59 ± 2.87	32	14.08 ± 4.34	0.572
HCY in Vitamin B6 recommendations (umol/l)	58	13.31 ± 3.48	18	15.53 ± 3.41	**0.032**
HCY in Vitamin B12 recommendations (umol/l)	75	13.78 ± 3.58	1	15.15 ± N.A	N.A
HCY in Vitamin C recommendations (umol/l)	63	13.91 ± 3.79	13	13.28 ± 2.09	0.593
HCY in Vitamin A recommendations (umol/l)	49	13.52 ± 3.98	27	14.32 ± 2.65	0.379
HCY in Vitamin D recommendations (umol/l)	8	15.22 ± 6.62	68	13.66 ± 3.17	0.312
HCY in Vitamin E recommendations (umol/l)	1	8.16 ± N.A	75	13.89 ± 3.52	N.A
HCY in Vitamin K recommendations (umol/l)	53	12.79 ± 2.51	23	16.19 ± 4.50	**0.002**

Values are expressed as mean ± SD. Significant differences appeared in bold. HCY, Homocysteine; N.A, not applied; TAC, Total Antioxidant Capacity.

Study 2: Sex-specific relationship between physical activity and sedentary behaviour with oxidative stress in older adults

The sociodemographic characteristics of the participants are presented in **Table 10.**

Mean values of PA, SB, and oxidative stress outcomes are presented in **Table 11** with sex comparisons.

Most of the PA and SB behaviours were similar between the sexes, with no significant differences in self-reported PA or SB.

Similar to this, accelerometer data were similar between the sexes. However, women spent significantly more total time SB and 30 to 45 and 45 to 60 minutes SB compared to men (+95.39 ± 38.8, +21.53 ± 0.4, and +14.9 ± 3.7 minutes, respectively) whereas men spent significantly more time doing VPA (+3.29 ± 5.5 minutes). Respecting oxidative stress, men showed higher values ($p<0.001$) than women for antioxidants (+156.56 ± 10.8 μmol/l) and HCY (+3.16 ± 0.78 μmol/l), with both differences showing a large effect using Cohen's d coefficients.

Considering self-report PA, only 21 participants (31.8 %) met the PA recommendations, with 12 men (40.0 %) and 9 women (25 %). No significant differences were found in oxidative stress values between the participants who met the recommendations and those who did not (**Figure 5**). According to the accelerometer method, 32 participants (48.5 %) met the recommendations achieving >150 min/week of MVPA, 16 men (53.3 %) and 16 women (44.44 %). No significant differences were found in oxidative stress values between the participants who met the recommendations and those who did not (**Figure 6**).

Table 10. Sociodemographic characteristics by total sample and by sex.

	Total (n=66)	Men (n=30)	Women (n=36)	p
Marital Status				
Single (%)	4 (6.06)	0 (0)	4 (11.11)	0.370
Married or with a couple (%)	49 (74.24)	24 (80)	25 (69.44)	
Widowed (%)	8 (74.24)	3 (10)	5 (13.89)	
Legally separated or divorced (%)	5 (7.58)	3 (10)	2 (5.56)	
Level of education completed				
Without studies (%)	8 (12.12)	2 (6.67)	6 (16.67)	0.696
Primary (%)	26 (39.39)	11 (36.67)	15 (41.67)	
Secondary/Job Training (%)	22 (33.33)	13 (40)	10 (27.78)	
University Studies (%)	10 (15.15)	5 (16.67)	5 (13.89)	
Contributory pension				
Own quotation (%)	42 (63.64)	30 (100)	12 (33.33)	**0.001**
Quotation from another person (%)	2 (3.03)	0 (0)	2 (5.56)	
Both own and from another person (%)	0 (0)	0 (0)	0 (0)	
No quotation (%)	22 (33.33)	0 (0)	22 (61.11)	
Smoking status				
Current smoker (%)	4 (6.06)	4 (6.06)	0 (0)	**0.047**
Non-smoker (%)	62 (93.94)	26 (86.67)	36 (100)	
Alcohol status				
Regular drinker (%)	16 (24.20)	11 (36.7)	5 (13.9)	0.078
Occasional drinker (%)	29 (43.90)	12 (40.0)	17 (47.2)	
Abstemious (%)	21 (31.81)	7 (23.3)	12 (38.9)	

Table 11. Participant characteristics of the total sample and by sex.

	Total (n=66)	Men (n=30)	Women (n=36)	p	ES
General Characteristics					
Age (years)	68.68 ± 3.02	68.5 ± 3.02	68.82 ± 3.05	0.903	0.0
Height (cm)	159.08 ± 9.33	165.99 ± 7.45	153.31 ± 6.37	**<0.001**	1.8
Weight (kg)	74.24 ± 14.6	82.9 ± 14.36	67.02 ± 10.37	**<0.001**	1.3
BMI (kg/m^2)	29.24 ± 4.63	30.01 ± 4.27	28.6 ± 4.63	0.222	0.3
Self-reported PA and SB					
Self-reported MPA (min/day)	28.47 ± 56.00	24.26 ± 57.15	32.03 ± 55.67	0.560	-0.1
Self-reported VPA (min/day)	2.34 ± 11.34	1.84 ± 6.52	2.82 ± 14.68	0.701	-0.1
Self-reported MVPA (min/day)	24.16 ± 52.11	19.08 ± 52.01	29.1 ± 54.41	0.167	-0.2
Self-reported SB (min/day)	340.35 ± 148.32	370.41 ± 170.4	313.94 ± 122.44	0.136	0.4
Accelerometer-measured PA and SB levels					
Total LPA (min/day)	281.02 ± 138.94	298.55 ± 159.37	264.07 ± 119.69	0.650	0.2
>10 min LPA (min/day)	167.61 ± 146.19	194.49 ± 165.14	145.2 ± 146.19	0.349	0.3
5-10 min LPA (min/day)	55.69 ± 19.83	51.36 ± 20.15	59.3 ± 19.09	0.106	-0.4
1-5 min LPA (min/day)	53.31 ± 15.45	50.7 ± 14.94	55.49 ± 15.73	0.212	-0.3
Total MPA (min/day)	26.97 ± 22.10	30.64 ± 22.1	23.91 ± 16.41	0.181	0.3
Total VPA (min/day)	1.98 ± 5.71	3.77 ± 7.77	0.48 ± 2.34	**<0.001**	0.6
Total MVPA (min/day)	27.02 ± 19.99	30.52 ± 21.74	24.31 ± 18.4	0.211	0.3
>10 min MVPA (min/day)	15.32 ± 18.70	18.79 ± 23.34	12.42 ± 2.23	0.458	0.2
5-10 min MVPA (min/day)	6.12 ± 5.32	6.8 ± 5.44	5.56 ± 5.22	0.366	0.2
1-5 min MVPA (min/day)	7.13 ± 4.28	7.98 ± 4.27	6.41 ± 4.2	0.140	0.4
Total SB (min/day)	556.29 ± 155.48	504.27 ± 169.03	599.65 ± 130.25	**0.012**	-0.6
10-20 min SB (min/day)	61.63 ± 22.31	57.19 ± 22.17	65.34 ± 22.05	0.141	-0.4
20-30 min SB (min/day)	50.68 ± 20.03	50.62 ± 23.75	50.73 ± 16.65	0.983	0.0
30-40 min SB (min/day)	57.34 ± 25.64	45.81 ± 23.68	67.34 ± 23.23	**<0.001**	-0.9
45-60 min SB (min/day)	53.37 ± 28.84	45.24 ± 25.99	60.15 ± 29.67	**0.041**	-0.5
>60 min SB (min/day)	254.34 ± 148.68	228.29 ± 148.97	276.05 ± 146.98	0.252	-0.3
Accelerometer-measured PA and SB bouts					
>10 min LPA (bouts)	6.07 ± 3.98	6.67 ± 4.19	5.56 ± 3.79	0.427	0.2
5-10 min LPA (bouts)	8.35 ± 2.92	7.67 ± 2.83	8.92 ± 2.91	0.084	-0.4
1-5 min LPA (bouts)	30.57 ± 8.86	29.36 ± 8.59	31.57 ± 8.86	0.309	-0.3
>10 min MVPA (bouts)	0.60 ± 0.68	0.74 ± 0.82	0.49 ± 0.53	0.588	0.4
5-10 min MVPA (bouts)	1.17 ± 0.95	1.28 ± 0.98	1.07 ± 0.93	0.342	0.2
1-5 min MVPA (bouts)	5.29 ± 2.98	5.99 ± 3.33	4.71 ± 2.57	0.069	0.5
10-20 min (bouts)	4.56 ± 1.65	4.29 ± 1.64	4.79 ± 1.64	0.216	-0.3
20-30 min SB (bouts)	2.13 ± 0.83	2.13 ± 0.98	2.12 ± 0.69	0.960	0.1
30-40 min SB (bouts)	1.63 ± 0.73	1.31 ± 0.69	1.9 ± 0.65	**<0.001**	-0.9
45-60 min SB (bouts)	1.06 ± 0.57	0.91 ± 0.51	1.19 ± 0.59	**0.041**	-0.5
>60 min SB (bouts)	2.43 ± 1.22	2.13 ± 1.27	2.69 ± 1.13	0.064	-0.5

		Fragmentation metrics			
TPSB2PA (%)	6.51 ± 2.40	6.45 ± 2.54	6.55 ± 2.39	0.877	0.0
TPSB2LPA (%)	6.44 ± 2.50	6.28 ± 2.4	6.55 ± 2.55	0.682	-0.1
TPSB2MVPA (%)	0.03 ± 0.05	0.04 ± 0.06	0.03 ± 0.04	0.604	0.2
TPPA2SB (%)	17.66 ± 6.90	16.13 ± 7	18.95 ± 6.5	0.098	-0.4
Sedentary Gini Index	0.67 ± 0.04	0.65 ± 0.04	0.67 ± 0.04	**0.026**	-0.6
PA Gini Index	0.57 ± 0.06	0.59 ± 0.05	0.56 ± 0.06	**0.019**	0.6
SB Gini Index	0.67 ± 0.04	0.65 ± 0.04	0.67 ± 0.04	**0.026**	-0.6
		Oxidative Stress			
TAC (µmol/l)	967.55 ± 184.30	1053.17 ± 171.52	896.61 ± 171.51	**<0.001**	1.0
HCY (µmol/l)	13.89 ± 3.54	15.61 ± 3.32	12.46 ± 2.54	**<0.001**	1.0

Values are expressed as mean ± SD, BMI, Body Mass Index; ES, Effect Size (Cohen's D); HCY, Homocysteine; LPA, light physical activity; MPA, Moderate physical activity; MVPA, Moderate to vigorous physical activity; SB, Sedentary behaviour; TAC, Total Antioxidant Capacity; TPPA2SB, Probability to transicionate from physical activity to sedentary; TPSB2LPA, Probability to transicionate from sedentary to light physical activity; TPSB2MVPA, Probability to transicionate from sedentary to moderate to vigorous physical activity; TPSB2PA, Probability to transicionate from sedentary to physical activity; VPA, Vigorous physical activity.

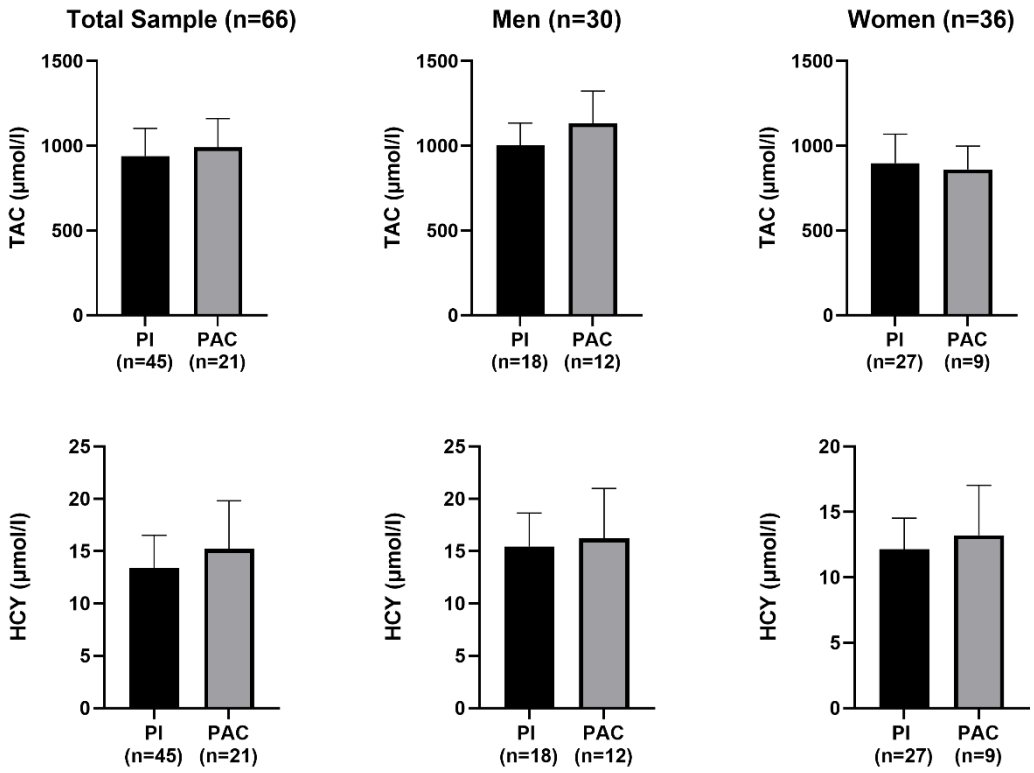

Figure 5. Differences in TAC and HCY levels between physically inactive (PI, <150 minutes of MVPA per week) and physically active (PAC, >150 minutes of MVPA per week) participants based on the Global Physical Activity Questionnaire in the total sample and separately by sex. TAC: Total Antioxidant Capacity; HCY: Homocysteine.

