UNIVERSIDAD DE GRANADA

SLEEP QUALITY AS A HEALTH AND AGING MARKER IN SEDENTARY MIDDLE-AGED ADULTS.

THE FIT-AGEING STUDY

DOCTORAL PROGRAM IN BIOMEDICINE

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International Doctoral Thesis / Tesis Doctoral Internacional

Sleep quality as a health and aging marker in sedentary middle-aged adults.
The FIT-AGEING study

Calidad del sueño como marcador de salud y envejecimiento en adultos sedentarios de mediana edad.
Estudio FIT-AGEING

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"Wherever you go, go with all your heart."
-Confucius-

"Dondequiera que vayas, ve con todo tu corazón."
-Confucio-

To Mom, Dad, Grandma, and Siblings,
for so much love and dedication

To people with magic,
especially Ángeles Almendros

A Mamá, Papá, Abuelita y Hermanos, por
tanto amor y dedicación

A las personas con magia,
en especial a Ángeles Almendros

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RESEARCH PROJECT AND FUNDING

The present International Doctoral Thesis was mainly performed under the framework of the FIT-AGEING study, which was funded by the "Junta de Andalucía" (B-CTS-363-UGR18).

ABBREVIATIONS

BCHox: Basal carbohydrate oxidation

BEDCA: "Base de Datos Española de Composición de Alimentos"

BFox: Basal fat oxidation

BMAL1: Brain and muscle ARNT- like protein 1

BMI: Body mass index

BMR: Basal metabolic rate

CEI: "Comité Ético de Investigación"

CI: Confidence interval

CLOCK: Circadian locomotor output cycles kaput

CMO: "Contenuto minerale osseo"

CRY: Cryptochrome

DMO: "Densità minerale ossea"

ENMO: Euclidean norm minus one

ES: "Efficienza del sonno"

FATmax: intensity of exercise that elicits MFO

FMI: Fat mass index

HDL-C: High-density lipoprotein cholesterol

HOMA: Homeostasis model assessment index

IC: Indirect calorimetry

IGF-1: Insulin-like growth factor 1

IL: Interleukin

IMC: "Indice di massa corporea"

IMG: "Indice di massa grassa"

IMM: "Indice di massa magra"

iMUDS: "Instituto Mixto Universitario Deporte y Salud"

ISAK: International Society for the Advancement of Kinanthropometry

LDL-C: Low-density lipoprotein cholesterol

LMI: Lean mass index

LPA: Light physical activity time

MedDiet: Mediterranean diet

MFO: Maximal fat oxidation

MPA: Moderate physical activity time

MPV: Mean platelet volume

MSLT: Multiple sleep latency test

MUFA: Monounsaturated fatty acids

MVPA: Moderate-vigorous physical activity time

NPAS2: Neuronal PAS domain protein 2

NREM: Non-rapid eye movements

ns: Nonsignificant

OSA: Obstructive sleep apnoea

PER: Period

PREDIMED: "Prevención con dieta mediterránea"

PSG: Polysomnography

PSQI: Pittsburgh Sleep Quality Index

PUFA: Polyunsaturated fatty acids

REM: Rapid eye movements

RVF: "Rapporto vita-fianchi"

S-Klotho: Secreted Klotho

SD: Standard deviation

SE: Sleep efficiency

SFA: Saturated fatty acids

SPSS: Statistical Package for Social Sciences

TST: Total sleep time

TTS: "Tempo totale di sonno"

TVI: "Tempo di veglia infrasonno"

USDA: United States Department of Agriculture

VCO_2: Carbon dioxide production

VO_2: Oxygen uptake

VO_2max: Maximal oxygen uptake

VPA: Vigorous activity time

WASO: Wake after sleep onset

WHO: World Health Organization

ABSTRACT

ABSTRACT

Current lifestyle choices—mainly characterized by novel technological and sociocultural rhythms—have led to altered sleep patterns which are related to an increased morbidity and mortality risk. The prevalence of sleep disorders in the general population has substantially increased in the last decade, becoming on an economic and clinical burden for the health system. Thereby, given that sleep is vital for restoration and preservation of multiple physiological systems, the development of both generalized and personalized sleep promotion strategies to encourage a healthy sleep pattern results crucial in order to avoid significant public health burdens. Thus, the role of sleep on health and aging-related markers needs to be deeply studied.

The main aim of this International Doctoral Thesis is to study the association of both subjective (measured by the Pittsburgh Sleep Quality Index questionnaire) and objective (assessed by accelerometry) sleep quantity and quality with health and aging-related markers (i.e., body composition, energy metabolism, cardiometabolic risk, hematological parameters, S-Klotho anti-aging protein, and physical activity and fitness) in sedentary middle-aged adults.

The results show that a poor subjective sleep quality is associated with an altered body composition status (i.e., decreased bone mineral density and lean mass, and increased fat mass), energy metabolism (i.e., lower basal fat oxidation), cardiometabolic risk profile (i.e., worse plasma lipid profile), hematological parameters (i.e., impaired hemostasis), S-Klotho anti-aging protein levels (i.e., decreased S-Klotho plasma levels), and physical activity and fitness (i.e., lower levels of overall physical activity, maximal oxygen uptake and muscular strength), all of them widely considered as aging biomarkers, in sedentary middle-aged adults. Interestingly, our results show that a poor objective sleep quantity is specifically associated with some altered cardiometabolic risk factors (i.e., greater waist circumference and higher levels of plasma glucose), and increased levels of sedentariness in sedentary middle-aged adults.

In summary, the results of this International Doctoral Thesis suggest that sleep plays a key role on diverse health and aging-related markers such as body composition, energy metabolism, cardiometabolic risk, hematological parameters, S-Klotho anti-aging protein, sedentariness, and physical activity and fitness. Thus, these findings suggest that sleep may be a modifiable risk factor of chronic diseases and the aging process.

RESUMEN

El estilo de vida actual, influido por nuevos e importantes cambios tecnológicos y socioculturales, ha desencadenado patrones de sueño alterados que se relacionan con un mayor riesgo de morbilidad y mortalidad. La prevalencia de los trastornos del sueño en la población general ha aumentado sustancialmente en la última década, convirtiéndose en una carga económica y clínica para el sistema de salud. Así, dado que el sueño es vital para la reparación y conservación de múltiples sistemas fisiológicos, resulta crucial desarrollar estrategias, tanto generalizadas como personalizadas, de promoción del sueño para fomentar un patrón de sueño saludable y evitar consecuencias significativas para la salud pública. Por lo tanto, el papel del sueño sobre diversos marcadores relacionados con la salud y el envejecimiento debe estudiarse en profundidad.

El objetivo principal de esta Tesis Doctoral Internacional es estudiar la asociación entre cantidad y calidad del sueño, evaluadas tanto subjetiva (medida con el cuestionario Índice de Calidad de Sueño de Pittsburgh) como objetivamente (medida mediante acelerometría), y marcadores relacionados con la salud y el envejecimiento (i.e., composición corporal, metabolismo energético, riesgo cardiometabólico, parámetros hematológicos, proteína antienvejecimiento S-Klotho y actividad y condición físicas) en adultos sedentarios de mediana edad.

Los resultados muestran que una mala calidad subjetiva del sueño se asocia a alteraciones en composición corporal (i.e., disminución de densidad mineral ósea y de masa magra, y aumento de masa grasa), metabolismo energético (i.e., menor oxidación basal de grasas), perfil de riesgo cardiometabólico (i.e., peor perfil lipídico), parámetros hematológicos (i.e., alteración de la hemostasia), niveles de proteína antienvejecimiento S-Klotho (i.e., reducción de niveles plasmáticos de S-Klotho) y actividad y condición físicas (i.e., niveles más bajos de actividad física general, de consumo máximo de oxígeno y de fuerza muscular), todos considerados biomarcadores de envejecimiento, en adultos sedentarios de mediana edad. Curiosamente, nuestros resultados muestran que una mala cantidad objetiva del sueño se asocia especificamente a ciertos factores de riesgo cardiometabólico (i.e., mayor circunferencia de cintura y mayores niveles de glucemia), así como a niveles mayores de sedentarismo en adultos sedentarios de mediana edad.

En resumen, los resultados de esta Tesis Doctoral Internacional sugieren que el sueño juega un papel clave en diversos marcadores relacionados con la salud y el envejecimiento como la composición corporal, el metabolismo energético, el riesgo cardiometabólico, los parámetros hematológicos, la proteína antienvejecimiento S-Klotho, el sedentarismo y la actividad y condición físicas. Por consiguiente, estos hallazgos sugieren que el sueño puede ser un factor de riesgo modificable de enfermedades crónicas y envejecimiento.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

As the French novelist François Sagan once said, "Happiness for me consists in enjoying good health, sleeping without fear and waking up without distress". In 1948, health was defined as "a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity" by the World Health Organization (WHO) [1]. However, Huber et al. [2] recently explained that this definition of health as entire wellbeing is no longer fit for purpose due to the significant increase of chronic disease, and suggest to change the emphasis towards the ability to adapt and self-manage in the face of social, physical, and emotional challenges.

Indeed, demographic changes in developed countries are leading to an aging population and an upsurge in vulnerability to morbidity and age-related chronic medical conditions among adults [3], becoming one of the most noteworthy global clinical and economic burdens for health systems and all aspects of society. Thus, the Global Burden of Disease Study 2017 [4], recognized 51.3% of all burdens among adults as age-related diseases, mostly including non-communicable diseases. Concretely, the WHO have described cardiovascular, endocrine, respiratory, and mental disorders, and neoplasms as the five major non-communicable diseases, all having a crucial impact on humans' health [5].

A preventive strategic plan to promote general health and to avoid, detect, and/or treat age-related chronic disease in the total population is actually necessary. Thus, the WHO developed a global strategy and action plan on aging and health in 2017 with aims and tactical purposes giving attention to health system alignment to the needs of the older population and the improvement of measurement, monitoring, and research to hold up healthy ageing [6,7]. Lifestyle behaviors in modern societies such as physical activity, diet, and sleep—the great forgotten "elixir" of health and physical recovery—are all well-recognized modifiable risk factors of chronic diseases, becoming, therefore, the spotlight.

SLEEP AS A KEY LIFE ELEMENT

Sleep throughout history

The medical concern and interest in sleep date back to ancient times. Diverse philosophers and scholars of ancient civilizations such as Greece (i.e., Alcmaeon of Croton, Hippocrates, Plato, Aristotle and Artemidorus of Daldis) and Rome (i.e., Asclepiades, Titus Lucretius Carus, Cicero, Galen, and Macrobius), and of the Middle Ages (i.e., Arnau de Vilanova, Lope de Barrientos, Avicenna and Maimonides) took care of sleep. Later, in the 16th and 17th centuries, Descartes and Willis proposed theories about the onset of sleep, as well as playwrights as Cervantes dealt with the topic of sleep. Lastly, diverse theories (vascular, neuronal, chemical and behavioral) regarding the origin of sleep were developed in the 19th century, which is also known due to the famous book published by Freud, "The interpretation of dreams". Another important event in this century was the invention of the electric light by Edison in 1879, a date which will suppose a change in the humanity since it would be easier to alter sleep rhythms, reduce total sleep time, and to break the synchronization with day-light leading to significant sleep disturbances [8].

However, until relatively recent dates, little has been known about sleep. Undoubtedly, an important fact is the moment in which the first electroencephalographic record is obtained by Berger in 1929, showing differences in brain activity throughout vigil and sleep. Nonetheless, the event that definitely marked a before and after in the study of sleep was the discover of sleep associated to rapid eye movements (REM) sleep by Aserinsky and Kleitman in 1953. After that, the hypothalamus was identified as a pacemaker of the sleep-wake cycle, and "A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects" was edited by Rechtschaffen and Kales in 1968. It was not until 1972 when the European Sleep Research Society was founded, and until 1987 when The World Federation of Sleep Research & Sleep Medicine Societies were created. Moreover, the first medical journal exclusively dedicated to sleep, under the name of "Sleep", was edited by Guilleminault and Dement in 1977, and the first sleep medicine treatise "Principles and Practice of Sleep Medicine" was born in 1989. Interestingly, the current 21st century is accompanied by the certification of the first specialists in sleep medicine in Europe [8].

Sleep architecture and physiology – age-related changes

Far from a simple absence of wakefulness, sleep is an actively regulated process notably modulated by homeostatic influences that accumulate during ongoing wakefulness and dissipate during sleep, and by circadian effects entrained to the 24-hour day [9]. Definitively, sleep is a broadly accepted key component of physiological restitution [10,11] essential for mental and physical health, and thus general well-being [12,13], including one third part of the life of the human being [14].

Hence, knowing the (i) anatomy/architecture and (ii) physiology of normal sleep and sleep homeostasis results mandatory to ensure a general good health, and to understand and treat possible alterations in sleep patterns and, consequently, sleep disturbances. On the one hand, sleep has a particular architecture characterized by a rhythmic alternation between REM and non-REM (NREM) stages. In addition, NREM sleep is composed by three stages (N1-N3) of progressively deepening sleep (it differs from the old Rechtschaffen and Kales' sleep staging nomenclature describing four NREM stages) [15]. Through a normal night, the sleep process is cyclical, with sleep onset being followed by a rapid descent to deep stage N3 sleep in the first hour. After that, cyclical alternations between NREM and REM sleep take place every 60–90 minutes during the rest of the night. Typically, most N3 sleep occurs during the first half-night, whereas most REM sleep appears during the second half-night (see Figure 1). Skeletal muscle activity exhibits successively decreasing amplitude with transitions from wakefulness to N1, N2, and N3 sleep, and REM sleep is associated with the lowest skeletal muscle tone. Transitions among sleep/wake passages are organized by a well-defined subcortical network of brain structures [9,16].

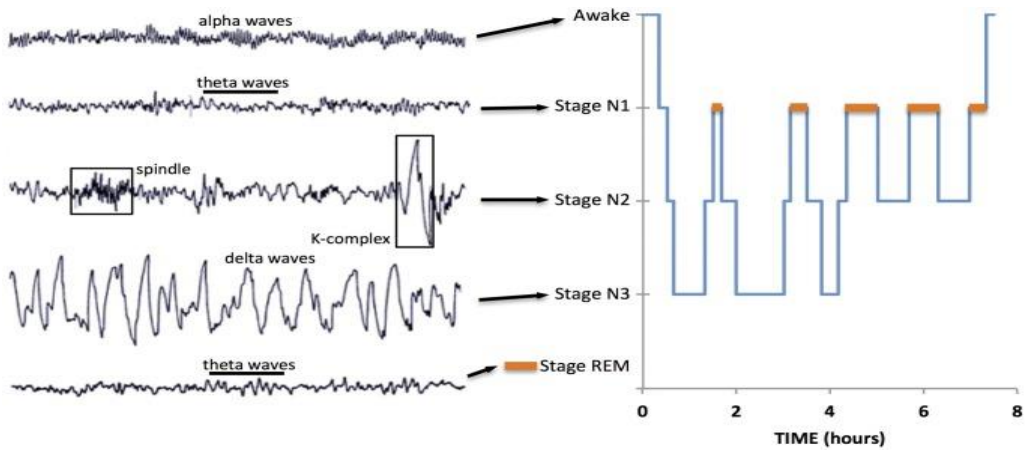

Figure 1. Electroencephalographic features of sleep/wake stages (left) and typical temporal organization of healthy nocturnal sleep in an adult (right). Adapted from "Physiology of Sleep" by Carley et al. [9].

As previously mentioned, alterations in the typical architecture of sleep could lead to sleep disturbances. In this sense, and given the high prevalence of sleep disorders in the current society [17–21], a detailed measurement of sleep is recommended as a routine component of geriatric management and as a major component of research involving older adults [22]. Screening and evaluation tools for sleep disorders can be mainly divided into two types: (i) subjective sleep instruments as questionnaires, scales, and diaries; and (ii) objective sleep instruments, including actigraphy, in-lab polysomnography (PSG), in-home PSG, and multiple sleep latency test (MSLT) [22]. However, psychological constructs, such as perceived sleep quality, are difficult to define for a whole group, as people have their own individual interpretation strategies regarding the course of the night and when answering sleep-related questions. Thus, the association among perceived sleep quality and actigraphy-based sleep characteristics is only modest [23]. In this sense, this International Doctoral Thesis was aimed at investigating the association of subjectively (i.e., self-administrated Pittsburgh Sleep Quality Index [PSQI] scores) and objectively (i.e., wrist-worn accelerometry) measured sleep quantity and quality with several health and aging-related outcomes in sedentary middle-aged adults. Apart from that, undoubtedly, a good sleep evaluation starts with a correct and personalized anamnesis.

On the other hand, regarding physiology, sleep is a vital physiological process with substantial and essential restorative functions for optimal day-time functioning [24]. Thereby, poor sleep quality has been linked with neurocognitive impairments, end-organ dysfunction and chronic health conditions, as well as raised mortality [24–57]. Importantly, changes in sleep quantity and quality are a well-established fact that occurs with ageing. In this respect, it is important to distinguish between normal age-associated changes in sleep patterns and treatable complaints [24]. Sleep is linked to the aging process in adults through diverse mechanisms and physiological pathways as oxidative stress [58] and chronic inflammation [59,60] —leading molecular mechanisms behind many age-related consequences [61–64]. Thus, aging is related to a decline in the amount of slow wave sleep and an increase in stage N1 and N2, often attributed to an increment in the number of spontaneous arousals taken place in the elderly (see Figure 2). Moreover, elderly people use to go to sleep earlier in the evening and wake earlier as a result of a phase advance in their normal circadian sleep cycle [24]. Hence, this International Doctoral Thesis also investigated whether age-related changes in sleep quantity and quality are associated with diverse health disturbances which also tends to appear or aggravate during the aging process. Therefore, studying aging-related molecules like the anti-aging protein S-Klotho in a

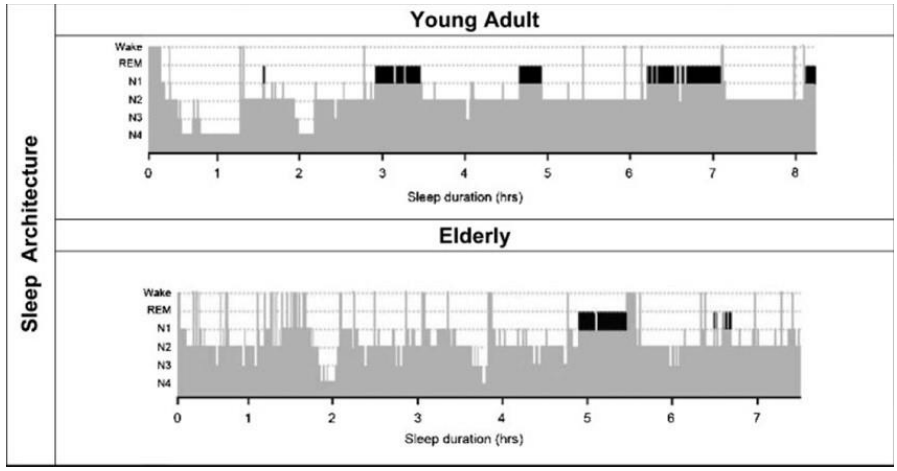

Figure 2. Effect of aging on the architecture of sleep. Typical hypnogram from a healthy young adult aged 24 years and a healthy elderly individual aged 72 years. Adapted from "Aging and Sleep: Physiology and Pathophysiology" by Edwards et al. [24].

middle-aged adults' cohort is of great interest.

Effectively, circadian sleep cycle suffers from some modifications in the elderly. Normally, most people have sleep–wake and activity rhythms slightly longer than 24 hours. Thus, circadian rhythms are near-24-hour oscillations observed in basically every physiological process in human brain and body. The suprachiasmatic nucleus placed in the hypothalamus acts as the master pacemaker synchronizing the timing of rhythms by the control of neuronal activity, body temperature, and hormonal signals (see Figures 3 and 4) [65,66]. In individual cells, molecular rhythms are generated by a transcriptional-translational feedback loop implying core transcriptional activators—circadian locomotor output cycles kaput (CLOCK), the closely related neuronal PAS domain protein 2 (NPAS2), and brain and muscle ARNT- like protein 1 (BMAL1)—that set the expression of diverse genes including those encoding period (PER) and cryptochrome (CRY), that inhibit their own transcription after being translated. Several more proteins (i.e., kinases, phosphatases, and other transcriptional cofactors) regulate this core molecular clock (see Figure 3) [65]. Importantly, both circadian gene variants and environmental factors (e.g., light exposure, social cues, meal times, and work schedules) can affect the period, phase and amplitude of these rhythms [65].

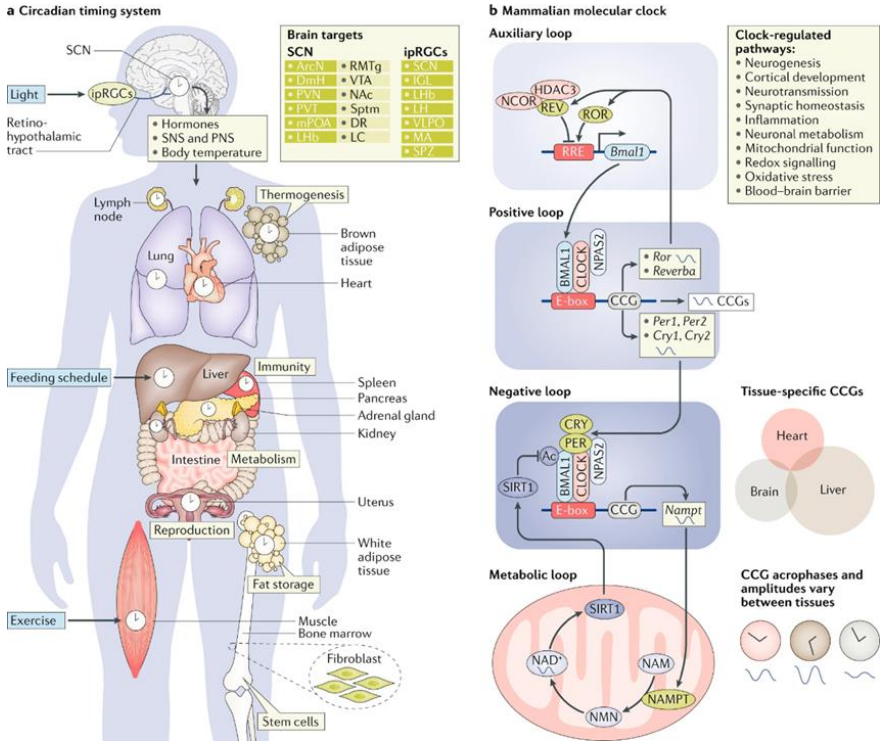

Figure 3. The circadian timing system. (a) The circadian timing system synchronizes clocks over the whole body to adapt and optimize physiology to changes in our environment. (b) The mammalian molecular clock is formed by transcriptional and translational feedback loops that oscillate with a near-24-hour cycle. Adapted from "Rhythms of life: circadian disruption and brain disorders across the lifespan" by Logan et al. [65].

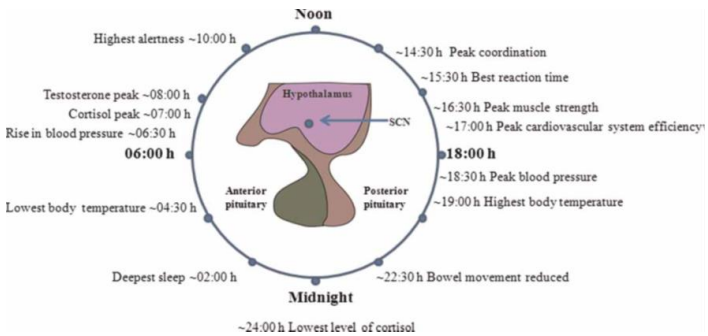

Figure 4. The location of the suprachiasmatic nucleus and an overview of some biochemical and physiological events associated with circadian rhythms. Adapted from "Interactions of cortisol, testosterone, and resistance training: influence of circadian rhythms" by Hayes et al. [66].

Sleep and physiological systems – sleep-related dysfunctions

Sleep, as an important component of human life, plays an important role in numerous vital functions as development, energy conservation, brain waste clearance, modulation of immune responses, cognition, performance, vigilance, disease, and psychological state [67].

Sleep and cardiovascular system

Sleep is a significant regulator of cardiovascular function, both in physiological conditions and in disease states. Thus, sleep may have an important impact on the autonomic nervous system, systemic hemodynamics, coagulation, and cardiac and endothelial function in individuals without a primary sleep disorder [25].

Altered sleep quantity and quality influence dietary pattern and physical activity levels developing diverse physiological disturbances such us greater oxidative stress, systemic inflammation, endothelial dysfunction, altered hormonal secretion, and sympathetic systemic activation, leading to increase cardiometabolic risk [26]. Several studies have suggested the possible causal link between sleep disturbances (e.g., sleep restriction, sleep-disordered breathing, or shift work) and cardiovascular disease (i.e., hypertension, atherosclerosis, stroke, heart failure, cardiac arrhythmias, or sudden death) [27–31]. In addition, sleep problems may be caused due to numerous medical conditions as obesity or chronic heart failure, contributing, therefore, to cardiovascular morbidity linked to these conditions [25].

Sleep and metabolic and endocrine system

Sleep has been postulated as a major modulator of hormonal release and glucose regulation. During slow wave sleep, somatotropin—the anabolic hormone—and prolactin are secreted, while cortisol—the stress hormone—is inhibited. Both somatotropin and cortisol have important functions in glucose metabolism. Moreover, sleep seems to be key in the control of blood glucose levels, as well as in the regulation of leptin and ghrelin levels—hormones involved in the central control of appetite and energy expenditure— [32].

Variability through days in duration of sleep and sleep schedules may, therefore, promote a mismatch between behavioural cycles and innate circadian rhythms leading to a dysregulation of metabolic and endocrine functions [33]. Thus, sleep curtailment has been postulated as a possible important, yet modifiable, risk factor for numerous metabolic and endocrine-related health complications (e.g., decreased glucose tolerance, decreased insulin sensitivity, elevated sympathovagal balance, increased evening concentrations of cortisol, increased levels of ghrelin, decreased levels of leptin, and increased hunger and appetite, among others) [34,35], linked, therefore, to obesity, type 2 diabetes, and metabolic syndrome [33,36–38],

increasing all-cause morbidity and mortality risk.

Sleep and reproductive system

Sleep is crucial in creating general signatures to conduct the synthesis, secretion, and metabolism of hormones needed for reproduction. In this sense, the reproductive function is modulated through diverse sex hormones secreted in synergy with the circadian rhythm of the body [39,68–70].

Sleep deprivation could thereupon influence reproductive hormones levels, key players in determining the tendencies of both male and female fertility. Insomnia leads to physiological disturbances similar to oxidative stress, stimulating the activation of the hypothalamus-pituitary-adrenal axis and inhibiting the hypothalamus-pituitary-gonadal axis, resulting therefore in an increased corticosteroids plasma level which are implicated in numerous cases of infertility in both men and women. In addition, circadian disruption caused by altered work schedules could affect reproductive health by deregulation of sex steroids, gonadotropins, and prolactin production. Surprisingly, it has been shown that sleep curtailment could not only have damage effects on reproduction functions but also transcend to the offspring by impairing their sexual performances [39].

Sleep and excretory system

The sleep-arousal cycle affects diverse physiological processes considered significant for renal function and the production of urine. The transition from wakefulness to sleep is linked to an important decline in diuresis, a required physiological process that let sleeping without interruptions [40].

