Association of physical activity with cardiovascular risk factors and all-cause mortality in older Spanish individuals

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To my parents, my sisters, and Gaspar
who despite the distance, are with me every day.

To my loving husband,
my daily company and best friend.

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Abstract

Introduction Among older adults, there is limited and inconsistent evidence on the prospective associations of physical activity (PA) with cardiovascular risk factors and mortality. This thesis aimed to: i) Analyse the prospective associations of leisure-time physical activity (LTPA) with body mass index (BMI), waist circumference (WC), and incidence of obesity. ii) Assess the independent and joint associations of LTPA and adherence to the Mediterranean diet (MedDiet) with all-cause mortality. iii) Determine the association between changes in PA and changes in the inflammatory profile. iv) Assess the effectiveness of a 1-year PA intervention aiming to increase PA levels and decrease BMI and WC.

Methods This thesis used data from two Spanish randomized trials. Both of these trials assessed the effect of different interventions on cardiovascular outcomes in older adults at high risk of cardiovascular disease (CVD). The PREDIMED study (N=7447 / 55 to 80 years old) compared the effect of two MedDiets - one supplemented with extra virgin olive oil and one supplemented with nuts - with a control group on a low-fat diet. The PREDIMED-Plus study (N=6874 / 55 to 75 years old) compared the effects of an intensive lifestyle intervention, that included an energy-restricted MedDiet, PA promotion and behavioural support, with a control intervention, based on usual health care and a MedDiet with no energy restriction.

Results The articles included in this thesis showed: i) After 6.8 years of follow-up, there was an inverse association of total LTPA with BMI, WC, and incidence of general and abdominal obesity. Light LTPA was inversely associated with BMI and incidence of general obesity, while moderate-to-vigorous PA (MVPA) was inversely associated with all four outcomes. ii) After 6.8 years of follow-up, higher levels of LTPA and adherence to a MedDiet were independently and jointly associated with a lower risk of all-cause mortality. After replacing total LTPA with light and MVPA, a similar independent and joint association with all-cause mortality was observed. Combined levels of high adherence to a MedDiet and high levels of PA (total, light and MVPA) showed the strongest association with all-cause mortality. iii) After 1 year of follow-up, increases in total PA were associated with decreases in interleukin-18, monocyte chemo-attractant protein-1, C-peptide, and in the inflammatory profile. Moderate and MVPA showed an inverse association with the inflammatory score. iv) Total, light and MVPA increased in the intervention and control group. The magnitude of the increase in total and MVPA was significantly higher in the intervention group than in the control group, regardless of sex, age, obesity and education. Additionally, increasing levels of MVPA were associated with decreases in BMI and WC in both, control and intervention group. The magnitude of these decreases was stronger in the intervention group.

Conclusion Increasing total and MVPA were inversely associated with surrogate markers of fat accumulation, the inflammatory

profile, and incidence of general and abdominal obesity. High adherence to the MedDiet, together with high levels of PA, regardless of PA intensities (light and MVPA), were associated with the lowest risk of all-cause mortality. Finally, the 1 –year PA intervention was effective in increasing total and MVPA and decreasing surrogate markers of fat, independently of potential moderators. The findings that light PA was inversely associated with the incidence of general obesity and all-cause mortality, and that increases in moderate PA were associated with decreases in the inflammatory profile, are relevant because these intensities of PA provides a feasible option for older adults. These conclusions are important for PA recommendations and public health prevention strategies.

Resumen

Introducción En adultos mayores, la evidencia sobre las asociaciones prospectivas de la actividad física (AF) con factores de riesgo cardiovascular y mortalidad es limitada e inconsistente. El objetivo de esta tesis fue: i) Analizar las asociaciones prospectivas de la actividad física durante el tiempo libre (AFTL) con el índice de masa corporal (IMC), la circunferencia de cintura (CC), y la incidencia de obesidad. ii) Estudiar las asociaciones independientes y conjuntas de la AFTL y la adherencia a la dieta Mediterránea (DM) con la mortalidad por todas las causas. iii) Determinar la asociación entre cambios en la AF y cambios en el perfil inflamatorio. iv) Evaluar la efectividad de una intervención con AF

de un año de duración. Esta intervención tenía como objetivo aumentar los niveles de AF y disminuir el IMC y la CC.

Métodos Los datos de esta tesis provienen de dos ensayos clínicos realizados en España. Estos ensayos estudiaron el efecto de diferentes intervenciones en la incidencia de eventos cardiovasculares en adultos mayores con alto riesgo de enfermedades cardiovasculares (ECV). El estudio PREDIMED (n= 7447 / 55 a 80 años de edad) comparó los efectos de dos MDs – una suplementada con aceite de oliva virgen extra y otra suplementada con frutos secos – con una dieta control baja en grasas. El estudio PREDIMED–Plus (n=6874 / 55 a 75 años de edad) comparó los efectos de una intervención intensiva de estilo de vida, que incluyó una DM hipocalórica, promoción de AF y apoyo conductual, con un grupo control, intervenido con cuidados de salud habitual un una DM sin restricción energética.

Resultados Los artículos incluidos en esta tesis mostraron: i) Después de 6.8 años de seguimiento, se encontró una asociación inversa entre la AFTL total con el IMC, la CC, y la incidencia de obesidad general y abdominal. La AFTL ligera mostró una asociación inversa con el IMC y la incidencia de obesidad general, mientras que la AFTL moderada –intensa mostró una asociación inversa con las 4 variables. ii) Después de 6.8 años de seguimiento, mayores niveles de AFTL y de adherencia a la DM mostraron una asociación independiente y conjunta con un riesgo de mortalidad por todas las causas más bajo. Después de remplazar la AFTL total

con la AFTL ligera y moderada-intensa, se observó una asociación independiente y conjunta similar con la mortalidad por todas las causas. Niveles combinados de una alta adherencia a la DM y altos niveles de APTL (total, ligera y moderada-intensa) mostraron la asociación más fuerte con mortalidad por todas las causas. iii) Después de un año de seguimiento, aumentos en la AF total estuvieron asociados con disminuciones en los niveles de interleucina-18, proteína quimioatrayente de monocitos 1, péptido C, y en el perfil inflamatorio. La AF moderada y la AF moderada-intensa tuvieron una asociación inversa con el perfil inflamatorio. iv) La AF total, ligera, y moderada –intensa aumentaron en el grupo intervención y grupo control. La magnitud del aumento en la ACF total y moderada-intensa fue significativamente más alto en el grupo de intervención que en el grupo control, independientemente del sexo, edad, presencia de obesidad y nivel de educativo. Adicionalmente, el aumento en la AF moderada-intensa estuvo asociada con disminuciones en el IMC y CC en el grupo control y en el grupo intervención. Estas disminuciones fueron de mayor magnitud en el grupo de intervención.

Conclusión Los aumentos en la AF total y en la AF moderada-intensa estuvieron inversamente asociados con marcadores de grasa corporal, con el perfil inflamatorio, y con la incidencia de obesidad abdominal y general. Una alta adherencia a la DM, en conjunto con altos niveles de AF (total, ligera y moderada-intensa), estuvieron asociados con un riesgo de mortalidad por todas las causas bajo. Finalmente, la intervención con AF aumentó la AF total y la

moderada-intensa y disminuyó los marcadores de grasa, independientemente de moderadores potenciales. Los hallazgos que mostraron que la AF ligera estaba inversamente asociada con la incidencia de obesidad general y mortalidad por todas las causas, y que aumentos en la actividad física moderada estuvieron asociados con disminuciones en el perfil inflamatorio, son relevantes, porque estas intensidades de AF proporcionan una opción factible para muchos adultos mayores. Estas conclusiones son importantes para la formulación de recomendaciones de AF y de estrategias de prevención en salud pública.