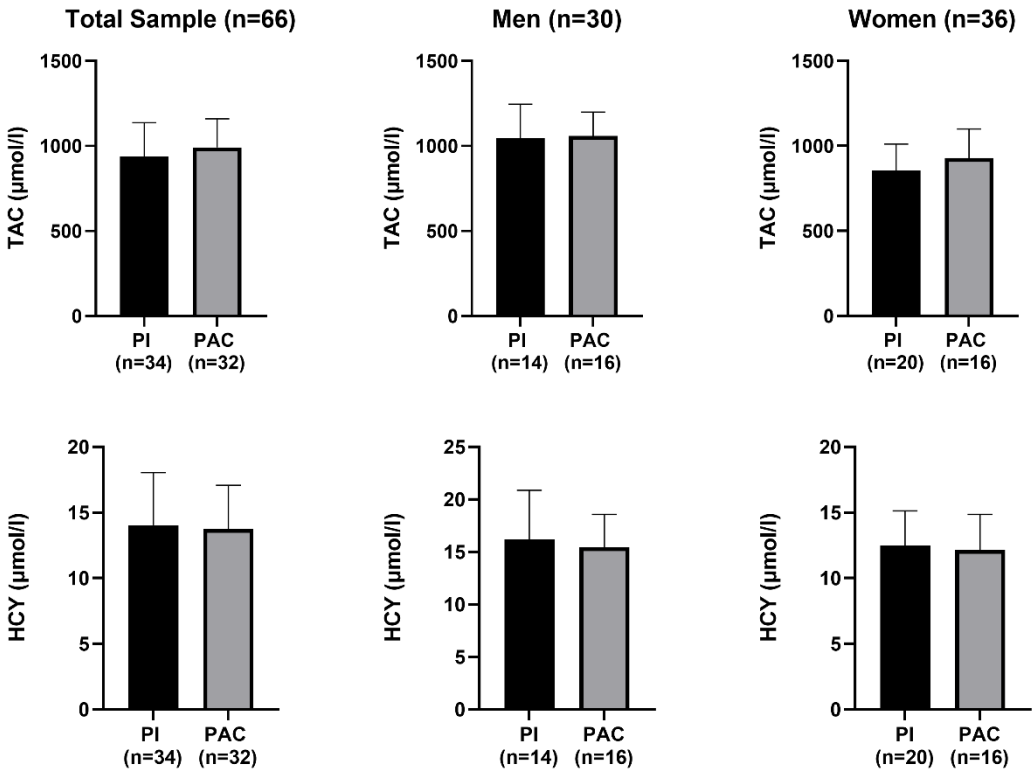

Figure 6. Differences in TAC and HCY levels between physically inactive (PI, <150 minutes of MVPA per week) and physically active (PAC, >150 minutes of MVPA per week) and participants based on accelerometer measured data in the total sample and separately by sex. TAC: Total Antioxidant Capacity; HCY: Homocysteine.

Associations of PA and SB with levels of oxidative stress markers.

The relationship between self-reported PA levels and SB with oxidative stress markers (TAC and HCY) is shown in **Table 12** and **Table 13**. No significant associations were found between any of the self-reported behaviours (PA and SB) and oxidative stress values.

Regarding accelerometer-measured variables, the associations between PA and SB levels and TAC can be found in **Table 14** and **Table 15**, respectively. Only total MVPA levels were positively associated with TAC in men whereas MVPA levels between 1 and 10 minutes were positively associated with TAC in women. No significant associations were found neither for PA or SB bouts, except for MVPA bouts between 5 and 10 minutes for women, nor fragmentation metrics with TAC in both sexes (**Table 16 and Table 17**).

Concerning HCY, LPA time between 1 and 5 minutes was significantly inversely associated with HCY in men whereas total LPA and LPA longer than 10 minutes were inversely associated with HCY in women (**Table 18)**. No significant associations between SB levels and HCY were found (**Table 19).** Regarding PA or SB bouts, only bouts of LPA time between 1 and 5 minutes remained significantly associated with HCY in men after adjusting by age **(Table 20).**

Additionally, the probability of transitioning from PA behaviour to SB was positively associated with HCY **(Table 21).**

Dietary assessment

Regarding the dietary assessment, no differences were found between macro and micro intakes in both sexes, except for Vitamin C (p=0.015) (**Table 21**). When the TAC and HCY levels were compared between meeting or not the dietary recommendations groups, there were only differences in TAC and HCY levels between the participants who met the nutritional recommendations of Vitamin B6 and Vitamin K, similar to **Study 1** (**Table 22**). Consequently, the three significant variables were introduced in simple linear regressions to find any associations with TAC, but no significant associations were found, supporting that those statistically significant results which may appear in our participants were not due to dietary imbalances.

Table 12. Associations of self-reported PA and SB levels with TAC (μmol/l) separately by sex.

	TAC (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Self-reported MPA (min/day)								
Model 1	-0.004	0.128	-0.673 ; 1.286	0.526	-0.444	-0.138	-1.633 ; 0.746	0.452
Model 2	0.326	0.136	-0.730 ; 1.381	0.530	-0.220	-0.069	-1.349 ; 0.907	0.692
Self-reported VPA (min/day)								
Model 1	-0.555	-0.023	-8.761 ; 7.650	0.892	-0.440	-0.038	-4.334 ; 3.455	0.820
Model 2	-0.543	-0.022	-8.812 ; 7.726	0.895	-1.001	-0.086	-4.665 ; 2.660	0.583
Self-reported MVPA (min/day)								
Model 1	0.065	0.021	-0.963 ; 1.093	0.899	-0.299	-0.091	-1.386 ; 0.788	0.580
Model 2	0.007	0.002	-1.043 ; 1.057	0.989	-0.140	-0.043	-1.170 ; 0.891	0.785
Self-reported SB (min/day)								
Model 1	0.162	0.159	-0.236 ; 0.560	0.410	0.029	0.024	-0.422 ; 0.481	0.896
Model 2	0.172	0.168	-0.227 ; 0.571	0.388	0.066	0.054	-0.308 ; 0.441	0.720

B, regression coefficient; β means standardized coefficient; CI, confidence interval; MPA, Moderate physical activity; MVPA, Moderate to vigorous physical activity; SB, Sedentary behaviour TAC: Total Antioxidant Capacity; VPA, Vigorous physical activity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 13. Associations of self-reported PA and SB levels with HCY (μmol/l) separately by sex.

	HCY (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Self-reported MPA (min/day)								
Model 1	-0.008	-0.168	-0.029 ; 0.124	0.411	-0.044	-0.099	-0.021 ; 0.012	0.591
Model 2	-0.004	-0.075	-0.026 ; 0.018	0.725	-0.002	-0.045	-0.018 ; 0.014	0.801
Self-reported VPA (min/day)								
Model 1	0.047	0.106	-0.104 ; 0.198	0.532	-0.027	-0.144	-0.087 ; 0.034	0.382
Model 2	0.047	0.106	-0.102 ; 0.199	0.537	-0.031	-0.170	-0.092 ; 0.029	0.303
Self-reported MVPA (min/day)								
Model 1	-0.006	-0.102	-0.025 ; 0.013	0.547	-0.008	-0.147	-0.024 ; 0.009	0.372
Model 2	-0.004	-0.069	-0.023 ; 0.015	0.674	-0.006	-0.124	-0.236 ; 0.011	0.453
Self-reported SB (min/day)								
Model 1	-0.005	-0.338	-0.100 ; 0.006	0.078	0.000	-0.006	-0.008 ; 0.008	0.974
Model 2	-0.005	-0.352	-0.105 ; 0.000	0.065	0.000	0.005	-0.008 ; 0.008	0.977

B, regression coefficient; β means standardized coefficient; CI, confidence interval; HCY, Homocysteine; MPA, Moderate physical activity; MVPA, Moderate to vigorous physical activity; SB, Sedentary behaviour; VPA, Vigorous physical activity. Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 14. Associations of accelerometer-measured PA levels with TAC (μmol/l) separately by sex.

	TAC (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Total LPA (min/day)								
Model 1	-0.083	-0.087	-0.448 ; 0.283	0.646	0.652	0.047	-0.415 ; 0.545	0.784
Model 2	-0.079	-0.083	-0.449 ; 0.291	0.666	0.092	0.067	-0.335 ; 0.519	0.665
>10 min LPA (min/day)								
Model 1	-0.070	-0.076	-0.423 ; 0.284	0.689	0.084	0.065	-0.37 ; 0.539	0.708
Model 2	-0.065	-0.071	-0.423 ; 0.293	0.711	0.084	0.064	-0.321 ; 0.488	0.676
5-10 min LPA (min/day)								
Model 1	0.484	0.065	-2.412 ; 3.380	0.735	-0.978	-0.113	-3.971 ; 2.015	0.511
Model 2	0.386	0.051	-2.56 ; 2.508	0.790	-0.219	-0.025	-2.947 ; 2.508	0.871
1-5 min LPA (min/day)								
Model 1	-0.890	-0.088	-4.787 ; 3.007	0.643	-0.960	-0.092	-4.599 ; 2.680	0.595
Model 2	-1.005	-0.099	-4.959 ; 2.949	0.606	-0.490	-0.047	-3.754 ; 2.770	0.762
Total MPA (min/day)								
Model 1	-0.219	-0.030	-2.966 ; 2.527	0.871	1.752	0.166	-1.885 ; 5.389	0.334
Model 2	-0.674	-0.095	-3.689 ; 2.340	0.650	1.135	0.107	2.150 ; 4.420	0.487
Total VPA (min/day)								
Model 1	-13.871	-0.269	-33.079 ; 5.338	0.150	13.123	0.211	-8.028 ; 34.274	0.216
Model 2	-14.850	-0.288	-34.316 ; 4.616	0.129	8.964	0.144	-10.249 ; 28.178	0.349

Total MVPA (min/day)								
Model 1	2.678	0.439	0.421 ; 4.935	**0.022**	1.449	0.159	-1.733 ; 4.631	0.361
Model 2	2.637	0.432	0.181 ; 5.093	**0.036**	0.924	0.102	-1.928 ; 3.776	0.514
>10 min MVPA (min/day)								
Model 1	0.362	0.056	-2.139 ; 2.863	0.769	0.041	0.003	-4.254 ; 4.335	0.985
Model 2	0.137	0.021	-2.514 ; 2.788	0.916	-1.149	-0.093	-5.024 ; 2.727	0.551
5-10 min MVPA (min/day)								
Model 1	1.611	0.058	-9.110 ; 12.332	0.761	10.268	0.324	-0.161 ; 20.697	**0.050**
Model 2	1.004	0.036	-10.040 ; 12.055	0.853	10.037	0.317	0.886 ; 19.180	**0.033**
1-5 min MVPA (min/day)								
Model 1	3.615	0.102	-9.994 ; 17.220	0.591	10.768	0.274	-2.39 ; 23.926	0.105
Model 2	1.700	0.048	-14.843 ; 18.240	0.835	12.853	0.327	1.485 ; 24.225	**0.028**

B, regression coefficient; β means standardized coefficient; CI, confidence interval; MPA, Moderate physical activity; MVPA, Moderate to vigorous physical activity; TAC, Total Antioxidant Capacity; VPA, Vigorous physical activity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 15. Associations of accelerometer-measured SB levels with TAC (μmol/l) separately by sex.