Acute sleep deprivation leads to higher sympathetic activity and elevated blood pressure, heart rate, and catecholamine excretion. In this sense, nights deprived of sleep are distinguished by natriuresis, osmotic diuresis, and a dramatic urine production increase. Blood pressure dipping is attenuated, and the renin-angiotensin-aldosterone system is clearly suppressed [40].

Sleep and respiratory system

Sleep and arousal from sleep are related to great changes in respiratory function [71]. Sleep causes a withdrawal of the wakefulness stimuli and a start of dynamic sleep processes that interact with the respiratory system, resulting in a ventilation mainly determined by metabolic demand. Therefore, a slight hypoventilation is induced through sleep, as well as a maintenance of eucapnia [41]. The control of the diverse parts of the respiratory system is heterogeneous: upper airway muscle activity appears to be more dependent on waking mechanisms to carry on activity than is the diaphragm. Moreover, the areas of the brain stem that participate in the maintenance of sleep state also seem to contribute to diverse responses to a variety of respiratory stimuli (i.e., hypercapnia, hypoxia, and a diverse upper airway reflexes) [72].

Sleep constitutes a vulnerable state for aggravating hypoventilation and gives rise to respiratory failure in patients with chronic lung diseases or neuromuscular weakness [41].

Sleep and musculoskeletal system

Sleep is characterized by an elevated vagal activity and reduced muscle tonus. In this sense, sleep could act as a natural muscle relaxation agent. Sleep is preserved by several brain structures which play also a role in pain modulation [42].

Inadequate sleep could disturb the relaxation and/or recovery of muscle activity. Moreover, sleep disturbance produce an activation of the hypothalamus-pituitary-adrenal axis, which could be linked to new onset of chronic general pain [42]. In addition, sleep curtailment generates elevated levels of pro-inflammatory cytokines and modifications in central pain processing [43]. Thereby, sleep deprivation leads to harmful effects on diverse clinical outcomes in patients with musculoskeletal pain by aggravating pain levels, psychological health status, and physical functionality [44].

Sleep and gastrointestinal system

Circadian rhythms are involved in digestive functioning, including epithelial turnover, immune function, hepatic metabolism, intestinal permeability, colonic motility, and gut peristalsis. Sleep evokes several changes in the digestive system (e.g., changes in swallowing and salivation, in esophageal and gastric acidity, in sphincters pressures, and in mobility patterns) [45,73,74].

Alterations in sleep cause diverse physiological changes which direct and indirectly affect gastrointestinal functioning, leading to a strong impact in symptom manifestation and the pathogenesis of digestive diseases as gastroesophageal reflux disease, irritable bowel syndrome, inflammatory bowel disease, non-alcoholic fatty liver disease, and colorectal cancer [45,46]. Moreover, sleep disturbances are linked to gut dysbiosis, maybe due to activation of the hypothalamus pituitary adrenal-axis [47].

Sleep and central nervous system

Sleep is vital to keep the central nervous system—the main body information highway—functioning properly. Each phase of the sleep cycle restores and rejuvenates the brain for an adequate function, through a reorganization and a toxic waste byproducts disposal accumulated throughout the day. Sleep improves memory recall, regulate metabolism, and reduce mental fatigue. Thus, healthy sleep is needed for proper cognitive and behavioral function [48].

Sleep deprivation produces a dysfunction of the active process of the glymphatic system, which lead to an increased accumulation of toxic metabolites, that could, therefore, have an impact on motor functions, cognitive abilities, and behavioral patterns, emphasizing the

possible both short and long term dangerous repercussions (i.e., Alzheimer's disease and dementia) [48,49].

Sleep and immune system

Sleep and the circadian system exert a strong regulatory influence on immune functions. Indeed, it has been showed that differentiated immune cells with immediate effector functions (i.e., cytotoxic natural killer cells, and terminally differentiated cytotoxic T lymphocytes) peak through daytime wakefulness, allowing an efficient and fast combat of intruding antigens and reparation of tissue damage, which tends to take place during the active phase of the organism. However, undifferentiated or less differentiated cells (i.e., naïve and central memory T cells) peak through early nocturnal sleep, when the more slowly evolving adaptive immune response is started [50].

Prolonged sleep shortening and the subsequent stress response lead to a persistent unspecific production of pro-inflammatory cytokines, considered as chronic low-grade inflammation, and also produce immunodeficiency, both of them causing deleterious effects on health [50,51].

Taking into account the crucial role of sleep on multiple physiological systems and considering the controversial findings previously reported regarding the potential link between sleep alterations and health, it is of notorious interest to investigate the relationship of sleep quantity and quality with diverse health and aging-related outcomes in a sedentary but still healthy middle-aged adults' cohort.

SLEEP HEALTH PROMOTION: SLEEP AS A MEDICINE

Current lifestyle choices—mainly characterized by novel technological and sociocultural rhythms (i.e., greater access to television and internet or work demands)—have led to altered sleep patterns which are related to an increased morbidity and mortality risk [75]. The prevalence of sleep disorders in the general population has substantially increased in the last decade [17–19], becoming on an economic and clinical burden for the health system, reaching insomnia a total of $100 billion USD per year [76] . The most common sleep disorders are insomnia and obstructive sleep apnoea, with a prevalence of 10–40% and 9–38% in the overall population [20,21], respectively, both related to the development and worsening of a broad-spectrum of chronic medical conditions [52–57].

Thereby, given that sleep is vital for restoration and preservation of multiple physiological systems, the development of both generalized and personalized sleep promotion strategies (i.e., healthier habits including adequate environment conditions and schedules, fit diet, and/or physical fitness and activity, among others) to encourage a healthy sleep pattern results crucial in order to avoid significant public health burdens. In this sense, evaluating people particular circumstances and identifying those behaviors most likely to induce sleep disturbance could be of great interest. Moreover, sleep hygiene education could be a key approach for a sleep upgrade in the general population [77].

After all, sleep is integral to life [28], and, paraphrasing the famous Descartes' quote, the human being could say "I sleep, therefore I am".

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AIMS & HYPOTHESIS

AIMS

The overall aim of this International Doctoral Thesis is to study the link of sleep with health and aging related-markers in sedentary middle-aged adults. This overall aim is addressed in six studies.

Chapter 1: Sleep and body composition

Specific objective 1: To examine the relationship of sleep quantity and quality with anthropometric and body composition parameters in sedentary middle-aged adults **(Study 1)**.

Chapter 2: Sleep and energy metabolism

Specific objective 2: To study the relationship of sleep quantity and quality with energy metabolism related parameters in sedentary middle-aged adults **(Study 2)**.

Chapter 3: Sleep and cardiometabolic risk

Specific objective 3: To examine the relationship of sleep quantity and quality with cardiometabolic risk in sedentary middle-aged adults **(Study 3)**.

Chapter 4: Sleep and hematological parameters

Specific objective 4: To study the relationship of sleep quantity and quality with selected hematological parameters in sedentary middle-aged adults **(Study 4)**.

Chapter 5: Sleep and the S-Klotho anti-aging protein

Specific objective 5: To examine the relationship of sleep quantity and quality with S-Klotho anti-aging protein in sedentary middle-aged adults **(Study 5)**.

Chapter 6: Physical activity, fitness and sleep

Specific objective 6: To study the role of physical activity and fitness on sleep quantity and quality in sedentary middle-aged adults **(Study 6)**.

HYPOTHESIS

The overall hypothesis of this International Doctoral Thesis is that sleep is associated with different health and aging-related markers such as body composition, energy metabolism, cardiometabolic risk, immune function and hemostasis, S-Klotho anti-aging protein, and physical activity and fitness in sedentary middle-aged adults.

Chapter 1: Sleep and body composition

Specific hypothesis 1: Both subjective and objective sleep quantity and quality would be (i) negatively associated with body mass index, waist-hip ratio, fat mass and visceral adipose tissue, and (ii) positively associated with bone mineral content and density, and lean mass in sedentary middle-aged adults **(Study 1)**.

Chapter 2: Sleep and energy metabolism

Specific hypothesis 2: Both subjective and objective sleep quantity and quality would be (i) positively associated with specific energy metabolism related parameters such as basal metabolic rate, basal fat oxidation, and maximal fat oxidation, and (ii) negatively associated with basal carbohydrate oxidation in sedentary middle-aged adults **(Study 2)**.

Chapter 3: Sleep and cardiometabolic risk

Specific hypothesis 3: Both subjective and objective sleep quantity and quality would be (i) negatively associated with cardiometabolic risk factors including blood pressure, insulin resistance, glucose, insulin, total cholesterol, LDL-C, and triglycerides plasma levels, and (ii) positively associated with HDL-C plasma levels in sedentary middle-aged adults **(Study 3)**.

Chapter 4: Sleep and hematological parameters

Specific hypothesis 4: Both subjective and objective sleep quantity and quality would be associated with a healthier blood cell count, particularly in terms of leukocytes and platelets, thus reflecting a better immune function and hemostasis in sedentary middle-aged adults **(Study 4)**.

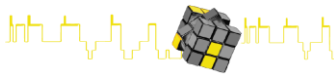
Chapter 5: Sleep and the S-Klotho anti-aging protein

Specific hypothesis 5: Both subjective and objective sleep quantity and quality would be positively associated with S-Klotho plasma levels in sedentary middle-aged adults **(Study 5)**.

Chapter 6: Physical activity, fitness and sleep

Specific hypothesis 6: Physical activity, cardiorespiratory fitness, and muscular strength would be positively associated with both subjective and objective sleep quantity and quality in sedentary middle-aged adults **(Study 6)**.

RESULTS & DISCUSSION



Chapter 1

Association of sleep with body composition in sedentary middle-aged adults: The FIT-AGEING study **(Study 1)**

"Associazione tra sonno e composizione corporea in adulti sedentari di mezza età: Studio FIT-AGEING"

SOMMARIO

L'invecchiamento è associato a cambiamenti sia nel pattern di sonno che nella composizione corporea, i quali sono correlati a diverse malattie. Scopo dello studio è stato quello di analizzare l'associazione tra quantità e qualità del sonno e un ampio insieme di parametri relativi alla composizione corporea (indice di massa corporea, rapporto vita-fianchi, contenuto minerale osseo [CMO], densità minerale ossea [DMO], massa magra, indice di massa magra [IMM], massa grassa, percentuale di massa grassa, indice di massa grassa e tessuto adiposo viscerale) in adulti sedentari di mezza età.

Sono stati reclutati un totale di 74 (39 donne) adulti sedentari di mezza età (40–65 anni). La quantità e la qualità soggettiva del sonno sono state determinate attraverso il Pittsburgh Sleep Quality Index (PSQI). Un punteggio PSQI globale superiore a 5 indica una scarsa qualità del sonno. I parametri oggettivi della quantità e la qualità del sonno sono stati invece valutati utilizzando un accelerometro da polso. Il peso, l'altezza, la circonferenza vita e i fianchi sono stati misurati, e l'indice di massa corporea e il rapporto vita-fianchi sono anche stati calcolati. La composizione corporea è stata valutata con uno scanner d'assorbimetria a raggi X a doppia energia. Il punteggio PSQI globale è stato associato negativamente con il CMO, la DMO, la massa magra e l'IMM (tutti $p \leq 0.008$), mentre è stato associato positivamente con la percentuale di massa grassa ($p = 0.009$) in adulti sedentari di mezza età. Questi risultati suggeriscono che una buona qualità del sonno potrebbe svolgere un ruolo significativo nella prevenzione delle alterazioni dello stato della composizione corporea (cioè, aumento della densità minerale ossea e della massa magra e diminuzione della massa grassa).

Parole chiave: indice di massa corporea; densità minerale ossea; massa magra; massa grassa; actigrafia.

INTRODUZIONE

I cambiamenti legati all'età nella composizione corporea, caratterizzati da una diminuzione della densità minerale ossea e della massa muscolare e un aumento della massa grassa, sono una problematica della società anziana [1]. Infatti, sarcopenia, obesità e osteopenia/osteoporosi sono tre malattie croniche frequenti nella popolazione anziana, anche se queste condizioni sono progressive e iniziano in giovane età [2,3]. Questi cambiamenti nella composizione corporea sono legati a una diminuzione della qualità della vita e ad un aumento del rischio di mortalità [4], producendo un significativo aggravio sia per il benessere dell'individuo che per la salute pubblica [3].

Un'altra problematica significativa associata all'invecchiamento è il cambiamento dei pattern del sonno [5], tra cui una diminuzione della quantità e la qualità del sonno [5]. È stato stimato che circa il 50% degli anziani lamenta difficoltà ad iniziare o mantenere il sonno [6]. Una peggiore qualità del sonno potrebbe aumentare l'infiammazione, diminuire la produzione di melatonina e interrompere i ritmi circadiani [7], i quali sono coinvolti in diverse malattie legate al processo di invecchiamento, come la malattia coronarica [8], il diabete di tipo 2 [9], l'obesità [10], la depressione [11], e di conseguenza l'aumento del rischio di mortalità [6].

Alcuni studi hanno proposto una relazione tra la qualità del sonno e la densità minerale ossea [12], la massa muscolare [13] e la massa grassa [14]. Una peggiore qualità del sonno compromette il ciclo catabolico /anabolico aumentando il rischio di osteoporosi [15], induce un'elevata resistenza all'insulina aumentando il rischio di sarcopenia [16] e produce delle alterazioni metaboliche ed endocrine aumentando il rischio di obesità [17]. Tuttavia, questi studi hanno utilizzato questionari soggettivi per la valutazione della qualità del sonno. Sebbene questi questionari siano stati precedentemente validati, non ci sono studi che valutino l'associazione tra la quantità e la qualità del sonno misurate mediante l'accelerometria e i parametri di composizione corporea.

Scopo di questo studio è stato quello di analizzare l'associazione tra la quantità e la qualità del sonno (misurata tramite metodi sia soggettivi che oggettivi) e un ampio set di parametri di composizione corporea (indice di massa corporea [IMC], rapporto vita-fianchi [RVF], contenuto minerale osseo [CMO], densità minerale ossea [DMO], massa magra, indice di massa magra [IMM], massa grassa, percentuale di massa grassa, indice di massa grassa [IMG] e tessuto adiposo viscerale) in adulti sedentari di mezza età. Abbiamo ipotizzato che i partecipanti con una scarsa quantità e qualità del sonno abbiano una bassa densità minerale ossea, una bassa massa magra e alti livelli di massa grassa.

MATERIALI E METODI

Protocollo di studio e partecipanti

Un totale di 74 adulti sedentari di mezza età (39 donne, 40–65 anni) sono stati reclutati per il presente studio. I partecipanti sono stati arruolati nello studio FIT-AGING [18], uno studio controllato randomizzato basato sull'esercizio (clinictrial.gov: ID: NCT03334357). Tutti i partecipanti hanno riferito di essere non attivi fisicamente (< 20 minuti di attività fisica di intensità moderata su 3 giorni/settimana), di aver mantenuto un peso stabile (variazioni di peso < 3 kg) negli ultimi 3 mesi, di non avere malattie e di non assumere alcun farmaco. Lo studio è stato approvato dal Comitato Etico sulla Ricerca Umana dell'Università di Granada e dal Servicio Andaluz de Salud (CEI-Granada) [0838-N-2017] (25 settembre 2017). I protocolli di studio e il disegno sperimentale sono stati applicati in conformità con i principi della Dichiarazione di Helsinki. Tutti i partecipanti hanno firmato un consenso informato. Tutte le variabili sono state valutate al basale dello studio FIT-AGING [18].

Misurazioni

Antropometria e composizione corporea

Le misurazioni del peso corporeo e dell'altezza sono state eseguite senza scarpe e con indumenti leggeri, utilizzando una scala elettronica e uno stadiometro pre-validati (modello 799, bilancia digitale a colonna, Amburgo, Germania). L'IMC è stato calcolato come $Peso\ (kg)\ /\ Altezza^2 (m^2)$ [19].

La circonferenza della vita nel punto medio tra l'ultima costa e la parte superiore della cresta iliaca e la circonferenza dell'anca attorno alla parte più ampia dei glutei sono state misurate (tre volte) utilizzando un nastro non elastico (Seca 200, MWS Ltd., Scalesmart, Leicester, Regno Unito) con l'approssimazione di 0.1 cm. Il RVF è stato calcolato dividendo la circonferenza della vita per la circonferenza dell'anca.

Uno scanner d'assorbimetria a raggi X a doppia energia (Discovery Wi, Hologic, Inc., Bedford, MA, Stati Uniti d'America) è stato utilizzato per misurare la composizione corporea seguendo le raccomandazioni del produttore. La scansione del corpo intero è stata considerata per ottenere tutti i parametri di valorazione della composizione corporea (CMO, massa magra, massa grassa e tessuto adiposo viscerale). I controlli di qualità, il posizionamento dei partecipanti e le analisi dei risultati sono stati condotti seguendo le raccomandazioni del produttore. Una segmentazione automatica delle regioni anatomiche è stata eseguita dal software APEX 4.0.2. Per il controllo della qualità, i fantocci della colonna vertebrale sono stati scansionati giornalmente. La DMO è stata calcolata come il CMO in grammi diviso per la superficie ossea totale in cm^2. L'IMM è stato calcolato come la massa magra in kg divisa per l'altezza al quadrato in m^2. Allo stesso modo, l'IMG è stato calcolato come la massa grassa in kg divisa per l'altezza al quadrato in m^2. La massa grassa è

stata espressa anche come percentuale rispetto alla massa corporea totale.

Quantità e qualità del sonno

La quantità e la qualità soggietiva del sonno dei partecipanti sono state valutate attraverso la somministrazione del Pittsburgh Sleep Quality Index (PSQI), una scala di 19 item suddivise in 7 sottoscale che valutano: (i) qualità soggettiva del sonno, (ii) latenza del sonno, (iii) durata del sonno, (iv) efficacia abituale del sonno, (v) disturbi del sonno, (vi) uso di farmaci per dormire e (vii) disfunzione diurna [20]. La somma dei punteggi delle 7 componenti da il punteggio globale, che ha un range compreso fra 0 e 21 [20]. Un punteggio PSQI globale superiore a 5 risulta indicativo di cattiva qualità del sonno [20].

Le caratteristiche oggettive dei pattern sonno-veglia sono state misurate con un accelerometro da polso (ActiSleep, Actigraph, Pensacola, FL, Stati Uniti d'America) durante 7 giorni consecutivi (24 ore/giorno) [18]. I partecipanti hanno ricevuto delle informazioni dettagliate su come indossare l'accelerometro ed è stato chiesto a loro di rimuoverlo solo durante le attività in acqua. I partecipanti hanno anche registrato l'ora in cui sono andati a letto ogni notte, si sono svegliati ogni mattina e hanno rimosso il dispositivo ogni giorno. I dati sono stati elaborati utilizzando il software ActiLife v. 6.13.3 (ActiGraph, Pensacola, FL, Stati Uniti d'America). Gli accelerometri sono stati inizializzati per memorizzare le accelerazioni grezze a una frequenza di campionamento di 100 Hz [21]. Le seguenti variabili sono state analizzate: tempo totale di sonno (TTS; minuti di sonno tra ora di andare a dormire e ora di veglia), tempo di veglia infrasonno (TVI; minuti di veglia tra l'inizio del sonno e l'ora della veglia) ed efficienza del sonno (ES; percentuale di tempo di sonno a letto) [22].

Analisi statistica

La dimensione del campione e i calcoli sulla potenza sono stati effettuati sulla base dei dati di uno studio pilota [18]. Il test Shapiro-Wilk, l'analisi degli istogrammi, Q-Q e box-plots sono stati utilizzati per verificare la distribuzione di tutte le variabili. I parametri descrittivi sono stati riportati come media e deviazione standard. Sono stati eseguiti *t*-test per campioni indipendenti per determinare le differenze tra sessi.

Modelli di regressione lineare semplice sono stati condotti per esaminare l'associazione tra la quantità e la qualità del sonno (punteggio PSQI globale, TTS, TVI, ed ES) e i parametri di composizione corporea (IMC, RVF, CMO, DMO, massa magra, IMM, massa grassa, percentuale di massa grassa, IMG e tessuto adiposo viscerale).

Modelli di regressione lineare multipla sono stati anche condotti per testare queste associazioni dopo aggiustamento per sesso, per età, e sia per sesso che per età.

Tutte le analisi sono state condotte utilizzando il Pacchetto Statistico per le Scienze Sociali (SPSS, v. 23.0, IBM SPSS Statistics, IBM Corp., Armonk, NY, Stati

Uniti d'America) e il livello di significatività è stato fissato a < 0.05. Le rappresentazioni grafiche sono state realizzate utilizzando GraphPad Prism 5 (GraphPad Software, San Diego, CA, Stati Uniti d'America).

RISULTATI

La Tabella 1 mostra le caratteristiche del campione di studio. Differenze significative in IMC, RVF, CMO, DMO, massa magra, IMM, percentuale di massa grassa, tessuto adiposo viscerale e TTS sono stati osservati tra uomini e donne (tutti $p \leq 0.001$). Una scarsa qualità soggettiva del sonno (punteggio PSQI globale > 5) é stata identificata nel 40.3% della nostra coorte. La Figura 1 riporta la media e deviazione standard di parametri relativi alla composizione corporea dopo aver classificato i partecipanti del presente studio come dormitori "buoni" (punteggio PSQI globale ≤ 5) o "cattivi" (punteggio PSQI globale > 5). Tra questi gruppi di punteggio PSQI globale non sono state osservate differenze statisticamente significative nei parametri oggettivi della quantità e la qualità del sonno (TTS, TVI ed ES, tutti $p \geq 0.05$, Figura 2).

La Figura 3 mostra le associazioni tra la quantità e qualità del sonno e l'IMC e il RVF. Il punteggio PSQI globale è stato associato negativamente con l'IMC ($\beta = -0.269$, $R^2 = 0.072$, $p = 0.028$, Figura 3A), questa associazione scomparsa dopo aggiustamento per sesso, per età, e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2). Il punteggio PSQI globale non è stato associato con il RVF ($p \geq 0.05$, Figura 3E), né dopo correzione per sesso, per età, e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2). Non è stata trovata alcuna associazione tra il TTS e l' IMC ($p \geq 0.05$, Figura 3B), né dopo aggiustamento

Tabella 1. Caratteristiche descrittive dei partecianti.

	N	Totale		N	Maschi		N	Femmine	
Età (anni)	74	53.66	(5.14)	35	54.39	(5.27)	39	53.01	(5.00)
Parametri di composizione corporea									
Indice di massa corporea (kg/m²)	74	26.72	(3.76)	35	28.32	(3.61)	39	25.27	(3.31) *
Rapporto vita-fianchi	74	0.91	(0.08)	35	0.97	(0.07)	39	0.86	(0.06) *
Contenuto minerale osseo (g)	74	2258.1	(453.5)	35	2633.6	(301.2)	39	1921.1	(259.8) *
Densità minerale ossea (g/cm²)	74	1.10	(0.10)	35	1.16	(0.08)	39	1.05	(0.09) *
Massa magra (kg)	74	43.47	(11.68)	35	53.90	(6.50)	39	34.11	(5.84) *
Indice di massa magra (kg/m²)	74	15.21	(2.88)	35	17.49	(2.02)	39	13.17	(1.80) *
Massa grassa (kg)	74	30.01	(8.43)	35	30.85	(9.77)	39	29.25	(7.06)
Massa grassa (%)	74	39.90	(9.06)	35	34.75	(7.99)	39	44.52	(7.36) *
Indice di massa grassa (kg/m²)	74	10.75	(3.13)	35	10.03	(3.23)	39	11.39	(2.93)
Tessuto adiposo viscerale (g)	74	789.7	(387.1)	35	972.4	(392.0)	39	625.8	(303.4) *
Quantità e qualità del sonno									
Punteggio PSQI globale	67	5.61	(3.47)	31	4.77	(3.15)	36	6.33	(3.62)
Tempo totale di sonno (min)	71	359.9	(48.85)	34	337.9	(46.30)	37	380.1	(42.44) *
Tempo di veglia infrasonno (min)	71	63.90	(27.44)	34	65.80	(32.45)	37	62.15	(22.19)
Efficienza del sonno (%)	71	85.01	(6.29)	34	83.88	(7.53)	37	86.06	(4.75)

I dati si presentano come media (deviazione standard). *Differenze significative tra sessi ottenute da t-test per campioni indipendenti ($p < 0.05$). PSQI: Pittsburgh Sleep Quality Index.

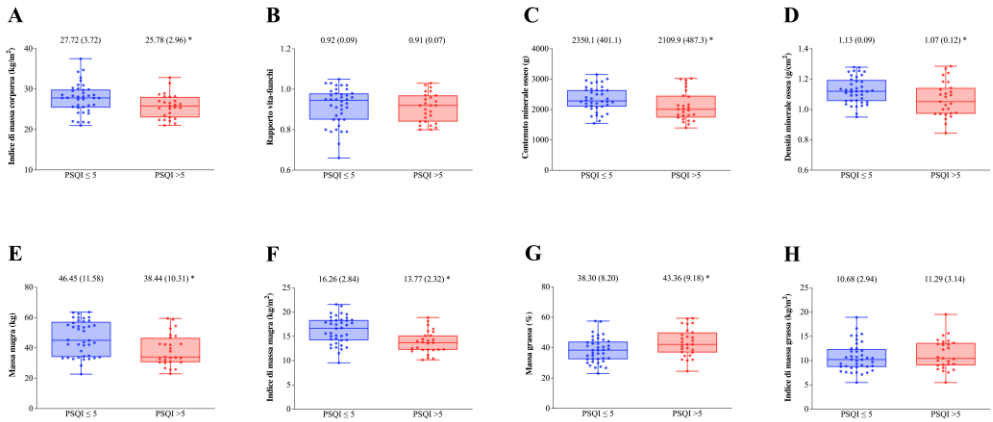


Figura 1: Media (deviazione standard) di parametri relativi alla composizione corporea dopo aver classificato i partecipanti come dormitori "buoni" (punteggio PSQI globale ≤ 5) o "cattivi" (punteggio PSQI globale > 5). *Differenze significative tra i gruppi di punteggio PSQI globale ottenute da t-test per campioni indipendenti (p < 0.05). PSQI: Pittsburgh Sleep Quality Index.

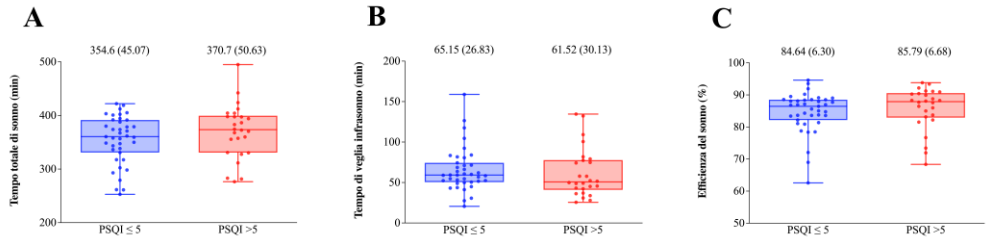


Figura 2: Media (deviazione standard) di parametri oggettivi della quantità e qualità del sonno dopo aver classificato i partecipanti come dormitori "buoni" (punteggio PSQI globale ≤ 5) o "cattivi" (punteggio PSQI globale > 5). *Differenze significative tra i gruppi di punteggio PSQI globale ottenute da t-test per campioni indipendenti (p < 0.05). PSQI: Pittsburgh Sleep Quality Index.