Preface

The worldwide population is facing an increase in the proportion of older adults, a modification in the prevalence of cardiovascular risk factors and a consequent increase in the burden of cardiovascular diseases. Under this situation, public health researchers have begun to study in depth the complex associations between different lifestyle factors and cardiovascular outcomes.

Physical activity (PA) is a lifestyle factor that has been extensively linked with improvements in general health and has shown to be an important factor in the prevention of chronic diseases. This thesis aims to provide additional evidence of the associations of total PA - and its different intensities - with cardiovascular risk factors and all-cause mortality. Additionally, the present thesis aims to determine the effectiveness of a PA intervention, as one of the main components of a lifestyle intervention, within the framework of the PREDIMED- Plus study.

This thesis was developed in the Cardiovascular Risk and Nutrition group (CARIN), under the direction of Dr. Helmut Schröder. CARIN is part of the Epidemiology and Public Health research program of the Hospital del Mar Medical Research Institute (IMIM), located at Barcelona, Spain. CARIN research includes the study of diet and PA practice and its associations with the cardiovascular risk.

This thesis was made in accordance with the regulations of the PhD Programme in Biomedicine of the Department of Experimental and Health Sciences at the Pompeu Fabra University. It includes an abstract, an introduction, a methodology, the results, a general discussion, and a final conclusion. The results section is composed by a series of four scientific articles, which were written between 2016 and 2018. These articles were made in the framework of two large–scales, randomized trials, called PREDIMED and PREDIMED-Plus. These two trials assess the effect of lifestyle-related interventions, including the promotion of the Mediterranean Diet and PA, on cardiovascular outcomes in older Spanish individuals at high risk of cardiovascular disease.

The PhD student contribution included the revision and analysis of the data and the writing of the manuscripts. It also included the assistance to writing and statistical courses, medical conferences, research meetings and field training. Additionally, the PhD student assisted to fieldwork of studies related to the thesis subject.

Abbreviations

In order of appearance:

Cardiovascular Risk and Nutrition group (CARIN)

Hospital del Mar Medical Research Institute (IMIM)

Girona Heart Registry group (REGICOR)

Physical activity =PA

Leisure-time physical activity= LTPA

Body mass index= BMI

Waist circumference= WC

Mediterranean diet= MedDiet

Cardiovascular disease= CVD

PREDIMED study

PREDIMED-Plus study

Moderate-to-vigorous physical activity= MVPA

World Health Organization= WHO

DALYs= Disability-adjusted life- years

Metabolic equivalent task= MET

Metabolic equivalent task per minutes per day= METs·min/d

Interleukin 6= IL-6

Interleukin 8= IL-8

Regulated on activation, normal T-cell expressed and secreted chemokine= RANTES Interleukin 18= IL-18

Monocyte chemo-attractant protein-1= MCP-1

High-sensitivity C-reactive protein= hs-CRP

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Chapter 1
INTRODUCTION

INTRODUCTION

In the latest decades, there is been a worldwide increase in the prevalence of older adults. Consequently, ageing-related diseases have become one of the most burdensome public health challenges. In this context, research regarding the prevention of these diseases and the control of their risk factors has become imperative for the creation and managing of public strategies.

1.1 The population is ageing

The world population is ageing, in part, due to a continue increase in life expectancy [1]. According to the World Health Organization (WHO), global life expectancy at birth has increase by 5.5 years between the years 2000 and 2016, being the fastest increase since the 1960s [2]. The Global Burden Disease study reported that in Spain, from 1990 to 2016, life expectancy at birth increased from 80 to 85 years in females and from 73 to 80 years in males [3].

This increase in life expectancy has been a tremendous achievement in the health system. However, the population is now facing other health issues. As the population ages, there has been a rise in the burden of chronic non-communicable diseases and in the prevalence of chronic health problems in older adults [4, 5].

Moreover, there has been a critical increase in the disability-adjusted life-years (DALYs) from non-communicable diseases [3].

This measure represents the years of life lost due to premature mortality and disability. It has been reported that from 1990 to 2016, the DALYs due to neoplasm and cardiovascular diseases (CVD) increased by 40.7% and 32.4%, respectively [3].

The main mechanism that could explain the connection between the increase in life expectancy and the increase in the burden of chronic diseases are the changes that underlie ageing. Several physiological and physical changes occurring in the ageing process are involved in the pathogenesis of chronic conditions, including CVD. Some of these changes involve modifications in the vascular structure, including arterial stiffening and endothelial dysfunction [6]. There is also a loss of bone tissue and muscle strength, and an increase in fat mass [7]. Moreover, when comparing older with middle age and younger adults, an increase in the prevalence of overweight, general obesity and abdominal obesity is observed [8, 9]. Additionally, an important proportion of older adults tend to have an increased level of chronic inflammation [10, 11]. All these age-related changes contribute to an increase in the risk of CVD [6].

1.2 Cardiovascular diseases

Cardiovascular diseases are a group of conditions that affect the heart and blood vessels [12]. The most prevalent CVD are coronary heart disease and cerebrovascular disease. Coronary heart disease, also called heart attack, affects the blood vessels that supply the

heart muscle while the cerebrovascular disease, also called stroke, affects the blood vessels that supply the brain [13].