	TAC (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Total SB (min/day)								
Model 1	0.023	0.057	-0.418 ; 0.465	0.915	0.023	0.018	-0.418 ; 0.465	0.915
Model 2	0.075	0.084	-0.280 ; 0.430	0.667	0.083	0.066	-0.311 ; 0.477	0.671
10-20 min SB (min/day)								
Model 1	-0.392	-0.058	-3.025 ; 2.241	0.763	-0.973	-0.130	-3.558 ; 1.613	0.450
Model 2	-0.094	-0.014	-2.959 ; 2.770	0.947	-0.456	-0.061	-2.797 ; 1.884	0.694
20-30 min SB (min/day)								
Model 1	0.200	0.031	-2.260 ; 2.660	0.869	0.449	0.045	-3.001 ; 3.899	0.793
Model 2	0.265	0.042	-2.232 ; 2.762	0.830	1.571	0.158	-1.532 ; 4.673	0.310
30-40 min SB (min/day)								
Model 1	-0.290	-0.045	-2.757 ; 2.177	0.811	0.958	0.135	-1.496 ; 3.411	0.433
Model 2	-0.109	-0.017	-2.679 ; 2.461	0.931	1.444	0.203	-0.721 ; 3.609	0.184
45-60 min SB (min/day)								
Model 1	-0.502	-0.086	-2.743 ; 1.739	0.650	0.354	0.064	-1.58 ; 2.289	0.712
Model 2	-0.590	-0.101	-2.869 ; 1.689	0.600	-0.268	-0.048	-2.037 ; 1.501	0.760

>60 min SB (min/day)								
Model 1	0.083	0.082	-0.308 ; 0.474	0.667	0.026	0.024	-0.365 ; 0.418	0.892
Model 2	0.107	0.106	-0.293 ; 0.508	0.588	0.047	0.042	-0.300 ; 0.396	0.784

B, regression coefficient; β means standardized coefficient; CI, confidence interval; SB, Sedentary behaviour; LPA, light physical activity TAC, Total Antioxidant Capacity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 16. Associations of accelerometer-measured PA and SB bouts with TAC (μmol/l) separately by sex.

	TAC (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
>10 min LPA (bouts)								
Model 1	2.160	0.059	-11.767 ; 16.092	0.753	-0.359	-0.008	-15.552 ; 14.830	0.962
Model 2	2.513	0.070	-11.610 ; 16.639	0.718	0.994	0.022	-12.558 ; 14.551	0.882
5-10 min LPA (bouts)								
Model 1	0.351	0.007	-20.310 ; 21.010	0.973	-4.612	-0.081	-24.310 ; 15.085	0.637
Model 2	-0.431	-0.008	-21.483 ; 20.620	0.967	-0.220	-0.004	-18.046 ; 17.608	0.980
1-5 min LPA (bouts)								
Model 1	-1.624	-0.092	-8.403 ; 5.155	0.627	-0.088	-0.047	-7.365 ; 5.602	0.784
Model 2	-1.921	-0.109	-8.822 ; 4.980	0.573	0.054	0.003	-5.757 ; 5.865	0.985
>10 min MVPA (bouts)								
Model 1	9.401	0.051	-62.211 ; 81.030	0.790	3.290	0.010	-106.709 ; 113.281	0.952
Model 2	3.321	0.017	-72.116 ; 78.758	0.929	-30.880	-0.097	-130.570 ; 68.800	0.533
5-10 min MVPA (bouts)								
Model 1	5.620	0.036	-53.788 ; 65.045	0.848	57.737	0.324	-0.967 ; 116.442	**0.050**
Model 2	1.260	0.008	-60.560 ; 63.101	0.967	52.220	0.293	0.031 ; 104.420	**0.050**

1-5 min MVPA (bouts)								
Model 1	5.312	0.117	-12.128 ; 22.752	0.538	16.339	0.254	-5.343 ; 38.021	0.135
Model 2	3.494	0.077	-16.290 ; 23.257	0.721	15.518	0.241	-3.677 ; 34.714	0.110
10-20 min SB (bouts)								
Model 1	-5.550	-0.060	-41.196 ; 30.078	0.752	-12.408	-0.124	-47.109 ; 22.290	0.472
Model 2	-1.561	-0.017	-40.350 ; 37.230	0.935	-7.480	-0.074	-38.672 ; 23.710	0.629
20-30 min SB (bouts)								
Model 1	5.852	0.038	-53.900 ; 65.612	0.842	5.887	0.025	-77.803 ; 89.577	0.887
Model 2	7.627	0.050	-53.027 ; 68.371	0.797	33.867	0.141	-41.710 ; 109.451	0.369
30-40 min SB (bouts)								
Model 1	-7.420	-0.034	-92.324 ; 77.48	0.859	46.342	0.184	-40.156 ; 132.840	0.284
Model 2	-1.586	-0.007	-89.620 ; 86.440	0.971	58.660	0.232	-17.261 ; 134.587	0.125
45-60 min SB (bouts)								
Model 1	-15.457	-0.052	-129.560 ; 98.650	0.783	18.223	0.065	-79.230 ; 115.680	0.706
Model 2	-21.400	-0.073	-138.030 ; 95.220	0.709	-9.180	-0.032	-97.870 ; 79.500	0.834
>60 min SB (bouts)								
Model 1	-1.771	-0.015	-47.741 ; 44.199	0.938	6.748	0.046	-44.139 ; 57.636	0.789
Model 2	1.578	0.013	-46.117 ; 49.274	0.946	8.640	0.059	-36.628 ; 53.910	0.700

B, regression coefficient; β means standardized coefficient; CI, confidence interval; LPA, light physical activity; MVPA, Moderate to vigorous physical activity; SB, Sedentary behaviour; TAC, Total Antioxidant Capacity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 17. Associations of accelerometer-measured fragmentation metrics with TAC (µmol/l) separately by sex.

	TAC (µmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
TPPA2SB (%)								
Model 1	59.892	0.028	-765.910 ; 885.700	0.883	96.181	0.038	-776.940 ; 969.770	0.824
Model 2	51.023	0.023	-785.278 ; 887.325	0.901	145.161	0.057	-631.949 ; 922.270	0.706
TPSB2PA (%)								
Model 1	486.831	0.083	-2030.989 ; 3004.650	0.693	-819.642	-0.117	-3285.120 ; 1645.837	0.504
Model 2	394.464	0.067	-2245.043 ; 3033.971	0.760	-436.517	-0.062	-2651.556 ; 178.523	0.691
TPSB2LPA (%)								
Model 1	542.137	0.091	-2029.783 ; 3114.057	0.667	-813.729	-0.126	-3053.195 ; 1425.738	0.465
Model 2	454.820	0.076	-2232.949 ; 3142.597	0.729	-532.328	-0.082	-2541.841 ; 1477.184	0.594
TPSB2MVPA (%)								
Model 1	-7558.092	-0.031	-103973.100 ; 88856.930	0.873	35276.660	0.096	-98404.770 ; 168958.100	0.594
Model 2	-21896.950	-0.089	-127352.500 ; 83558.610	0.673	20018.420	0.054	-98273.270 ; 138310.100	0.732
PA Gini Index								
Model 1	392.200	0.133	-788.991 ; 1573.390	0.501	-106.532	-0.040	-17.340 ; 804.280	0.814
Model 2	389.018	0.132	-808.423 ; 1586.590	0.510	-271.500	-0.104	-1084.119 ; 541.120	0.501

SB Index								
Model 1	664.474	0.188	-731.188 ; 2060.137	0.337	158.637	0.031	-1598.091 ; 1915.365	0.855
Model 2	612.856	0.174	-826.781 ; 2052.490	0.389	-102.455	-0.020	-1675.957 ; 1471.047	0.895

B, regression coefficient; β means standardized coefficient; CI, confidence interval; TAC, Total Antioxidant Capacity; TPPA2SB, Probability to transicionate from physical activity to sedentary; TPSB2LPA, Probability to transicionate from sedentary to light physical activity; TPSB2PA, Probability to transicionate from sedentary to physical activity; TPSB2MVPA, Probability to transicionate from sedentary to moderate to vigorous physical activity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 18. Associations of accelerometer-measured PA levels with HCY (μmol/l) separately by sex.

	HCY (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Total LPA (min/day)								
Model 1	0.000	0.010	-0.007 ; 0.008	0.959	-0.009	-0.386	-0.016 ; -0.014	**0.020**
Model 2	0.000	0.005	-0.007 ; 0.007	0.980	-0.008	-0.381	-0.016 ; -0.001	**0.022**
>10 min LPA (min/day)								
Model 1	0.001	0.081	-0.006 ; 0.009	0.677	-0.008	-0.381	-0.015 ; -0.001	**0.022**
Model 2	0.001	0.073	-0.006 ; 0.008	0.700	-0.008	-0.381	-0.015 ; -0.001	**0.022**
5-10 min LPA (min/day)								
Model 1	-0.048	-0.318	-0.105 ; 0.009	0.093	-0.021	-0.154	-0.068 ; 0.026	0.368
Model 2	-0.043	-0.286	-0.100 ; 0.013	0.128	-0.018	-0.131	-0.066 ; 0.030	0.456
1-5 min LPA (min/day)								
Model 1	-0.100	-0.480	-0.172 ; -0.028	**0.008**	0.008	0.048	-0.050 ; 0.066	0.781
Model 2	-0.094	-0.453	-0.166 ; -0.022	**0.012**	0.011	0.063	-0.048 ; 0.069	0.718
Total MPA (min/day)								
Model 1	-0.015	-0.107	-0.072 ; 0.041	0.579	-0.037	-0.220	-0.094 ; 0.020	0.198
Model 2	0.002	0.013	-0.060 ; 0.064	0.951	-0.041	-0.242	-0.099 ; 0.017	0.159
Total VPA (min/day)								
Model 1	0.100	0.098	-0.303 ; 0.503	0.614	0.034	0.035	-0.311 ; 0.381	0.839
Model 2	0.140	0.136	-0.258 ; 0.537	0.476	0.013	0.013	-0.338 ; 0.365	0.939

Total MVPA (min/day)								
Model 1	-0.009	-0.063	-0.070 ; 0.052	0.758	-0.024	-0.166	-0.075 ; 0.026	0.339
Model 2	0.006	0.041	-0.060 ; 0.072	0.852	-0.028	-0.190	-0.078 ; 0.023	0.277
>10 min MVPA (min/day)								
Model 1	-0.017	-0.134	-0.068 ; 0.033	0.487	-0.013	-0.068	-0.080 ; 0.055	0.693
Model 2	-0.007	-0.054	-0.060 ; 0.046	0.786	-0.020	-0.101	-0.090 ; 0.050	0.566
5-10 min MVPA (min/day)								
Model 1	-0.027	-0.047	-0.250 ; 0.196	0.808	-0.124	-0.244	-0.294 ; 0.047	0.151
Model 2	0.007	0.013	-0.217 ; 0.232	0.947	-0.125	-0.247	-0.296 ; 0.047	0.148
1-5 min MVPA (min/day)								
Model 1	-0.176	-0.246	-0.451 ; 0.098	0.198	-0.129	-0.206	-0.344 ; 0.084	0.228
Model 2	-0.094	-0.131	-0.436 ; 0.249	0.579	-0.121	-0.193	-0.337 ; 0.095	0.264

B, regression coefficient; β means standardized coefficient; CI, confidence interval; HCY, Homocysteine; MPA, Moderate physical activity; MVPA, Moderate to vigorous physical activity VPA, Vigorous physical activity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 19. Associations of accelerometer-measured SB levels with HCY (μmol/l) separately by sex.

	HCY (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Total SB (min/day)								
Model 1	0.000	-0.001	-0.007 ; 0.007	0.997	0.005	0.259	-0.002 ; 0.012	0.128
Model 2	-0.001	-0.060	-0.008 ; 0.006	0.758	0.006	0.276	-0.001 ; 0.012	0.106
10-20 min SB (min/day)								
Model 1	-0.015	-0.109	-0.069 ; 0.039	0.572	0.012	0.100	-0.030 ; 0.053	0.562
Model 2	-0.032	-0.234	-0.087 ; 0.023	0.244	0.015	0.125	-0.027 ; 0.057	0.475
20-30 min SB (min/day)								
Model 1	-0.007	-0.052	-0.058 ; 0.044	0.790	0.152	0.439	-0.030 ; 0.079	0.376
Model 2	-0.009	-0.067	-0.058 ; 0.042	0.726	0.031	0.195	-0.022 ; 0.087	0.269
30-40 min SB (min/day)								
Model 1	0.009	0.069	-0.041 ; 0.059	0.723	0.016	0.140	-0.023 ; 0.055	0.414
Model 2	0.000	0.002	-0.050 ; 0.050	0.990	0.019	0.164	-0.020 ; 0.058	0.346
45-60 min SB (min/day)								
Model 1	-0.040	-0.346	-0.085 ; 0.003	0.066	0.028	0.310	-0.002 ; 0.057	0.066
Model 2	-0.038	-0.325	-0.081 ; 0.005	0.080	0.026	0.291	-0.005 ; 0.056	0.095

>60 min SB (min/day)								
Model 1	0.003	0.162	-0.005 ; 0.011	0.401	0.002	0.130	-0.004 ; 0.009	0.448
Model 2	0.002	0.113	-0.006 ; 0.010	0.560	0.002	0.136	-0.004 ; 0.009	0.429

B, regression coefficient; β means standardized coefficient; CI, confidence interval; HCY, Homocysteine; LPA, light physical activity; SB, Sedentary behaviour. Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 20. Associations of accelerometer-measured PA and SB bouts with HCY (μmol/l) separately by sex.