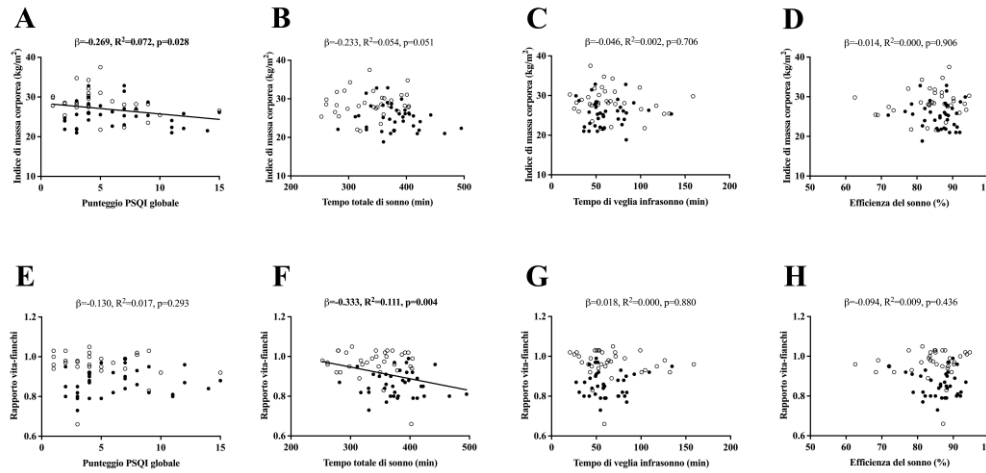


Figura 3: Associazione tra quantità e qualità soggettiva e oggettiva del sonno e parametri di composizione corporea (indice di massa corporea e rapporto vita-fianchi) in adulti sedentari di mezza età. β (coefficiente di regressione standardizzato), R^2, e p ottenuti da un'analisi di regressione lineare semplice. I valori p significativi (< 0.05) sono in grassetto. I cerchi aperti rappresentano i maschi, i cerchi pieni rappresentano le femmine. PSQI: Pittsburgh Sleep Quality Index.

per sesso e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2), mentre si è evidenziata un'associazione negativa dopo correzione per età ($\beta = -0.244$, $R^2 = 0.083$, $p = 0.040$, Tabella 2). Il TTS è stato associato negativamente con il RVF ($\beta = -0.333$, $R^2 = 0.111$, $p = 0.004$, Figura 3F), questa associazione rimasta dopo correzione per età ($\beta = -0.325$, $R^2 = 0.126$, $p = 0.006$, Tabella 2), mentre scomparsa dopo aggiustamento per sesso e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2). Non sono state osservate associazioni tra il TVI e l'ES con l'IMC e il RVF (tutti $p \geq 0.05$, Figure 3C e 3D, rispettivamente per l'IMC, e Figure 3G e 3H, rispettivamente per il RVF), né dopo correzione per sesso, per età, e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2).

La Figura 4 mostra le associazioni tra la quantità e qualità del sonno e il CMO e la DMO. Il punteggio PSQI globale è stato associato negativamente con il CMO e la DMO ($\beta = -0.327$, $R^2 = 0.107$, $p = 0.007$, Figura 4A; $\beta = -0.444$, $R^2 = 0.197$, $p < 0.001$, Figura 4E, rispettivamente), queste associazioni rimaste dopo aggiustamento per sesso ($\beta = -0.159$, $R^2 = 0.634$, $p = 0.045$; $\beta = -0.334$, $R^2 = 0.419$, $p = 0.001$, rispettivamente; Tabella 2) e per età ($\beta = -0.328$, $R^2 = 0.107$, $p = 0.009$; $\beta = -0.448$, $R^2 = 0.197$, $p < 0.001$, rispettivamente; Tabella 2). Tuttavia, dopo correzione sia per sesso che per età, l'associazione tra il punteggio PSQI globale e il CMO è scomparsa ($p \geq 0.05$, Tabella 2), mentre è rimasta con la DMO ($\beta = -0.316$, $R^2 = 0.424$, $p = 0.003$, Tabella 2). Il TTS si è associato negativamente con il CMO e la DMO ($\beta = -0.384$, $R^2 = 0.148$, $p = 0.001$, Figura 4B; $\beta = -0.387$, $R^2 = 0.150$, $p = 0.001$, Figura 4F, rispettivamente), queste associazioni rimaste dopo aggiustamento per età ($\beta = -0.389$, $R^2 = 0.152$, $p = 0.001$; $\beta = -0.394$; $R^2 = 0.159$, $p = 0.001$, rispettivamente; Tabella 2), mentre scomparse dopo correzione per sesso e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2). Non sono state trovate associazioni tra il TVI e l'ES con il CMO e la DMO (tutti $p \geq 0.05$, Figure 4C e 4D, rispettivamente per BMC, e Figure 4G e 4H, rispettivamente per BMD), né dopo aggiustamento per sesso, per età, e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2).

La Figura 5 mostra le associazioni tra la quantità e qualità del sonno e la massa magra e l'IMM. Il punteggio PSQI globale è stato associato negativamente con la massa magra e l'IMM ($\beta = -0.321$, $R^2 = 0.103$, $p = 0.008$, Figura 5A; $\beta = -0.440$, $R^2 = 0.193$, $p < 0.001$, Figura 5E, rispettivamente), associazioni rimaste dopo aggiustamento per sesso ($\beta = -0.138$, $R^2 = 0.725$, $p = 0.044$; $\beta = -0.285$, $R^2 = 0.640$, $p < 0.001$, rispettivamente; Tabella 2) e per età ($\beta = -0.297$, $R^2 = 0.115$, $p = 0.016$; $\beta = -0.399$, $R^2 = 0.228$, $p = 0.001$, rispettivamente; Tabella 2). Tuttavia, dopo correzione per sesso e per età, l'associazione tra il punteggio PSQI globale e la massa magra è scomparsa ($p \geq 0,05$, Tabella 2), mentre rimasta con l'IMM ($\beta = -0.201$, $R^2 = 0.740$, $p = 0.004$, Tabella 2). Il TTS è stato associato negativamente con la massa magra e l'IMM ($\beta = -0.384$, $R^2 = 0.148$, $p = 0.001$, Figura 5B;

Tabella 2. Associazione tra quantità e qualità soggettiva e oggettiva del sonno e parametri di composizione corporea (Modello 0) dopo correzione per sesso (Modello 1), per età (Modello 2), e sia per sesso che per età (Modello 3).

	Punteggio PSQI globale			**Tempo totale di sonno**			**Tempo di veglia infrasonno**			**Efficienza del sonno**		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Indice di massa corporea (kg/m²)												
Modello 0	–0.269	0.072	**0.028**	–0.233	0.054	0.051	–0.046	0.002	0.706	–0.014	0.000	0.906
Modello 1	–0.184	0.204	0.112	–0.060	0.182	0.622	–0.074	0.184	0.502	0.061	0.183	0.583
Modello 2	–0.236	0.094	0.057	–0.244	0.083	**0.040**	–0.040	0.025	0.739	–0.020	0.024	0.869
Modello 3	–0.126	0.252	0.279	–0.065	0.224	0.587	–0.068	0.225	0.528	0.058	0.223	0.597
Rapporto vita-fianchi												
Modello 0	–0.130	0.017	0.293	–0.333	0.111	**0.004**	0.018	0.000	0.880	–0.094	0.009	0.436
Modello 1	0.002	0.345	0.982	0.079	0.391	0.455	–0.023	0.386	0.807	0.015	0.386	0.878
Modello 2	–0.170	0.050	0.178	–0.325	0.126	**0.006**	0.013	0.020	0.912	–0.089	0.028	0.460
Modello 3	–0.018	0.351	0.867	–0.077	0.396	0.467	–0.025	0.391	0.791	0.016	0.391	0.869
Contenuto minerale osseo (g)												
Modello 0	–0.327	0.107	**0.007**	–0.384	0.148	**0.001**	0.029	0.001	0.811	–0.130	0.017	0.281
Modello 1	–0.159	0.634	**0.045**	–0.047	0.639	0.565	–0.025	0.637	0.737	0.010	0.637	0.895
Modello 2	–0.328	0.107	**0.009**	–0.389	0.152	**0.001**	0.030	0.003	0.803	–0.131	0.019	0.279
Modello 3	–0.124	0.651	0.121	–0.050	0.656	0.532	–0.021	0.655	0.771	0.008	0.654	0.917
Densità minerale ossea (g/cm²)												
Modello 0	–0.444	0.197	**<0.001**	–0.387	0.150	**0.001**	0.071	0.005	0.558	–0.159	0.025	0.185
Modello 1	–0.334	0.419	**0.001**	–0.171	0.352	0.120	0.033	0.329	0.745	–0.061	0.332	0.545
Modello 2	–0.448	0.197	**<0.001**	–0.394	0.159	**0.001**	0.073	0.010	0.546	–0.162	0.031	0.180
Modello 3	–0.316	0.424	**0.003**	–0.174	0.371	0.110	0.036	0.348	0.714	–0.063	0.350	0.528
Massa magra (kg)												
Modello 0	–0.321	0.103	**0.008**	–0.384	0.148	**0.001**	0.065	0.004	0.587	–0.164	0.027	0.171
Modello 1	–0.138	0.725	**0.044**	–0.015	0.734	0.825	0.008	0.734	0.897	–0.016	0.734	0.807
Modello 2	–0.297	0.115	**0.016**	–0.397	0.186	**0.001**	0.072	0.034	0.551	–0.171	0.058	0.152
Modello 3	–0.069	0.792	0.259	–0.022	0.805	0.718	0.016	0.805	0.772	–0.020	0.805	0.716
Indice di massa magra (kg/m²)												
Modello 0	–0.440	0.193	**<0.001**	–0.369	0.136	**0.002**	0.095	0.009	0.432	–0.184	0.034	0.125
Modello 1	–0.285	0.640	**<0.001**	–0.054	0.563	0.544	0.045	0.563	0.580	–0.055	0.564	0.502
Modello 2	–0.399	0.228	**0.001**	–0.391	0.237	**<0.001**	0.105	0.096	0.365	–0.194	0.123	0.092
Modello 3	–0.201	0.740	**0.004**	–0.063	0.707	0.393	0.055	0.707	0.406	–0.061	0.708	0.365
Massa grassa (kg)												
Modello 0	0.120	0.014	0.333	–0.027	0.001	0.826	–0.169	0.029	0.159	0.147	0.022	0.222
Modello 1	0.145	0.026	0.255	0.038	0.019	0.775	–0.179	0.049	0.136	0.175	0.047	0.150
Modello 2	0.121	0.014	0.346	–0.020	0.010	0.870	–0.173	0.040	0.151	0.150	0.032	0.212
Modello 3	0.151	0.027	0.256	0.040	0.026	0.765	–0.181	0.057	0.132	0.177	0.055	0.148
Massa grassa (%)												
Modello 0	0.316	0.100	**0.009**	0.254	0.065	**0.033**	–0.137	0.019	0.254	0.197	0.039	0.099
Modello 1	0.199	0.353	0.058	0.033	0.275	0.774	–0.103	0.285	0.321	0.109	0.286	0.298
Modello 2	0.299	0.106	**0.016**	0.268	0.109	**0.022**	–0.144	0.058	0.224	0.204	0.079	0.084
Modello 3	0.153	0.383	0.150	0.039	0.340	0.724	–0.110	0.351	0.269	0.113	0.351	0.261
Indice di massa grassa (kg/m²)												
Modello 0	0.135	0.018	0.277	0.085	0.007	0.482	–0.134	0.018	0.264	0.153	0.023	0.204
Modello 1	0.083	0.068	0.506	–0.001	0.039	0.993	–0.122	0.054	0.307	0.122	0.053	0.313
Modello 2	0.133	0.018	0.297	0.091	0.016	0.451	–0.138	0.027	0.253	0.156	0.032	0.196
Modello 3	0.070	0.070	0.588	0.002	0.052	0.991	–0.125	0.067	0.294	0.124	0.067	0.306
Tessuto adiposo viscerale (g)												
Modello 0	–0.037	0.001	0.764	–0.260	0.068	**0.028**	–0.004	0.000	0.971	–0.058	0.003	0.631
Modello 1	0.063	0.188	0.591	–0.072	0.221	0.548	–0.036	0.218	0.741	0.024	0.217	0.827
Modello 2	–0.052	0.006	0.687	–0.255	0.073	**0.033**	–0.008	0.008	0.951	–0.055	0.011	0.651
Modello 3	0.066	0.188	0.583	–0.071	0.222	0.555	–0.037	0.219	0.735	0.024	0.218	0.824

β (coefficiente di regressione standardizzato), R^2, e *p* ottenuti da analisi di regressione lineare semplice e multipla. I valori *p* significativi (< 0.05) sono in grassetto. PSQI: Pittsburgh Sleep Quality Index.

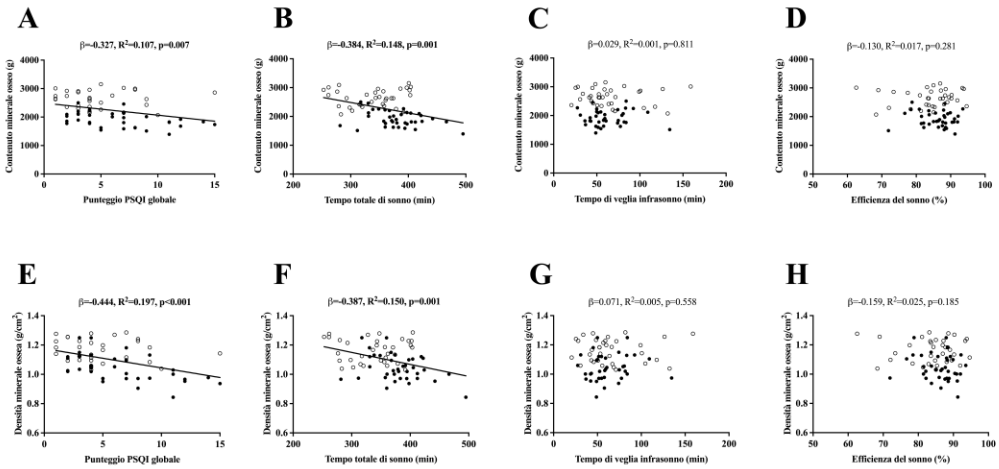


Figura 4: Associazione tra quantità e qualità soggettiva e oggettiva del sonno e parametri di composizione corporea (contenuto minerale osseo e densità minerale ossea) in adulti sedentari di mezza età. β (coefficiente di regressione standardizzato), R^2, e p ottenuti da un'analisi di regressione lineare semplice. I valori p significativi (< 0.05) sono in grassetto. I cerchi aperti rappresentano i maschi, i cerchi pieni rappresentano le femmine. PSQI: Pittsburgh Sleep Quality Index.

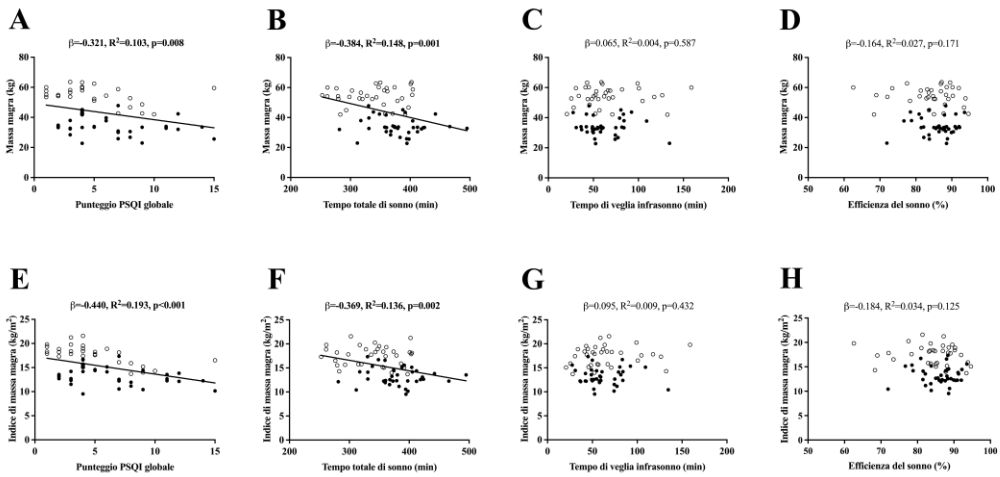


Figura 5. Associazione tra quantità e qualità soggettiva e oggettiva del sonno e parametri di composizione corporea (massa magra e indice di massa magra) in adulti sedentari di mezza età. β (coefficiente di regressione standardizzato), R^2, e p ottenuti da un'analisi di regressione lineare semplice. I valori p significativi (< 0.05) sono in grassetto. I cerchi aperti rappresentano i maschi, i cerchi pieni rappresentano le femmine. PSQI: Pittsburgh Sleep Quality Index.

β = –0.369, R^2 = 0.136, p = 0.002, Figura 5F, rispettivamente), queste associazioni rimaste dopo aggiustamento per età (β = –0.397, R^2 = 0.186, p = 0.001; β = –0.391; R^2 = 0.237, p < 0.001, rispettivamente; Tabella 2), mentre scomparse dopo correzione per sesso e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2). Non sono state osservate associazioni tra il TVI e l'ES con la massa magra e l'IMM (tutti $p \geq 0.05$, Figure 5C e 5D, rispettivamente per la massa magra, e Figure 5G e 5H, rispettivamente per l'IMM), né dopo aggiustamento per sesso,

per età, e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2).

La Figura 6 mostra le associazioni tra la quantità e qualità del sonno e la percentuale di massa grassa e l'IMG. Il punteggio PSQI globale è stato associato positivamente con la percentuale di massa grassa (β = 0.316, R^2 = 0.100, p = 0.009, Figura 6A), questa associazione rimasta dopo aggiustamento per età (β = 0.299, R^2 = 0.106, p = 0.016, Tabella 2), mentre scomparsa dopo correzione per sesso e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2). Non è stata osservata alcuna associazione tra il punteggio PSQI globale e l'IMG ($p \geq 0.05$, Figura 6E), né dopo correzione per sesso, per età, e sia per sesso che per età (tutti $p \geq 0.05$; Tabella 2). Il TTS è stato associato positivamente con la percentuale di massa grassa (β = 0.254, R^2 = 0.065, p = 0.033, Figura 6B), associazione rimasta dopo aggiustamento per età (β = 0.268, R^2 = 0.109, p = 0.022, Tabella 2), mentre scomparsa dopo correzione per sesso e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2). Non è stata osservata alcuna associazione tra il TTS e l'IMG ($p \geq 0.05$, Figura 6F), né dopo aggiustamento per sesso, per età, e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2, rispettivamente). Non sono state trovate associazioni tra il TVI e l'ES con la percentuale di massa grassa e l'IMG (tutti $p \geq 0.05$, Figure 6C e 6D, rispettivamente per la percentuale di massa grassa, e Figure 6G e 6H, rispettivamente per l'IMG), né dopo correzione per sesso, per età, e sia per sesso che per età (tutti $p \geq 0.05$, Tabella 2).

DISCUSSIONE

Scopo di questo studio è stato quello di analizzare l'associazione tra quantità e qualità del sonno e un ampio insieme di parametri relativi alla composizione corporea in adulti sedentari di mezza età. Il presente studio ha mostrato che una scarsa qualità soggettiva del sonno è stata associata negativamente con il CMO, il DMO, la massa magra e l'IMM, mentre è stata associata positivamente con la percentuale di massa grassa in adulti sedentari di mezza età.

L'osteoporosi è una malattia prevalente legata all'età caratterizzata da bassa massa ossea e un deterioramento microarchitettonico del tessuto osseo [15]. Una scarsa qualità del sonno potrebbe essere un fattore di rischio per l'osteoporosi e l'osteopenia negli adulti di mezza età [23]. In questo senso, una inadeguata qualità del sonno potrebbe generare diversi effetti fisiologici che influenzano negativamente la DMO: (i) alterazione dei ritmi circadiani che compromettono la microstruttura ossea [24]; (ii) aumento delle citochine proinfiammatorie [25], le quale sono legate all'osteoporosi [26]; (iii) secrezione anormale di melatonina [27], i cui bassi livelli sono legati ad anomalie e malattie delle ossa [28]; (iv) aumento del cortisolo [25] che diminuisce sia la crescita delle cellule ossee che la DMO [29]; e (v) diminuzione dei livelli di leptina [25], i cui adeguati livelli sono stati positivamente correlati con la BMD [30]. Tutti questi meccanismi descritti potrebbero influire

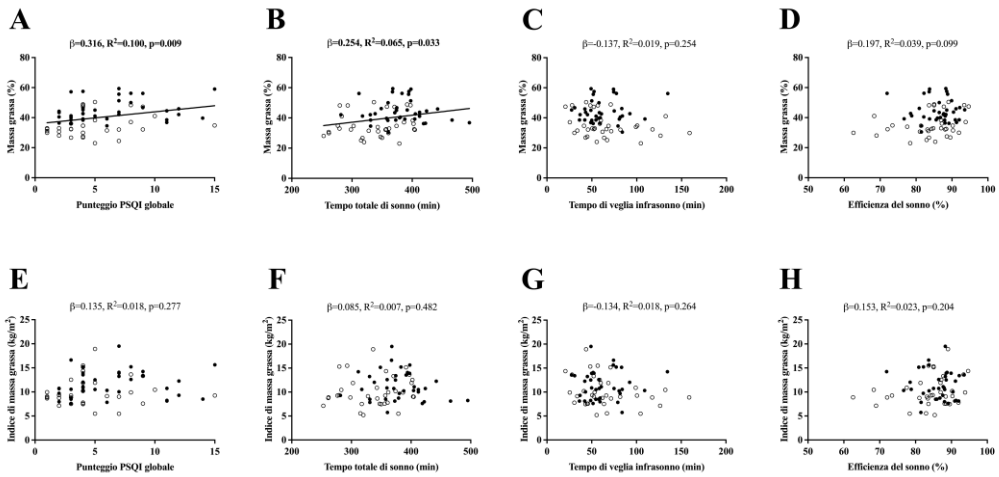


Figura 6: Associazione tra quantità e qualità soggettiva e oggettiva del sonno e parametri di composizione corporea (percentuale di massa grassa e indice di massa grassa) in adulti sedentari di mezza età. *β* (coefficiente di regressione standardizzato), R^2, e *p* ottenuti da un'analisi di regressione lineare semplice. I valori *p* significativi (< 0.05) sono in grassetto. I cerchi aperti rappresentano i maschi, i cerchi pieni rappresentano le femmine. PSQI: Pittsburgh Sleep Quality Index.

negativamente sulla DMO, aumentando dunque il rischio di sviluppare osteoporosi. I nostri risultati concordano con altri studi in cui si è dimostrato che una scarsa qualità soggettiva del sonno potrebbe essere associata a una bassa DMO nelle donne di mezza età [12], nei giovani uomini [31], e nei soggetti più anziani [32].

La sarcopenia è il progressivo declino della massa muscolare scheletrica legato all'età [16]. È stato dimostrato che una inadeguata qualità del sonno è un fattore di rischio per la sarcopenia legata all'età [16]. Esistono molteplici meccanismi che potrebbero spiegare l'influenza negativa di una scarsa qualità del sonno sulla massa magra: (i) aumento della secrezione dell'ormone catabolico cortisolo [33], noto per stimolare la degradazione e inibire la sintesi delle proteine muscolari [34]; (ii) interruzione del ritmo fisiologico dell'ormone anabolico di crescita dell'insulina 1 (IGF-1) [35], il quale ha un ruolo chiave nella stimolazione della sintesi proteica muscolare [36]; (v) incremento delle citochine proinfiammatorie [25], le quali sono legati all'atrofia muscolare [36]; e (vi) aumento della resistenza all'insulina [37], che può limitare la sintesi proteica muscolare [38]. In relazione alla massa magra, i nostri risultati concordano con quelli ottenuti da uno studio trasversale che include 1.196 partecipanti anziani [13].

L'obesità è caratterizzata da un eccessivo accumulo di adiposità, molto prevalente nel processo di invecchiamento [39]. Una povera qualità del sonno (i) aumenta l'infiammazione [25], che è significativamente elevata nell'obesità [40]; (ii) altera i ritmi circadiani aumentando il grasso attraverso diversi meccanismi come l'alterazione delle abitudini alimentari o i disturbi ormonali, tra

altri [41]; (iii) altera la secrezione di melatonina [27], la quale ha dimostrato di essere il mediatore chiave per l'ottimizzazione del bilancio energetico e la regolazione del peso corporeo [42], direttamente legato al grasso e l'obesità [42]; (iv) si associa a bassa leptina e alta grelina, le quali possono aumentare l'appetito e, di conseguenza, il rischio di obesità [25]; (v) riduce i livelli di adiponectina [43], che è inversamente associata all'adiposità [44]; e (vi) causa una resistenza insulinica negli individui sani [37], che si associa ad un aumento dell'adiposità [45]. Alla luce di tutto ciò, una inadeguata qualità del sonno, attraverso tutti i meccanismi descritti, potrebbe aumentare l'adiposità e, pertanto, il rischio di sviluppare obesità. In linea con i nostri risultati, studi precedenti hanno riportato una relazione tra scarsa qualità del sonno e maggiore massa grassa negli adulti di mezza età [46] e nei giovani adolescenti [47].

Quando si tiene conto del sesso o dell'età, la mancanza di associazione, in alcuni casi, tra i parametri del sonno e i parametri della composizione corporea potrebbe essere spiegata da: (i) le differenze di sesso nel sonno e nei disturbi del sonno tra donne e uomini [48], che possono essere basate sulle differenze nelle condizioni fisiologiche tra i sessi come il ciclo mestruale, gli ormoni maschili e femminili [49] e differenze nei ritmi circadiani [50]; e (ii) i cambiamenti del sonno legati all'età e i disturbi del sonno tipici che si verificano con l'avanzare dell'età [6].

È interessante sottolineare che sono stati osservati dei risultati diversi quando la valutazione dell'associazione tra sonno e composizione corporea è stata eseguita misurando la quantità e la qualità del sonno in maniera soggettiva con un questionario PSQI rispetto a quando è stata valutata oggettivamente da un accelerometro. Differenze tra le valutazioni soggettive e oggettive del sonno sono già state riportate. Quindi, studi precedenti hanno mostrato delle correlazioni deboli o incoerenti di misure soggettive (punteggi PSQI) con misure oggettive (actigrafia e polisonnografia) [51,52]. Uno studio precedente sui giovani ha osservato che il PSQI e l'accelerometro possono misurare diversi attributi del sonno, riportando la inadeguata capacità dell'accelerometria di identificare la veglia, probabilmente codificando come sonno il tempo sdraiato a letto sveglio ma immobile [53]. A causa di questa limitazione, si raccomanda di utilizzare metodi di valutazione complementari (sia soggettivi che oggettivi) per ottenere informazioni dettagliate oltre ai limitati dati derivati dai movimenti del corpo [54]. Il PSQI può riflettere lo stato psicologico generale della persona, piuttosto che la quantità o la qualità effettiva del sonno [51]. Inoltre, non è ancora ben definito cosa comporti effettivamente una "buona notte di sonno" nella percezione del dormiente e si evidenzia che molti fattori giocano un ruolo nel giudicare la qualità del sonno [55]. Tuttavia, la validità del PSQI è ulteriormente supportata da differenze simili tra i gruppi

che utilizzano PSQI o polisonnografia ed è stata utilizzata in un'ampia gamma di studi basati sulla popolazione e clinici [52].