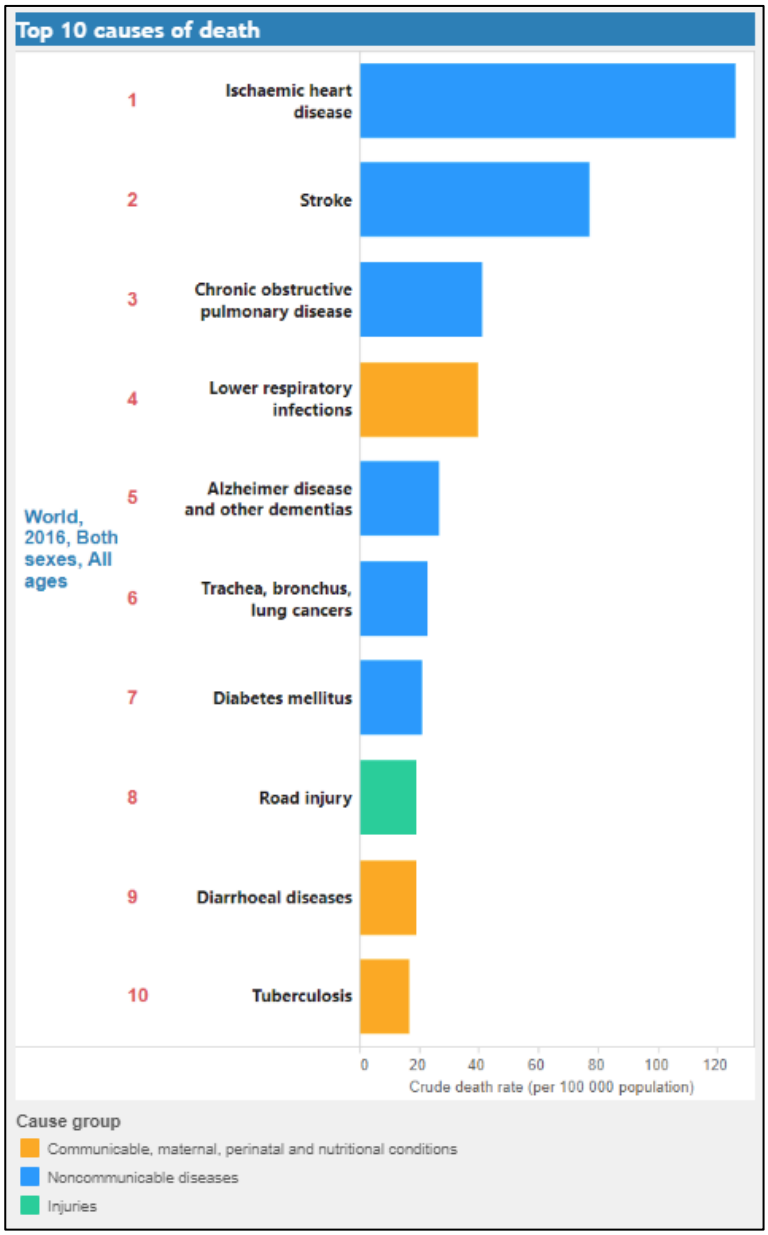

Figure 1: Top 10 causes of death in the world, year 2016 Global Health Observatory data [14].

In people aged 60 years and older, CVD has become the leading contributor to disease burden and still is the number one cause of death worldwide (Figure 1) [14, 15]. It has been estimated that 17.9 million people die every year from CVD and that an 85% of this deaths are from heart attacks and strokes [12].

In this context, it is paramount to investigate all prevention strategies that could be associated with a decrease in the risk of CVD, especially in older adults. In the same line, as the population continues aging, it is imperative to search for health actions aiming to prevent premature death and to increase the quality of life in older adults. The promotion of physical activity (PA) has emerged as an alternative approach in the prevention of CVD and premature mortality.

1.3 Physical activity

Physical activity is defined as any bodily movements produced by skeletal muscles that result in energy expenditure [16]. Some examples of PA are walking, gardening and swimming. Exercise is a term often confused with PA, and it refers to a subcategory of PA that includes structured, repetitive, and planned activities such as weights lifting and aerobic classes.

Physical activity is described by four dimensions [17]. These dimensions are: 1) mode or type, 2) frequency, 3) duration, and 4) intensity. The mode or type of PA is related to the specific activity

performed (e.g. walking, gardening, and climbing stairs) or to the physiological and mechanical demands that PA involves (e.g. aerobic versus anaerobic, resistance or strength training, and balance training). The frequency is the number of times per day or per week in which a specific type of PA is performed, and the duration is the minutes or hours the activity lasts. The intensity is related to the metabolic demand of a specific activity. It is usually measured in metabolic equivalent task (MET). One MET is equal to 1.0 kcal.kg.h and corresponds to the energy cost of an individual at rest. The METs assigned to a specific activity corresponds to the multiples of energy expenditure at rest [18]. Total energy expenditure during PA can be classified in different intensities, including light (≤4 METs), moderate (4 METs to 5.5 METs) and vigorous PA (≥ 6 METs).

Physical activity is further divided in four domains, including occupational (related to work), domestic (related to house work, yard work, and to shopping), transportation (related to the purpose of going somewhere), and leisure time PA (LTPA) (related to recreational activities) [17].

In public health, PA has not only been studied as a therapeutic tool, but also as a toll of prevention. In the general population, PA has been extensively linked with improvements in both, mental and physical health [19–21]. Moreover, it has been associated with a decrease in the incidence of chronic diseases and mortality [20, 22–24]. Similarly, among the older adults, there is strong evidence

linking PA with improvements in several health outcomes and with a decrease in the risk of various chronic non-communicable conditions, including CVD [25–28].

In epidemiological setting, especially in large cohorts, PA questionnaires are the most feasible way to measure PA [29]. Although this is a subjective tool, PA questionnaires are of low cost and there are specific questionnaires validated to different populations. In the articles presented in this thesis, two PA questionnaires where used: The Minnesota Leisure Time Physical Activity Questionnaire and The REGICOR Questionnaire.

The Minnesota Leisure Time Physical Activity Questionnaire was used in the PREDIMED study (PREvención con DIeta MEDiterránea). This questionnaire has been extensively used for decades, and it has been validated in the Spanish population [30, 31]. It quantifies the energy expenditure during LTPA. It collects the number of days and minutes per day in which 67 different types of activities are performed during the previous week and year. The unit used to estimate PA levels was metabolic equivalent task per minutes per day (METs·min/d). This unit was calculated by obtaining the product of the intensity of each activity (METs) and its duration (minutes per day).

The PREDIMED-Plus study (PREvención con DIeta MEDiterránea-Plus) measured PA using the REGICOR Short Physical Activity Questionnaire. This questionnaire has been

recently released and validated in the Spanish population [32]. It collects information on the frequency (days per month), duration (minutes per day), and intensity (METs assigned to each activity) in which 6 different types of activities are performed. The METs·min/d was calculated by summing the products of the intensity, frequency, and duration of each activity, and then dividing by 30.

In the latest decades, the study of PA and its associations with cardiovascular risk factors and mortality has gain special relevance. In the next lines a brief introduction of the cardiovascular risk factors included in this thesis and mortality will be exposed.

1.4 Cardiovascular risk factors and mortality

1.4.1 Obesity

Obesity and overweight are defined as an abnormal or excessive accumulation of body fat that may impair health [33]. Overweight and obesity diagnosis are often determined by body mass index (BMI), which is calculated by dividing the weight (kilograms) by the square of the height (meters^2) (Table 1). Having a BMI greater than or equal to 25 and 30 kg/m^2 indicates overweight and obesity, respectively. Waist circumference (WC) is a less used but effective surrogate marker of intra-abdominal fat, and it is strongly associated with CVD and its risk factors [34, 35]. A WC above 88 cm in women and 102 cm in men is an indicator of abdominal obesity.

Obesity has been associated with an increased risk of CVD [36, 37]. In the older population obesity has been also linked with an increased risk of cancer, frailty, and mobility disabilities [38–40].

Table 1 Body mass index categories [41]

Body mass index [a]	Weight Status
Below 18.5	Underweight
18.5 – 24.9	Normal or Healthy Weight
25.0 – 29.9	Overweight
30.0 and Above	Obese

[a] Body mass index is calculated dividing the weight in kilograms by the square of the height in meters.

Despite these adverse associations, the incidence of obesity is still growing. Among older adults, the prevalence of obesity is especially high. In Europe, recent data of individuals aged 50 years or older indicated that the prevalence of obesity increased between 2005 and 2013, from 17.5% to 19.2% [42]. The ENPE study (Spanish acronym for Nutritional Study of the Spanish Population) reported that in individuals aged 55 to 64 years old, the prevalence of general and abdominal obesity was 33.4% and 48.1%, respectively [9].

In the frame of an ageing population and increased obesity rates, it is imperative to conduct epidemiological studies to assess factors that could contribute to control this pandemic, and therefore to

prevent obesity related health problems. One factor that has been linked to obesity is PA. In the general population, there is strong evidence showing that PA is prospectively associated with a decrease BMI and WC [43–45]. However, in older individuals these associations have not yet been clarified. Intervention studies assessing the effect of PA on surrogate markers of fat have had inconsistent results [46–49]. Moreover, most of these trials included structured sessions of resistance and strength exercises, which are difficult for older individuals to adhere in a long term basis [50, 51]. Prospective studies assessing these variables in older adults are limited and inconsistent [52, 53].