	HCY (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
>10 min LPA (bouts)								
Model 1	-0.069	-0.096	-0.353 ; 0.210	0.620	-0.220	-0.316	-0.451 ; 0.010	0.060
Model 2	-0.081	-0.113	-0.36 ; 0.197	0.553	-0.215	-0.307	-0.447 ; 0.017	0.069
5-10 min LPA (bouts)								
Model 1	-0.370	-0.342	-0.771 ; 0.032	0.069	-0.106	-0.117	-0.498 ; 0.207	0.497
Model 2	-0.334	-0.309	-0.736 ; 0.677	0.099	-0.086	-0.094	-0.406 ; 0.234	0.588
1-5 min LPA (bouts)								
Model 1	-0.172	-0.479	-0.297 ; -0.048	**0.009**	0.008	0.028	-0.095 ; 0.112	0.872
Model 2	-0.161	-0.447	-0.286 ; -0.035	**0.014**	0.013	0.044	-0.09 ; 0.118	0.800
>10 min MVPA (bouts)								
Model 1	-0.261	-0.700	-1.728 ; 1.200	0.718	-0.691	-0.136	-2.432 ; 1.051	0.426
Model 2	0.047	0.012	-1.466 ; 1.560	0.950	-0.897	-0.177	-2.677 ; 0.883	0.313
5-10 min MVPA (bouts)								
Model 1	-0.329	-0.107	-1.537 ; 0.879	0.581	-0.726	-0.255	-1.686 ; 0.233	0.133
Model 2	-0.126	-0.040	-1.357 ; 1.106	0.836	-0.759	-0.266	-1.720 ; 0.203	0.118
1-5 min MVPA (bouts)								
Model 1	-0.209	-0.227	-0.561 ; 0.144	0.235	-0.253	-0.246	-0.600 ; 0.094	0.148
Model 2	-0.116	-0.126	-0.517 ; 0.286	0.142	-0.258	-0.250	-0.605 ; 0.090	0.142

10-20 min SB (bouts)								
Model 1	-0.236	-0.127	-0.967 ; 0.495	0.513	0.167	0.104	-0.389 ; 0.723	0.546
Model 2	-0.473	-0.253	-1.222 ; 0.276	0.206	0.194	0.121	-0.396 ; 0.755	0.485
20-30 min SB (bouts)								
Model 1	-0.145	-0.046	-1.397 ; 1.108	0.814	0.715	0.186	-0.600 ; 2.030	0.277
Model 2	-0.200	-0.063	-1.433 ; 0.031	0.740	0.893	0.232	-0.451 ; 2.23	0.186
30-40 min SB (bouts)								
Model 1	0.216	0.049	-1.500 ; 1.932	0.798	0.648	0.161	-0.741 ; 2.036	0.350
Model 2	-0.050	-0.011	-1.782 ; 1.680	0.952	0.714	0.177	-0.683 ; 2.110	0.306
45-60 min SB (bouts)								
Model 1	-2.300	-0.385	-4.488 ; -0.122	**0.039**	1.488	0.332	0.050 ; 2.960	**0.048**
Model 2	-2.130	-0.355	-4.310 ; 0.048	0.055	1.409	0.314	-0.112 ; 2.92	0.068
>60 min SB (bouts)								
Model 1	0.026	0.011	-0.910 ; 0.963	0.954	0.199	0.085	-0.613 ; 1.011	0.621
Model 2	-0.136	-0.057	-1.081 ; 0.810	0.771	0.209	0.089	-0.606 ; 1.023	0.606

B, regression coefficient; β means standardized coefficient; CI, confidence interval; HCY, Homocysteine; LPA, Light physical activity; SB, Sedentary behaviour; MVPA, Moderate to vigorous physical activity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 21. Associations of accelerometer-measured fragmentation metrics with HCY (µmol/l) separately by sex.

	HCY (µmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
TPPA2SB (%)								
Model 1	-7.247	-0.113	-23.703 ; 9.209	0.374	14.041	0.350	0.952 ; 27.131	**0.036**
Model 2	-6.811	-0.161	-22.990 ; 9.370	0.395	14.308	0.357	1.207 ; 27.410	**0.033**
TPSB2PA (%)								
Model 1	-34.685	-0.264	-90.816 ; 21.447	0.213	-23.997	-0.214	-62.789 ; 14.795	0.217
Model 2	-25.555	-0.194	-83.193 ; 32.083	0.367	-22.400	-0.199	-61.698 ; 17.012	0.256
TPSB2LPA (%)								
Model 1	-35.416	-0.264	-92.645 ; 21.810	0.213	-19.740	-0.191	-55.170 ; 15.689	0.265
Model 2	-26.616	-0.198	-85.05 ; 31.828	0.354	-18.458	-0.178	-54.271 ; 17.350	0.302
TPSB2MVPA (%)								
Model 1	164.474	0.033	-1804.872 ; 2133.819	0.865	-209.435	-0.038	-2249.647 ; 1830.778	0.836
Model 2	840.221	0.172	-1247.760 ; 2928.202	0.415	-309.338	-0.056	-2344.919 ; 1726.244	0.758
PA Gini Index								
Model 1	9.775	0.177	-12.670 ; 32.221	0.378	-6.478	-0.155	-20.770 ; 7.921	0.367
Model 2	9.729	0.157	-11.688 ; 31.147	0.358	-7.407	-0.177	-21.931 ; 7.118	0.307

SB Gini Index								
Model 1	-14.595	-0.209	-42.770 ; 13.585	0.296	5.999	0.040	-22.024 ; 34.024	0.666
Model 2	-11.341	-0.162	-38.530 ; 15.850	0.398	4.740	0.059	-23.594 ; 33.080	0.735

B, regression coefficient; β means standardized coefficient; CI, confidence interval; HCY, Homocysteine; TPPA2SB, Probability to transicionate from physical activity to sedentary; TPSB2LPA, Probability to transicionate from sedentary to light physical activity; TPSB2PA, Probability to transicionate from sedentary to physical activity; TPSB2MVPA, Probability to transicionate from sedentary to moderate to vigorous physical activity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 22. Nutritional Intake by total sample and by sex.

	Total (n=66)	Men (n=30)	Women (n=36)	p
Energy (kcal)	1764.49 ± 379.04	1743.60 ± 379.04	1786.95 ± 341.11	0.586
Water (ml)	1150.12 ± 273.05	1101.95 ± 260.95	1201.90 ± 279.50	0.096
Protein (g)	76.72 ± 19.42	76.41 ± 21.12	77.05 ± 17.69	0.882
Carbohydrates (g)	162.34 ± 38.30	159.27 ± 41.83	165.65 ± 34.33	0.452
Lipids (g)	81.03 ± 21.09	79.30 ± 20.38	82.89 ± 21.93	0.442
Cholesterol (mg)	280.08 ± 113.25	283.32 ± 122.06	276.6 ± 104.39	0.789
Vitamin B1 (mg)	1.15 ± 0.32	1.18 ± 0.32	1.19 ± 0.32	0.306
Vitamin B2 (mg)	1.43 ± 0.34	1.38 ± 0.32	1.48 ± 0.38	0.209
Vitamin B6 (mg)	1.93 ± 0.62	1.88 ± 0.67	1.99 ± 0.56	0.400
Vitamin B12 (mg)	5.30 ± 3.97	4.95 ± 2.49	5.68 ± 5.11	0.405
Vitamin C (mg)	134.98 ± 63.12	118.88 ± 57.92	153.3 ± 64.59	**0.015**
Vitamin A (µg)	831.82 ± 502.78	750.88 ± 553.77	918.83 ± 431.59	0.129
Retinol (µg)	369.02 ± 285.37	358.45 ± 285.28	380.37 ± 288.67	0.729
Carotenes (µg)	2486.84 ± 2481.83	2100.91 ± 2718.64	2901.73 ± 2156.48	0.143
Vitamin D (µg)	4.40 ± 9.07	3.03 ± 3.47	5.88 ± 12.48	0.152
Vitamin E (µg)	7.83 ± 2.93	7.48 ± 2.77	8.21 ± 3.08	0.256
Vitamin K (µg)	137.13 ± 63.74	138.14 ± 72.91	136.05 ± 53.07	0.883

Values are expressed as mean ± SD. Significant differences appeared in bold.

Table 23. TAC and HCY levels by meeting sex-specific nutritional recommendations.

	n	Meet the recommendations	n	Not meeting the recommendations	p
TAC in Water recommendations (umol/l)	3	977.04 ± 187.23	63	1114.85 ± 1116.80	0.100
TAC in Protein recommendations (umol/l)	62	958.90 ± 187.29	4	1017.20 ± 180.05	0.300
TAC in Carbohydrates recommendations (umol/l)	4	947.82 ± 378.90	62	975.50 ± 172.2	0.900
TAC in Lipids recommendations (umol/l)	6	950.39 ± 245.96	60	957.36 ± 181.79	0.662
TAC in Cholesterol recommendations (umol/l)	40	910.50 ± 180.46	26	975.61 ± 169.32	0.500
TAC in Vitamin B6 recommendations (umol/l)	56	920.45 ± 190.02	10	1056.21 ± 146.19	**0.025**
TAC in Vitamin B12 recommendations (umol/l)	65	980.78 ± 189.06	1	1095.53 ± n.s	N.A
TAC in Vitamin C recommendations (umol/l)	58	960.56 ± 179.29	8	956.34 ± 220.34	0.910
TAC in Vitamin A recommendations (umol/l)	46	922.75 ± 180.75	20	1020.80 ± 180.84	0.100
TAC in Vitamin D recommendations (umol/l)	5	1040.45 ± 225.69	61	949.85 ± 180.60	0.250
TAC in Vitamin E recommendations (umol/l)	1	1015.55 ± n.s	65	970.45 ± 179.85	N.A
TAC in Vitamin K recommendations (umol/l)	46	925.36 ± 170.36	20	100.00 ± 203.15	**0.029**
HCY in Water recommendations (umol/l)	3	11.84 ± 2.26	63	13.73 ± 3.20	0.318
HCY in Protein recommendations (umol/l)	62	13.34 ± 3.28	4	14.30 ± 2.65	0.322
HCY in Carbohydrates recommendations (umol/l)	4	13.83 ± 3.09	62	13.62 ± 3.20	0.905
HCY in Lipids recommendations (umol/l)	6	12.67 ± 3.43	60	13.77 ± 3.14	0.395
HCY in Cholesterol recommendations (umol/l)	40	13.67 ± 2.91	26	13.60 ± 3.58	0.935
HCY in Vitamin B6 recommendations (umol/l)	56	13.06 ± 2.78	10	15.94 ± 3.67	**0.004**
HCY in Vitamin B12 recommendations (umol/l)	65	13.61 ± 3.19	1	15.15 ± N.A	N.A
HCY in Vitamin C recommendations (umol/l)	58	13.74 ± 3.32	8	13.09 ± 2.20	0.580
HCY in Vitamin A recommendations (umol/l)	46	13.29 ± 3.29	20	14.34 ± 2.89	0.228
HCY in Vitamin D recommendations (umol/l)	5	12.56 ± 1.45	61	13.74 ± 3.27	0.435
HCY in Vitamin E recommendations (umol/l)	1	8.16 ± N.A	65	13.73 ± 3.11	0.081
HCY in Vitamin K recommendations (umol/l)	46	12.83 ± 2.56	20	15.85 ± 3.68	**0.002**

Values are expressed as mean ± SD. Significant differences appeared in bold. HCY, Homocysteine; N.A, not applied; TAC, Total Antioxidant Capacity.

Study 3: Sex-specific associations of sleep parameters with oxidative stress in older adults.

The sociodemographic and nutritional characteristics of the participants are presented in **Study 2 (Table 10, Table 22** and **Table 23).**

Mean values of reported sleep behaviour, accelerometer sleep behaviour, and oxidative stress outcomes are presented in **Table 24** with sex comparisons.

No significant sex differences were found for any PSQI component (**Table 25**). In contrast, men participants obtained better scores for reported sleep efficiency and PSQI final score than women ($p<0.050$) (**Table 24**).

Regarding accelerometer sleep parameters, men spent significantly more time awake during the night than women (+17.08 ± 7.46 minutes per night, $p<0.01$) and also obtained lower percentages in the sleep regularity index and sleep efficiency than women (-5.42 ± 1.33 and -4.23 ± 0.85, respectively, $p<0.03$).

Men showed significantly higher resting values of TAC and HCY than women (both $p<0.001$).