Questo studio ha alcune limitazioni che dobbiamo considerare: in primo luogo, non è possibile chiarire con questo studio trasversale se la quantità e la qualità del sonno contribuiscono al mantenimento di variabili di composizione corporea adeguate (cioè alta DMO, bassa massa grassa e alta massa magra), o se lo stato della composizione corporea ha un'influenza positiva sulla quantità e la qualità del sonno. Per questo sono necessari studi longitudinali per chiarire la direzione dell'associazione. In secondo luogo, il nostro studio ha incluso solo adulti sedentari di mezza età, quindi non possiamo estrapolare i nostri risultati su individui più anziani, più giovani e / o fisicamente attivi. In terzo luogo, la dimensione del campione di questo studio è relativamente piccola. Infine, la mancanza dei parametri ematici citati sopra, non consente di confermare che la relazione sia dovuta ai meccanismi fisiologici proposti. In terzo luogo, la misurazione oggettiva del sonno è stata valutata attraverso accelerometria anche se la polisonnografia è l'esame diagnostico considerato il gold standard. Nonostante le suddette limitazioni, il fatto che la quantità e la qualità del sonno siano state misurate sia in modo soggettivo che oggettivo, risulta essere effettivamente il punto di forza di questo studio, poiché simili studi precedenti non hanno misurato in maniera oggettiva la quantità e la qualità del sonno [12–14,31,32,46].

CONCLUSIONI

In conclusione, il nostro studio ha mostrato che una scarsa qualità soggettiva del sonno è stata associata negativamente con CMO, DMO, massa magra e IMM, mentre è stata associata positivamente con la percentuale di massa grassa in adulti sedentari di mezza età. Pertanto, i nostri risultati suggeriscono che una buona qualità del sonno potrebbe svolgere un ruolo significativo nella prevenzione delle alterazioni dello stato della composizione corporea (cioè, aumento della densità minerale ossea e della massa magra e diminuzione della massa grassa).

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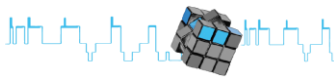

Chapter 2

Association between sleep and energy metabolism in sedentary middle-aged adults: The FIT-AGEING study **(Study 2)**

ABSTRACT

Sleep is linked to metabolism. However, there are no studies testing the associations of both subjective and objective sleep quantity and quality with basal metabolic rate (BMR) and fuel oxidation in basal conditions and during exercise. Moreover, there is a lack of evidence investigating the mediating role of dietary intake in the relationship of sleep with energy metabolism. The aim of the present study was to investigate the relationship of sleep quantity and quality with BMR and fuel oxidation in basal conditions and during exercise in sedentary middle-aged adults. The mediating role of dietary intake and adherence to the traditional Mediterranean diet in the relationship between sleep and energy metabolism parameters was also studied.

A total of 70 sedentary middle-aged adults (40–65 years old) participated in the present study. Sleep quantity and quality were assessed using the Pittsburgh Sleep Quality Index (PSQI) and wrist accelerometers (ActiSleep, Actigraph, Pensacola, Florida, USA) for 7 consecutive days. BMR was measured with indirect calorimetry and fuel oxidation was estimated through stoichiometric equations. Maximal fat oxidation was determined by a walking graded exercise and dietary intake with 24-hour recalls. Adherence to the traditional Mediterranean diet was assessed through the "PREvención con DIeta MEDiterránea" (PREDIMED) questionnaire. A higher global PSQI score (poor sleep quality) was associated with lower basal fat oxidation (BFox), both expressed in g/min, and as a percentage of BMR (all $p < 0.001$), independently of covariates. No mediating role of the dietary intake or PREDIMED total score was observed in the association of global PSQI score and BFox. The main findings of the present study showed that a poor subjective sleep quality was associated with lower BFox, which is not mediated by dietary intake in sedentary middle-aged adults. Thus, improving sleep quality could be considered a potential prevention and/or treatment pathway to reduce metabolism alterations related to lower basal fat oxidation, independently of dietary patterns.

Keywords: basal metabolic rate; basal fat oxidation; maximal fat oxidation; dietary intake; macronutrient intake; fiber intake; actigraphy

INTRODUCTION

Cardiometabolic diseases and obesity are the leading causes of death in developed countries, becoming an epidemic in the last years [1,2]. Unhealthy diets represent one of the top risk factors for cardiometabolic diseases and obesity, developing a positive energy balance [3]. Simultaneously, a low basal metabolic rate (BMR), an impaired meal-induced thermogenesis and low physical activity levels could result in a reduced total energy expenditure [4]. This low total energy expenditure coupled to high energy intake could produce a gradual weight gain and visceral adipose tissue deposition, increasing the risk of cardiometabolic diseases and obesity [5].

The ability to oxidize fat as a fuel is considered an important metabolic health parameter [6]. An impaired ability to oxidize fat is associated with an increased risk of obesity, type 2 diabetes mellitus, cardiovascular disease, metabolic syndrome, cancer and systemic inflammation [7]. Therefore, fat oxidation in basal conditions (BFox) and maximal fat oxidation during exercise (MFO) are considered markers of metabolic health [8–10].

Sleep pattern variations, including a decrease in the quantity and quality of sleep, have been shown to be also a risk factor for the development of obesity and cardiometabolic diseases [11]. These changes in sleep quantity and quality disrupt the circadian rhythms and may have deleterious consequences on people health [12]. Previous studies have provided a causal link between short sleep duration and poor sleep quality with pathological metabolic consequences due to the disruption in the circadian rhythms and increasing levels of adiposity [11,13]. Metabolic regulation is not an output function of the circadian system [12]. However, nutrient, energy and redox levels signal back to cellular clocks to reinforce circadian rhythms and to adapt physiology (i.e., hormones, body temperature, and nervous system) to temporal tissue-specific requirements [11,13]. In this sense, previous studies have demonstrated that poor sleep quantity and quality may decrease BMR and BFox [14], while sleep deprivation may not affect MFO in young adults [15]. However, there are no studies testing the associations of both subjective and objective sleep quantity and quality with BMR and fuel oxidation in basal conditions and during exercise.

One of the possible causes of the relationship of sleep parameters with BMR and fuel oxidation could be dietary modifications. In this sense, unhealthy sleep patterns could increase food consumption and, consequently, energy intake through several previously-explained potential mechanisms [16]. Previous studies have shown that dietary intake could influence BFox and MFO [8,17,18]. Concretely, a high-fat, low-carbohydrate intake could increase BFox and MFO [6,19]. In addition, the deficiency of sleep

can increase the consumption of high fat energy-dense foods [16], which theoretically may modify BFox and MFO. However, there is a lack of evidence investigating the mediating role of dietary intake in the relationship of sleep outcomes with energy metabolism parameters.

Therefore, the aim of the present study was to investigate the relationship of both subjective and objective sleep quantity and quality with BMR and fuel oxidation in basal conditions and during exercise in sedentary middle-aged adults. We also aimed to study the mediating role of dietary intake and adherence to the traditional Mediterranean diet between sleep and energy metabolism parameters in sedentary middle-aged adults. We hypothesized that sleep quantity and quality would be (i) positively associated with energy metabolism related parameters such as basal metabolic rate, basal fat oxidation, and maximal fat oxidation, and (ii) negatively associated with basal carbohydrate oxidation.

MATERIALS AND METHODS

Study protocol and participants

A total of 70 (36 women) sedentary middle-aged adults (40–65 years old) participated in this cross-sectional study. The participants were enrolled in the FIT-AGEING study [20], an exercise-based randomized controlled trial (clinicaltrial.gov: ID: NCT03334357). Data for these subjects were collected at baseline data collection in the FIT-AGEING study. All of them declared: (i) to be non-physically active (< 20 minutes of moderate-intensity physical activity on 3 days/week), (ii) to have a stable weight (weight changes < 3 kg) over the last 3 months, (iii) to be healthy and (iv) to be free of medication (medication for thyroid, betablockers, benzodiazepines, glucose lowering medication, or cardiovascular medication) during the last 3 months. All participants gave their oral and written informed consent before the beginning of the intervention. The study was approved by the Ethics Committee on Human Research of the University of Granada and the Andalusian Health Service (CEI-Granada) [0838-N-2017]. The study protocols and experimental design were applied according to the last revised ethical guidelines of the Declaration of Helsinki. All assessments were made at the Sport and Health University Research Institute (iMUDS, Granada, Spain) during September and October 2016 and September and October 2017.

Measurements

Anthropometry and body composition

Anthropometric variables were measured by a certified anthropometrist (by the International Society for the Advancement of Kinanthropometry [ISAK]) following the ISAK guidelines [21]. Both body weight and height were assessed using an electronic scale and stadiometer (Seca model 799, Hamburg, Germany), and the body mass index (BMI) was calculated as $Body\ weight\ (kg)\ /$

$Height^2\ (m^2)$. A dual-energy X-ray absorptiometry scanner (Discovery Wi, Hologic, Inc., Bedford, MA, USA) was used to measure body composition following the manufacturer's recommendations. We conducted the quality controls, the positioning of the participants and the analyses of the results following the manufacturer's recommendations. An automatic delineation of the anatomic regions was performed by the software APEX 4.0.2. We acquired spine phantom quality control scans on each study day. The results displayed lean mass and fat mass, and the lean mass index (LMI) and the fat mass index (FMI), which were calculated as: $Lean\ mass\ (kg)\ /\ Height^2\ (m^2)$ and $Fat\ mass\ (kg)\ /\ Height^2\ (m^2)$, respectively.

Sleep quantity and quality

Subjective sleep quantity and quality were assessed using the Pittsburgh Sleep Quality Index (PSQI), a self-report tool which consists of a 19-item scale that provides 7 component scores (ranges 0–3): (i) subjective sleep quality, (ii) sleep latency, (iii) sleep duration, (iv) habitual sleep efficiency, (v) sleep disturbances, (vi) use of sleeping medication, and (vii) daytime dysfunction; with a global score ranging from 0 to 21 [22]. A global PSQI score higher than 5 indicates poor sleep quality [22].

Objective characteristics of sleep-wake cycles were monitored with a wrist-worn accelerometer (ActiSleep, Actigraph Pensacola, Florida, USA) for 7 consecutive days (24 hours/day) [20]. Participants received detailed information on how to wear the accelerometer and were asked to remove it only for water activities. It was also recorded the times in which participants went to bed every night, woke up every morning and removed the device every day. The accelerometers used an epoch length of 1 second and a frequency rate of 100 Hz to store raw accelerations [23]. The raw accelerations were exported in ".csv" format using ActiLife v. 6.13.3 software (ActiGraph, Pensacola, FL, USA) and processed using the GGIR package (v. 1.6-0, https://cran.r-project.org/web/packages/GGIR/index.html) [24] in R (v. 3.1.2, https://www.cran.r-project.org/). We derived the Euclidean Norm Minus One G (ENMO) as $\sqrt{(x^2 + y^2 + z^2)}$ -1G (where 1G ~ 9.8 m/s^2) with the accelerometer's z angle to describe sleep patterns. We used a previously published algorithm combining data from the accelerometers and diary reports to detect sleep period time [25,26]. According to this algorithm, sleep was defined as any period of sustained inactivity, in which there were minimal changes in the arm angle (i.e., as much 5 degrees for 5-minute periods) during a period recorded as sleep by the participant in their diary reports [25]. The following variables were analyzed: total sleep time (TST; minutes slept between bedtime and wake time), wake after sleep onset (WASO; minutes awake between sleep onset and wake time), and sleep efficiency (SE; percentage of time asleep while in bed). It is to note that only the

participants wearing the accelerometers for ≥ 16 hours/day for at least 4 days (including at least 1 weekend day) were included in the final analyses [23]. The mean accelerometer wear-time for the participants included in the final analyses were 6.7 days (4.3% of non-wear time).

Basal metabolic rate and fuel oxidation in basal conditions

Subjects were told to arrive at the laboratory in fasting condition of at least 8 hours in a motor vehicle and to avoid any moderate/vigorous physical activity in the previous 24/48 hours, respectively. All were required to confirm that they had met these conditions. The evening meal consumed by the subjects prior to fasting was previously standardized: an egg omelet with fried tomato and boiled rice.

BMR and fuel oxidation in basal conditions were measured through indirect calorimetry (IC) following the current scientific consensus [27]. All tests were conducted in the same quiet room with controlled room temperature (i.e., 22–24 ^{o}C) and humidity (i.e., 35–45%). IC measurements were performed during 30-minute periods with a CPX Ultima CardiO2 system (Medical Graphics Corp, St Paul, MN, USA) employing a neoprene face-mask with no external ventilation [27].

The Ultima CardiO2 metabolic cart device assessed oxygen consumption (VO_2) using a galvanic fuel cell, and carbon dioxide production (VCO_2) via non-dispersive infrared analysis using a breath-by-breath system [28]. A gas calibration using 2 standard gas concentrations and a flow calibration using a 3-L calibration syringe were performed following the manufacturer's recommendations. Prior to the start of the BMR assessment, the subjects reclined on a bed for ~30 minutes in a comfortable supine position covered by a sheet [29,30]. During the assessment, participants laid on a bed in a supine position and were instructed to breathe normally and not to talk, fidget or sleep.

The first 5 minutes of each measurement were discarded and the most stable 5-minute period that met steady state criteria (i.e., coefficient of variation < 10% in VO_2, CO_2, minute ventilation, and coefficient of variation < 10% in respiratory exchange ratio) was considered for further analyses following previous studies [29–32]. The Weir's abbreviated equation [33] was used to estimate the BMR expressed in kcal/day and also calculated with respect to the lean mass (BMR_{LM}). The Frayn's equation was used to estimate BFox and basal carbohydrate oxidation (BCHox) expressed in g/min [34]. The BFox and BCHox were also expressed as a percentage of the BMR.

Maximal fat oxidation during exercise

MFO and the intensity that elicits MFO (FATmax) were assessed in a different day of the BMR and BFox/BCHox test (i.e., interval 3 to 15 days). Participants were asked

to arrive at the laboratory in a fasted state of 6 hours and to avoid any physical activity both moderate (24 hours) and vigorous intensity (48 hours) before the measurement.

A walking graded test on a treadmill (H/P/cosmos pulsar, H/P/cosmos sports & medical GmbH, Nussdorf-Traunstein, Germany) was performed to calculate MFO and FATmax following a previously validated methodology [35]. Briefly, the protocol started assessing the maximal walking speed of each participant [35–37]. After ~3 minutes resting, the walking graded test started with a 3-minute warm up at 3.5 km/h. Subsequently, the treadmill speed was increased 1 km/h every 3 minutes until the maximal walking speed was reached. Thereafter, the treadmill gradient was increased 2% every 3 minutes until the respiratory exchange ratio was above 1.0. An automated gas analysis system (CPX Ultima CardiO2; Medical Graphics Corp, St Paul, MN) was used to record breath-by-breath gas exchange measurements. Participants wore an oronasal mask (model 7400, Hans Rudolph Inc, Kansas City, MO, USA) equipped with a prevent™ metabolic flow sensor (Medgraphics Corp, Minnesota, USA). Gas analysis systems were calibrated following the manufacturer's recommendations. VO_2 and VCO_2 were averaged over and the last 60 seconds of each graded exercise protocol stage. Frayn's equation was used to estimate fat oxidation rates [34]. These fat oxidation values were plotted against the relative-exercise intensity, expressed as the percentage of maximum oxygen uptake (VO_2max); a third-degree polynomial curve was built to determine MFO and FATmax [38]. MFO was also expressed as MFO_{LM} in order to relativize it to the lean mass. Maximal carbohydrate oxidation was not included in the analyses since it is not a key factor of energy metabolism during exercise [39]. Indeed, our recent systematic review has analyzed a total of 112 studies which included data about fuel oxidation during exercise [37]. None of those studies reported maximal carbohydrate oxidation during exercise.

Cardiorespiratory fitness

VO_2max was determined using a maximum treadmill (H/P/Cosmos Pulsar treadmill, H/P/Cosmos Sport & Medical GMBH, Germany) exercise test following the modified Balke protocol, which has been extensively validated [40]. In short, the warm up consisted in walking at 3 km/h for 1 minute followed by 2 minutes at 4 km/h. The incremental protocol started at a speed of 5.3 km/h (0% grade), which was kept constant with the gradient increasing by 1% every minute until the participants reached their volitional exhaustion. We used the same indirect calorimetry and software as in the MFO assessment.

Dietary intake

Diet was assessed using three 24-hour recalls carried out on 3 separate days (2 weekdays and 1 weekend day) by a qualified

and trained research dietitian. Dietary recalls were done on different days than the MFO and VO_2 assessments.

In the face-to-face interviews, the participants were asked to recall all food consumed during the previous day. The interviews involved a detailed assessment and description of the food consumption using colored photographs of different-size portions of food to improve the participants' accuracy of food quantification [41]. These data were introduced by two independent qualified and trained dietitians in the EvalFINUT software. Energy, macronutrient, fiber, lipid profile and ethanol intake data were obtained by EvalFINUT, which is based on the USDA (United States Department of Agriculture) and BEDCA ("Base de Datos Española de Composición de Alimentos") databases.

Dietary energy density was calculated by dividing the energy contained in food and beverages (excluding water) by the total weight of daily food and beverages (expressed as kcal/g) [42]. Energy and weight data of daily food and beverages were obtained from the 24-hour recalls.

The traditional Mediterranean diet is associated with a lower prevalence of chronic diseases (i.e., obesity, metabolic syndrome, cardiovascular diseases, cancer) and mortality [43]. The adherence to the traditional Mediterranean diet (MedDiet) was estimated by using the 14-point questionnaire of adherence to the MedDiet used and validated in the PREDIMED trial [44]. The "PREvención con DIeta MEDiterránea" (PREDIMED) questionnaire includes 12 questions related to frequency intake of key foods and 2 questions related to specific dietary habits of the MedDiet. Each question scores 0 or 1 point. The total score ranges from 0 to 14, being 0 points null adherence and 14 points complete adherence to the MedDiet. The PREDIMED questionnaire proved to be very useful in a large Spanish cohort for a quick adherence estimation to the traditional MedDiet [44].

Statistical analysis

The sample size and power calculations were made based on the data of a pilot study of the FIT-AGEING study [20]. This study aimed to compare the influence of different exercise programs on BMR, BFox and MFO in sedentary middle-aged adults. We based the sample size calculations on a minimum predicted change in MFO of 0.05 g/min between the intervention groups and the control group, and a standard deviation for this change of 0.05 g/min. A sample size of 17 participants was predicted to provide a statistical power of 80%, considering a type I error of 0.05. Assuming a maximum loss of 25% at follow-up, we decided to recruit at least 20 participants for each group (N = ~80 individuals). The present study is based on a secondary analysis using baseline data from the FIT-AGEING study, and therefore a specific sample size calculation was not conducted.

Shapiro-Wilk test, visual check of histograms, Q-Q and box plots were used to verify all variable distributions. The descriptive parameters were reported as mean and standard deviation. Given that no sex interactions were observed, the analyses were conducted including men and women together. Simple linear regressions were performed to examine the association of sleep quantity and quality (global PSQI score, TST, WASO, and SE) with BMR, BMR_{LM}, BFox, BCHox, MFO, MFO_{LM} and FATmax. Multiple linear regression models were also conducted to test these associations after adjusting by sex (Model 1), by both sex and age (Model 2), and by sex, age, and FMI (Model 3).

Pearson correlation was performed to assess the association between sleep parameters and dietary outcomes. Effect modification analyses were conducted to test the joint effects of dietary intake (dietary intake outcome * sleep outcome) and sleep quality on energy metabolism. Mediation analyses were conducted to quantify the mediating role of dietary intake (i.e., energy, macronutrient, fiber, ethanol and lipid profile intake, and PREDIMED total score) in the relationship of sleep parameters with BMR and fuel oxidation [45]. We used the PROCESS macro version 3.3, model 4 with 5,000 bias-corrected bootstrap samples and 95% confidence intervals. Bootstrapping is a nonparametric resampling procedure that does not require the assumption of normality of the sampling distribution [46]. The mediation was estimated using the indirect effect, which indicates the change in the effect of the independent variable on the outcome that can be endorsed to the proposed mediator. Indirect effects (a * b paths) with confidence intervals not including zero are interpreted as statistically significant [47] which could occur regardless of the significance of the total effect (c path, effect of the independent variable on the dependent variable) and the direct effect (c′ path, effect on the dependent variable when both the independent and the mediator variables are included as independent variables) [45]. To quantify how much of the total effect was due to the mediation, we calculated the percentage of mediation ([indirect effect / total effect] * 100) provided when the total effect was larger than the indirect effect with the same direction [45] .

All analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 25.0, IBM SPSS Statistics, IBM Corporation) and the level of significance was set at < 0.05. Graphical presentations were prepared using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

RESULTS

The characteristics of the study sample are shown in Table 1. Significant differences in BMI, lean mass, LMI, BMR, BFox, BCHox, MFO, energy, fat, ethanol, saturated fatty acids, monounsaturated fatty acids, and TST were observed between men and women (all $p < 0.05$). A poor subjective

sleep quality (global PSQI score > 5) was identified in 40.3% of our cohort. Figure 1 reports mean and standard deviation of energy metabolism related parameters after categorizing participants in our study as either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. Between these two groups, no statistically significant differences were observed in objective sleep quantity and quality parameters (i.e., TST, WASO, and SE, all $p \geq 0.05$).

Table 2 shows the association of sleep quantity and quality with BMR and BMR_{LM}. We observed an inverse association between TST and BMR ($\beta = -0.459$, $R^2 = 0.211$,

Table 1. Descriptive parameters of participants.

	N	All		N	Men		N	Women	
Age (years)	70	53.4	(4.9)	34	54.2	(5.2)	36	52.7	(4.7)
Anthropometry and body composition									
Body mass index (kg/m²)	70	26.8	(3.8)	34	28.5	(3.5)	36	25.3	(3.4) *
Lean mass (kg)	70	44.0	(11.7)	34	54.2	(6.3)	36	34.4	(5.7) *
Lean mass index (kg/m²)	70	15.3	(2.9)	34	17.5	(2.0)	36	13.2	(1.8) *
Fat mass (kg)	70	30.2	(8.4)	34	31.3	(9.5)	36	29.2	(7.3)
Fat mass index (kg/m²)	70	10.8	(3.1)	34	10.2	(3.2)	36	11.3	(3.0)
Basal metabolic rate and fuel oxidation in basal oxidation									
BMR (kcal/day)	70	1511.3	(366.3)	34	1805.5	(244.8)	36	1233.5	(211.0) *
BMR_{LM} ($kcal/kg_{leanmass}$/day)	70	35.2	(7.3)	34	33.6	(5.3)	36	36.8	(8.5)
BFox (g/min)	70	0.05	(0.04)	34	0.06	(0.05)	36	0.04	(0.02) *
BFox (%BMR)	70	45.0	(29.7)	34	45.6	(32.7)	36	44.4	(27.1)
BCHox (g/min)	70	0.11	(0.1)	34	0.14	(0.11)	36	0.10	(0.07) *
BCHox (%BMR)	70	42.4	(31.5)	34	44.0	(34.7)	36	40.9	(28.6)
Fuel oxidation during exercise									
MFO (g/min)	70	0.29	(0.09)	34	0.35	(0.09)	36	0.24	(0.04) *
MFO_{LM} ($g/kg_{leanmass}$/day)	70	6.8	(1.6)	34	6.4	(1.5)	36	7.1	(1.7)
FATmax (%VO_2max)	70	43.1	(10.5)	34	41.6	(10.3)	36	44.4	(10.7)
Dietary intake									
Energy (kcal/day)	70	2147.7	(739.8)	34	2206.8	(965.2)	36	2061.0	(459.0) *
Dietary energy density (kcal/g/day)	70	1.2	(0.5)	34	1.3	(0.7)	36	1.1	(0.2)
Fat (g/day)	70	88.6	(25.4)	34	90.3	(26.1)	36	87.3	(25.4) *
Protein (g/day)	70	90.0	(39.6)	34	92.5	(48.8)	36	85.9	(27.1)
Carbohydrate (g/day)	70	227.4	(120.0)	34	233.9	(158.8)	36	215.2	(61.6)
Fiber (g/day)	70	31.7	(28.5)	34	31.3	(26.1)	36	33.4	(32.6)
Ethanol (g/day)	70	9.9	(11.3)	34	11.3	(12.3)	36	8.2	(9.9) *
SFA (g/day)	70	22.2	(7.4)	34	25.5	(6.7)	36	19.5	(7.0) *
MUFA (g/day)	70	41.2	(13.9)	34	47.0	(12.7)	36	37.0	(13.3) *
PUFA (g/day)	70	12.8	(5.0)	34	14.3	(5.3)	36	11.8	(4.5)
Cholesterol (mg/day)	70	279.2	(106.0)	34	295.9	(118.9)	36	261.7	(94.4)
PREDIMED total score	70	9.5	(1.9)	34	9.0	(2.0)	36	9.8	(1.8)
Sleep quantity and quality									
Global PSQI score	67	5.6	(3.5)	34	4.8	(3.2)	36	6.3	(3.6)
Total sleep time (min)	70	360.0	(48.9)	34	337.9	(46.3)	36	381.3	(41.8) *
Wake after sleep onset (min)	70	62.2	(25.9)	34	65.8	(32.5)	36	58.7	(17.0)
Sleep efficiency (%)	70	84.1	(11.9)	34	83.9	(7.5)	36	84.3	(14.9)

Values are expressed as mean (standard deviation). *Significant differences between sexes obtained from an independent samples *t*-test ($p < 0.05$). BMR: basal metabolic rate; BMR_{LM}: basal metabolic relativized to the lean mass; BFox: basal fat oxidation; BCHox: basal carbohydrate oxidation; MFO: maximal fat oxidation; MFO_{LM}: maximal fat oxidation relativized to the lean mass; FATmax: intensity of exercise that elicits MFO; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PREDIMED: PREvención con DIeta MEDiterránea; PSQI: Pittsburgh Sleep Quality Index.

p < 0.001, Table 2), which disappeared after including sex, age, and FMI in the model (all $p \geq 0.05$, Table 2). No association was found between the remaining subjective or objective sleep parameters and both BMR and BMR_{LM} (all $p \geq 0.05$, Table 2), neither when we accounted for covariates.