1.4.2 Inflammation

The chronic, systemic, low-level inflammation, often detected in older adults, is called “inflammageing”. This process is characterized by high levels of pro-inflammatory biomarkers in blood and other tissues, in absence of infection [10, 11].

This age-related inflammation contributes to the pathogenesis of several chronic diseases, including CVD, diabetes, chronic kidney disease, cancer, depression, dementia, osteoporosis, sarcopenia, and anaemia [10, 11, 54–57]. Chronic inflammation is also related with an increase in the risk of typical conditions associated with ageing, including frailty and multimorbidity, and with a decrease in cognitive and physical functioning [10].

In view of the ageing of the population and the increase state of chronic inflammation in older adults, epidemiological studies has been focusing in assessing effective prevention tools that could decrease the inflammatory profile. One lifestyle factor that has been linked to inflammation is PA. In the general population strong evidence shows the effect of PA in decreasing inflammatory markers [58–63]. However, in older adults this effect is less strong and is mostly based on short-term interventions [64–67]. As in the evidence regarding the effect of PA on obesity, interventions studies have been mainly composed by structured sessions of aerobic, resistance, and strength exercises, which are difficult to perform and to adhere in a long term basis, especially in overweight or obese older adults. Longitudinal studies in older individuals are limited and show contrary results [68–71].

1.4.3 Mortality

As the population ages, epidemiology research has started to increase their interest in the association of different lifestyle factors and mortality in older adults [72–74]. In the general population, numerous and convincing evidence has shown that higher levels of PA are associated with decreases in all-cause mortality [75–80]. In older adults, there is also evidence showing this association [81–83]. However, this evidence is more limited and provides few data regarding different intensities of PA.

The level of adherence to a healthy diet is other lifestyle factor that has been linked to all-cause mortality. An increased adherence to a healthy diet has been extensively associated with lower rates of all-cause mortality in the general [84, 85] and older population [83, 86, 87]. The Mediterranean diet (MedDiet) is a good example of a healthy diet pattern. It is characterized by a high consumption of plant-based foods (fruits, vegetables, nuts and cereals) and olive oil, a moderate consumption of fish and poultry, a low consumption of dairy products and red meat, and a moderate intake of wine [88, 89]. This diet pattern has been previously associated with improvements in the general health of the population and reductions in the incidence of CVD, type two diabetes, depression, metabolic syndrome, and cognitive decline [90, 91]. Moreover, a higher adherence to this diet patter has been repeatedly associated with a decrease in all- cause mortality [83, 86, 87].

Several current public health programs includes both, PA and diet related recommendations, including the WHO Global Strategy on Diet, Physical Activity and Health [92] and the Global Non-communicable Diseases Action Plan 2013-2020 [93]. This integrative approach is also taken by several national dietary guidelines that additionally include PA recommendations [94–96]. Although evidence shows a strong independent association of PA and levels of adherence to the MedDiet with all-cause mortality, there is limited evidence of the joined association between this two lifestyle factors and this outcome [97]. By assessing this two

lifestyle factors together, an integrative and real perspective of their association with mortality could be provided.

1.5 Physical activity interventions

Physical activity has been extensively recognized as a contributing factor to overall health, even when becoming physical active late in life [98]. This is why the WHO, several country governments, and different health institutions have formally released PA guidelines. The general PA recommendations for adults older than 65 years old includes the following: to do at least 150 minutes per week of moderate aerobic PA, or 75 minutes of vigorous aerobic PA per week, in bouts of at least 10 minutes [99].

Regardless of the scientific evidence showing the benefits of PA, only around half of the Spanish older adults meet the PA guidelines [100]. Moreover, in Spain the amounts of PA performed by older adults have decreased along the years [5]. Furthermore, only in the year 2010, physical inactivity and low levels of PA accounted for 3.2 million deaths worldwide [101]. Therefore, it seems critical to include effective interventions aiming to increase levels of PA, especially in this vulnerable age-range population.

Several studies have asses the effectiveness of lifestyle interventions aiming to improve PA levels in older adults [102–105]. However, the variability in the study design and in the types

of the interventions makes difficult to obtain clear conclusions of the characteristics that an effective intervention should have.

Chapter 2
OBJECTIVES

OBJECTIVES

In line with the antecedents, the general and specific objectives of this thesis are the followings:

PART I: THE PROSPECTIVE ASSOCIATION OF PHYSICAL ACTIVITY WITH CARDIOVASCULAR RISK FACTORS AND MORTALITY

1. General objective I To study the prospective association between LTPA and obesity in Spanish older adults at high risk of CVD. *(Article I)*

   Specific objectives:

   a) To analyse the association between LTPA - total, light and moderate-to-vigorous PA (MVPA) - and BMI, WC, and incidence of general and abdominal obesity.
   b) To analyse the dose-effect association of MVPA with BMI and WC.

2. General objective II To determine the individual and joint association between LTPA and adherence to a MedDiet with all-cause mortality in older adults at high risk of CVD. *(Article II)*

   Specific objectives:

a) To assess the individual and joint association between LTPA (total, light and MVPA) and adherence to a MedDiet with all-cause mortality.
b) To analyse the dose-effect association of all-cause mortality with adherence to a MedDiet and total, light, and MVPA.

3. General objective III To determine the associations between changes in PA and changes in the inflammatory profile in older adults with overweight or obesity and metabolic syndrome. *(Article III)*

   Specific objectives:

   a) To analyse the associations between changes in PA (total, light and MVPA) and changes in inflammatory biomarkers- interleukin 6 (IL-6); interleukin 8 (IL-8); regulated on activation, normal T-cell expressed and secreted chemokine (RANTES); interleukin 18 (IL-18); monocyte chemo-attractant protein-1 (MCP-1); C-peptide; high-sensitivity C-reactive protein (hs-CRP); and leptin-.
   b) To determine the associations of changes in an inflammatory score, composed by 8 inflammatory biomarkers, with changes in total, light, moderate, vigorous and MVPA.
   c) To analyse the dose-effect association of light and MVPA with the inflammatory score.

PART II: PHYSICAL ACTIVITY INTERVENTIONS

4. General objective IV To study the effectiveness of a one-year PA intervention, as part of the multicomponent PREDIMED-Plus program, in older adults with overweight or obesity and metabolic syndrome. *(Article IV)*

   Specific objectives:

   a) To study the effectiveness of a one-year PA intervention by measuring the change in PA levels (total, light and MVPA).
   b) To determine the influence of potential effect moderators, including sex, age, education, and obesity, in the effectiveness of the PA intervention.
   c) To analyse the dose-effect association of changes in light and MVPA with changes in BMI and WC, in the intervention and control group.

Chapter 3
METHODS

1. METHODS

This chapter includes a brief description of the main characteristics of the trials from which the data of this thesis was obtained. The articles presented in this thesis were made in the framework of two large, multicentre, randomized clinical trials called the PREDIMED [106] and the PREDIMED-Plus (Table 2). The complete protocols of both articles are presented in their official websites (www.predimed.es and www.predimedplus.com) and inside the articles of this thesis. These trials were design to assess the effect of different lifestyle interventions, based mainly on the promotion of the MedDiet, on cardiovascular outcomes. These cardiovascular outcomes included stroke, myocardial infarction, and cardiovascular death in Spanish older adults.

3.1 PREDIMED study

The PREDIMED study was a 3-arm trial that ran from 2003 to 2008 and continues to date as a cohort. This study recruited 7447 participants from 11 Spanish health centres. They were randomly distributed in one of the following groups: MedDiet supplemented with extra virgin olive oil, MedDiet supplemented with nuts, and a control group with low-fat diet.