Table 24. Participant characteristics of the total sample and by sex.

	Total (n=66)	Men (n=30)	Women (n=36)	p	ES
General Characteristics					
Age (years)	68.68 ± 3.02	68.50 ± 3.02	68.82 ± 3.05	0.903	0.0
Height (cm)	159.08 ± 9.33	165.99 ± 7.45	153.31 ± 6.37	**<0.001**	1.8
Weight (kg)	74.24 ± 14.60	82.90 ± 14.36	67.02 ± 10.37	**<0.001**	1.3
BMI (kg/m^2)	29.24 ± 4.63	30.01 ± 4.27	28.60 ± 4.63	0.222	0.3
Self-reported sleep quality					
Self-reported sleep time (hrs)	6.52 ± 1.14	6.71 ± 1.05	6.32 ± 1.22	0.130	0.3
Self-reported sleep latency (min)	16.06 ± 19.53	20.68 ± 19.53	10.00 ± 5.73	0.107	0.4
Self-reported time in bed (hrs)	8.08 ± 1.09	8.00 ± 1.15	8.17 ± 1.04	0.570	-0.2
Self-reported sleep efficiency	82.45 ± 12.80	85.31 ± 11.14	79.66 ± 13.79	**0.049**	0.4
Self-reported sleep score	6.26 ± 2.81	5.20 ± 1.98	7.21 ± 3.12	**0.003**	-0.7
Accelerometer measured sleep behaviour					
Time in bed (hrs)	7.51 ± 1.14	7.54 ± 0.85	7.49 ± 1.33	0.863	0.0
Sleep time (hrs)	6.62 ± 1.12	6.49 ± 0.88	6.72 ± 1.29	0.399	-0.2
Wake after sleep onset (min)	52.21 ± 20.59	61.63 ± 22.59	44.55 ± 15.13	**0.002**	0.8
Sustained inactivity time (hrs)	2.50 ± 0.80	2.69 ± 0.80	2.35 ± 0.78	0.101	0.4
Sustained inactivity time (bouts)	14.00 ± 4.09	14.84 ± 4.86	13.30 ± 3.22	0.175	0.3
Number of awakenings (n)	13.56 ± 3.15	13.89 ± 3.10	13.30 ± 3.22	0.471	0.2
Time in SIB of 15 min (hrs)	1.36 ± 0.67	1.52 ± 0.75	1.23 ± 0.58	0.096	0.4
Sleep onset (hours)	0:45:36 ; 1:21:36	0:32:24 ;1:36:36	0:57:00 ; 1:06:00	0.438	-0.3
Wake-up time (hrs)	8:22:48 ; 1:32:24	8:08:24 ; 1:50:24	8:34:48 ;1:50:24	0.438	-0.3
Sleep Regularity Index	48.96 ± 9.56	45.95 ± 9.96	51.36 ± 8.63	**0.024**	-0.6
Sleep efficiency (%)	88.24 ± 4.44	85.92 ± 4.38	90.15 ± 3.54	**<0.001**	-1.1
Oxidative Stress					
TAC (µmol/l)	967.55 ± 184.30	1053.17 ± 171.52	896.61 ± 171.51	**<0.001**	1.0
HCY (µmol/l)	13.89 ± 3.54	15.61 ± 3.32	12.46 ± 2.54	**<0.001**	1.0

Values are expressed as mean ± SD, BMI, Body Mass Index; ES, Effect Size (Cohen's D); HCY, Homocysteine; SIB: Sustained Inactivity Bouts; TAC, Total Antioxidant Capacity.

Table 25. Pittsburgh sleep quality index components by sex.

	Total (n=66)	Men (n=30)	Women (n=36)	p
Reported Sleep Quality				
Very bad (%)	1 (1.52)	0 (0)	1 (2.78)	
Fairly Bad (%)	9 (13.64)	1 (3.33)	7 (19.44)	
Fairly Good (%)	50 (76.75)	24 (80)	27 (75)	0.098
Very good (%)	6 (9.09)	5 (16.67)	1 (2.78)	
Reported sleep latency				
Less than 15 minutes (%)	22 (33.33)	14 (46.67)	8 (22.22)	
Between 16 and 30 minutes (%)	24 (36.36)	7 (23.33)	18 (50)	0.119
Between 31 and 60 minutes (%)	15 (22.73)	7 (23.33)	7 (19.44)	
More than 60 minutes (%)	5 (7.58)	2 (6.67)	3 (8.33)	
Reported sleep duration				
Less than 5 hours (%)	1 (1.52)	0 (0)	1 (2.78)	
Between 5 and 6 hours (%)	35 (53.03)	15 (50)	21 (58.33)	0.657
Between 6 and 7 hours (%)	15 (22.73)	8 (26.67)	7 (19.44)	
More than 7 hours (%)	15 (22.73)	7 (23.33)	7 (19.44)	
Habitual sleep efficiency				
Less than 65% (%)	8 (12.12)	4 (13.33)	4 (11.11)	
Between 65 and 74% (%)	7 (10.61)	0 (0)	8 (22.22)	0.073
Between 75 and 84% (%)	21 (31.82)	10 (33.33)	11 (30.56)	
More than 85% (%)	30 (45.45)	16 (53.33)	14 (38.89)	
Sleep disturbances				
Not in the last month (%)	17 (25.76)	9 (30)	8 (22.22)	
Less than once a week (%)	48 (72.73)	21 (70)	27 (75)	0.464
Once or twice a week (%)	1 (1.52)	0	1 (2.78)	
Use of sleep medication				
Not in the last month (%)	44 (66.67)	22 (73.33)	21 (58.33)	
Less than once a week (%)	4 (6.06)	2 (6.67)	3 (8.33)	0.551
Once or twice a week (%)	2 (3.03)	1 (3.33)	1 (2.78)	
Three or more times per week (%)	16 (24.24)	5 (16.67)	1 (2.78)	
Daytime dysfunction				
Not in the last month (%)	50 (75.76)	25 (83.3)	25 (69.44)	
Less than once a week (%)	13 (19.70)	5 (16.67)	8 (22.22)	0.241
Once or twice a week (%)	3 (4.55)	0 (0)	3 (8.33)	

Both men's and women's mean values are considered as poor quality sleepers, and only a limited number of participants 16 participants (7 women) obtained a score to be considered as good quality sleepers. When oxidative values were compared between sleep quality groups, no significant differences were found for TAC whereas poor-quality sleepers obtained significantly higher values of HCY but when divided by sex, these differences only remained in women (13.02 ± 2.56 μmol/l for poor-quality sleepers and 9.78 ± 1.56 μmol/l for good-quality sleepers, p=0.002 with a large effect size (1.32)) (**Figure 7**).

Associations of sleep behaviour with oxidative stress markers.

The relationships between reported sleep behaviour and accelerometer sleep behaviour outcomes with oxidative stress markers (TAC and HCY) are shown in tables from **Table 26** to **Table 29**.

Regarding reported sleep behaviour, sleep latency was positively associated with HCY (Model 1), and remained significant after adjusting by age (Model 2) only in men (**Table 27**).

Regarding accelerometer measures, WASO and sleep onset were negatively associated with TAC only in women (model 1), but only WASO remained significant after adjusting by age (model 2) (**Table 28)**. In men, the Sleep Regularity Index was negatively and significantly associated with HCY in the unadjusted model, however, it became non-significant but maintained a trend to signification after adjusting by age (**Table 29**).

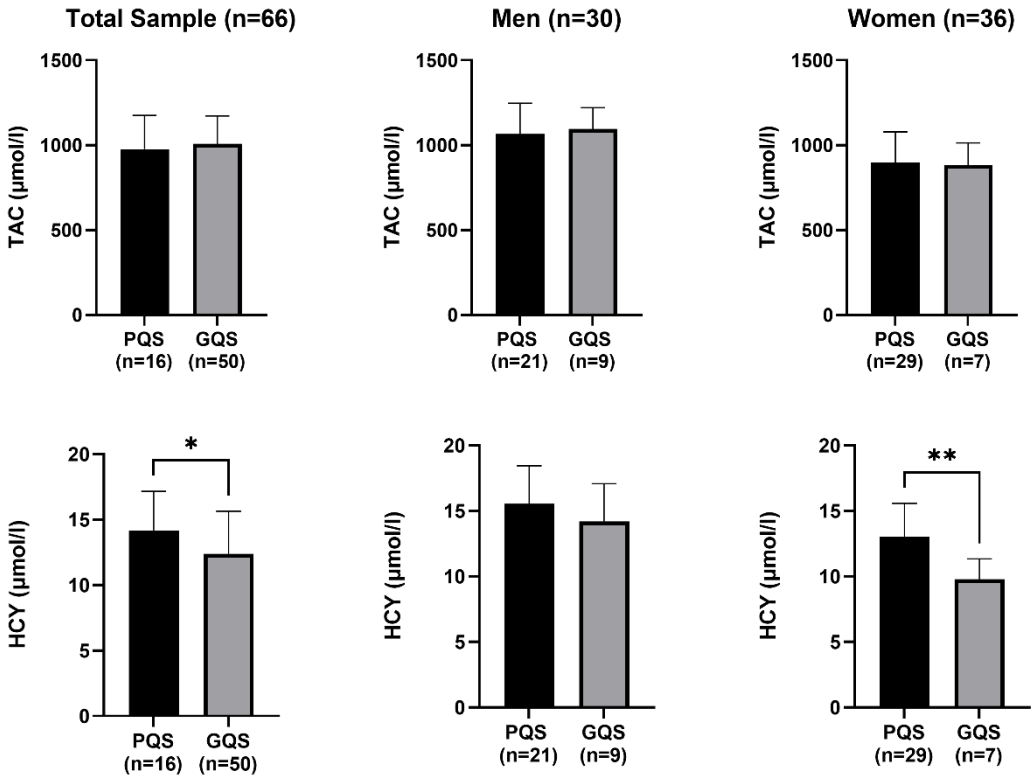

Figure 7. Differences in TAC and HCY levels between PSQI poor-quality sleepers (PQS) and PSQI good-quality sleepers (GQS) participants based on accelerometer-measured data in the total sample and separately by sex. TAC: Total Antioxidant Capacity; HCY: Homocysteine. * $p<0.033$, **, $p<0.002$.

Table 26. Associations of self-reported sleep behaviour components with TAC (μmol/l) separately by sex.

	TAC (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Self-reported sleep time (hrs)								
Model 1	8.397	0.052	-45.023 ; 61.817	0.752	-16.555	-0.119	-63.099 ; 29.989	0.475
Model 2	9.066	0.056	-44.913 ; 63.045	0.735	-18.267	-0.132	-62.907 ; 26.373	0.412
Self-reported sleep latency (min)								
Model 1	1.604	0.192	-1.095 ; 4.307	0.237	-3.741	-0.125	-15.446 ; 7.963	0.517
Model 2	1.563	0.192	-1.225 ; 4.351	0.263	-4.996	-0.167	-16.182 ; 6.189	0.367
Self-reported time in bed (hrs)								
Model 1	18.145	0.124	-30.315 ; 66.605	0.453	-45.946	-0.278	-99.574 ; 7.681	0.091
Model 2	17.530	0.120	-31.480 ; 66.539	0.473	-39.080	-0.237	-90.441 ; 12.280	0.131
Self-reported sleep efficiency								
Model 1	2.518	0.177	-2.206 ; 7.242	0.287	-1.035	-0.083	-5.166 ; 3.096	0.615
Model 2	2.749	0.176	-2.028 ; 7.526	0.251	-0.533	-0.197	-4.437 ; 3.372	0.784
Self-reported sleep score								
Model 1	-4.024	-0.053	-30.885 ; 22.837	0.762	-4.647	-0.084	-22.909 ; 13.613	0.609
Model 2	-5.254	-0.069	-31.890 ; 21.383	0.691	-3.318	-0.058	-20.367 ; 14.009	0.710

B, regression coefficient; β means standardized coefficient; CI, confidence interval; TAC, Total antioxidant capacity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 27. Associations of self-reported sleep behaviour components with HCY (μmol/l) separately by sex.