Table 3 shows the association of sleep quantity and quality with BFox and BCHox (both expressed in g/min, and as %BMR). An inverse association was detected between global PSQI score and BFox (expressed in g/min, and as %BMR) (β = −0.475, R^2 = 0.225, p < 0.001; β = −0.480,

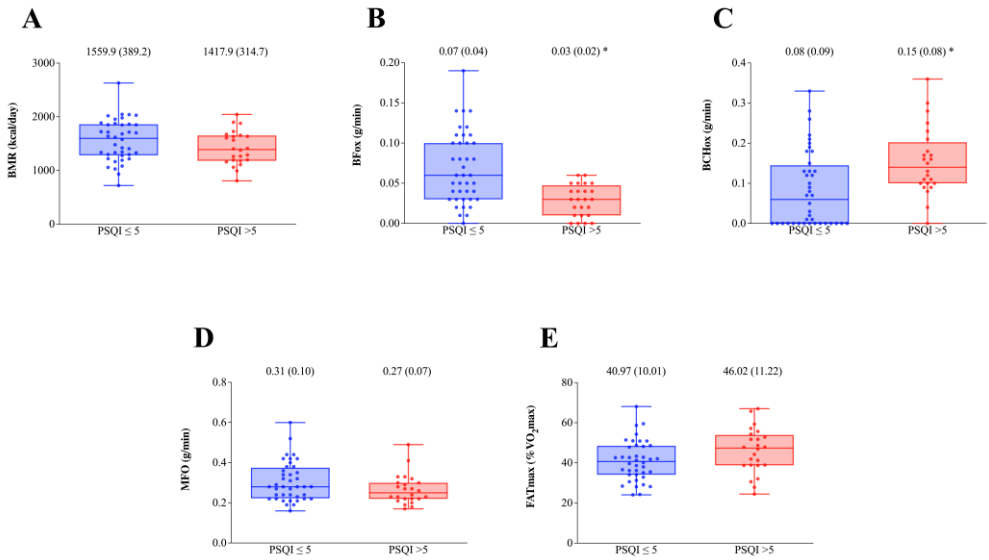

Figure 1. Mean (standard deviation) of energy metabolism related parameters after categorizing participants as either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. *Significant differences between global PSQI score groups obtained from an independent samples t-test (p < 0.05). PSQI: Pittsburgh Sleep Quality Index; BMR: basal metabolic rate; BMR_{LM}: basal metabolic relativized to the lean mass; BFox: basal fat oxidation; BCHox: basal carbohydrate oxidation; MFO: maximal fat oxidation; MFO_{LM}: maximal fat oxidation relativized to the lean mass; FATmax: intensity of exercise that elicits MFO.

Table 2. Association of sleep quantity and quality with BMR and BMR_{LM} (Model 0) adjusted by sex (Model 1), by both sex and age (Model 2), and by sex, age, and fat mass index (Model 3).

	Global PSQI score			Total sleep time (min)			Wake after sleep onset (min)			Sleep efficiency (%)		
	β	R^2	p	β	R^2	p	β	R^2	p	β	R^2	p
BMR (kcal/day)												
Model 0	−0.178	0.032	0.158	−0.459	0.211	**<0.001**	0.219	0.048	0.071	−0.077	0.006	0.525
Model 1	−0.006	0.598	0.943	−0.136	0.629	0.109	0.113	0.627	0.141	−0.063	0.622	0.408
Model 2	0.026	0.616	0.760	−0.132	0.641	0.118	0.103	0.638	0.177	−0.077	0.006	0.528
Model 3	0.019	0.625	0.826	−0.135	0.657	0.105	0.126	0.658	0.098	−0.074	0.007	0.550
BMR_{LM} (Kcal/$kg_{leanmass}$/day)												
Model 0	0.222	0.049	0.078	0.035	0.001	0.775	−0.047	0.002	0.700	−0.018	0.000	0.879
Model 1	0.182	0.081	0.155	−0.079	0.053	0.557	0.079	0.054	0.516	−0.023	0.048	0.850
Model 2	0.143	0.107	0.269	−0.088	0.102	0.507	0.101	0.105	0.401	−0.019	0.094	0.874
Model 3	0.117	0.212	0.339	−0.095	0.216	0.444	0.159	0.233	0.163	−0.054	0.211	0.630

β (standardized regression coefficient), R^2, and p-value were obtained from the linear regression analyses. Significant p-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; BMR: basal metabolic rate; BMR_{LM}: basal metabolic rate relativized to the lean mass.

R^2 = 0.230, p < 0.001, respectively, Table 3), which remained significant after including sex, age, and FMI in the model (all $p \leq 0.002$, Table 3). We did not find any significant association between any objective sleep parameter and BFox (all $p \geq 0.05$, Table 3). Global PSQI score was positively associated with BCHox (expressed in g/min, and as %BMR) (β = 0.003, R^2 = 0.138, p = 0.002; β = 0.458, R^2 = 0.210, p < 0.001, respectively, Table 3), even after controlling for sex, age, and FMI (all $p \leq 0.002$, Table 3). We observed an inverse association between TST and BCHox (expressed in g/min) (β = −0.268, R^2 = 0.072, p = 0.026, Table 3), which disappeared after including sex, age, and FMI in the model (all $p \geq 0.05$, Table 3). No association was found between the remaining objective sleep parameters and BCHox (expressed in g/min, and as %BMR) (all $p \geq 0.05$, Table 3), neither when we accounted for covariates.

Table 4 shows the association of sleep quantity and quality with MFO, MFO_{LM}, and FATmax (expressed as $\%VO_2max$). We showed an inverse association between TST and MFO (expressed in g/min) (β = −0.318, R^2 = 0.101, p = 0.008, Table 4), which disappeared when the model includes sex, age, and FMI (all $p \geq 0.05$, Table 4). No association was found between the remaining sleep parameters and both MFO and MFO_{LM} (all $p \geq 0.05$, Table 4). A positive association was detected between global PSQI score and FATmax (expressed as $\%VO_2max$) (β = 0.282, R^2 = 0.080, p = 0.024, Table 4), which remained significant after

Table 3. Association of sleep quantity and quality with BFox and BCHox (both expressed in g/min and in %BMR) (Model 0) adjusted by sex (Model 1), by both sex and age (Model 2), and by sex, age, and fat mass index (Model 3).

	Global PSQI score			Total sleep time (min)			Wake after sleep onset (min)			Sleep efficiency (%)		
	β	R^2	p	β	R^2	p	β	R^2	p	β	R^2	p
BFox (g/min)												
Model 0	−0.475	0.225	**<0.001**	−0.047	0.002	0.699	0.099	0.010	0.417	0.012	0.000	0.919
Model 1	−0.426	0.271	**<0.001**	0.100	0.089	0.450	−0.061	0.085	0.608	0.085	0.018	0.879
Model 2	−0.345	0.391	**0.002**	0.117	0.281	0.322	0.020	0.271	0.853	0.010	0.274	0.924
Model 3	−0.345	0.391	**0.002**	0.118	0.282	0.325	0.019	0.271	0.861	0.011	0.274	0.918
BFox (%BMR)												
Model 0	−0.480	0.230	**<0.001**	0.092	0.008	0.453	0.044	0.002	0.717	0.044	0.002	0.718
Model 1	−0.494	0.234	**<0.001**	0.122	0.012	0.375	0.043	0.002	0.727	0.044	0.002	0.718
Model 2	−0.417	0.342	**<0.001**	0.140	0.199	0.265	0.003	0.184	0.981	0.037	0.150	0.743
Model 3	−0.417	0.342	**<0.001**	0.140	0.200	0.266	−0.002	0.185	0.985	0.040	0.188	0.723
BCHox (g/min)												
Model 0	0.003	0.138	**0.002**	−0.268	0.072	**0.026**	0.002	0.049	0.687	−0.071	0.005	0.561
Model 1	0.439	0.224	**0.001**	−0.197	0.092	0.138	0.016	0.061	0.896	−0.066	0.064	0.578
Model 2	0.374	0.301	**0.002**	−0.212	0.235	0.085	0.052	0.201	0.648	−0.059	0.202	0.591
Model 3	0.370	0.303	**0.002**	−0.214	0.244	0.083	0.068	0.212	0.552	−0.070	0.212	0.532
BCHox (%BMR)												
Model 0	0.458	0.210	**<0.001**	−0.133	0.018	0.275	−0.038	0.001	0.759	−0.051	0.003	0.677
Model 1	0.485	0.224	**<0.001**	−0.136	0.018	0.323	−0.046	0.005	0.712	−0.050	0.005	0.684
Model 2	0.407	0.335	**0.001**	−0.153	0.206	0.220	−0.005	0.188	0.964	−0.042	0.191	0.705
Model 3	0.406	0.335	**0.001**	−0.154	0.209	0.220	0.002	0.190	0.987	−0.047	0.193	0.674

β (standardized regression coefficient), R^2, and p-value were obtained from the linear regression analyses. Significant p-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; BFox: basal fat oxidation; BCHox: basal carbohydrate oxidation.

including sex in the model (p = 0.038, Table 4), but disappeared when age and FMI were included in the model (all $p \geq 0.05$, Table 4). We did not find any significant association between any objective sleep parameter and FATmax (all $p \geq 0.05$, Table 4).

We repeated all previous associations controlling for menopausal status (pre- or post-menopausal) in order to avoid the possible cofounder of female hormones, and the results did not change (data not shown).

Tables 5 and 6 show the association of PSQI components scores (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction) with BMR, BFox, BCHox, MFO, and FATmax.

We observed only a negative association of global PSQI score with fiber intake, and TST and WASO were negatively and positively associated with cholesterol intake, respectively (Table 7). However, we observed a modification effect of different dietary factors (i.e., fiber and ethanol intake, Table 8). Despite this modification effect of dietary factors and the several associations between dietary factors and sleep parameters, we did not find a mediating effect of energy, dietary energy density, fat, protein, carbohydrate, fiber intake, lipid profile intake, ethanol intake, and PREDIMED total score on the association of the global PSQI score and BFox both expressed in g/min, and as %BMR (Figures 2 to 5).

DISCUSSION

The aim of the present study was to investigate the relationship of both subjective and objective sleep quantity and quality with BMR and fuel oxidation in basal conditions and during exercise in sedentary middle-

Table 4. Association of sleep quantity and quality with MFO, $MFO_{LM,}$ and FATmax (Model 0) adjusted by sex (Model 1), by both sex and age (Model 2), and by sex, age, and fat mass index (Model 3).

	Global PSQI score			Total sleep time (min)			Wake after sleep onset (min)			Sleep efficiency (%)		
	β	R^2	p	β	R^2	p	β	R^2	p	β	R^2	p
MFO (g/min)												
Model 0	–0.173	0.030	0.171	–0.318	0.101	**0.008**	0.136	0.018	0.266	–0.022	0.000	0.856
Model 1	–0.036	0.390	0.727	–0.051	0.386	0.637	0.052	0.386	0.598	–0.010	0.389	0.913
Model 2	–0.020	0.394	0.851	–0.048	0.393	0.661	0.044	0.393	0.658	–0.022	0.001	0.857
Model 3	–0.021	0.394	0.847	–0.049	0.399	0.651	0.057	0.400	0.570	–0.017	0.003	0.893
MFO_{LM} ($g/kg_{leanmass}$/day)												
Model 0	0.125	0.016	0.324	0.111	0.012	0.365	0.010	0.000	0.935	0.033	0.001	0.783
Model 1	0.087	0.043	0.499	0.025	0.042	0.852	0.039	0.043	0.752	0.030	0.040	0.804
Model 2	0.036	0.092	0.784	0.016	0.090	0.902	0.060	0.093	0.617	0.037	0.035	0.757
Model 3	0.020	0.130	0.876	0.011	0.147	0.932	0.101	0.157	0.393	0.008	0.113	0.944
FATmax (%VO_2max)												
Model 0	0.282	0.080	**0.024**	0.174	0.030	0.153	0.022	0.000	0.859	–0.041	0.002	0.735
Model 1	0.267	0.085	**0.038**	0.145	0.034	0.288	0.040	0.018	0.744	–0.044	0.020	0.719
Model 2	0.206	0.150	0.104	0.132	0.133	0.310	0.071	0.124	0.547	–0.035	0.089	0.765
Model 3	0.230	0.238	0.060	0.138	0.192	0.277	0.033	0.178	0.777	–0.014	0.129	0.901

β (standardized regression coefficient), R^2, and p-value were obtained from the linear regression analyses. Significant p-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; MFO: maximal fat oxidation; MFO_{LM}: maximal fat oxidation relativized to the lean mass; FATmax: intensity of exercise that elicits MFO.

Table 5. Association of PSQI components scores with BMR, BFox, and BCHox (Model 0) adjusted by sex (Model 1), by both sex and age (Model 2), and by sex, age, and fat mass index (Model 3).

	BMR (kcal/day)			BMR_{LM} ($Kcal/kg_{leanmass}/day$)			BFox (g/min)			BFox (%BMR)			BCHox (g/min)			BCHox (%BMR)		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Subjective sleep quality (Component 1)																		
Model 0	–0.216	0.047	0.087	0.265	0.070	**0.034**	–0.361	0.130	**0.003**	–0.351	0.123	**0.004**	0.235	0.055	0.061	0.339	0.115	**0.006**
Model 1	0.048	0.600	0.580	0.215	0.090	0.102	–0.288	0.172	**0.023**	–0.378	0.129	**0.004**	0.341	0.144	**0.009**	0.388	0.134	**0.003**
Model 2	0.076	0.621	0.382	0.183	0.118	0.166	–0.213	0.322	0.068	–0.307	0.266	**0.013**	0.281	0.242	**0.024**	0.316	0.271	**0.010**
Model 3	0.057	0.627	0.519	0.112	0.210	0.384	–0.220	0.323	0.068	–0.313	0.267	**0.014**	0.276	0.242	**0.032**	0.321	0.272	**0.011**
Sleep latency (Component 2)																		
Model 0	–0.033	0.001	0.795	0.203	0.041	0.107	–0.391	0.153	**0.001**	–0.456	0.208	**<0.001**	0.390	0.152	**0.001**	0.454	0.206	**<0.001**
Model 1	0.007	0.598	0.934	0.192	0.086	0.122	–0.376	0.239	**0.001**	–0.455	0.209	**<0.001**	0.402	0.202	**0.001**	0.456	0.207	**<0.001**
Model 2	0.048	0.618	0.567	0.148	0.109	0.248	–0.276	0.354	**0.013**	–0.364	0.307	**0.002**	0.325	0.271	**0.006**	0.363	0.307	**0.002**
Model 3	0.031	0.625	0.719	0.084	0.206	0.497	–0.284	0.355	**0.013**	–0.371	0.308	**0.002**	0.321	0.271	**0.009**	0.369	0.307	**0.002**
Sleep duration (Component 3)																		
Model 0	–0.060	0.004	0.636	–0.023	0.001	0.860	–0.309	0.096	**0.013**	–0.323	0.105	**0.009**	0.252	0.063	**0.045**	0.292	0.086	**0.019**
Model 1	–0.044	0.600	0.591	–0.027	0.050	0.828	–0.303	0.190	**0.011**	–0.323	0.106	**0.010**	0.256	0.106	**0.038**	0.293	0.086	**0.020**
Model 2	–0.036	0.617	0.657	–0.040	0.090	0.749	–0.278	0.360	**0.009**	–0.297	0.273	**0.009**	0.235	0.229	**0.043**	0.267	0.257	**0.020**
Model 3	–0.037	0.626	0.641	–0.046	0.202	0.695	–0.277	0.360	**0.010**	–0.297	0.274	**0.010**	0.233	0.234	**0.046**	0.267	0.258	**0.021**
Habitual sleep efficiency (Component 4)																		
Model 0	–0.245	0.060	0.051	0.072	0.005	0.570	–0.337	0.114	**0.006**	–0.293	0.086	**0.019**	0.165	0.027	0.194	0.251	0.063	**0.046**
Model 1	–0.035	0.599	0.679	0.012	0.049	0.925	–0.271	0.166	**0.029**	–0.303	0.087	**0.020**	0.238	0.093	0.065	0.276	0.071	**0.036**
Model 2	–0.022	0.616	0.797	–0.008	0.089	0.949	–0.229	0.332	**0.042**	–0.262	0.248	**0.029**	0.203	0.212	0.096	0.234	0.235	0.052
Model 3	–0.011	0.624	0.896	0.031	0.201	0.802	–0.234	0.333	**0.041**	–0.269	0.251	**0.027**	0.214	0.222	0.081	0.242	0.240	**0.047**
Sleep disturbances (Component 5)																		
Model 0	–0.230	0.053	0.068	0.067	0.005	0.597	–0.374	0.140	**0.002**	–0.354	0.125	**0.004**	0.240	0.058	0.056	0.343	0.118	**0.005**
Model 1	–0.072	0.603	0.385	0.022	0.050	0.863	–0.323	0.198	**0.008**	–0.360	0.126	**0.005**	0.295	0.124	**0.019**	0.362	0.126	**0.004**
Model 2	–0.013	0.616	0.889	–0.088	0.095	0.533	–0.155	0.302	0.215	–0.203	0.216	0.127	0.158	0.193	0.240	0.204	0.217	0.124
Model 3	–0.004	0.624	0.968	–0.056	0.202	0.680	–0.158	0.302	0.213	–0.208	0.219	0.122	0.167	0.201	0.219	0.210	0.221	0.118
Use of sleeping medication (Component 6)																		
Model 0	–0.095	0.009	0.456	0.339	0.115	**0.006**	–0.249	0.062	**0.048**	–0.224	0.050	0.076	0.220	0.048	0.081	0.222	0.049	0.078
Model 1	0.026	0.599	0.749	0.311	0.144	**0.012**	–0.205	0.139	0.094	–0.222	0.050	0.084	0.258	0.106	**0.040**	0.230	0.052	0.074
Model 2	0.033	0.617	0.682	0.302	0.177	**0.014**	–0.183	0.316	0.096	–0.200	0.224	0.087	0.240	0.230	**0.041**	0.208	0.228	0.075
Model 3	0.032	0.625	0.691	0.298	0.286	**0.010**	–0.183	0.316	0.099	–0.200	0.225	0.091	0.239	0.236	**0.043**	0.208	0.229	0.078
Daytime dysfunction (Component 7)																		
Model 0	0.056	0.003	0.662	0.083	0.007	0.514	–0.226	0.051	0.073	–0.278	0.077	**0.026**	0.283	0.080	**0.024**	0.288	0.083	**0.021**
Model 1	0.025	0.599	0.762	0.092	0.058	0.462	–0.239	0.155	**0.047**	–0.280	0.080	**0.026**	0.275	0.116	**0.026**	0.287	0.083	**0.022**
Model 2	0.030	0.617	0.710	0.084	0.096	0.495	–0.222	0.332	**0.040**	–0.263	0.254	**0.022**	0.261	0.242	**0.024**	0.271	0.259	**0.018**
Model 3	0.023	0.625	0.773	0.060	0.203	0.609	–0.222	0.332	**0.042**	–0.262	0.254	**0.024**	0.257	0.245	**0.028**	0.269	0.259	**0.020**

β (standardized regression coefficient), R^2, and *p*-value were obtained from the linear regression analyses. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; BMR: basal metabolic rate; BFox: basal fat oxidation; BCHox: basal carbohydrate oxidation.

Table 6. Association of PSQI components scores with MFO and FATmax (Model 0) adjusted by sex (Model 1), by both sex and age (Model 2), and by sex, age, and fat mass index (Model 3).

	MFO (g/min)			MFO_{LM} ($g/kg_{leanmass}$/day)			FATmax (%VO_2max)		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Subjective sleep quality (Component 1)									
Model 0	−0.192	0.037	0.128	0.154	0.024	0.223	0.305	0.093	**0.014**
Model 1	0.018	0.389	0.865	0.103	0.045	0.442	0.294	0.094	**0.027**
Model 2	0.033	0.395	0.760	0.060	0.094	0.651	0.243	0.163	0.061
Model 3	0.033	0.395	0.769	0.015	0.130	0.909	0.322	0.276	**0.011**
Sleep latency (Component 2)									
Model 0	−0.057	0.003	0.653	0.109	0.012	0.390	0.213	0.045	0.092
Model 1	−0.025	0.389	0.801	0.100	0.046	0.428	0.206	0.059	0.102
Model 2	−0.005	0.394	0.963	0.037	0.092	0.773	0.130	0.127	0.305
Model 3	−0.007	0.394	0.950	−0.003	0.130	0.982	0.194	0.224	0.118
Sleep duration (Component 3)									
Model 0	0.045	0.002	0.726	0.099	0.010	0.438	−0.025	0.001	0.846
Model 1	0.058	0.392	0.564	0.095	0.045	0.453	−0.027	0.018	0.829
Model 2	0.063	0.398	0.534	0.081	0.097	0.515	−0.047	0.114	0.703
Model 3	0.063	0.398	0.538	0.077	0.136	0.529	−0.041	0.192	0.725
Habitual sleep efficiency (Component 4)									
Model 0	−0.193	0.037	0.127	0.054	0.003	0.669	0.239	0.057	0.057
Model 1	−0.023	0.389	0.825	0.003	0.036	0.985	0.220	0.062	0.093
Model 2	−0.016	0.394	0.883	−0.022	0.091	0.866	0.190	0.145	0.132
Model 3	−0.015	0.394	0.890	0.001	0.130	0.991	0.160	0.214	0.193
Sleep disturbances (Component 5)									
Model 0	−0.204	0.042	0.106	0.042	0.002	0.740	0.337	0.113	**0.007**
Model 1	−0.078	0.394	0.447	0.003	0.036	0.981	0.324	0.117	**0.011**
Model 2	−0.055	0.396	0.635	−0.133	0.104	0.347	0.227	0.151	0.101
Model 3	−0.054	0.396	0.642	−0.114	0.140	0.416	0.201	0.221	0.134
Use of sleeping medication (Component 6)									
Model 0	−0.189	0.036	0.135	0.116	0.013	0.363	0.215	0.046	0.088
Model 1	−0.094	0.397	0.354	0.088	0.043	0.489	0.199	0.056	0.119
Model 2	−0.090	0.402	0.376	0.076	0.096	0.541	0.184	0.145	0.134
Model 3	−0.090	0.402	0.379	0.074	0.135	0.548	0.187	0.225	0.113
Daytime dysfunction (Component 7)									
Model 0	−0.039	0.002	0.760	−0.033	0.001	0.798	0.079	0.006	0.533
Model 1	−0.064	0.392	0.523	−0.025	0.036	0.843	0.085	0.024	0.506
Model 2	−0.061	0.398	0.544	−0.034	0.092	0.782	0.073	0.117	0.552
Model 3	−0.062	0.398	0.542	−0.049	0.132	0.687	0.094	0.199	0.425

β (standardized regression coefficient), R^2, and *p*-value were obtained from the linear regression analyses. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; MFO: maximal fat oxidation; MFO_{LM}: maximal fat oxidation relativized to the lean mass; FATmax: intensity of exercise that elicits MFO.

Table 7. Association of dietary factors with sleep parameters.

	Global PSQI score		Total sleep time		Wake after sleep onset		Sleep efficiency	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Energy (kcal)	−0.120	0.365	0.058	0.646	−0.167	0.186	0.133	0.290
Dietary energy density (kcal/g/day)	−0.165	0.195	−0.053	0.669	−0.006	0.962	0.020	0.872
Fat (g/day)	−0.141	0.287	−0.070	0.583	−0.020	0.874	0.020	0.873
Protein (g/day)	−0.105	0.430	0.110	0.388	−0.196	0.120	0.036	0.774
Carbohydrate (g/day)	−0.092	0.489	0.114	0.368	−0.199	0.116	0.183	0.144
Fiber (g/day)	−0.281	**0.031**	0.128	0.315	−0.103	0.416	0.069	0.584
Ethanol (g/day)	0.040	0.762	−0.108	0.396	−0.008	0.947	0.057	0.651
SFA (g/day)	−0.092	0.473	−0.163	0.185	−0.126	0.307	−0.016	0.898
MUFA (g/day)	0.000	0.998	−0.205	0.094	0.027	0.827	0.044	0.718
PUFA (g/day)	0.023	0.856	−0.131	0.287	−0.132	0.282	0.122	0.317
Cholesterol (mg/day)	0.109	0.394	−0.326	**0.007**	0.367	**0.002**	−0.163	0.180
PREDIMED total score	0.091	0.480	0.150	0.226	−0.027	0.827	0.006	0.960

Pearson correlations were performed. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PREDIMED: PREvención con DIeta MEDiterránea.

Table 8. Effect modification analyses of dietary intake and global PSQI score on basal fat oxidation.

	BFox (g/min)			BFox (%BMR)		
	β	R^2	*p*	*β*	R^2	*p*
Energy (kcal) * PSQI interaction	−0.297	0.088	**0.022**	−0.327	0.107	**0.011**
Dietary energy density (kcal/g/day) * PSQI interaction	−0.405	0.164	**0.001**	−0.440	0.194	**<0.001**
Fat (g/day) * PSQI interaction	−0.330	0.109	**0.008**	−0.386	0.149	**0.002**
Protein (g/day) * PSQI interaction	−0.296	0.088	**0.017**	−0.324	0.105	**0.009**
Carbohydrate (g/day) * PSQI interaction	−0.349	0.122	**0.005**	−0.385	0.148	**0.002**
Fiber (g/day) * PSQI interaction	−0.039	0.002	0.768	−0.061	0.004	0.646
Ethanol (g/day) * PSQI interaction	−0.142	0.020	0.275	−0.185	0.034	0.154
SFA (g/day) * PSQI interaction	−0.312	0.097	**0.013**	−0.390	0.152	**0.002**
MUFA (g/day) * PSQI interaction	−0.326	0.106	**0.009**	−0.378	0.143	**0.002**
PUFA (g/day) * PSQI interaction	−0.261	0.068	**0.039**	−0.295	0.087	**0.019**
Cholesterol (mg/day) * PSQI interaction	−0.313	0.098	**0.013**	−0.341	0.117	**0.006**
PREDIMED total score * PSQI interaction	−0.469	0.220	**0.000**	−0.465	0.216	**<0.001**

β (standardized regression coefficient), R^2, and *p*-value were obtained from the linear regression analyses. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; BMR: basal metabolic rate; BFox: basal fat oxidation; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PREDIMED: PREvención con DIeta MEDiterránea.