This study included men (aged 55 to 80 years) and women (aged 60 -80 years) at high risk of CVD. Participants had type II diabetes or at least 3 of the following cardiovascular risk factors: current

smoking, hypertension, low high-density lipoprotein-cholesterol, elevated low-density lipoprotein-cholesterol, overweight or obesity and family history of premature coronary heart disease.

3.2 PREDIMED-Plus study

The PREDIMED-Plus study is a randomized trial that started in 2013 and is expected to run for 6 years. This trial recruited 6874 participants from 23 Spanish health centres. They were randomized into an intervention or control group. The intervention group consisted in an intensive weight loss program, including advice on a hypocaloric MedDiet, PA promotion and behavioural support. The control group included usual health care and advice on a MedDiet without energy restriction.

Participants were men (aged 55 to 75 years) and women (aged 60 to 75 years) who had overweight or obesity (BMI $\geq$ 27 or $<$ 40 km/m^2). Additionally, participants had to meet at least three components of the metabolic syndrome [107].

During the first year of the PA intervention, education and PA promotion was delivered during 12 face-to-face individual sessions, 12 telephone calls and 12 group sessions (3 out of the 12 sessions were regarding to PA). During the individual sessions of the first 6 months, participants in the intervention group received personalized recommendations aiming to increase their levels of aerobic MVPA to at least 150 minutes/week. The goal was to walk at least 45

minutes per day for 6 days/week. These aerobic recommendations were based mainly on walking. A pedometer was given to each participant to use a motivation tool. They were advice to walk 10.000 steps per day. Further recommendations were made on strength, flexibility, and balance exercises. During the rest of the year the aim was to continue to increase their levels of PA.

Table 2 Characteristics of the PREDIMED and PREDIMED-Plus studies

	PREDIMED	PREDIMED-Plus
Participants	N= 7447	N= 6874
Inclusion criteria	.-Age 55 to 80 years (men) .-Age 60 to 80 years (women) .-Presence of type II diabetes or at least 3 cardiovascular risk factors	.-Age 55-75 years (men) .-Age 60-75 years (women) .-Overweight or obese .- Presence of at least 3 components of the metabolic syndrome
Date	From 2003 to 2008	From 2013, still on-going (Expected to run for 6 years)
Groups	i) MedDiet + extra virgin olive oil ii) MedDiet + nuts iii) Control group: Low-fat diet	i) Hypocaloric MedDiet, PA promotion and behavioural support. ii) Control group: MedDiet without energy restriction and usual health care.

MedDiet= Mediterranean diet

Chapter 4

RESULTS

4. RESULTS

The results chapter includes four articles that were developed in the framework of this doctoral thesis. The articles and their current status are summarized in table 3. The principal characteristics of each article are summarized in table 4. The first and second part of this thesis includes three and one article respectively.

PART I: THE PROSPECTIVE ASSOCIATION OF PHYSICAL ACTIVITY WITH CARDIOVASCULAR RISK FACTORS AND MORTALITY

The first article (Article I), named "Association of physical activity with body mass index, waist circumference, and incidence of obesity in older adults", was a prospective cohort study published in the European Journal of Public Health. It analysed the prospective associations between levels of LTPA (total, light and MVPA) and anthropometric markers in older adults at high risk of CVD. It also exposed the associations between levels of LTPA and the incidence of general and abdominal obesity.

The second article (Article II), called "Multiple approaches to associations of physical activity and adherence to the Mediterranean diet with all-cause mortality in older adults: the PREvención con DIeta MEDiterránea study", was a prospective cohort published in the European Journal of Nutrition. It studied the independent and

the joint association of LTPA and adherence to the MedDiet with all-cause mortality in older adults at high risk of CVD.

The third article (Article III), named "Prospective association of physical activity and inflammatory biomarkers in older adults from the PREDIMED-Plus Study with overweight or obesity and metabolic syndrome", was a prospective cohort study which is now under revision in the Journal of Gerontology: Medical Sciences. It assessed the associations of changes in PA (total, light, moderate, vigorous and MVPA) with changes in the inflammatory profile and in the following markers of inflammation: IL-6, IL-8, RANTES, IL-18, MCP-1, C-peptide, hs-CRP and leptin.

PART II: PHYSICAL ACTIVITY INTERVENTIONS

The fourth and final article (Article IV), called "Effectiveness of the physical activity intervention program in the PREDIMED-Plus study: A randomized controlled trial" was published in the International Journal of Behavioural Nutrition and Physical Activity. It aimed to determine the effectiveness of a PA intervention in individuals with overweight or obesity and metabolic syndrome. This PA intervention was part of a multicomponent program, which also included advice on a MedDiet and behavioral support.

Table 3 Articles included in the thesis and its current publication status

	Title of the article	**Data source**	**Status**
Part I	Article I Association of physical activity with body mass index, waist circumference and incidence of obesity in older adults	PREDIMED study	Published in the European Journal of Public Health in March 15 of 2018. (2017 Impact factor 2.782, 1st quartile)
	Article II Multiple approaches to associations of physical activity and adherence to the Mediterranean diet with all-cause mortality in older adults: the PREvención con DIeta MEDiterránea study	PREDIMED study	Published in European Journal of Nutrition in April 25 of 2018 (2017 Impact factor 4.423, 1st quartile)
	Article III Prospective association of physical activity and inflammatory biomarkers in older adults from the PREDIMED-Plus Study with overweight or obesity and metabolic syndrome	PREDIMED-Plus study	Submitted to the Journal of Gerontology: Medical Sciences in November 2 of 2018

	Title of the article	**Data source**	**Status**
Part II	Article IV Effectiveness of the physical activity intervention program in the PREDIMED-Plus study: A randomized controlled trial	PREDIMED-Plus study	Published in the International Journal of Behavioural Nutrition and Physical Activity in November 15 of 2018 (2017 Impact factor 5.548, 1st quartile)

Table 4 Characteristics of the articles included in this thesis

Article	Design	Follow-up	N	Sample	Independent variable	Dependent variable
Article I	Prospective cohort	6.8 years	7144	Older adults at high risk of CVD	.-cumulative average of LTPA (total, light and MVPA)	Cumulative average of BMI and WC and incidence of general and abdominal obesity
Article II	Prospective cohort	6.8 years	7356	Older adults at high risk of CVD	.- cumulative average LTPA (total, light and MVPA) .- cumulative average of the adherence to the MedDiet	All-cause mortality

Article	Design	Follow-up	N	Sample	Independent variable	Dependent variable
Article III	Prospective cohort	1 year	492	Older adults with overweight or obesity and metabolic syndrome	.-Changes in PA (total, light, moderate, vigorous and MVPA)	Changes in IL-6, IL-8, RANTES, IL-18, MCP-1, C-peptide, hs-CRP, leptin and inflammatory score.
Article IV	Randomized control trial	1 year	6059	Older adults with overweight or obesity and metabolic syndrome	.-PA intervention as part of a multicomponent program	Changes in PA , BMI and WC

CVD = Cardiovascular disease, LTPA= Leisure time physical activity, MVPA = Moderate-to-vigorous physical activity, MedDiet= Mediterranean diet, PA= physical activity, BMI=body mass index, WC = waist circumference, IL-6= interleukin 6, IL-8= interleukin 8, RANTES= regulated on activation normal T-cell expressed and secreted chemokine, IL-18=interleukin 18, MCP-1=monocyte chemo-attractant protein-1, and hs-CRP= high-sensitivity C-reactive protein.