	HCY (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Self-reported sleep time (hrs)								
Model 1	-0.100	-0.029	-1.234 ; 1.054	0.862	-0.179	-0.080	-0.938 ; 0.580	0.635
Model 2	-0.118	-0.034	-1.270 ; 1.033	0.836	-19.505	-0.087	-0.952 ; 0.562	0.604
Self-reported sleep latency (min)								
Model 1	0.064	0.348	0.004 ; 0.120	**0.035**	0.031	0.062	-0.165 ; 0.227	0.748
Model 2	0.072	0.394	0.014 ; 0.131	**0.017**	0.028	0.056	-0.173 ; 0.229	0.778
Self-reported time in bed (hrs)								
Model 1	0.867	0.264	-0.198 ; 1.871	0.110	0.364	0.139	-0.510 ; 1.235	0.405
Model 2	0.884	0.279	-0.145 ; 1.913	0.090	0.438	0.168	-0.429 ; 1.305	0.312
Self-reported sleep efficiency								
Model 1	0.010	-0.039	-0.079 ; 0.094	0.815	-0.029	-0.146	-0.093 ; 0.036	0.374
Model 2	0.004	0.013	-0.086 ; 0.092	0.936	-0.025	-0.128	-0.090 ; 0.040	0.440
Self-reported sleep score								
Model 1	0.140	0.094	-0.395 ; 0.674	0.598	0.229	0.265	-0.049 ; 0.508	0.104
Model 2	0.161	0.108	-0.374 ; 0.695	0.545	0.242	0.279	-0.034 ; 0.519	0.084

B, regression coefficient; β means standardized coefficient; CI, confidence interval; HCY, Homocysteine; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 28. Associations of accelerometer-measured sleep behaviour components with TAC (µmol/l) separately by sex.

	TAC (µmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Bedtime (hrs)								
Model 1	-65.210	-0.346	-137.952 ; 7.533	0.077	-24.946	-0.210	-66.854 ; 16.963	0.234
Model 2	-65.170	0.007	-139.513 ; 9.174	0.083	-21.051	0.015	-60.820 ; 18.722	0.289
Sleep time (hrs)								
Model 1	-59.289	-0.323	-130.784 ; 12.205	0.100	-18.899	-0.154	-62.541 ; 24.745	0.384
Model 2	-59.263	-0.323	-132.329 ; 13.803	0.896	-11.224	-0.092	-53.230 ; 30.788	0.590
Wake after sleep onset (min)								
Model 1	-8.492	-0.200	-177.56 ; 160.575	0.919	-207.215	-0.312	-434.575 ; 20.145	0.073
Model 2	-7.704	0.256	-180.66 ; 165.252	0.928	-274.440	-0.300	-467.395 ; -81.488	**0.007**
Sustained inactivity time (hrs)								
Model 1	-4.073	-0.023	-78.010 ; 69.863	0.910	52.760	0.250	-19.601 ; 125.123	0.147
Model 2	-3.330	-0.019	-79.129 ; 72.469	0.928	27.946	0.132	-41.522 ; 97.414	0.419
Sustained inactivity time (bouts)								
Model 1	11.347	0.218	-9.538 ; 32.233	0.274	-12.580	-0.245	-30.199 ; 5.039	0.156
Model 2	11.282	0.217	-10.144 ; 32.709	0.288	-14.878	-0.290	-30.452 ; 0.696	0.060
Number of awakenings (n)								
Model 1	11.347	0.218	-9.500 ; 32.233	0.274	-12.580	-0.245	-30.199 ; 5.040	0.156
Model 2	11.282	0.217	-10.144 ; 32.708	0.288	-14.878	-0.290	-30.452 ; 0.696	0.060

Time in SIB of 15 min (hrs)								
Model 1	-6.425	-0.197	-82.965 ; 70.115	0.864	50.851	-0.090	-48.846 ; 150.548	0.307
Model 2	-5.158	-0.028	-83.743 ; 73.420	0.893	39.758	0.139	-50.882 ; 130.398	0.378
Sleep onset (hours)								
Model 1	-13.915	-0.130	-55.688 ; 27.857	0.500	60.059	0.403	11.717 ; 1008.400	**0.016**
Model 2	-12.416	-0.116	-56.396 ; 31.563	0.567	43.789	0.294	-3.458 ; 91.035	0.068
Wake-up time (hrs)								
Model 1	4.086	0.044	-32.785 ; 40.956	0.822	-8.193	-0.062	-54.983 ; 38.596	0.724
Model 2	5.970	0.064	-32.308 ; 44.248	0.751	-2.848	-0.022	-45.303 ; 39.607	0.892
Sleep Regularity Index								
Model 1	-0.073	-0.005	-6.460 ; 6.324	0.982	-1.580	-0.083	-8.334 ; 5.175	0.637
Model 2	-0.198	-0.012	-6.892 ; 6.495	0.952	0.904	0.047	-5.440 ; 7.250	0.774
Sleep efficiency (%)								
Model 1	-186.683	-0.048	-1730.725 ; 1357.36	0.806	421.931	0.090	-1225.684 ; 2069.545	0.606
Model 2	-158.586	-0.041	-1736.272 ; 1417.099	0.837	1337.228	0.295	-143.663 ; 2900.123	0.074

B, regression coefficient; β means standardized coefficient; CI, confidence interval; SIB: Sustained Inactivity Bouts TAC, Total antioxidant capacity; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

Table 29. Associations of accelerometer-measured sleep behaviour components with HCY (μmol/l) separately by sex.

	HCY (μmol/l)							
	Men (n=30)				Women (n=36)			
	B	**β**	**95%CI**	**p**	**B**	**β**	**95%CI**	**p**
Time in bed (hrs)								
Model 1	-0.474	-0.106	-2.311 ; 1.364	0.600	0.245	0.129	-0.432 ; 0.922	0.466
Model 2	-0.483	-0.108	-2.226 ; 1.259	0.572	0.236	0.125	-0.455 ; 0.927	0.490
Sleep time (hrs)								
Model 1	-0.363	-0.083	-2.156 ; 1.431	0.681	0.109	0.056	-0.595 ; 0.812	0.755
Model 2	-0.369	-0.084	-2.071 ; 1.334	0.659	0.090	0.046	-0.635 ; 0.815	0.801
Wake after sleep onset (min)								
Model 1	-1.395	-0.135	-5.519 ; 2.729	0.493	2.898	0.286	-0.603 ; 6.400	0.102
Model 2	-1.592	-0.154	-5.544 ; 0.394	0.419	2.996	0.295	-0.622 ; 6.615	0.101
Sustained inactivity time (hrs)								
Model 1	0.092	0.029	-1.229 ; 1.412	0.887	0.827	0.255	-0.284 ; 1.939	0.140
Model 2	0.022	0.007	-1.275 ; 1.320	0.972	0.869	0.268	-0.307 ; 2.040	0.142
Sustained inactivity time (bouts)								
Model 1	0.021	0.016	-0.505 ; 0.547	0.935	0.116	0.147	-0.161 ; 0.392	0.400
Model 2	0.053	0.042	-0.459 ; 0.565	0.832	0.115	0.145	-0.168 ; 0.397	0.414
Number of awakenings (n)								
Model 1	0.020	0.016	-0.505 ; 0.547	0.935	0.116	0.147	-0.161 ; 0.392	0.400
Model 2	0.053	0.042	-0.459 ; 0.565	0.832	0.115	0.145	-0.160 ; 0.397	0.414

Time in SIB of 15 min (hrs)								
Model 1	0.420	0.103	-1.235 ; 2.074	0.606	0.252	0.057	-1.304 ; 1.808	0.744
Model 2	0.258	0.064	-1.322 ; 1.838	0.739	0.243	0.055	-1.340 ; 1.830	0.757
Sleep onset (hours)								
Model 1	-0.234	-0.098	-1.170 ; 0.703	0.612	-0.234	0.064	-0.664 ; 0.957	0.716
Model 2	-0.443	-0.186	-1.371 ; 0.486	0.336	0.139	0.060	-0.722 ; 1.000	0.744
Wake-up time (hrs)								
Model 1	0.146	0.037	-1.478 ; 1.731	0.851	0.115	0.056	-0.605 ; 0.835	0.748
Model 2	-0.081	-0.020	-1.348 ; 0.227	0.914	0.121	-0.332	-0.614 ; 0.855	0.740
Sleep Regularity Index								
Model 1	-0.148	-0.378	-0.293 ; -0.002	**0.047**	-0.075	-0.259	-0.176 ; 0.025	0.138
Model 2	-0.127	-0.325	-0.274 ; 0.021	0.089	-0.079	-0.268	-0.185 ; 0.025	0.141
Sleep efficiency (%)								
Model 1	0.267	0.003	-34.264 ; 34.799	0.987	-20.661	-0.288	-45.034 ; 3.713	0.094
Model 2	-1.989	-0.023	-35.695 ; 31.717	0.904	-22.903	-0.319	-49.387 ; 3.569	0.088

B, regression coefficient; β means standardized coefficient; CI, confidence interval; HCY, Homocysteine; SIB: Sustained Inactivity Bouts; Model 1, Unadjusted model; Model 2, Analyses adjusted for age.

DISCUSSION

DISCUSSION

The discussion of this International Doctoral thesis is divided into three specific discussions for each study. The specific discussions for each study are shown below.

Study 1: Sexual differences in the association of physical fitness components with oxidative stress in older adults

Our results showed that there are sexual differences in oxidative stress markers for older adults, with men showing higher values of TAC and HCY compared to women with a large effect on both. Additionally, the associations of body composition and fitness components with these markers also manifest sexual differences. Regarding body composition, no significant associations were found with TAC however, higher levels of BMI and thigh circumference were associated with higher levels of HCY, in women, while in men the association was only found with fat-free mass. Concerning physical fitness, faster normal gait speed was associated with TAC and higher performance in upper limb strength, flexibility, and agility was associated with lower levels of HCY only in women. Moreover, higher levels of CRF were associated with lower plasma HCY concentrations in both sexes.

It has been reported that adult women show a better antioxidant capacity compared to men, displaying a protective effect of estrogens (1). However, in our sample older women had significantly lower antioxidant capacity compared to men, similar to what other authors have found (2). It has been hypothesized that the differences in fat mass could affect these differences. Excessive fat accumulation increases ROS production and depletes the antioxidant sources, decreasing the antioxidant capacity in this population (3,4). Nevertheless, even though our women participants showed higher levels of fat mass, no associations between any of the body composition variables and TAC were found, neither in men nor women, which does not support this suggested mechanism. Another possible explanation for this decreased capacity in women is that after menopause, the diminished levels of estrogens could also carry the decline in the levels of TAC. This idea is supported by studies that have shown that post-menopausal women had lower values of TAC, and these values could be increased to pre-menopausal levels using hormonal therapy (5).

When the associations between physical fitness and TAC were studied, our data only reported a positive association between normal gait speed and antioxidant capacity in women. Given the fact that lower gait speed is one of the main indicators of frailty (6,7), our results support the hypothesis that older women closer to frailty would have lower levels of antioxidant capacity. On one hand, some authors support this theory suggesting that lower levels of plasma antioxidants or antioxidant enzymes are identified as frailty indicators (8,9). On the other hand, a study in noninstitutionalized Spanish older adults found no associations between gait speed with serum antioxidant capacity (10). This discrepancy might be explained by the different methods used to evaluate the antioxidant capacity (plasma TAC using FRAP vs. uric acid in serum) and the gait speed distance (6 meters vs. 4.57 meters). However, it is worth mentioning that when a similar distance was used (4 meters) the association between gait speed and TAC disappeared when age was included as a cofounder. In addition to this, no significant differences were found between SPPB groups in TAC values. Therefore, even though our results showed a promising association between gait speed and TAC, there is still some controversy, so more studies are needed to clarify this association.

Concerning the sex differences in HCY, with men reporting higher values of this pro-oxidant marker, our findings are similar to what was found in other older adult populations (11). These sex differences might be related to the distinct metabolism of the HCY (11). Men have shown lower concentrations of vitamin B12 and folate compared to women (12). Additionally, men tend to have increased levels of creatinine due to their higher muscle mass, which is one of the two amino acids involved in the biosynthesis of the HCY (13). This need for higher values of creatinine influences the production of HCY through transmethylation through the guanidinoacetate methyltransferase (13). This suggested mechanism is also supported by other findings from this International Doctoral Thesis, which showed that higher levels of fat-free mass are directly associated with plasma HCY in men independently of age. In women, despite the lack of a relationship found with fat-free mass, direct associations were found for BMI and thigh circumference with HCY concentrations. In fact, adipose tissue may be an important source of circulating HCY (14). In line with this, the association between BMI and HCY levels has been previously stated (15,16) as well as with waist circumference (17), however, this is

the first article to our knowledge reporting an association between thigh circumference and HCY concentrations in women. This sex-specific association between thigh circumference and HCY concentrations could be related to different fat patterning (android *vs.* gynoid). This sex-specific fat accumulation makes women with gynoid pattern store more fat mass in the thighs (18) which could lead to a higher thigh circumference. Given this, a higher thigh circumference in women may imply a higher fat accumulation and consequently higher levels of obesity, which has been shown to increase plasma HCY levels (15).