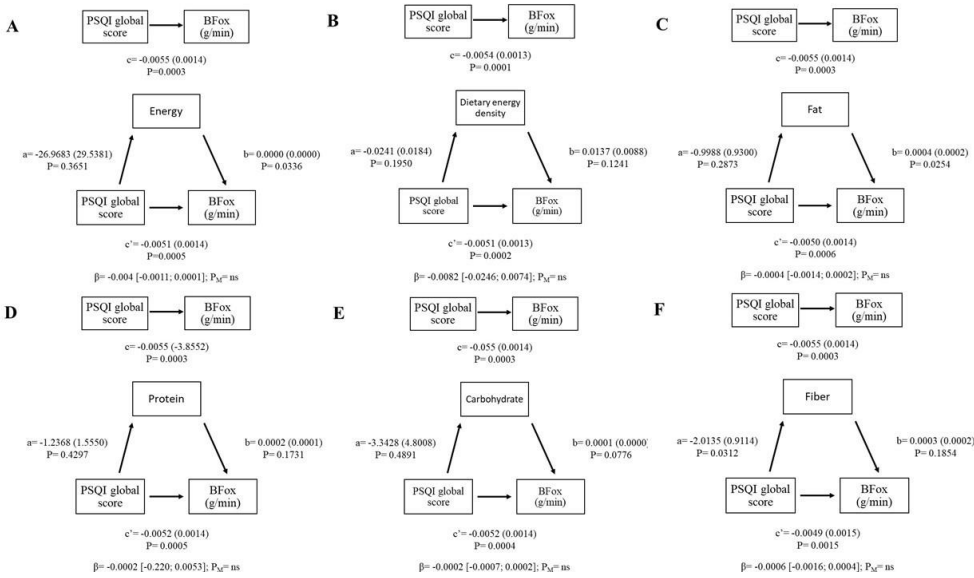

Figure 2. Mediation models of the relationship between global PSQI score and BFox with (A) energy intake, (B) dietary energy density intake, (C) fat intake, (D) protein intake, (E) carbohydrate intake, and (F) fiber intake included as mediator variables. Paths a, b, c and c′ are presented as unstandardized coefficients (SE). *β* = indirect effect (a * b paths) [lower-limit CI; upper-limit CI], lower and upper levels for bias-corrected 95% Cis of the indirect effect based on 5,000 bootstraps. PSQI: Pittsburgh Sleep Quality Index; BFox: basal fat oxidation; CI: confidence interval; ns:

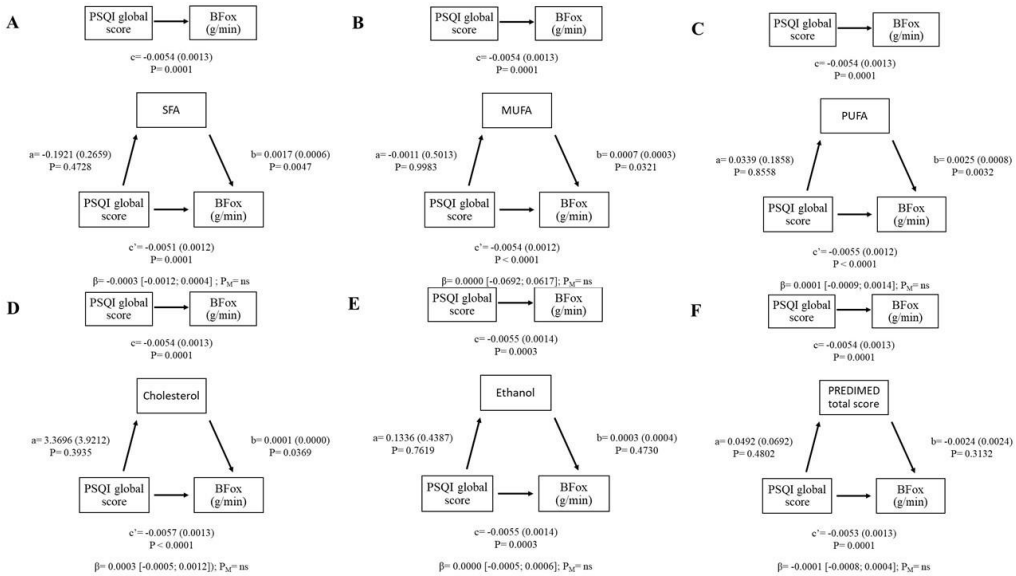

Figure 3. Mediation models fiber intake of the relationship between global PSQI score and BFox with (A) SFA intake, (B) MUFA intake, (C) PUFA intake, (D) cholesterol intake, (E) ethanol intake, and (F) PREDIMED total score included as mediator variables. Paths a, b, c and c′ are presented as unstandardized coefficients (SE). β = indirect effect (a * b paths) [lower-limit CI; upper-limit CI], lower and upper levels for bias-corrected 95% Cis of the indirect effect based on 5,000 bootstraps. PSQI: Pittsburgh Sleep Quality Index; BFox: basal fat oxidation; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PREDIMED: PREvención con DIeta MEDiterránea; CI: confidence interval; ns: nonsignificant.

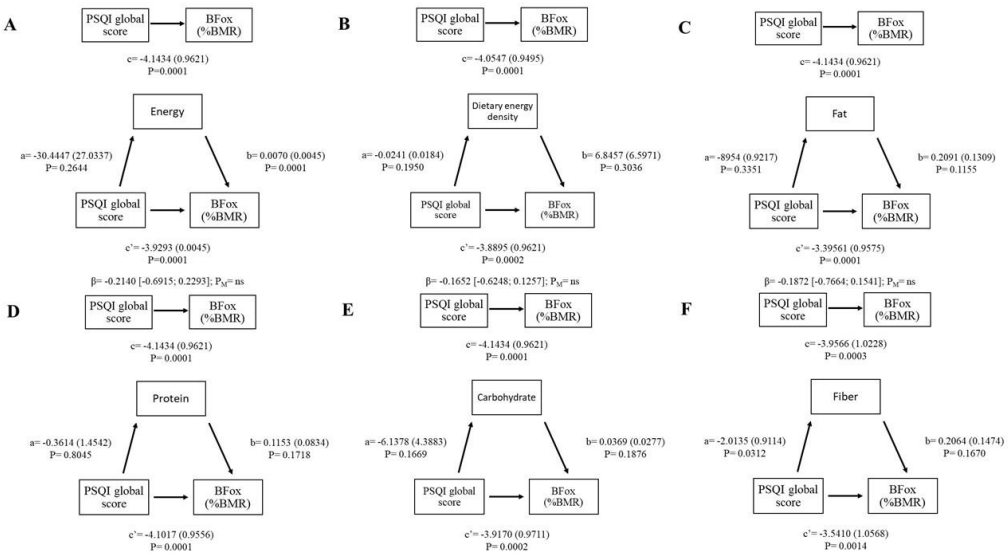

Figure 4. Mediation models of the relationship between global PSQI score and BFox expressed as percentage of BMR with (A) energy intake, (B) dietary energy density intake, (C) fat intake, (D) protein intake, (E) carbohydrate intake, and (F) fiber intake included as mediator variables. Paths a, b, c and c′ are presented as unstandardized coefficients (SE). β = indirect effect (a * b paths) [lower-limit CI; upper-limit CI], lower and upper levels for bias-corrected 95% Cis of the indirect effect based on 5,000 bootstraps. PSQI: Pittsburgh Sleep Quality Index; BMR: basal metabolic rate; BFox: basal fat oxidation; CI: confidence interval; ns: nonsignificant.

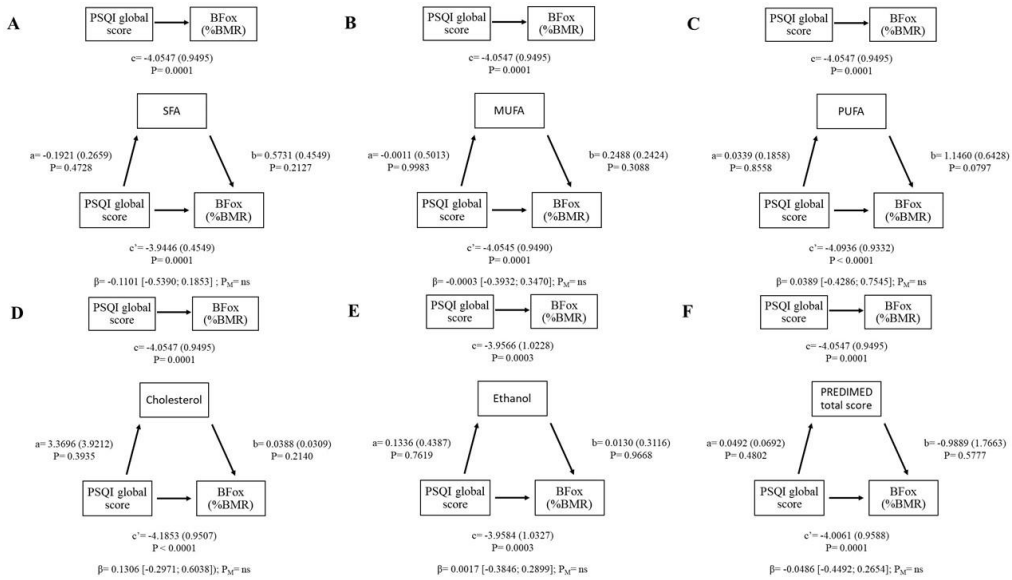

Figure 5. Mediation models fiber intake of the relationship between global PSQI score and BFox with (A) SFA intake, (B) MUFA intake, (C) PUFA intake, (D) cholesterol intake, (E) ethanol intake, and (F) PREDIMED total score included as mediator variables. Paths a, b, c and c' are presented as unstandardized coefficients (SE). β = indirect effect (a * b paths) [lower-limit CI; upper-limit CI], lower and upper levels for bias-corrected 95% Cis of the indirect effect based on 5,000 bootstraps. PSQI: Pittsburgh Sleep Quality Index; BFox: basal fat oxidation; BMR: basal metabolic rate; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PREDIMED: PREvención con DIeta MEDiterránea; CI: confidence interval; ns: nonsignificant.

aged adults. We also aimed to study the mediating role of dietary intake and adherence to the traditional Mediterranean diet between sleep and energy metabolism parameters in sedentary middle-aged adults. The main finding of the present study is that a poor subjective sleep quality was associated with lower BFox independently of sex, age, and body composition outcomes, in sedentary middle-aged adults. Moreover, our results indicated that the association of global PSQI score with BFox was not mediated by dietary intake and MedDiet adherence.

Several physiological mechanisms could explain the relationship between sleep quality and BFox. Sleep restriction is associated with insulin resistance characterized by a decreased insulin-mediated glucose uptake [48], which could develop metabolic inflexibility characterized by an impaired BFox [49]. Short sleep duration and sleep fragmentation are also related to low leptin levels or leptin resistance [50] which are associated with an impaired fatty acid oxidation [51]. Sleep disruption (discontinuity of sleep) can lead to the disruption of circadian rhythms [13], which orchestrate crucial physiological and behavioral functions, one of them being the regulation of carbohydrate and fatty acid metabolism [12]. Higher sleep duration and quality are associated with a healthier gut microbiome [52], which could suppress insulin signaling, increase β-oxidation and inhibit fat oxidation

derived from the production of short-chain fatty acids [53]. Furthermore, sleep disruption (discontinuity of sleep) could decrease melatonin production [13], which has important metabolic functions, such as lipolysis, regulating the energy flow [54]. An increase in the production of pro-inflammatory cytokines and reactive oxygen species is observed in impaired sleep patterns [13]. Both inflammation and oxidative stress could modulate metabolic flexibility, specifically fat oxidation [55,56]. Therefore, based on the above-mentioned mechanisms, a healthy sleep pattern could improve metabolic health via the increment of BFox and viceversa.

In addition, an impaired sleep pattern, determined by a low sleep duration could increase energy intake through several potential mechanisms: increment of time and opportunities for eating, psychological distress, sensitivity to food reward, energy needed to sustain wakefulness, hunger hormones and decrease dietary restraint [16]. A lack of sleep or low sleep quality could increase the intake of high energy-dense foods, high fat and sugary snacks, which are low in fiber [16]. In this sense, although we did not find any association between energy and macronutrient intake, we observed that fiber intake was negatively associated with global PSQI score. Fiber intake could have different metabolic effects (i.e., insulin sensitivity and glycemia improvement) [57], that could have a potential role in the regulation of fat oxidation. However, we did not find any mediating role of dietary intake in the association of global PSQI score with BFox. The lack of a mediating role may be due to specific issues: (i) since dietary outcomes were assessed in a specific time point, it could be that the dietary intake was insufficiently maintained over time to modify BFox; (ii) the possible lower and upper threshold for when dietary intake (i.e., fat intake) could modify fat oxidation [58]; (iii) the inter-individual variability, body composition and metabolic status influence on fat oxidation [8]; (iv) a sleep patterns insufficiently maintained over time.

Regarding BMR and MFO, we observed an inverse association between TST and BMR which disappeared after controlling for covariates. The energy expenditure is minimum during sleep, therefore a high TST is related with a prolonged period of the lowest energy expenditure [59]. Sleep deprivation could increase energy expenditure since energy expenditure is reduced during sleep [59]. Sharma et al. proposed that these reduction in energy expenditure could be influenced by circadian rhythm, body temperature and muscle temperature [59]. However, the results should be interpreted with caution because this association disappeared after controlling for sex, age, and FMI. We also observed an inverse association between TST and MFO. A previous study of Konishi et al. observed that a night of sleep deprivation did not

affect MFO in healthy young men [15]. It has been reported several detrimental effects of long sleep for optimal health [60]. In addition, long sleep could increase fatigue, physiological deprivation, which could influence insulin resistance and hormonal imbalance [61]. Although the mechanisms are not clear, the above-mentioned mechanisms could have influenced this relationship. Nevertheless, these findings should be cautiously interpreted since the association did not remain after adjusting by sex, age, and FMI. The lack of association of other sleep outcomes with BMR and MFO could be explained by different factors. Sleep is a complex phenomenon influenced by behavioral and physiological mechanisms (i.e., homeostatic, circadian, and metabolic control) under the participant's natural sleep environment that we have not investigated [62]. These factors could influence the relationship of sleep parameters with BMR and MFO.

Surprisingly, different results were observed when the association of sleep quantity and quality with energy metabolism was performed considering subjective instead of objective measures of sleep. Differences between subjective and objective sleep assessments have been previously reported. Indeed, previous studies have shown weak or inconsistent correlations of subjective measures (i.e., PSQI scores) with objective measures (e.g., actigraphy and polysomnography) [63,64]. It has been previously reported that PSQI and accelerometer records measure different attributes of sleep, highlighting the bias of accelerometry to register wakefulness, thus lying in bed awake but motionless is likely to be coded as sleep [65]. Therefore, it is recommended to use both methods to obtain complementary information additionally to the body movements [66]. PSQI may reflect the overall psychological state of the person, rather than actual quantity or quality of sleep [63]. In addition, it is still not well-defined what a "good night's sleep" actually involves in the perception of the sleeper and stand out that many factors play a role when judging sleep quality [67]. However, PSQI validity is further supported by similar differences between groups using PSQI or polysomnographic sleep measures, and has been used in a wide range of population-based and clinical studies [64]. These differences in measurement of sleep attributes could explain the different results of the associations between sleep quality and energy metabolism.

Despite accelerometer records and subjective measurements are a valid and extensively used measure of sleep quality [26,68] they cannot differentiate between rapid eye movement sleep (REM) and non-rapid eye movement sleep (NREM), restricting the detailed assessment of the real biologic process of sleep. REM and NREM phases are metabolically different [69]. In REM sleep glucose uptake is increased, leading to

anaerobic glucose metabolism [70,71], therefore sleep quality in each phase could be differently associated with energy metabolism. Future studies that examine the relationship of REM and NREM sleep using polysomnography records with BMR and fuel oxidation in basal conditions and during exercise are needed.

The present study should be interpreted with caution; the study has a cross-sectional design that does not allow to establish causal relationship. Therefore, experimental studies should manipulate BMR and fuel oxidation and/or sleep (e.g., sleep deprivation) under well-controlled lab conditions in order to establish causal relationship. Furthermore, sleep and dietary parameters were assessed only in a specific timepoint, which do not allow us to extrapolate our results to chronic sleep or dietary patterns. Our study only included sedentary middle-aged adults and, consequently, we cannot extrapolate our results to older, younger, and/or physically active individuals. The difficulty of an accurate dietary evaluation with possible underreporting or misclassification should be considered, as in all cross-sectional studies.

CONCLUSIONS

In conclusion, our study showed that a poor subjective sleep quality was associated with lower BFox in sedentary middle-aged adults. Moreover, our findings indicated that the association of global PSQI score with BFox was not mediated by dietary intake and MedDiet adherence. Thus, improving sleep quality could be considered a potential prevention and/or treatment pathway to reduce metabolism alterations related to lower basal fat oxidation, independently of dietary patterns.

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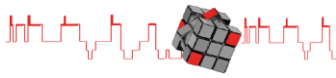

Chapter 3

Sleep and cardiometabolic risk in sedentary middle-aged adults: The FIT-AGEING study

(Study 3)

ABSTRACT

Sleep and cardiometabolic risk share some common mechanisms and physiological pathways. However, the relationship of sleep quantity and quality (measured by both subjective and objective methods) with cardiometabolic risk factors in still healthy sedentary middle-aged adults has been poorly investigated. The purpose of this study was to examine the relationship of subjective and objective sleep quantity and quality with cardiometabolic risk factors in sedentary middle-aged adults.

A total of 74 volunteers (52.7% women; aged 53.7 ± 5.1; 26.7 ± 3.8 kg/m^2) were recruited. Subjective sleep quantity and quality were assessed by the Pittsburgh Sleep Quality Index (PSQI; higher scores indicate worse sleep quality), while objective sleep quantity and quality parameters (total sleep time [TST], wake after sleep onset [WASO], and sleep efficiency [SE]) were determined using a wrist-worn accelerometer. Waist circumference, blood pressure, glucose, insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides plasma levels were selected as cardiometabolic risk factors and assessed following standard procedures. Insulin resistance was measured by the homeostasis model assessment index (HOMA). Worse perceived sleep quality was associated with higher total cholesterol, LDL-C, and triglycerides (all $p \leq 0.016$), and lower HDL-C ($p = 0.015$). Significant negative associations were found between TST and both waist circumference ($p = 0.033$) and glucose plasma levels ($p = 0.020$). The main findings of the present study showed that a poor subjective sleep quality was related to worse plasma lipid profile in sedentary middle-aged adults. Interestingly, a poor objective sleep quantity was associated with greater waist circumference and higher levels of plasma glucose in sedentary middle-aged adults. These results confirm that a healthy sleep pattern could play an important role in the prevention of an unhealthy body fat distribution, as well as blood glucose and lipid profiles alterations.

Keywords: waist circumference; blood pressure, HOMA, HDL-C, triglycerides, actigraphy.

INTRODUCTION

Current sociocultural, technological and lifestyle trends in modern societies, such as irregular work schedules or electronic media exposure, have led to altered sleep patterns which, in turn, are related to an increased morbidity and mortality risk [1]. As reported by epidemiological studies, the prevalence of sleep disorders in the global population has seriously increased in the last decade [2–4], insomnia and obstructive sleep apnoea being at the top of the most common sleep disorders with a prevalence of 10–40% and 9–38% in the overall population, respectively [5,6]. Given that sleep is essential for restoration and preservation of multiple physiological systems, including energy metabolism [7], developing healthier habits results crucial in order to avoid significant public health burdens.

Lifestyle behaviours in modern societies and the continuous increment of life expectancy in the last decades have triggered a subsequent rise of cardiometabolic diseases' incidence, which is currently considered the first cause of mortality [8]. Concretely, highly prevalent cardiometabolic disorders are diabetes mellitus (6–22%), impaired fasting glucose (7–26%), impaired glucose tolerance (17%), obesity (19–32%), metabolic syndrome (34–39%), hypercholesterolemia (17%), low high-density lipoprotein cholesterol (HDL-C) (37%), and hypertriglyceridemia (30%), among others [9]. Moreover, it is estimated that the sanitary cost derived from these cardiometabolic diseases in the United States was ~$555 billion in 2015, and it is expected to be intensified to $1.1 trillion by 2035 [10]. In this context, several public health strategies are needed to promote the prevention, detection, and/or treatment of cardiometabolic risk factors, including lifestyle habits such as physical activity, diet, and sleep—the forgotten "elixir" of health and physical recovery—, all of them well-recognised modifiable risk factors of cardiometabolic disease [10,11].

Previous studies have postulated that sleep and cardiometabolic health share some common mechanisms and physiological pathways [12]. An altered sleep quantity and quality affect dietary pattern and physical activity levels causing several physiological disturbances such as greater oxidative stress, systemic inflammation, endothelial dysfunction, altered hormonal secretion and sympathetic systemic activation leading to increase cardiometabolic risk via hypertension, dyslipidaemia or obesity [11]. Several studies have shown that sleep quantity and quality are closely related to various cardiometabolic risk factors [13–16]. However, they have commonly measured sleep-related outcomes using subjective measures (i.e., self-reported questionnaires) and applying different type of analysis in heterogeneous populations obtaining controversial findings [13–16]. To the best of our knowledge, the relationship of

sleep quantity and quality (measured by both subjective and objective methods) with cardiometabolic risk factors in still healthy sedentary middle-aged adults has been poorly investigated. Understanding the link between sleep and cardiometabolic risk factors in sedentary middle-aged adults is of clinical interest since the early identification and management of sleep alterations could be a potential strategy to prevent sleep disorders linked to cardiometabolic risk.

Hence, the objective of the present study was to analyse the relationship of both subjective and objective sleep quantity and quality (i.e., global Pittsburgh Sleep Quality Index [PSQI] score, total sleep time [TST], wake after sleep onset [WASO], and sleep efficiency [SE]) with cardiometabolic risk factors (i.e., waist circumference, blood pressure, glucose, insulin, insulin resistance, total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], and triglycerides) in sedentary middle-aged adults. Considering the common mechanisms and physiological pathways shared by both sleep and cardiometabolic processes, we hypothesized that individuals with worse sleep quantity and quality levels would present an increased cardiometabolic risk.

MATERIALS AND METHODS

Study protocol and participants

The FIT-AGEING study [17] was an exercise-based randomised controlled trial (clinicaltrial.gov: ID: NCT03334357), approved by the Human Research Ethics Committee of the Regional Government of Andalucía [0838-N-2017]. The study complied with the ethical principles described in the Declaration of Helsinki. An extended explanation of the study methodology can be found elsewhere [17]. The study population consisted of 74 sedentary middle-aged volunteers (52.7% women, 53.7 ± 5.1 years old, 26.7 ± 3.8 kg/m^2). Eligible participants were adults (i) aged 40–65 years, (ii) with a body mass index (BMI) between 18.5 and 35 Kg/m^2, (iii) having a stable weight (less weight changes than 3 kg over the last 3 months), (iv) non-physically active (i.e., self-reported < 20 minutes of vigorous-intensity physical activity on < 3 days a week), and (v) being a non-smoker. The exclusion criteria were: (i) diagnosis of any physical or psychological disease, (ii) taking some medication, as well as (iii) being pregnant. Upon meeting the inclusion criteria, all participants received a full explanation of the study, signed a written informed consent form, and underwent a complete medical and physical examination before enrolment. All assessments were performed at the Sport and Health University Research Institute (iMUDS) (Granada, southern Spain) during the months of September and October in 2016 and 2017.

Measurements

Anthropometry and body composition

Anthropometric variables were measured by a certified anthropometrist following the International Society for the Advancement of Kinanthropometry (ISAK) guidelines [18]. Waist circumference was assessed at the midpoint between the bottom of the rib cage and the iliac crest at the end of normal expiration (mean of three measurements). Body weight and height were measured using a pre-validated electronic scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany), and BMI was calculated as $Body\ weight\ (kg)\ /\ Height^2\ (m^2)$ [19].

Body composition was determined by a dual-energy X-ray absorptiometry scanner (Discovery Wi, Hologic, Inc., Bedford, MA, USA), and fat mass index (FMI) calculated as: $Fat\ mass\ (kg)\ /\ Height^2\ (m^2)$.

Sleep quantity and quality

Subjective sleep quantity and quality were assessed using the PSQI scale [20], a self-report tool which consists of a 19-item scale that provides 7 component scores (ranges 0–3): (i) subjective sleep quality, (ii) sleep latency, (iii) sleep duration, (iv) habitual sleep efficiency, (v) sleep disturbances, (vi) use of sleeping medication, and (vii) daytime dysfunction. Global PSQI score is obtained by the sum of the 7 components (ranged from 0 to 21). Lower global score denotes a healthier sleep quality, whereas a global PSQI score higher than 5 indicates poor sleep quality.

Objective sleep quantity and quality were determined by a wrist-worn accelerometer (ActiGraph GT3X+, Pensacola, FL, US) during 7 consecutive days (24 hours/day) [17]. Participants received detailed instructions to wear the accelerometer on the non-dominant wrist, and to remove it only during water activities such as swimming or bathing. They were provided with a 7-day sleep diary to record bed-time, wake up time, and the time they removed the device each day. The accelerometer was initialised to store raw accelerations at a sampling frequency of 100 Hz [21]. ActiLife software (version 6.13.3, ActiGraph, Pensacola, FL, US) was used to export and convert to the ".csv" format the raw accelerations. The raw ".csv" files were then processed with GGIR package (version 1.5-12, https://cran.r-project.org/web/packages/GGIR/) in R (version 3.1.2, https://www.cran.r-project.org/). Signal processing included autocalibration according to the local gravity [22], detection of sustained abnormal high accelerations, detection of the non-wear time, calculation of the Euclidean Norm Minus One (ENMO), identification of waking and sleeping hours with an automatized algorithm [23], and imputation of detected non-wear time and abnormal high values. TST (total amount of time spent in bed minus sleep onset latency), WASO (the sum of wake times from sleep onset to the final awakening), and SE (percentage of sleep time over the bedtime) were obtained by accelerometry [24]. Only the participants who

registered ≥ 16 hours/day of wear time during at least 4 of 7 possible days (including 1 weekend day) were included in the final analysis.

Blood pressure

Blood pressure was measured after ~30 minutes lying on a reclined bed with a validated digital automatic blood pressure monitor (Omron HEM 705 CP, Health-care Co, Kyoto, Japan) according to the updated European Heart Society guidelines [25]. A total of 3 trials separated by 1 minute were performed in the right arm, and the average of systolic and diastolic blood pressure was considered in all cases. Mean blood pressure was defined as diastolic blood pressure plus 1/3 of the difference between systolic and diastolic blood pressure [25].

Blood sampling

Blood for analysis was obtained from the antecubital vein applying standard techniques after an overnight fast and a minimum of 10 minutes rest in a supine position. Blood samples were collected in the morning (8:30 AM – 10:00 AM) in prechilled ethylene diamine tetra-acetic acid-containing tubes (Vacutainer SST, Becton Dickinson, Plymouth, UK) and immediately centrifuged at 4000 rpm at 4 ^{o}C for 7 minutes, processed in a controlled-temperature room (22 ± 0.5 °C), and stored in a −80 °C freezer until analysis. Plasma glucose and insulin were respectively assessed using a model AU5800 spectrophotometer (Beckman Coulter, Brea, CA, USA) and by chemiluminescence immunoassay involving UniCel DxI 800 paramagnetic particles (Beckman Coulter, Brea, CA, USA), and the results were expressed in mg/dL, and uIU/mL, respectively. Total cholesterol, HDL-C, and triglycerides were assessed using the same spectrophotometric apparatus, and LDL-C was calculated as $(Total\ cholesterol) - (HDL - C) - 0.45 * (Triglycerides)$. These results were expressed in mg/dL. Subjects were instructed not to consume any drugs and/or caffeine, to eat a pre-established dinner (i.e., boiled rice, tomato sauce, and plain egg omelet) before sampling, and to avoid any physical activity of moderate (24 hours before) and/or vigorous intensity (48 hours before).

Insulin resistance

The homeostasis model assessment index (HOMA) was calculated as [26]:

$$\frac{Plasma\ insulin\ \left(\frac{UI}{mL}\right) * \ Plasma\ glucose\ \left(\frac{nmol}{L}\right)}{22.5}$$

Dietary intake

Dietary intake assessment was conducted through 3 non-consecutive 24-hour recalls (one weekend day included) performed by an experienced and qualified nutritionist [17]. Energy consumption and macronutrient intake (fat, carbohydrate and protein intakes) [27] were determined using EvalFINUT software (Granada, Spain), which includes the USDA (U.S. Department of Agriculture) and BEDCA ("Base de Datos

Española de Composición de Alimentos") databases.

Statistical analysis

Descriptive parameters are expressed as mean and standard deviation. Data were checked for normality with the use of distribution plots (i.e., visual check of histograms, Q-Q plots, and box plots) and the Shapiro-Wilk test. Sex differences were determined using independent samples *t*-test.

Simple linear regression models were built to investigate the association of sleep quantity and quality (i.e., TST, WASO, SE, and global PSQI score) with cardiometabolic risk factors. Multiple linear regression models were also conducted in order to test these associations after adjusting by age, by sex, and by FMI. Potential covariates were selected based on theoretical bases and statistical procedures (i.e., stepwise regressions).

All analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 23.0, IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA), and graphical presentations were prepared using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). *P* values less than 0.05 were considered statistically significant.