4.1 ARTICLE I Association of physical activity with body mass index, waist circumference and incidence of obesity in older adults

Cárdenas-Fuentes G, Bawaked RA, González MÁM, Corella D, Cachinero IS, Salas-Salvadó J, et al. Association of physical activity with body mass index, waist circumference and incidence of obesity in older adults. Eur J Public Health. 2018;28(5):944–50. DOI: 10.1093/eurpub/cky030

4.2 ARTICLE II Multiple approaches to associations of physical activity and adherence to the Mediterranean diet with all-cause mortality in older adults: the PREvención con DIeta MEDiterránea study

Cárdenas-Fuentes G, Subirana I, Martinez-Gonzalez MA, Salas-Salvadó J, Corella D, Estruch R, et al. Multiple approaches to associations of physical activity and adherence to the Mediterranean diet with all-cause mortality in older adults: the PREvención con DIeta MEDiterránea study. Eur J Nutr. 2019 Jun 1;58(4):1569–78. DOI: 10.1007/s00394-018-1689-y

4.3 ARTICLE III Prospective association of physical activity and inflammatory biomarkers in older adults from the PREDIMED-Plus Study with overweight or obesity and metabolic syndrome

Cárdenas-Fuentes G, Castañer O, Warnberg J, Subirana I, Buil-Cosiales P, Salas-Salvadó J, et al. Prospective association of physical activity and inflammatory biomarkers in older adults from the PREDIMED-Plus study with overweight or obesity and metabolic syndrome. Clin Nutr. 2020 Feb 3;S0261-5614(20)30038-8. DOI: 10.1016/j.clnu.2020.01.015

4.4 ARTICLE IV Effectiveness of the physical activity intervention program in the PREDIMED-Plus study: a randomized controlled trial

Schröder H, Cárdenas-Fuentes G, Martínez-González MA, Corella D, Vioque J, Romaguera D, et al. Effectiveness of the physical activity intervention program in the PREDIMED-Plus study: A randomized controlled trial. Int J Behav Nutr Phys Act. 2018 Nov 13; 15(1):110. DOI: 10.1186/s12966-018-0741-x

Chapter 5
DISCUSSION

5. DISCUSSION

5.1 Main findings

The first article of this thesis included 7144 older adults at high risk of CVD from the PREDIMED study. This article analysed the prospective association of the cumulative average of LTPA with the cumulative average of surrogate markers of fat and incidence of obesity. This article showed that after 6.8 years of follow-up, total LTPA was inversely associated with BMI, WC, and incidence of general and abdominal obesity. Moderate-to-vigorous LTPA showed similar results while light LTPA was associated with BMI and incidence of general obesity.

The second article analysed the independent and joint associations of LTPA and adherence to the MedDiet with all-cause mortality. It included 7356 older adults at high risk of CVD from the PREDIMED study. This article showed that after 6.8 years of follow-up, higher levels of LTPA and adherence to the MedDiet were independently and jointly associated with a lower risk of all-cause mortality. Similar associations were found when replacing total LTPA with light and MVPA.

The third article of this thesis analysed the association of changes in PA with changes in eight inflammatory biomarkers and in the inflammatory profile. This article included 492 older adults with overweight or obesity and metabolic syndrome from the

PREDIMED-Plus study. This article showed that after one year of follow-up, changes in total PA were inversely associated with changes in IL-18, MCP-1, C-peptide, and in the inflammatory profile. Similarly, moderate and MVPA showed an inverse association with the inflammatory score.

The fourth article analysed the effectiveness of a one-year PA intervention within the framework of the PREDIMED-Plus study. It included 6059 older adults with overweight or obesity and metabolic syndrome. This article showed an increase in total, light, and MVPA in the control and intervention group. However, the magnitude of the increase in total and MVPA was significantly higher in the intervention group. The effect of the PA intervention was not affected by sex, age, education or obesity stratification. Finally, increasing levels of MVPA were associated with decreases in BMI and WC in both of the groups, being this decrease of a greater magnitude in the intervention group.

5.2 Update on recent evidence

5.2.1 Physical activity and obesity

Prospective evidence in the general population has shown a strong association between higher levels of PA and lower values of surrogate markers of fat [43–45]. In adults aged 25 to 79 years old, Ekelund and colleagues found that after a mean follow-up of 5.1 years, there was an inverse association between baseline levels

of PA and annual changes in WC, independently of age, baseline BMI, and changes in body weight [43]. They also found that, in those participants aged 50 years or older, there was an inverse association between baseline PA and the annual change in BMI. However, this association was only found in women who had obesity at baseline, and not in those who had normal or overweight. In the PREDIMED cohort the inverse association of total LTPA with BMI and WC was similarly found only in obese individuals (Article I). This is relevant because individuals belonging to this BMI category are at greater risk of CVD compared to their normal weight and overweight pairs [108], and therefore are the most important immediate target of PA interventions. Also in the general adult population, Bell and colleagues showed that, compared to participants in the lower tertile of PA, those in the higher tertile had a 30% and 33% reduction in the incidence of obesity after 5 and 10 years, respectively [44]. Additionally, Hamer and colleagues found that during 10 years of follow-up, individuals who increased their levels of PA to at least 2.5 hr/week, compared to individuals who kept stable levels of PA, showed a lower BMI (0.40 kg/m^2) and a lower WC (1.10 cm) [45].

In older adults prospective evidence on the association between PA and body fat is scarce and show inconsistent results [52, 53]. A 6-year follow-up study reported an inverse association between PA and changes in fat mass in postmenopausal women aged 60 to 70 years old. However, in those aged 70 years or older, they found no association between these two variables [52]. Another study

reported an inverse association between PA and changes in weight in women age 55 to 64, however they did not observed a significant association in women aged 65 years or older [53]. In the PREDIMED cohort we found a strong prospective association between PA and surrogate markers of fat, independently of age stratification (Article I). This finding indicates that PA could have an impact in BMI and WC at all age ranges of the older population.

5.2.2 Physical activity and all-cause mortality

In the general population there are numerous evidence of the inverse association between PA and all-cause mortality [75–80]. In older adults there is less but consistent evidence showing this association [81–83]. A meta-analysis including individuals aged 60 years or older, reported that after 10 years of follow-up, even lower doses of the recommended levels of PA were associated with a 22% reduction in the risk of all- cause mortality [81]. Additionally, in those individuals who meet PA recommendations there was a 28% reduction in this outcome. In the same line, Esrud and colleges studied the association between objectively measured PA and the all-cause in older adults [82]. They found that, after 4.5 years of follow-up, lower levels of light and moderate PA were similarly associated with a higher risk of all-cause mortality, with moderate PA showing a slightly stronger association.

A high adherence to the MedDiet has also been linked to all-cause mortality, not only in the general population but also in older adults

[83, 86, 87]. Limongi and colleagues found that compared to elderly individuals with a low adherence to the MedDiet (first tertile), those with a high adherence (highest tertile) had a 34% lower risk of all-cause mortality after 7.1 years of follow-up [86]. This reduction in the mortality risk is lower than in our study (53%) (Article II). This difference could be due to the older age-range of their participants.

Although there is strong evidence of the independent association of PA and adherence to the MedDiet with all-cause mortality, there is limited evidence on the combined association of these two factors with this outcome [97]. To our knowledge, only Behrens and colleagues studied this combined association on a large cohort of older adults. They found that meeting the recommended levels of PA and having a high adherence to the MedDiet were independently and jointly associated with a lower risk of all-cause mortality. They found that the joint association between these two variables with all-cause mortality was stronger than the independent association. The magnitudes of these associations were lees stronger than in the PREDIMED cohort (Article II).