When the associations of physical components with the pro-oxidative markers were studied, we found an inverse association of cardiorespiratory fitness in both men and women. Cardiorespiratory fitness is the main indicator of physical function, our results back and extend previous investigations which suggested that physical decline in older adults could be an important predictor of elevated plasma HCY levels (19) as both CRF tests (6 minutes walk test for both sexes and the Bruce test for the men) were associated with the pro-oxidant marker, however, sex should be considered as a mediation characteristic due to the lack of association with the Bruce Test (20). In addition to this, our findings showed that women with higher levels of upper limb strength and flexibility also showed lower levels of HCY plasma concentrations, similar to what other cross-sectional studies with middle-aged and older participants have found (21). The lack of association in men is not fully understood yet. However, it is believed that sex-specific hormones could play a role, with testosterone being associated with higher levels of muscle strength and plasma HCY levels (22). To our knowledge, this is the first time that an association between flexibility and HCY in women is observed. This association might be explained by the important role of flexibility in stabilizing and maintaining joint movement and increasing muscular function (23), allowing our women participants to have higher levels of upper limb muscular strength which is associated with reduced HCY levels. Finally, better performance in agility or the dynamic balance (8-foot Up and Go Test) was associated with lower levels of pro-oxidative markers. Our findings support previously published results (24), which suggest that decreased gait function was related to higher levels of HCY through impaired muscle, vascular, and nerve function. This could also explain the lack of association in men, due to the protective effect of testosterone on muscle function (25).

Hence, different components of anthropometry, body composition and physical fitness seem to be involved in the redox status of older adults and it seems that sex-specific intervention should by applied. Meanwhile, physical fitness components are not significantly associated with TAC in men, older women might benefit from an improvement in gait speed which could enhance their antioxidant status. Regarding HCY, interventional programs focused on improving the CRF of older adults might help decrease the HCY levels in older men. Concerning older women, interventions designed to reduce the BMI and thigh perimeter of women in conjunction with upper body strength and flexibility, gait ability, and CRF improvements might reduce the levels of HCY.

Study 2: Sex-specific relationship between physical activity and sedentary behaviour with oxidative stress in older adults

In the present study, older adults present sex differences in oxidative stress; specifically, men have higher TAC but also higher HCY than women, which emphasizes the importance of the sex-specific analysis. PA and SB were estimated using both a questionnaire and an accelerometer, analysing sex-specific associations with oxidative stress levels (TAC and HCY) in older adults. The main findings of this study are the associations between PA and oxidative stress in older adults, with accelerometer-measured MVPA being directly associated with TAC, with total time in this behaviour and time accumulated in bouts shorter than 10 minutes being associated with an increased antioxidant capacity in men and women respectively. Similar to this, accelerometer-measured LPA is associated with HCY, with LPA levels accumulated in shorter bouts and total LPA time being associated with lower values of HCY in men and women, respectively. However, no associations between self-reported PA and SB and accelerometer-measured SB and oxidative stress were found for our older adults when age was included as a cofounder.

Higher MVPA seems to be related to a higher antioxidant capacity, estimated through plasma TAC, which is the measure of the number of free radicals scavenged by antioxidants through a test solution (26). These antioxidants can have endogenous or exogenous origins, while the first one is modified mostly by the diet (27), the second one can be altered due to many factors including PA (28,29). As stated in the results no associations were found between the dietary analysis and the TAC, showing that the associations observed in our study between MVPA and TAC are independent of dietary imbalances. In this line, and, accordingly to our results, a previous study conducted on older adults reported a relationship between MVPA and skeletal muscle expression of endogenous antioxidants, including superoxide dismutase, catalase, and glutathione peroxidase (30) or total antioxidant potential (31). As the main function of antioxidants is to protect the body against oxidative damage, older adults can benefit from regular MVPA with reduced oxidative damage. However, there were sex-specific associations between MVPA and TAC. Men participants with a more total time of MVPA showed an increased

TAC, regardless of the volume whereas women who spent more time in bouts between 1 to 5 and 5 to 10 minutes showed an increased TAC, showing that both sexes can benefit from the different accumulation of this behaviour.

Several studies have evaluated the importance of LPA in general health. Recently it has been shown to reduce the risk of suffering from cardiometabolic diseases or another impaired status such as frailty (32) or maintained cognitive function (33) in older adults. However, the influence of this intensity on other biological markers such as low-grade inflammation is not clear (34). In this International Doctoral Thesis, we showed that higher levels of LPA are associated with lower HCY levels in both sexes. Nevertheless, this fact does not indicate that a higher intensity of PA increases oxidative stress compared with LPA since oxidative stress occurs when there is an imbalance between the production of ROS and the ability to neutralize them or repair the resulting damage by antioxidants (35). MVPA was positively related to antioxidant levels in our data, and the available evidence suggests that it appears unlikely that vigorous exercise results in an oxidative stress level that indices harmful effects on human health in both untrained humans and animal models (28). Notwithstanding, our results showed that LPA might prevent oxidative stress by reducing HCY. Moreover, low-intensity aerobic exercise increased antioxidants and reduced oxidative stress in older adults (36), which may help to understand the influence of LPA on reducing mortality risk in older adults (32). Therefore, lifelong regular PA could maintain the antioxidant defence and prevent age-related increases in oxidative stress (37). Similar to MVPA, LPA showed different sex-specific associations in how LPA was accumulated, with men who spent more time in bouts of 1 to 5 minutes of LPA showing a decreased HCY, whereas this occur in women who spent more time in bouts between 5 to 10 and longer than 10 minutes of LPA.

The SB and oxidative stress are involved in many diseases and, consequently, are predictors of mortality, as well as being related to sarcopenia and frailty (35), which compromise the health and quality of life of older adults. It has been suggested that an extended time in SB could produce an environment inclined to oxidative stress (38). A sedentary lifestyle has been associated with higher levels of oxidative stress in post-menopausal women (39). Nonetheless, in the present study, surprisingly, the

time accumulated in bouts between 45 and 60 minutes of SB was associated with lower HCY in men; which is contrary to what the literature has shown, with the older adults who did not meet the WHO recommendations and consequently spent more time in a sedentary behaviour usually showed higher levels of HCY (40). In addition to this, the time accumulated in bouts between 45 and 60 minutes of SB was also associated in women, but in the opposite direction, with higher levels of SB being associated with higher levels of HCY, similar to previous results (40). This difference emphasizes the importance of analysing older adults divided by their sex. Nevertheless, it is important to note that these associations were not significant when adjusting by age. Therefore, this confounding variable must be included in future analyses aimed to investigate the relationship between SB and oxidative stress.

The transition probability from PA to SB was positively associated with HCY levels in women. This association between the probability of entering into SB and oxidative stress could be explained due to the low number of women older adults who met the WHO guidelines, only 25% - 44 % (Global Physical Activity Questionnaire - accelerometer, respectively), the higher levels of SB of women compared to men and women and the higher levels (with a trend to signification) of transition from PA to SB.

The associations between PA and SB with oxidative stress were found only when these outcomes were estimated through accelerometers. Therefore, accelerometer methods to measure PA and SB are encouraged when the prediction of oxidative stress in older adults is pretended.

For all these reasons, different intensities of PA could be used to promote active ageing, consequently preventing the negative consequences of ageing, which could lead to an impaired status such as frailty. In addition to this, since the interest in LPA has increased in recent years due to the recently found benefits on parameters, it would be recommended to add it to physical activity questionnaires considering LPA is not included in most of the questionnaires (41). Thus, active ageing is encouraged to prevent age-related increases in oxidative stress by including not only LPA but also MVPA and the accumulation type may differ depending on the sex.

Study 3: Sex-specific associations of sleep parameters with oxidative stress in older adults.

The present study estimated sleep behaviour through accelerometry and a reported questionnaire, investigating the associations with oxidative stress markers in older adults. Older adults present sex differences in these oxidative markers, with men, having higher values of pro-oxidants (HCY) as well as higher values of antioxidant capacity (TAC) than women, as well as differences in sleep behaviour, with men spending more time awake during night time, obtaining a worse sleep regularity index and sleep efficiency than women, when sleep was measured through accelerometry. Nevertheless, women obtained worse self-reported sleep efficiency and score than men, with self-reported poor-quality women sleepers having more HCY levels than good-quality sleepers. The main findings of the associations are that there are sex-specific associations between sleep behaviour, especially sleep disorders, and oxidative stress in older adults, with the more time awake during the night the lower TAC in resting blood in women, whereas the latency being associated with HCY in men, with longer latencies being associated with higher levels of HCY.

There were significant differences in oxidative markers between men and women. Despite women having shown a better antioxidant capacity than men in other populations, possibly due to the antioxidant effect of estrogens (1), men showed an increased TAC than women in our results, similar to what other authors have found (2). These differences could be explained by two possible causes. Firstly, women tend to accumulate more fat than men, and excessive accumulation of fat has been associated with the depletion of antioxidant resources, leading to a diminished TAC (3,4). The other cause could be that men had higher levels of pro-oxidant markers leading to increased TAC levels in this population to counteract the deleterious effects of the imbalance between pro-oxidants and antioxidants since it has been shown that the antioxidant capacity increased its levels as an adaptation to increased oxidative stress (33). This possible cause is supported by our results, with men showing significantly higher values of HCY than women. These increased pro-oxidant concentrations may be related to sex-specific metabolism and biosynthesis of HCY, with men tending to have higher concentrations due to the higher levels of creatinine produced by their higher muscle mass (13).

Previous findings have shown that men experienced a greater sleep fragmentation, usually measured with the number of awakenings during the night, which directly leads to less consolidated sleep than women (42). Despite there were no differences in the number of awakenings between sexes in our sample, the greater sleep fragmentation in men of our sample could be supported by our data since men displayed significantly more time awake during the night than women, and consequently, men had directly affected their sleep efficiency with a significantly lower efficiency than women. This behaviour could also affect the sleep regularity index which also showed to have lower values in the men participants compared to their women counterparts. A less consolidated sleep during the night might derivate in a higher nap propensity during the day (42), which could lead to a disruption of the sleep schedule and reduce the index.

The antioxidant capacity could be mainly affected by several factors, for instance, the diet followed by the person, which in our data showed no statistical differences in most of the variables studied, or sleep habits (28,43). In our results, the time awake during the night seems to be associated with a lower antioxidant capacity measured with plasma TAC in our women participants. This could suggest that sleep disturbance, such as waking up during the night and staying awake for a long time could be decreasing the plasma antioxidant levels of older women. Despite not being significant, this hypothesis might be supported by the negative association between the number of awakenings and TAC found in our study, which had a trend to signification ($p=0.060$). This association has been found also in animal models, showing that chronic sleep deprivation reduces several antioxidant enzymes such as superoxide dismutase activity and, glutathione concentrations, as well as diminishing the catalase activity in murine models (44,45). Similar to this, this association has been also found in humans, with authors showing that sleep disorders decrease the antioxidant capacity in night workers (46) as well as older adults with mild cognitive impairment (47).

Despite no significant associations being found between sleep behaviour and the antioxidant capacity of our men participants, a significant positive association between reported latency and pro-oxidant levels was found in men. Our data showed that men with longer sleep latency times had higher levels of HCY. These

results support the data shown by other authors, who have found that higher levels of HCY were significantly correlated with poorer sleep quality in older adults (47). As stated by other authors higher sleep latencies are associated with poorer sleep quality in older adults (48), which could suggest that poorer sleep quality could increase the levels of HCY in men. Despite the cause of the relationship between sleep and HCY is not clear yet (49), it could be hypothesized that sleep disorders such as sleep latency (which directly affect sleep quality) or sleep apnea (50) might induce an increase in the pro-oxidants flux leading to an incremented levels of HCY in serum.

In addition to this, a significant association was found between sleep regularity index and HCY in men using the unadjusted model, however, this significant association disappeared when the association was adjusted by age but remained with a trend to signification (p=0.089). The sleep regularity index is a novel aspect of sleep quality (51). To our knowledge, this is the first article that has found an association between this novel aspect and oxidative stress. This could be suggesting that a more consistent sleep schedule could decrease the levels of pro-oxidant markers in men older adults. Previously sleep Regularity Index has been associated with cardiometabolic risk (52), impairing glucose metabolism and affecting the body composition of people with lower percentages of this aspect, and stress or depression in older adults (53). Therefore, changes in the sleep schedule could disrupt the circadian rhythm causing an impact on normal biological functioning (54), which could enhance the production of HCY. Similar to this, in our women participants, a trend of significant association was found between higher levels of sleep efficiency and lower levels of HCY. This trending association in conjunction with the higher HCY values obtained by the poor-quality women sleepers in comparison with their good-quality sleeper counterparts could imply that a good sleep quality night (without any disturbances such as awakenings) may have some influence on improving the oxidative stress of women older adults.