RESULTS

Table 1 shows the descriptive parameters of our study participants by sex. Significant differences in height, weight, waist circumference, BMI, systolic, diastolic, and mean blood pressure, HDL-C, TST, energy, fat, and ethanol were observed between men and women (all $p \leq 0.024$). A poor subjective sleep quality (global PSQI score > 5) was identified in 40.3% of our cohort. Figure 1 reports mean and standard deviation of cardiometabolic risk factors after categorizing participants in our study as either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. Between these two groups, no statistically significant differences were observed in objective sleep quantity and quality parameters (i.e., TST, WASO, and SE, all $p \geq 0.05$).

Figure 2 shows the association of sleep quantity and quality with cardiometabolic risk factors (i.e., waist circumference, systolic blood pressure, HDL-C, and triglycerides). A significant negative association of global PSQI score with HDL-C plasma levels, while a significant positive association of global PSQI score with triglycerides plasma levels was observed ($\beta = -0.299$, $R^2 = 0.089$, $p = 0.015$, Figure 2I; $\beta = 0.297$, $R^2 = 0.088$, $p = 0.016$, Figure 2M, respectively). Regarding objective sleep quantity and quality, there was a significant negative association between TST and waist circumference ($\beta = -0.254$, $R^2 = 0.064$, $p = 0.033$, Figure 2B). However, neither WASO nor SE were associated with waist circumference (all $p \geq 0.05$, Figures 2C and 2D). No associations of TST, WASO, or SE

Table 1. Descriptive characteristics of participants.

	N	All		N	Men		N	Women	
Age (years)	74	53.66	(5.14)	35	54.39	(5.27)	39	53.01	(5.00)
Antropometry and Body composition									
Height (cm)	74	167.8	(9.81)	35	175.8	(6.48)	39	160.7	(6.10) *
Weight (kg)	74	75.73	(14.98)	35	87.38	(10.95)	39	65.28	(9.32) *
Waist circumference (cm)	74	95.06	(11.70)	35	102.7	(8.76)	39	88.24	(9.67) *
Body mass index (kg/m^2)	74	26.72	(3.76)	35	28.32	(3.61)	39	25.27	(3.31) *
Fat mass index (kg/m^2)	74	10.75	(3.13)	35	10.03	(3.23)	39	11.39	(2.93)
Blood pressure									
Systolic blood pressure (mm Hg)	70	127.0	(15.49)	32	134.2	(13.62)	38	121.0	(14.49) *
Diastolic blood pressure (mm Hg)	70	81.20	(11.56)	32	85.38	(10.76)	38	77.68	(11.15) *
Mean blood pressure (mm Hg)	70	104.1	(12.93)	32	109.8	(11.52)	38	99.33	(12.21) *
Glycaemic metabolism									
Glucose (mg/dL)	72	92.93	(10.01)	33	93.52	(11.36)	39	92.44	(8.82)
Insulin (uIU/mL)	72	7.80	(4.73)	33	8.02	(4.99)	39	7.62	(4.56)
HOMA	72	1.82	(1.19)	33	1.88	(1.26)	39	1.76	(1.14)
Lipid metabolism									
Total cholesterol (mg/dL)	73	207.5	(33.48)	34	203.8	(36.74)	39	210.6	(30.49)
HDL-C (mg/dL)	73	59.12	(12.81)	34	55.53	(12.71)	39	62.26	(12.21) *
LDL-C (mg/dL)	73	126.6	(29.30)	34	127.8	(31.87)	39	125.5	(27.23)
Triglycerides (mg/dL)	73	136.0	(67.84)	34	147.4	(83.76)	39	126.1	(49.07)
Dietary intake									
Energy (kcal/day)	73	2133.9	(688.4)	35	2373.8	(838.5)	38	1912.9	(414.3) *
Fat (g/day)	73	87.00	(25.10)	35	96.72	(25.12)	38	78.06	(21.79) *
Carbohydrate (g/day)	73	225.7	(112.2)	35	252.1	(145.1)	38	201.4	(62.09)
Protein (g/day)	73	89.77	(38.55)	35	93.40	(38.79)	38	86.42	(38.53)
Ethanol (g/day)	73	11.24	(13.10)	35	16.24	(16.03)	38	6.63	(7.23) *
Sleep quantity and quality									
Global PSQI score	67	5.61	(3.47)	31	4.77	(3.15)	36	6.33	(3.62)
Total sleep time (min)	71	359.9	(48.85)	34	337.9	(46.30)	37	380.1	(42.44) *
Wake after sleep onset (min)	71	63.90	(27.44)	34	65.80	(32.45)	37	62.15	(22.19)
Sleep efficiency (%)	71	85.01	(6.29)	34	83.88	(7.53)	37	86.06	(4.75)

Data are presented as mean (standard deviation). *Significant differences between sexes obtained from an independent samples *t*-test ($p < 0.05$). HOMA: homeostasis model assessment index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; PSQI: Pittsburgh Sleep Quality Index.

with other cardiometabolic risk factors were found (all $p \geq 0.05$, Figures 2F to 2H, 2J to 2L, and 2N to 2P).

Figure 3 shows the association of sleep quantity and quality with cardiometabolic risk factors (i.e., glucose and insulin plasma levels and insulin resistance [HOMA]). No associations of global PSQI score with glucose, insulin plasma levels, nor HOMA (all $p \geq 0.05$, Figures 3A, 3E, and 3I) were observed. Regarding objective sleep quantity and quality, a significant negative association was found between TST and glucose plasma levels ($\beta = -0.280$, $R^2 = 0.079$, $p = 0.020$, Figure 3B). However, neither WASO nor SE were associated with glucose plasma levels (all $p \geq 0.05$, Figures 3C and 3D). No associations of TST, WASO, or SE with insulin or HOMA were found (all $p \geq 0.05$, Figures 3F to 3H, and 3J to 3L).

Table 2 shows the association of sleep quantity and quality with further cardiometabolic risk factors (i.e., waist circumference; systolic, diastolic, and mean

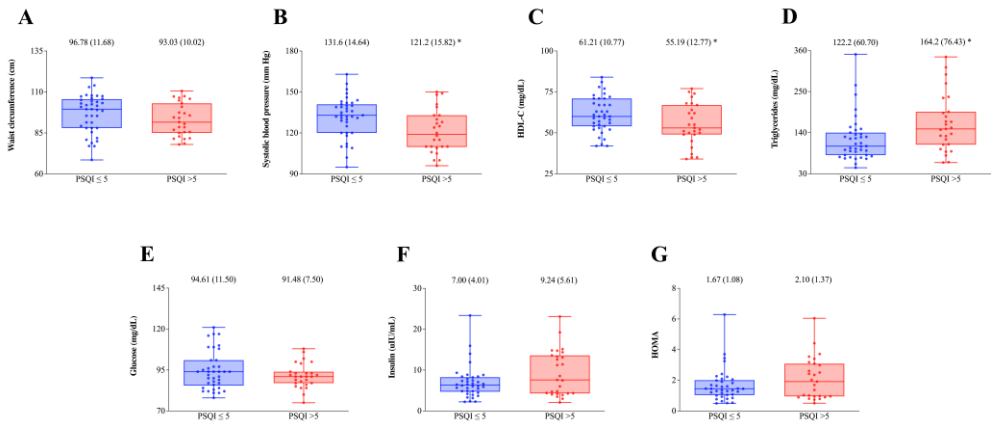

Figure 1. Mean (standard deviation) of cardiometabolic risk factors after categorizing participants as either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. *Significant differences between global PSQI score groups obtained from an independent samples *t*-test ($p < 0.05$). PSQI: Pittsburgh Sleep Quality Index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA: homeostasis model assessment index.

blood pressure; glucose and insulin plasma levels, and HOMA; and total cholesterol, HDL-C, LDL-C, and triglycerides plasma levels) after adjusting by age, by sex, and by FMI. Global PSQI score was negatively associated with waist circumference after adjusting by FMI, while positively associated with insulin plasma levels after adjusting by sex ($\beta = -0.233$, $R^2 = 0.191$, $p = 0.044$; $\beta = 0.262$, $R^2 = 0.068$, $p = 0.041$, respectively). Significant positive associations were found between global PSQI score and both total cholesterol and LDL-C ($\beta = 0.433$, $R^2 = 0.188$, $p < 0.001$; $\beta = 0.307$, $R^2 = 0.094$, $p = 0.012$, respectively).

DISCUSSION

Our study aimed to investigate the relationship of sleep quantity and quality with cardiometabolic risk factors in still healthy sedentary middle-aged adults. The main findings of the present study suggested that a poor subjective sleep quality was related to higher levels of total cholesterol, LDL-C, and triglycerides, as well as with lower levels of HDL-C in sedentary middle-aged adults. Interestingly, a poor objective sleep quantity was associated with greater waist circumference and higher levels of plasma glucose, but not to other markers of cardiometabolic risk in sedentary middle-aged adults. Therefore, these results suggest that maintaining adequate levels of sleep quantity and quality may be important to prevent abdominal fat accumulation and both glucose and lipid profiles alterations.

Sleep is currently considered one of the most representative responsible of several regulatory and maintenance functions in human physiology [28]. Variability across days in duration of sleep and sleep schedules may contribute to a mismatch

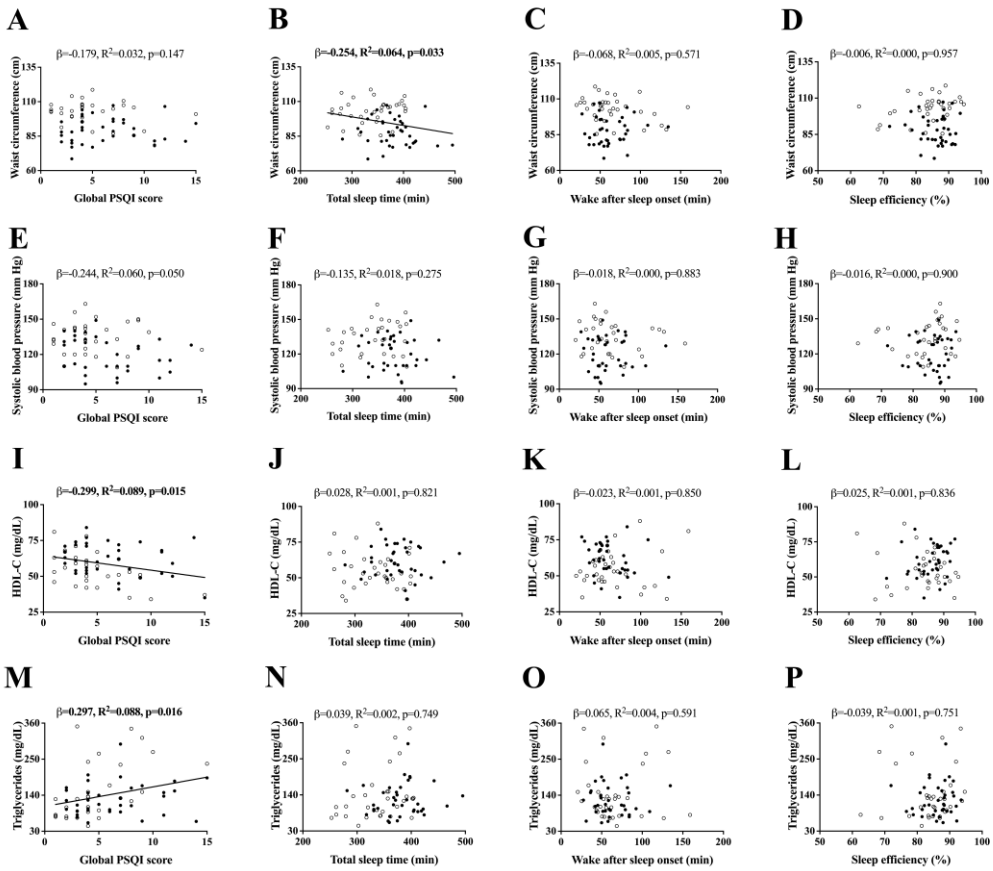

Figure 2. Association of sleep quantity and quality with cardiometabolic risk factors in sedentary middle-aged adults. *β* (standardized regression coefficient), R^2, and *p*-value from a simple linear regression analysis. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; HDL-C: high-density lipoprotein cholesterol.

between behavioural cycles and innate circadian rhythms causing a dysregulation of metabolic and endocrine functions [14]. In our study, we found that a higher global PSQI score was strongly related to higher plasma levels of total cholesterol, LDL-C, and triglycerides, but reduced levels of HDL-C. Considering objectively measured sleep parameters, our study did not find any link of sleep with total cholesterol, HDL-C, LDL-C, nor triglycerides, while lower TST was associated with greater waist circumference (an important determinant of cardiometabolic risk), thus finding different but complementary results between subjective and objective sleep assessments.

A previous meta-analysis [29] demonstrates a significant inverse association between sleep duration (mainly assessed by subjective methods) and waist circumference. However, another meta-analysis [30] regarding the relation of sleep quantity and quality (mostly self-reported) with blood lipids in the general population was unable to find supportive evidence of a relationship

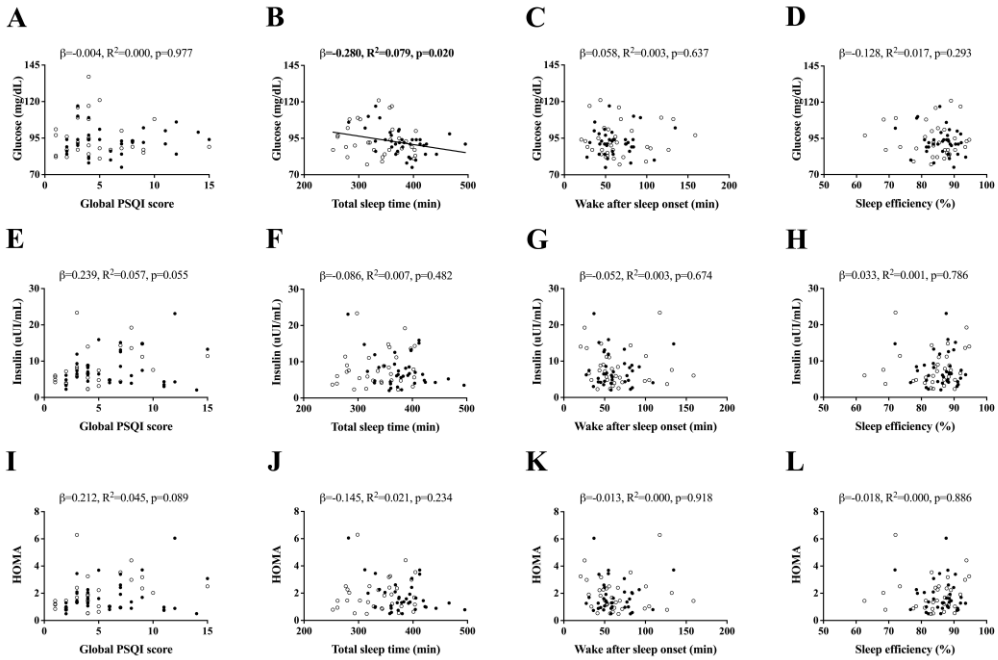

Figure 3. Association of sleep quantity and quality with insulin resistance in sedentary middle-aged adults. *β* (standardized regression coefficient), R^2, and *p*-value from a simple linear regression analysis. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; HOMA: homeostasis model assessment index.

between sleep duration and the development of dyslipidaemia. A recently published cross-sectional study by Matricciani et al. [11] analysed the association of actigraphy-derived sleep profiles with a metabolic syndrome severity score. Compared to overall good sleepers, adults with a late to bed sleep profile showed a higher score, while no associations were noted in short sleepers. These results concur with those obtained in the current study since it included a high number of short sleepers. Interestingly, a study conducted by Rey-López et al. [31] shows a direct relationship between sleep time during the weekend and the presence of metabolic risk in European adolescents. Short sleepers are thought to increase allostatic load, appetite for unhealthy food and cause fatigue limiting physical activity [11], behavioural activities that are commonly accentuated on the weekends [32].

Sleep is also an important modulator of neuroendocrine function and glucose metabolism [33,34]. Indeed, sleep restriction has been postulated as an important risk factor for numerous metabolic and endocrine alterations (e.g., decreased glucose tolerance, decreased insulin sensitivity, elevated sympathovagal balance, increased evening concentrations of cortisol, increased levels of ghrelin, decreased levels of leptin, and increased hunger and appetite, among others) [34,35]. Hence, sleep curtailment should be considered as an important but modifiable

Table 2. Association of sleep quantity and quality with cardiometabolic risk factors (Model 0) adjusted by age (Model 1), by sex (Model 2), and by fat mass index (Model 3).

	Global PSQI score			Total sleep time			Wake after sleep onset			Sleep efficiency		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Waist circumference (cm)												
Model 0	−0.179	0.032	0.147	−0.254	0.064	**0.033**	−0.068	0.005	0.571	−0.006	0.000	0.957
Model 1	−0.160	0.039	0.206	−0.260	0.074	**0.029**	−0.066	0.011	0.589	−0.009	0.007	0.938
Model 2	−0.042	0.385	0.681	0.030	0.412	0.770	−0.112	0.424	0.230	0.109	0.423	0.250
Model 3	−0.233	0.191	**0.044**	−0.291	0.261	**0.007**	−0.012	0.177	0.914	−0.072	0.182	0.516
Systolic blood pressure (mm Hg)												
Model 0	−0.244	0.060	0.050	−0.135	0.018	0.275	−0.018	0.000	0.883	−0.016	0.000	0.900
Model 1	−0.250	0.060	0.052	−0.139	0.020	0.269	−0.017	0.001	0.895	−0.018	0.001	0.889
Model 2	−0.164	0.220	0.155	0.052	0.195	0.675	−0.046	0.195	0.681	0.057	0.196	0.615
Model 3	−0.239	0.063	0.057	−0.134	0.018	0.283	−0.022	0.001	0.860	−0.012	0.001	0.924
Diastolic blood pressure (mm Hg)												
Model 0	−0.137	0.019	0.275	−0.146	0.021	0.237	−0.052	0.003	0.676	0.005	0.000	0.965
Model 1	−0.150	0.022	0.248	−0.147	0.022	0.240	−0.052	0.003	0.677	0.006	0.000	0.964
Model 2	−0.069	0.138	0.569	−0.016	0.107	0.902	−0.073	0.113	0.538	0.060	0.111	0.614
Model 3	−0.134	0.020	0.291	−0.147	0.022	0.240	−0.054	0.003	0.670	0.007	0.000	0.959
Mean blood pressure (mm Hg)												
Model 0	−0.208	0.043	0.097	−0.146	0.021	0.237	−0.034	0.001	0.783	−0.007	0.000	0.956
Model 1	−0.216	0.045	0.093	−0.149	0.022	0.235	−0.033	0.001	0.791	−0.008	0.000	0.949
Model 2	−0.129	0.198	0.269	0.024	0.168	0.849	−0.060	0.171	0.598	0.061	0.171	0.597
Model 3	−0.203	0.045	0.108	−0.146	0.021	0.243	−0.038	0.002	0.768	−0.004	0.000	0.972
Glucose (mg/dL)												
Model 0	−0.004	0.000	0.977	−0.280	0.079	**0.020**	0.058	0.003	0.637	−0.128	0.017	0.293
Model 1	0.020	0.012	0.877	−0.285	0.082	**0.019**	0.059	0.005	0.635	−0.129	0.018	0.293
Model 2	0.017	0.009	0.895	−0.318	0.084	**0.019**	0.054	0.006	0.664	−0.122	0.018	0.331
Model 3	−0.004	0.000	0.977	−0.285	0.084	**0.018**	0.068	0.007	0.588	−0.141	0.022	0.257
Insulin (UI/mL)												
Model 0	0.239	0.057	0.055	−0.086	0.007	0.482	−0.052	0.003	0.674	0.033	0.001	0.786
Model 1	0.176	0.137	0.149	−0.056	0.147	0.627	−0.059	0.148	0.604	0.043	0.146	0.710
Model 2	0.262	0.068	**0.041**	−0.078	0.008	0.573	−0.056	0.006	0.651	0.045	0.005	0.720
Model 3	0.223	0.070	0.077	−0.098	0.037	0.421	−0.027	0.028	0.829	0.006	0.027	0.962
HOMA												
Model 0	0.212	0.045	0.089	−0.145	0.021	0.234	−0.013	0.000	0.918	−0.018	0.000	0.886
Model 1	0.158	0.105	0.203	−0.119	0.129	0.307	−0.019	0.115	0.867	−0.009	0.115	0.936
Model 2	0.236	0.057	0.066	−0.147	0.021	0.284	−0.017	0.004	0.888	−0.006	0.004	0.961
Model 3	0.200	0.053	0.114	−0.156	0.043	0.201	0.009	0.019	0.941	−0.042	0.021	0.734
Total cholesterol (mg/dL)												
Model 0	0.433	0.188	**<0.001**	0.112	0.013	0.357	−0.051	0.003	0.672	0.055	0.003	0.651
Model 1	0.358	0.315	**0.001**	0.141	0.178	0.208	−0.061	0.161	0.589	0.065	0.162	0.566
Model 2	0.437	0.188	**<0.001**	0.042	0.033	0.754	−0.038	0.033	0.756	0.022	0.033	0.856
Model 3	0.426	0.191	**<0.001**	0.098	0.042	0.419	−0.025	0.033	0.835	0.026	0.033	0.832
HDL-C (mg/dL)												
Model 0	−0.299	0.089	**0.015**	0.028	0.001	0.821	−0.023	0.001	0.850	0.025	0.001	0.836
Model 1	−0.211	0.257	0.061	−0.009	0.261	0.930	−0.011	0.261	0.915	0.013	0.261	0.902
Model 2	−0.378	0.214	**0.002**	−0.097	0.068	0.459	−0.004	0.060	0.976	−0.021	0.061	0.859
Model 3	−0.294	0.090	**0.018**	0.029	0.001	0.811	−0.027	0.001	0.829	0.029	0.001	0.813
LDL-C (mg/dL)												
Model 0	0.307	0.094	**0.012**	−0.012	0.000	0.924	−0.084	0.007	0.489	0.056	0.003	0.648
Model 1	0.209	0.308	0.056	0.024	0.239	0.825	−0.095	0.248	0.371	0.067	0.243	0.529
Model 2	0.326	0.102	**0.010**	−0.029	0.001	0.831	−0.082	0.007	0.502	0.052	0.003	0.675
Model 3	0.307	0.094	**0.014**	−0.020	0.010	0.872	−0.071	0.014	0.563	0.041	0.011	0.743
Triglycerides (mg/dL)												
Model 0	0.297	0.088	**0.016**	0.039	0.002	0.749	0.065	0.004	0.591	−0.039	0.001	0.751
Model 1	0.217	0.229	0.060	0.067	0.159	0.550	0.056	0.157	0.618	−0.029	0.155	0.796
Model 2	0.353	0.151	**0.004**	0.126	0.034	0.350	0.054	0.024	0.656	−0.012	0.021	0.924
Model 3	0.293	0.089	**0.018**	0.029	0.017	0.814	0.086	0.023	0.484	−0.061	0.020	0.619

β (standardized regression coefficient), R^2, and *p*-value of simple and multiple-regression analysis. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; HOMA: homeostasis model assessment index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

risk factor for diabetes and obesity [36,37]. In the current study, no associations were observed of global PSQI score with glucose, insulin plasma levels, nor HOMA. However, regarding objectively measured sleep, lower TST was associated with higher levels of plasma glucose. Nevertheless, we observed non-significant associations of plasma insulin and HOMA (a surrogate marker of insulin resistance) with objective sleep quantity and quality in our study sample (i.e., healthy middle-aged adults). Our results partially agree with those provided by a review of 83 studies conducted by Arora et al. [16], which concluded the existence of a reasonably consistent role of sleep in the management of diabetes. However, it should be take into account that the previously mentioned manuscripts employed different methods for data collection and analyses (i.e., only 5 of the cross-sectional studies used objective methods [38–42]) and included participants with different biological conditions (i.e., healthy adults versus patients with type 2 diabetes or other pathologies, and young adults versus old adults).

Interestingly, Knutson et al. [38] concluded that objectively measured sleep duration using wrist actigraphy was not associated with markers of glucose metabolism assessed after an overnight fast in middle-aged adults with and without type 2 diabetes. Similarly, Harada et al. [39] did not find a significant relationship between sleep duration measured by actigraphy and fasting plasma glucose levels in individuals without diabetes. However, they found a significant inverse relationship between these outcomes when the entire sample of subjects (including both participants with and without type 2 diabetes) was considered [39]. Darukhanavala et al. [40] revealed that, compared with healthy young adults with parental history of type 2 diabetes and habitual sleep ≥ 6 hours/day, those who showed low sleep time registered high levels of insulin resistant. Furthermore, St-Onge et al. [41] found a significant inverse association between sleep efficiency (measured by in-home polysomnography) and fasting plasma glucose in obese participants with type 2 diabetes. Lastly, Vgontzas et al. [42] concluded that objective short sleep duration in the absence of a sleep complaint was associated with a nonsignificant increased odds for diabetes. More future research is needed to elucidate the potential link between sleep and glucose metabolism due to the above-mentioned inconclusive results of the mediating effect of sleep quantity and quality on carbohydrate metabolism control.

Differences between subjective and objective sleep assessments have been previously reported. Therefore, previous studies have shown weak or inconsistent correlations of subjective measures (i.e., PSQI scores) with objective measures (e.g., actigraphy and polysomnography) [30,43,44]. In this sense, a previous work conducted in

youth observed that the PSQI and the accelerometer may measure different attributes of sleep, reporting the inadequate capacity of accelerometry to detect wakefulness since lying in bed awake but motionless is likely to be coded as sleep [45]. Due to this limitation, using both complementary assessment methods to obtain detailed information beyond the limited data derived from body movements, is recommended [46]. PSQI may reflect the overall psychological state of the person, rather than actual quantity or quality or of sleep [43]. In addition, it is still not well-defined what a "good night's sleep" actually involves in the perception of the sleeper and stand out that many factors play a role when judging sleep quality [47]. However, PSQI validity is further supported by similar differences between groups using PSQI or polysomnographic sleep measures, and has been used in a wide range of population-based and clinical studies [44].

Several limitations should be acknowledged and addressed in further studies. The main limitation was the cross-sectional study design which does not allow determination of cause–effect relationships, so longitudinal studies are needed to indicate the direction of the association. The use of accelerometry, even considering that it is a validated objective tool to measure sleep quantity and quality, may have under/over-estimated sleep parameters and, therefore, the use of gold-standard methods to assess sleep quantity and quality (i.e., polysomnography) would be desirable. Finally, our sample only included healthy sedentary middle-aged adults, so these findings cannot be generalized to other populations.

CONCLUSIONS

The main findings of the present study showed that a poor subjective sleep quality was related to higher levels of total cholesterol, LDL-C, and triglycerides, as well as with lower levels of HDL-C in sedentary middle-aged adults. Interestingly, a poor objective sleep quantity was associated with greater waist circumference and higher levels of plasma glucose, but not to other markers of cardiometabolic risk in sedentary middle-aged adults. These results confirm that a healthy sleep pattern could play an important role in the prevention of abdominal fat accumulation and both glucose and lipid profiles alterations.

The strong decrease in sleep quantity and quality together with the increased prevalence of cardiometabolic diseases (mainly due to current lifestyle trends) in modern societies during the last decades is currently considered an important public health problem [36]. Our findings have, therefore, powerful clinical and research implications, underlining the important role of sleep in the prevention of unhealthy android fat distribution and both glucose and lipid profiles disturbances.