The highest tertile of total PA and of the adherence to the MedDiet were jointly associated with the greatest decrease in all-cause mortality. However, combined intermediate levels of the adherence to the MedDiet and of total PA reduced more than 50% of the risk of all-cause mortality. This is relevant, especially among those older adults who have a low level of PA and a low adherence to a MedDiet. Recommending them to achieve intermediate levels of

this two lifestyle factors, could be a more feasible and rational approach, especially at the begging of an intervention.

5.2.3 Physical activity and chronic low-level inflammation

In the adult population, longitudinal studies have shown that increases in PA levels predict lower levels in inflammatory biomarkers at follow-up [109, 110]. However, longitudinal studies in older individuals have shown inconsistent results. Lee et al found that in postmenopausal women there was a cross-sectional inverse association between levels of PA and inflammatory biomarkers (CRP, IL-6, leptin and resistin) at baseline and after 3 years of follow-up. However, they did not find a significant association between changes in PA and changes in these biomarkers [68]. Similarly, in older men, Parsons and colleagues reported cross-sectional and inverse associations of PA with levels of IL-6 and CRP at baseline and after one year of follow-up. However, they found no significant associations of levels of IL-6 and CRP with the 1-year changes in PA. They attributed this lack of longitudinal association to statistical power limitations [69]. While these two studies found no longitudinal associations between the studied variables, Wannamethee and colleagues did found an association between 20-year changes in PA and inflammation. They reported that compared to individuals who became or remained inactive, those who became or remained active had lower levels of CRP and white cell count [70]. Jefferis and colleagues also supported a

longitudinal association by assessing these variables in older adults at baseline and 4 years later. They reported that compared to individuals who were inactive at both time-points, those who were active showed significantly lower levels of CRP at follow-up [71]. In the PREDIMED-Plus cohort (Article III), changes in PA levels were associated with changes in three of the eight biomarkers. The non-significant associations were in the expected directions.

The confection of an inflammatory score aimed to obtain the overall inflammatory profile of older adults. This score represented the sum of the changes in the eight biomarkers. To our knowledge, only one study assessed the association between levels of PA and an inflammatory score (Nishida 2014 Preventive Medicine). This cross-sectional study in the adult population reported an inverse association between PA and an inflammatory score composed by 5 biomarkers.

5.2.4 Effectiveness of physical activity interventions

There is numerous evidence regarding the effectiveness of PA interventions that aims to increase the levels of PA in older adults [102, 103, 105, 111]. However, most of these interventions are short-term. Moreover, the high heterogeneity in the types and duration of interventions, and in the outcome variables, makes it difficult to obtain clear conclusions. In fact, it is still unclear which are the characteristics of the most effective PA interventions, to use as models when designing new health strategies [102, 111].

Nevertheless, most of these interventions have showed to be generally effective in increasing PA levels. Zubala and colleagues reported that interventions including behavioural change techniques in older people resulted in a significant increase in the levels of PA during the trials, which were mainly of 1-year duration [102]. They also found that tailored approaches to promote PA, with additional behavioural support, were one of the most effective techniques to improve levels of PA.

Olanrewaju and colleagues highlighted the need to include individualised interventions in order to keep long-term adherence to PA [105]. They also highlighted the importance of several individual factors that could act as barriers or facilitators of PA and should be taken into account during the personalized interventions. They identified barrier factors including health status, previews PA level and cultural sensibility, and facilitator factors including enjoyable activities and a flexible and convenient scheduling.

The characteristics of the PREDIMED-Plus PA intervention included individual goals setting for feasible PA types, mainly walking. The promotion of walking as the main component of the intervention seems a feasible approach because the intervention was applied to overweight and obese older individuals. Walking usually does not require special equipment and generally can be easily performed in any location and time at a daily basis. These factors might partially explain the success of the PA intervention. The use

of a pedometer as a motivational tool for meeting walking goals could also explain part of the intervention success.

Although in a small proportion, the control group also increased their levels of PA. This finding could be explained by the imminent contamination occurred between intervention groups. Additionally, the fact that the control group received advice on improving their adherence to a MedDiet, could have led to an increase in the adherence in other lifestyle factors, including PA.

In the PREDIMED–Plus cohort, increases in MVPA were linearly associated with decreases in BMI and WC in both, the intervention and control group (Article IV). Although the magnitude of this associations were similar in both of the groups, there was a higher increase of MVPA in the intervention group, which combined with the diet intervention, could have led to higher reductions in BMI and WC. The finding that surrogates markers of fat decreased with increasing levels of PA is in line with Murtagh and colleagues previews findings [112]. They performed a meta-analysis of randomized control trials assessing the effect of walking interventions on cardiovascular risk factors. They found a reduction in WC, BMI and in the proportion of body fat, highlighting the importance of walking on weight control interventions.

After the publication of our results (Article IV), Oja and colleges published an extensive meta-analysis assesing the effects of walking interventions on CVD risk factors in adults [113]. They found that

walking interventions were effective at reducing BMI and body fat -but not WC-. They acknowledge that there was not enough evidence to obtain clear conclusions on the frequency, intensity, duration and volume of walking interventions aiming to decrease CVD risk factors.

5.2.5 Physical activity recommendations

A considerable proportion of the PREDIMED and PREDIMED-Plus participants reported not meeting the recommended levels of MVPA. These is a relent issue if taken into account the large benefits of meeting the PA guidelines.

Hamer and colleagues found that compared to adults who meet PA recommendations ones or less through a 10-year follow-up, those who meet them in all 3 follow-up assessments, had a 0.43 kg/m2 lower BMI and a 2.50 cm lower WC [45]. In the same line, our results showed a linear dose-effect association of higher levels of the recommended amount of MVPA with lower WC and BMI. This results suggest that in older individuals, meeting PA recommendations is associated with lower surrogate markers of fat and therefore, with a decrease in the cardiovascular risk.

Other studies have also shown that even lower levels of the recommended amount of PA have beneficial effects in all-cause mortality. In older adults, meeting the recommended levels of PA has been associated with 28% decrease in all-cause mortality.

However, individuals who met 1-499 METs·min/week, showed a 22% decrease in all-cause mortality, concluding that older adults should be encouraged to perform even lower doses of MVPA in a daily basis [81]. This approach could lead to improvements in the adherence of regular PA practice among older adults.

5.2.6 Intensities of physical activity

Ageing is associated with a decline in the physical performance and with an increase in the risk of frailty [114, 115]. Similarly, in older adults an increased BMI and WC are associated with a higher risk of frailty and lower physical performance [116]. It has been demonstrated that being older and being obese are independently associated with lower odds of being physically active during the transition from midlife to old age [50]. Also, it has been observed that older adults usually tend to reduce their levels of vigorous PA [51]. Therefore, the recommendation of lower intensities of PA to older individuals might be a safer and a more feasible approach.

In older adults emerging evidence has linked light PA with several health outcomes, including a lower risk of coronary heart disease [71]. Our findings showed that light LTPA was prospectively associated with lower BMI, lower incidence of general obesity and lower rates of all-cause mortality.

Limited evidence has assessed the association of light PA with cardiovascular health outcomes. Recent cross-sectional studies have

assessed the association between light-intensity PA and anthropometric markers in older adults, finding inconsistent results [117, 118]. Additionally, a review of randomized trials showed little support for the role of light PA in reducing CVD risk factors, such as WC and the proportion of body fat [119]. However, the studies included in this review were mostly of short duration and many of them had a low to fair study design. Our results showed a strong association between the cumulative average of light PA and incidence of obesity in a large sample of older adults. These finding highlight one of the benefits of light PA in this age-range population.