For all of these reasons, our results suggest that correct sleep behaviour could be used to improve the oxidative markers of the older population with different interventions depending on the sex of the participants. In this regard, our results showed that men could benefit more from strategies related to reducing sleep

latency (which shows to increase HCY) and women with programs focused on reducing the time awake during the night (which was shown to decrease TAC). However, it could be hypothesized that a bidirectional association between these variables exists (55) since it has been also shown that oxidative stress can cause sleep disturbance (56). Nevertheless, it is important not only to focus on these possible bidirectional relationships but to focus on how these two variables could influence other key aspects of ageing. On one hand, a lower TAC capacity and higher levels of HCY have been associated with poorer cognitive performance (57). On the other hand, a disturbance in sleep has also been associated with cognitive decline in the elderly (58). Thus, programs focused on improving the sleep behaviour of older adults could be useful to benefit the oxidative stress in older adults, which could obtain other beneficial effects such as delaying cognitive impairment.

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LIMITATIONS AND STRENGTHS

LIMITATIONS AND STRENGTHS

The findings included in the current International Doctoral Thesis present some general and specific limitations that should be taken into consideration when interpreting the results. Nonetheless, this International Doctoral Thesis also presents several strengths. Therefore, these limitations in conjunction with the main strengths are displayed afterwards.

Limitations

- The cross-sectional design of the current International Doctoral Thesis does not allow us to determine causal relationships between the studied associations.
- Although the gender perspective focused particularly on sex-based differences is a strength of the study, this fact reduces the sample size of the study compromising external validity.
- Since the sample has been divided by sex, the sample size was reduced
- The INTERMAE project only included older adults in the age range of 65 and 75 years old. Despite the suitability of this fact due to the homogeneity of the sample in the age, it also limits the generalizability of these results to other ages among older adults.
- Even though TAC is a valid marker of antioxidant potential and synergetic interactions of body fluids (1), is the only antioxidant marker included in this International Doctoral Thesis. The inclusion of other antioxidants markers such as enzymes or plasmas antioxidants such as ascorbic acid to have a better knowledge of the associations found would be desirable.
- Similar to antioxidants, the only pro-oxidant marker included in this International Doctoral Thesis is HCY. Therefore, the inclusion of other pro-oxidant markers, for instance, Malondialdehyde, advanced oxidation protein products, or 8-hydroxydeoxyguanosine among others would be desirable.
- In **Study 1**, the body composition was not evaluated using a gold standard method such as dual-energy X-ray absorptiometry (2). The bioimpedance analysis has been shown to underestimate or overestimate to some extent the body composition parameters (3,4).

- Another limitation found in **Study 2** is the lack of well-established criteria to measure the daily physical activities (PA and SB) in older adults. There are several cut-points developed for this population (5), however, the differences in the classification of intensities may impair the comparability with other protocols.
- In **Study 3**, sleep is measured using a wrist-worn accelerometer which could be seen as a limitation due to the accelerometer estimating sleep from movement patterns and not asses the actual sleep. Nevertheless, wrist-worn accelerometers are a minimally invasive method with good validity in asses sleep behaviours in day-to-day life (6).

Strengths

- To evaluate if the antioxidant capacity of the diet could be influencing our results, a profound analysis of our participant's diet was performed, showing that in our sample, there were no associations between the antioxidants diet and plasma TAC.
- In **Study 1**, there is a deep investigation of several body composition and physical fitness variables through numerous tests, including two different validated physical batteries for older adults (7–9), to obtain an understanding of how can these be related to oxidative stress markers.
- The main strength of **Study 2** is the analysis of both self-reported, using a validated questionnaire (10), and accelerometer-measured PA and SB provided a deep evaluation of the PA and SB behaviours of our older adults.
- The main strength of **Study 3** is an estimation of sleep behaviour by using both a validated questionnaire, PSQI (11), and diary-guided accelerometer methods. In addition to this, the sex-specific associations of oxidative markers and sleep parameters are a novel finding which could help to individualise sleep behaviours programs in the older population.

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CONCLUSIONS

Conclusions

The conclusions of this International Doctoral Thesis obtained from the studies performed on older adults are explained below.

1) There is a sexual difference in basal oxidative stress markers (TAC and HCY) in older adults, with men showing higher values of both plasma TAC and serum HCY.
2) No significant associations between any of the anthropometrics or body composition variables with TAC were found for any of the sexes. Higher levels of BMI and thigh perimeter were associated with increased levels of HCY in women whereas higher levels of fat-free mass were associated with increased levels of HCY in men. In men, no significant associations were found between fitness variables and TAC. In older women, only a faster gait speed is associated with higher levels of TAC. Better upper body strength, flexibility and agility are associated with lower levels of HCY in women. Higher CRF is associated with lower levels of HCY in both sexes.
3) In older adults, the levels of PA seem to be more relevant to oxidative stress than SB, with higher MVPA levels being associated with higher TAC levels and higher LPA being associated with lower levels of HCY. It is noteworthy that more than half of our participants did not meet the PA recommendations guidelines. Therefore, our results suggest the benefits of being active in the elderly to reduce oxidative stress and that accelerometer-measured PA seems to be more sensitive to differences in oxidative stress than self-reported PA.
4) There were sex-specific associations of sleep disorders with oxidative stress. While the time awake during the night was inversely associated with TAC in women, reported sleep latency was inversely associated with HCY in men. In addition to this, women with a worse self-reported quality of sleep had higher levels of HCY compared to those who felt they were good-quality sleepers. It should be noted that almost three-quarters of older adult participants reported poor quality of sleep. Thus, our results suggest a good sleep quality could reduce oxidative stress levels in older adults. For this reason, public health actions are encouraged to increase the quality of sleep of older adults, especially among women.

FUTURE RESEARCH DIRECTIONS

FUTURE RESEARCH DIRECTIONS

Oxidative stress has been linked to the development of several age-related diseases. For this reason, we were especially interested in analysing the possible associations of daily life behaviours or activities such as physical fitness, physical activity or sleep behaviour as a first step in order to find effective strategies to improve oxidative stress in older adults. These associations have been found in the present International Doctoral Thesis within the INTERMAE project.

However, due to the design of the present International Doctoral Thesis, causal relationships cannot be established yet. Thus, we will aim to analyse the effects of a 5-month supervised exercise intervention with a special focus on a multi-component design including cardiovascular, strength, and cognitive training on TAC and HCY and also analyse if this possible effect is mediated by sex. In addition to this, we will investigate if the 5-month interventional program has an influence on physical fitness, PA and SB levels, and sleep behaviour of older adults. Consequently, we will analyse the effect of this 5-month interventional program on the associations found in the present International Doctoral Thesis with the objective of trying to find causal relationships.

Finally, we want to implement all of what we have learned during the process of this International Doctoral Thesis in the following projects. During the pre-doctoral period, we learned about new measuring techniques that could complement the data from previous projects and also about mistakes we have made. Therefore, this new knowledge can be transferred to our upcoming investigation projects to improve the quality of our research.

ANNEXES

ANNEX I: Supplementary Tables

Table S1. Sex-specific nutritional recommended intakes by age-adapted from Gil-Herández et al (2017).

	Age (years)	**Men**	Age (years)	**Women**
Water recommendations (ml/day)	18-100	2500.00	18-100	2000.00
Protein recommendations (g/kg/day)	60-100	0.66	60-100	0.66
Carbohydrates recommendations (%kcal/day)	60-100	45-60	60-100	45-60
Lipids recommendations (%kcal/day)	60-100	20-35	60-100	20-35
Cholesterol recommendations (mg/day)	60-100	<300	60-100	<300
Vitamin B6 recommendations (mg/day)	60-100	1.60	60-100	1.20
Vitamin B12 recommendations (µg/day)	60-100	2.00	60-100	2.00
Vitamin C recommendations (µg/day)	60-100	70.00	60-100	70.00
Vitamin A recommendations (mg/day)	60-100	700.00	60-100	600.00
Vitamin D recommendations (IU/day)	60-69	7.50	60-69	7.50
	70-100	10.00	70-100	10.00
Vitamin E recommendations (mg/day)	60-100	15.00	60-100	15.00
Vitamin K recommendations (mg/day)	60-100	120.00	60-100	90.00

ANNEX II: Short curriculum vitae

Personal information

Juan Corral Pérez (signature on articles: Juan Corral-Perez, Juan Corral-Pérez)

Born: September the 26th of 1994, Jerez de la Frontera, Cádiz, Spain.

Contact: juan.corral@uca.es, +34 690223121

Education

2012-2016 Bachelor's degree in Sport Sciences. Pablo de Olavide University, Seville, Spain.

2016-2017 Master's degree in Physical Activity and Health. University of Cádiz, Spain.

2018-2022 PhD Student in Health Sciences. University of Cádiz, Spain.

Previous and current positions

2017-2018 Research Support Technician, Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Spain.

2018-2019 Predoctoral FPI fellow. Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Spain.

2019-2020 Predoctoral FPU-UCA fellow. Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Spain.

2020-2023 Predoctoral FPU fellow. Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Spain.

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28. Jiménez-García JD, **Corral-Pérez J,** Ponce-González JG, Casals-Vázquez C. 2019. Utilización de las Redes Sociales como Medio de Difusión Científica en Trabajo Cooperativo por proyectos en alumnado del grado de Ciencias de la Actividad Física

y el Deporte. Innovación docente e investigación en ciencias, ingeniería y arquitectura.26. DYKINSON, SL.

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Research stays

2021 Center for Healthy Aging, Xlab, Department of Biomedical Sciences. University of Copenhagen, Denmark.

Prof: Jørn Wulff Helge

Duration: 3 months

2022 Nursing Department, Health Sciences Faculty. Universidad de Málaga, Spain.

Prof: María Ángeles Vázquez Sánchez

Duration: 1 month

2022 Pharmaceutic Faculty, CEU-San Pablo University, Madrid, Spain.

Prof: Martín Alcalá Díaz-Mor

Duration: 1 month

Research Experience

- Ensayo clínico aleatorizado para la evaluación del Programa FRAGSALUD para la prevención y tratamiento de la fragilidad en adultos mayores que viven en la

comunidad. i+D+I porgram of the operative program FEDER Andalucía, Spain (UMA20-FEDERJA-154. 2022-2023. 22,522.00€

- Papel de una estrategia educativa nutricional y de ejercicio físico en la regulación del apetito y composición corporal en función del perfil de exosomas en diabéticos tipo 2. Proyecto APETEX. i+D+I program of the Spanish Ministry Science and Innovation, The Government of Spain (ID2020-120034RA-I00). 2021-2024. 49.852,00 €.
- Eficacia de una estrategia de educación nutricional y el rol del ejercicio fisico en la modulación del metabolismo muscular a través de la microbiota intestinal en diabeticos (Estudio EDUGUTION). i+D+I program of the Spanish Ministry Science and Innovation, The Government of Spain (PID2019-110063RA-I00). 2020-2023. 36.300,00 €.
- Effect of supervised physical exercise at the cerebral, cognitive and metabolomics level in older adults with mild cognitive impairment. EFICCOM study. i+D+I program of the Spanish Ministry Science and Innovation, The Government of Spain (DEP201676123-R). 2017-2020. 120,000€.
- Influence of a physical exercise intervention on markers associated with aging, proteomic profile and fragility. INTERMAE project. Program for the financing of biomedical i+D+I and of health sciences in the province of Cadiz, Spain (PI-00022017). 2018-2021. 492,107.54 €.
- Promoting the shift sedentary Lifestyle towards active Ageing. LifeAge Study. Competitiveness ERASMUS+ SPORT 2018 (603121-EPP-1-2018-1-ES-SPO-SCP). 2019-2020. 389,830 €
- Evaluation of the effects of the nasal flow restriction device (feelbreath) by means of muscular oximetry and electromyography of the respiratory muscles. Southern Association of Pulmonology and Thoracic Surgery (4/2017). 2017-2018. 8915 €.
- THERAPEUTIC INSTRUMENTED BIKE WITH ADVANCED SENSORIZATION. Research transfer office contract, 'CDTI´Interconnect Program. 2017.
- Combined effects of the Mediterranean diet and physical exercise on cardiovascular disease risk factors in university Students. Funded by the University of Cádiz. 2016. 2,000 €.
- Mediating effect of physical activity, physical fitness, and nutrition on the influence of the FTO and PPARGC1A on adiposity and fat oxidation capacity during exercise:The NutAF Study. Funded by the University of Cádiz. 2016. 1,600 €.

- Diagnosis of the pattern of commuting and physical activity of students, teaching and research academic staff, and administration personnel of the University of Cádiz. 2017. Funded by the University of Cádiz. 3,400 €.

Awards

2020 Second best oral presentation at the congress international congress LIFEAGE: exercise prescription for healthy active ageing.

2021 First best oral communication at the XX lnternational Scientific anct Practical Conference of Young Researchers in English dedicated to the 100th anniversary of Evgeny Mikhallovich Chumakov.

Other merits

- Lecturer in the degree of Sport Sciences. Faculty of Education Sciences, University of Cádiz. A total of 180 hours (2019-present).
- Author and co-author of 54 congress communications (both national and international).
- Participating in 11 teaching innovations projects.
- Organizing committee in 1 international congress.

ANNEX III: Acknowledgements / Agradecimientos

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