Previous to the publication of our results (Article II), there was emerging evidence showing an inverse association between light PA and all-cause mortality [80, 120]. This evidence was later supported by our findings (Article II), which showed that even intermediate levels of light PA were associated with a 22% decrease in all-cause mortality. Additionally, it showed that intermediate levels of light PA joined with an intermediate adherence to the MedDiet were associated with a 58% decrease in all-cause mortality. The association between light PA and all-cause mortality disappeared after meeting 300 METs·min/d, indicating no further benefits with higher levels of light PA. It is especially interesting that the decreases in all-cause mortality associated with light PA were only slightly lower than those obtained with higher levels of MVPA. These findings suggest that the promotion of light PA among older adults is an alternative approach to prolong lifespan.

After the publication of our results (Article II) further evidence was released. Two reviews reported an inverse association of light PA and all-cause mortality in the adult population [20, 121]. This reviews also highlighted the need for more prospective evidence regarding cardiometabolic health parameters. A recently published study in older adults has also indicated an inverse association between light PA and all-cause mortality [122].

After the publication of our results (Article I and II), a relevant longitudinal study in the adult population was published. This article supported some of our findings, by showing an inverse association between light-intensity PA and all-cause mortality, WC, triglycerides, and prevalence of the metabolic syndrome [123]. Therefore, after revising the results of this thesis and the new evidence published, it seems that the promotion of light intensity PA could be an effective approach to prevent premature death and control several cardiovascular risk factors.

Surprisingly, we found that increases in light PA were not significantly associated with decreases in the inflammatory profile (Article III). Studies showing an inverse association between these two variables are few and mostly cross-sectional [69, 70, 124–127]. For instance, Nilsson and colleges recently found that light PA was transversally associated with lower fibrinogen, but not with lower CRP and adiponectin levels [126]. Therefore, if the aim among older adults is to decrease the inflammatory profile, the promotion

of MVPA seems to be appropriate. However, according to our findings, an increase only in moderate PA seems to be sufficient to decrease the overall inflammatory profile. Taken into account the decrease in the physical performance in older adults, it seems more appropriate to promote moderate-intensity PA when aiming to control low-level inflammation.

In summary, the findings in this thesis support the promotion of light and moderate PA. This is especially relevant for older adults who cannot perform other intensities of PA and for those older adults who feel more enjoyable at lower intensities of PA. As we observed in the PREDIMED study, the types of activities that older adults perform the most are gardening and walking, which are types of activities usually classified as light PA. It seems coherent to suggest that promoting light and moderate intensities of PA are more convenient for older adults. In fact, there is already sounding evidence that the tailored promotion of low and moderate intensities of PA, including walking, seems to be particularly effective in this population [102].

5.2.7 Mechanisms

The finding that PA was prospectively associated with surrogate markers of fat regardless of energy intake and diet quality could be explained by the physiological increase in the oxidation of carbohydrate and fat that takes place during exercise [128]. In older adults, repeated and prolonged exercises decrease the maximal

capacity of fat oxidation, probable due to a decrease in the availability of fatty acids in the plasma. However, during exercise, muscles tend to increase their lipolysis and increase their capacity to transport glucose leading to an increased contribution of exogenous glucose and endogenous fat to the body energy production [129].

The underlying mechanisms that could explain the association of PA and adherence to a MedDiet with mortality are varied. The strong impact of this this two lifestyle factors in the incidence of chronic diseases seems to be the main mechanism [22, 90, 91]. A higher adherence to the MedDiet has also been related with anti-inflammatory and antioxidant effects [54], which could contribute to attenuate the effects of ageing and therefore contribute to decrease mortality. On the other hand, it has been observed that among older adults PA contribute to preserve the function and structure of the heart, attenuating the age-related changes in the cardiovascular system and therefore decreasing the risk of cardiovascular mortality [130].

Smoking and increased age, BMI, and fat intake have been consistently associated with higher levels of inflammation [131, 132]. Changes in these pro-inflammatory factors could partially explain the inverse association between changes in PA and changes in the inflammatory profile. However, this association was true independently of these pro-inflammatory covariables. Similarly, Vella and colleagues found an inverse association between MVPA and inflammatory biomarkers, which was independent of

objectively measured subcutaneous and visceral fat [133].This findings could indicate the presence of underling mechanisms that could explain these results. Several studies have showed an association between PA and changes in the epigenetic. These studies have linked regular and moderate exercise with favourable epigenetic changes, including a down-regulation in the systemic inflammation [134–136].

Finally, all the mechanism explaining the results of this thesis could be connected to each other. The decrease in the incidence of general and abdominal obesity and the decrease in the inflammatory profile could be part of the mechanisms underlining the reduction in mortality. Recent evidence has shown a strong association between a higher inflammatory profile and a higher risk of all-cause mortality [137, 138]. Similarly, obesity has been linked for a long time with a significant increase in all-cause mortality [139, 140].

5.3 Strength and limitations

An important strength of this thesis was the use of data from two large-scale and well-design randomized clinical trials called the PREDIMED and the PREDIMED-Plus studies. The articles included in this theses had a prospective design, included a large sample size of older adults (especially in Articles I, II and IV) and used the repeated measures of different variables through follow-up.

A limitation of the articles included in this thesis was the use of self-reported PA data as the exposure variable. All registration tools involving recalling and reporting are sensitive to measurement errors and misclassifications. However, these errors are most likely to be random, which could attenuate the associations found in the presented articles.

5.4 Future research

The PREDIMED-Plus trail is a still on-going randomized control trial. During the progression of this trial and after it ends, a great number of evidence will be developed.

Studies will be related to the effects of the combined dietary and PA interventions on different health outcomes in a large population of older adults. Articles specifically related to PA could asses the effectiveness of the PREDIMED-Plus PA intervention after the trial finishes. This would allow obtaining conclusions of the long-term adherence of older individuals to these types of interventions.

Chapter 6
CONCLUSIONS

6. CONCLUSIONS

The present thesis analysed the prospective associations of PA levels with cardiovascular risk factors and all-cause mortality in older adults at high risk of CVD. It also analysed the effectiveness of a PA intervention as part of the PREDIMED-Plus multicomponent intervention program. The conclusions obtained were the following:

i. Total and MVPA had an inverse prospective association with BMI, WC, and incidence of general and abdominal obesity. Light PA was associated with BMI and the incidence of general obesity.
ii. Adherence to a MedDiet and LTPA (total, light and MVPA) had an inverse independent and joint association with all-cause mortality. The highest joined categories of the adherence to the MedDiet and PA (total, light and MVPA) showed the strongest association with the outcome.
iii. Changes in total, moderate, and MVPA had an inverse association with changes in the inflammatory profile, independently of inflammatory related cofounders.
iv. The 1-year PA intervention of the PREDIMED-Plus was effective at increasing levels of PA in adults with overweight or obesity and metabolic syndrome, independently of potential moderators.
v. The findings that light PA was inversely associated with BMI, incidence of general obesity and risk of all-cause

mortality, and that changes in moderate PA were inversely associated with changes the inflammatory profile, are relevant because this intensities of PA provides a feasible option for older adults.

These conclusions add evidence for the recommendations of light and moderate PA among older adults. The PA intervention of the PREDIMED-Plus might serve as a model for future public health prevention strategies aiming to increase PA levels in the older population.

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