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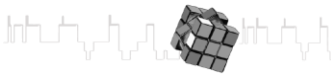

Chapter 4

Is there a relationship of sleep with leukocyte and platelet parameters in sedentary middle-aged adults? The FIT-AGEING study **(Study 4)**

ABSTRACT

Sleep has a general repairing effect in the body. Previous scientific research has shown that sleep alterations could modify the homeostasis of both immune and hemostatic systems. However, controversial findings have been reported. Therefore, this study aimed to investigate the association of both subjective and objective sleep quantity and quality with leukocyte and platelet parameters in sedentary middle-aged adults.

A total of 74 volunteers (52.7% women; 53.7 ± 5.1 years old, 26.7 ± 3.8 kg/m^2) were recruited for the present study. Subjective sleep quantity and quality were assessed by the Pittsburgh Sleep Quality Index (a higher score indicates worse sleep quality). Objective parameters of sleep quantity and quality (total sleep time, wake after sleep onset, and sleep efficiency) were determined using a wrist-worn accelerometer over seven consecutive days. Blood count of total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils, as well as platelet count and mean volume were measured by hematimetry. No significant associations of sleep quantity and quality with leukocyte parameters were found (all $p \geq 0.05$) in sedentary middle-aged adults. Global PSQI score was negatively associated with mean platelet volume (p = 0.024) in sedentary middle-aged adults. The main findings of the present study suggested that a poor subjective sleep quality was related to decreased mean platelet volume. Thus, maintaining an appropriate sleep quality may contribute to an optimal hemostasis.

Keywords: leukocyte count; platelet count; mean platelet volume; actigraphy.

INTRODUCTION

Sleep disturbances are considered one of the greatest threats to world population health and quality of life in the 21st century [1–3]. According to epidemiological studies, the prevalence of sleep disorders in worldwide population has dramatically increased in the last decade [4–6] (insomnia and obstructive sleep apnoea [OSA] being at the top of the most common sleep disorders with a prevalence of 10–40% and 9–38% in the overall population, respectively [7,8]) as a consequence of sociocultural and technological trends, and the lifestyle of modern societies [9–11]. Concretely, sleep alterations in developed countries can be explained by extended light exposure, the presence of shift works [9], the continued use of electronic devices [10], the high level of stress, and jet-lag [11]. This public health problem has a substantial economic impact, reaching insomnia a total of $100 billion USD per year [12]. Therefore, strategies to ensure a proper sleep pattern are of scientific and clinical interest [13].

Sleep is an active physiological process in which recovery and repairing of multiple body tissues occur allowing an optimal functioning during the awake hours [14]. Sleep is regulated by two important mechanisms: homeostasis and circadian rhythm [15,16]. Circadian rhythms are mainly regulated by the suprachiasmatic nucleus (called as the biological clock), which is located in the central nervous system, and synchronized by various factors, as the light-dark cycle. This central clock is continuously in contact with the rest of cells and tissues throughout the body via nervous connections and soluble mediators coordinating and adapting them to the circadian rhythm [9,17]. Such is the importance of circadian rhythms and their connection with all tissues that any alteration in the quantity and/or quality of sleep has been associated with increased incidence of several chronic diseases, such as depression, anxiety and eating disorders [18,19], Alzheimer's and Parkinson's diseases [20,21], type 2 diabetes, obesity and metabolic syndrome [22], breast, lung, colorectal and prostate cancer [23], cerebrovascular disease and acute myocardial infarction [24,25], acute infections, and chronic inflammatory diseases [16,26]. Interestingly, the last-mentioned pathologies could be explained by procoagulant and proinflammatory activity [27], and by an immune function dysregulation [26].

The role of the immune system in the functioning of the human body is essential for its protection against internal and external threats [16,28]. For that, it uses different structures (e.g., mucosal and skin epithelia) and blood cells synthetized in the bone marrow from stem cells (i.e., leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils) [16]. In addition, blood platelets (also generated in the bone marrow) have important functions within both the immune and hemostatic systems, preventing excessive blood loss when blood vessels are damaged [29]. Thus, any alteration of

these cells could impair the hemostatic balance towards either thrombosis or hemorrhage [29].

Previous studies have shown that sleep alterations could modify the homeostasis of immune and hemostatic systems [16,25,30]. However, controversial findings about this research topic have been reported. While some studies have suggested a significant association of sleep quantity and quality with leukocytes [31–35] and platelets [25,36,37] parameters, others have found no relationship with leukocytes [38] or platelets [34,38–42] parameters. Others researchers, however, have shown no relationship between short sleep duration and leukocytes whereas a significant association with long sleep duration has been described [43,44]. These uncertain results could be explained by: (i) diversity in the population recruited in the aforementioned studies, (ii) differences in the sleep measurement method, (iii) variability in the timing and methods of blood sample preparation; and (iv) the circadian nature of the immune and hemostatic systems. Considering these inconsistencies, it is of scientific and clinical interest to analyze the potential relationship between these parameters in a still healthy cohort of middle-aged adults.

The objective of the present study was to analyze the relationship of both subjective and objective sleep quantity and quality (i.e., global Pittsburgh Sleep Quality Index [PSQI] score, total sleep time [TST], wake after sleep onset [WASO], and sleep efficiency [SE]) with leukocyte and platelet parameters in sedentary middle-aged adults. Based on the current evidence, we hypothesized that sleep quantity and quality would be associated with a healthier blood cell count, particularly in terms of leukocytes and platelets, thus reflecting a better immune function and hemostasis.

MATERIALS AND METHODS

Study protocol and participants

The FIT-AGEING study [45] was an exercise-based randomised controlled trial (clinicaltrial.gov: ID: NCT03334357), approved by the Human Research Ethics Committee of the Regional Government of Andalucía [0838-N-2017], which complied with the ethical principles described in the Declaration of Helsinki. An extended explanation of the study methodology can be found elsewhere [45]. A total of 74 healthy sedentary middle-aged volunteers (52.7% women, 53.7 ± 5.1 years old, 26.7 ± 3.8 kg/m^2) were recruited for the present study. All participants received a full explanation of the study aims and procedures, signed an informed consent form, and underwent a complete history and physical examination prior to their participation in the study. Inclusion criteria were: (i) being aged from 40 to 65 years, (ii) having a body mass index (BMI) between 18.5 and 35 kg/m^2, (iii) having a stable weight (less weight changes than 3 kg over the last 3 months), (iv) being a non-smoker, and (v) being non-physically

active (less than 20 minutes of vigorous-intensity physical activity on less than 3 days a week). Exclusion criteria were: (i) having some acute or chronic illness, (ii) taking some medication, and (iii) being pregnant.

Measurements

Anthropometry

Body weight and height were measured by a pre-validated electronic scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany). BMI was calculated as $Body\ weight\ (kg)\ /\ Height^2\ (m^2)$ [46].

Sleep quantity and quality

Subjective sleep quantity and quality were assessed using the PSQI scale [47], a 19-item scale that provides 7 component scores with a range of 0–3 points: (i) subjective sleep quality, (ii) sleep latency, (iii) sleep duration, (iv) habitual sleep efficiency, (v) sleep disturbances, (vi) use of sleeping medication, and (vii) daytime dysfunction. A global PSQI score is obtained by the sum of the 7 components ranging from 0 to 21. Lower global scores denote a healthier sleep quality, whereas global scores greater than 5 is associated with poor sleep quality.

Objective sleep quantity and quality were determined using a wrist-worn accelerometer (ActiGraph GT3X+, Pensacola, FL, US) 24 hours/day during 7 consecutive days [45]. Participants received detailed instructions to wear the accelerometer on the non-dominant wrist, and to remove it only during water activities such as swimming or bathing. A 7-day sleep diary was provided to record bed-time, wake up time, and the time they removed the device each day. The accelerometer was initialized to store raw accelerations at a sampling frequency of 100 Hz [48]. ActiLife software (version 6.13.3, ActiGraph, Pensacola, FL, US) was used to process data. The GT3X+ files were subsequently converted to 1-second epoch csv files containing x, y, and z vectors to facilitate raw data processing. These files were processed in R (version 3.1.2, https://www.cran.r-project.org/) using GGIR package (version 1.5-12, https://cran.r-project.org/web/packages/GGIR/). Signal processing included: (i) autocalibration according to the local gravity [49], (ii) detection of sustained abnormal high accelerations, (iii) detection of the non-wear time, (iv) calculation of the Euclidean Norm Minus One (ENMO), (v) identification of waking and sleeping hours with an automatized algorithm [50], and (vi) imputation of detected non-wear time and abnormal high values. TST (total amount of time spent in bed minus sleep onset latency); WASO (the sum of wake times from sleep onset to the final awakening), and SE (percentage of sleep time over the bedtime) were obtained by accelerometry [51]. Adherence was defined as ≥ 16 hours/day of wear time for at least four of seven possible days of wear (including at least one weekend day).

Leukocyte and platelet parameters

A 10 mL peripheral blood sample was taken from the antecubital vein applying standard techniques after 12 hours fasting. Blood samples were collected in the morning (8:30 AM – 10:00 AM) in prechilled ethylene diamine tetra-acetic acid-containing tubes (Vacutainer SST, Becton Dickinson, Plymouth, UK), immediately centrifuged at 4000 rpm at 4 ^{o}C for 7 minutes, and processed in a controlled-temperature room (22 ± 0.5 °C). Leukocyte (i.e., blood count of leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils), and platelet (i.e., platelet count and mean platelet volume [MPV]) parameters were measured by Advia 120 Siemens Hematology System (Siemens Healthcare Diagnostics, Erlangen, Germany). The results were expressed in µL and fL, respectively. All participants were requested to abstain from drugs and/or caffeine (24 hours before), to eat a pre-established dinner (i.e., boiled rice, tomato sauce, and plain egg omelet) before sampling, and to avoid any physical activity of moderate (24 hours before) and/or vigorous intensity (48 hours before).

Statistical analysis

Descriptive parameters were reported as mean and standard deviation. Normal distribution of the variables was tested through the Shapiro-Wilk test and a visual check of histograms, Q-Q, and box plots. Sex differences were determined using independent samples *t*-test.

Simple linear regression models were conducted to examine the association of sleep quantity and quality (i.e., global PSQI score, TST, WASO, and SE) with leukocyte and platelet parameters (i.e., blood count of total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets, and MPV). Multiple linear regression models were also performed to test these associations after adjusting by age and by sex.

All analyses were conducted using the Statistical Package for Social Sciences (SPSS, version 26.0, IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA), and the level of significance was set at 5% ($p < 0.05$). Graphical presentations were prepared using GraphPad Prism 9.1.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Descriptive characteristics of study participants are shown in Table 1. Significant differences in height, weight, BMI, platelet count, and TST were observed between men and women (all $p \leq 0.001$). A poor subjective sleep quality (global PSQI score > 5) was identified in 40.3% of our cohort. Figure 1 shows mean and standard deviation of leukocyte and platelet parameters after categorizing the participants into either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. Between these two groups, no statistically significant differences were observed in objective sleep quantity and quality parameters (i.e., TST, WASO, and SE, all $p \geq 0.05$).

Figure 2 shows the association of subjective sleep quantity and quality with leukocyte parameters. No significant associations of global PSQI score with blood count of total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, and basophils were observed (all $p \geq 0.05$, Figures 2A to 2F).

Figure 3 shows the association of objective sleep quantity and quality with leukocyte parameters. No significant associations of TST, WASO, and SE with blood count of total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, and basophils were found (all $p \geq 0.05$, Figures 3A to 3Q).

Table 2 shows the association of both subjective and objective sleep quantity and quality with leukocyte parameters after including age and sex in the statistical models. All of the above-mentioned findings persisted after adjusting by age and by sex.

Figure 4 shows the association of both subjective and objective sleep quantity and quality with platelet parameters. No association was noted between global PSQI score and platelet count ($p \geq 0.05$, Figure 4A). By contrast, a significant negative association was observed between global PSQI score and MPV (β = –0.278, R^2 = 0.077, p = 0.024, Figure 4E). Regarding objective sleep quantity and quality, a significant positive association was observed between TST and platelet count (β = 0.297, R^2 = 0.088, p = 0.013, Figure 4B), while a significant negative association was noted between TST and MPV (β = –0.264,

Table 1. Descriptive characteristics of participants.

	N	All		N	Men		N	Women	
Age (years)	74	53.66	(5.14)	35	54.39	(5.27)	39	53.01	(5.00)
Anthropometry and body composition									
Height (cm)	74	167.8	(9.81)	35	175.8	(6.48)	39	160.7	(6.10) *
Weight (kg)	74	75.73	(14.98)	35	87.38	(10.95)	39	65.28	(9.32) *
Body mass index (kg/m²)	74	26.72	(3.76)	35	28.32	(3.61)	39	25.27	(3.31) *
Leukocyte parameters									
Total leukocytes (x10³/μL)	73	6.58	(1.60)	34	6.52	(1.80)	39	6.63	(1.43)
Neutrophils (x10³/μL)	73	3.59	(1.18)	34	3.68	(1.19)	39	3.50	(1.19)
Lymphocytes (x10³/μL)	73	2.26	(0.67)	34	2.13	(0.49)	39	2.38	(0.78)
Monocytes (x10³/μL)	73	0.39	(0.11)	34	0.41	(0.12)	39	0.38	(0.10)
Eosinophils (x10³/μL)	73	0.20	(0.13)	34	0.20	(0.13)	39	0.19	(0.14)
Basophils (x10³/μL)	73	0.04	(0.02)	34	0.04	(0.02)	39	0.04	(0.03)
Platelet parameters									
Platelets (x10³/μL)	73	234.4	(46.78)	34	215.7	(44.24)	39	250.7	(43.14) *
Mean platelet volume (fL)	73	8.31	(1.00)	34	8.49	(1.12)	39	8.15	(0.87)
Sleep quantity and quality									
Global PSQI score	67	5.61	(3.47)	31	4.77	(3.15)	36	6.33	(3.62)
Total sleep time (min)	71	359.9	(48.85)	34	337.9	(46.30)	37	380.1	(42.44) *
Wake after sleep onset (min)	71	63.90	(27.44)	34	65.80	(32.45)	37	62.15	(22.19)
Sleep efficiency (%)	71	85.01	(6.29)	34	83.88	(7.53)	37	86.06	(4.75)

Data are presented as mean (standard deviation). *Significant differences between sexes obtained from an independent samples t-test ($p < 0.05$). PSQI: Pittsburgh Sleep Quality Index.

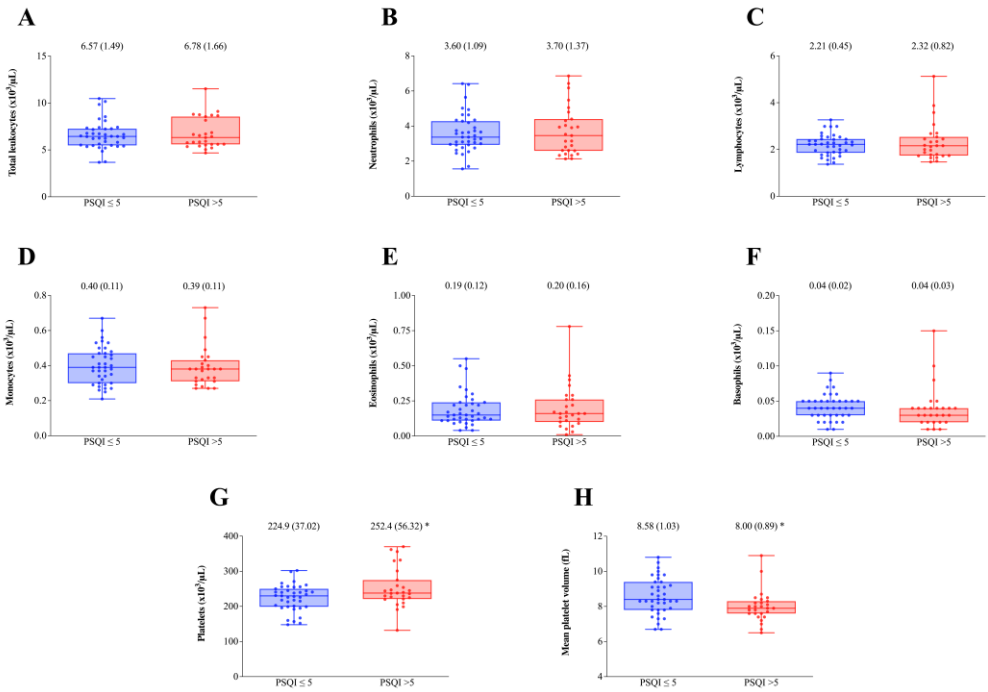

Figure 1. Mean (standard deviation) of leukocyte and platelet parameters after categorizing participants as either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. *Significant differences between global PSQI score groups obtained from an independent samples *t*-test ($p < 0.05$). PSQI: Pittsburgh Sleep Quality Index.

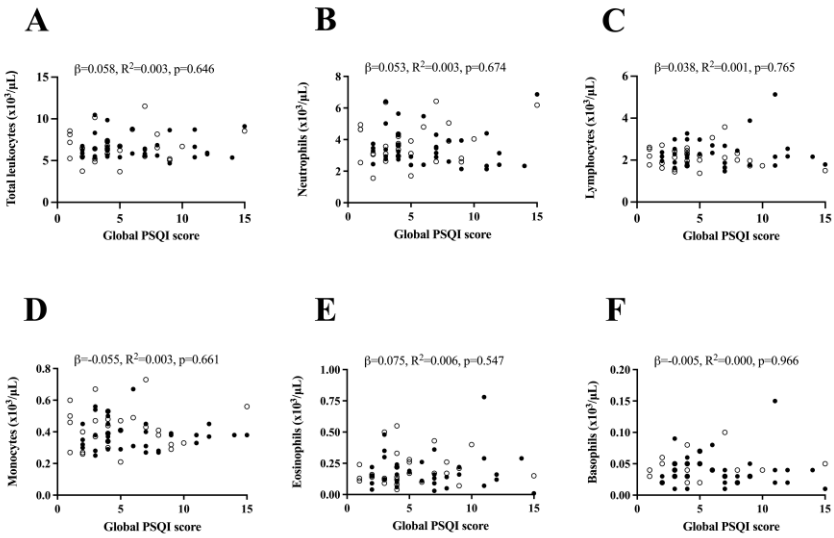

Figure 2. Association of subjective sleep quantity and quality with leukocyte parameters in sedentary middle-aged adults. β (standardized regression coefficient), R^2, and *p*-value from a simple linear regression analysis. Significant *p*-values (< 0.05) are in bold. Open circles represent men, and close circles represent women. PSQI: Pittsburgh Sleep Quality Index.

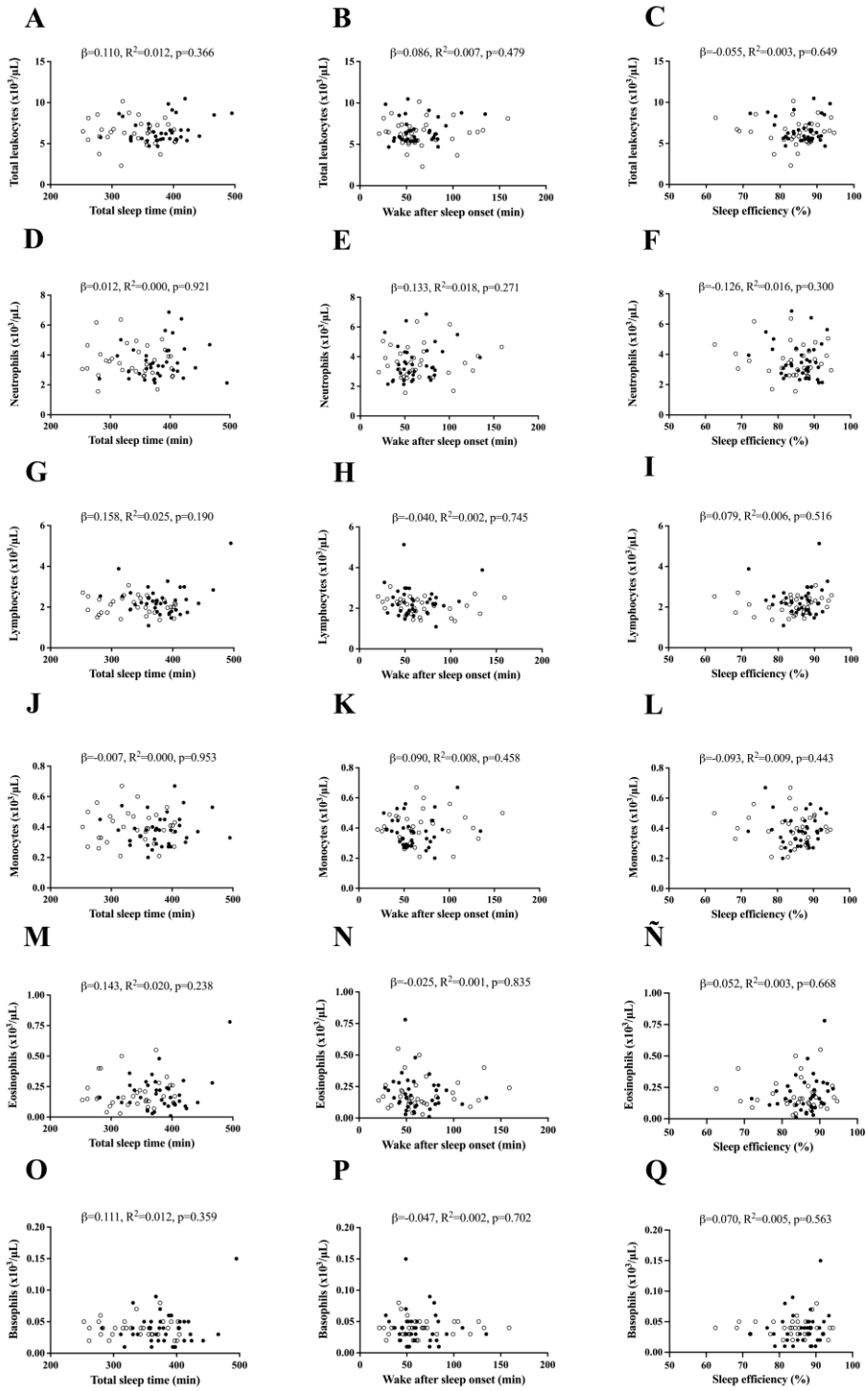

Figure 3. Association of objective sleep quantity and quality with leukocyte parameters in sedentary middle-aged adults. *β* (standardized regression coefficient), R^2, and *p*-value from a simple linear regression analysis. Significant *p*-values (< 0.05) are in bold. Open circles represent men, and close circles represent women.

Table 2. Association of sleep quantity and quality with leukocyte parameters (Model 0) adjusted by age (Model 1) and by sex (Model 2).

	Global PSQI score			Total sleep time			Wake after sleep onset			Sleep efficiency		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Total leukocytes (x10³/uL)												
Model 0	0.058	0.003	0.646	0.110	0.012	0.366	0.086	0.007	0.479	–0.055	0.003	0.649
Model 1	0.078	0.012	0.547	0.100	0.030	0.410	0.089	0.028	0.461	–0.059	0.023	0.628
Model 2	0.068	0.005	0.600	0.092	0.013	0.498	0.093	0.015	0.447	–0.073	0.012	0.556
Neutrophils (x10³/uL)												
Model 0	0.053	0.003	0.674	0.012	0.000	0.921	0.133	0.018	0.271	–0.126	0.016	0.300
Model 1	0.067	0.007	0.602	0.003	0.017	0.982	0.137	0.035	0.259	–0.129	0.033	0.288
Model 2	0.079	0.016	0.540	0.028	0.001	0.838	0.132	0.018	0.280	–0.125	0.016	0.313
Lymphocytes (x10³/uL)												
Model 0	0.038	0.001	0.765	0.158	0.025	0.190	–0.040	0.002	0.745	0.079	0.006	0.516
Model 1	0.049	0.004	0.707	0.154	0.028	0.205	–0.038	0.006	0.756	0.077	0.011	0.527
Model 2	0.001	0.028	0.993	0.092	0.044	0.490	–0.024	0.038	0.839	0.044	0.039	0.717
Monocytes (x10³/uL)												
Model 0	–0.055	0.003	0.661	–0.007	0.000	0.953	0.090	0.008	0.458	–0.093	0.009	0.443
Model 1	–0.017	0.034	0.891	–0.026	0.068	0.827	0.096	0.076	0.416	–0.100	0.077	0.400
Model 2	–0.015	0.034	0.904	0.042	0.010	0.758	0.083	0.016	0.497	–0.078	0.015	0.528
Eosinophils (x10³/uL)												
Model 0	0.075	0.006	0.547	0.143	0.020	0.238	–0.025	0.001	0.835	0.052	0.003	0.668
Model 1	0.082	0.007	0.525	0.142	0.021	0.246	–0.025	0.001	0.840	0.052	0.003	0.674
Model 2	0.090	0.010	0.487	0.175	0.025	0.195	–0.025	0.001	0.836	0.054	0.003	0.667
Basophils (x10³/uL)												
Model 0	–0.005	0.000	0.966	0.111	0.012	0.359	–0.047	0.002	0.702	0.070	0.005	0.563
Model 1	0.017	0.011	0.894	0.100	0.038	0.409	–0.043	0.030	0.724	0.066	0.032	0.583
Model 2	0.007	0.003	0.954	0.147	0.018	0.279	–0.048	0.003	0.694	0.076	0.006	0.540

β (standardized regression coefficient), R^2, and *p*-value of simple and multiple-regression analysis. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index.

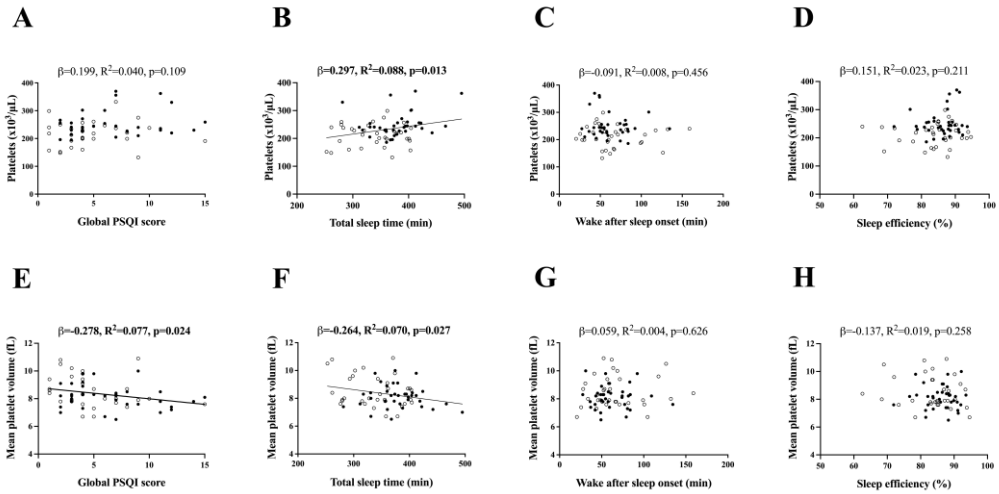

Figure 4. Association of both subjective and objective sleep quantity and quality with platelet parameters in sedentary middle-aged adults. *β* (standardized regression coefficient), R^2, and *p*-value from a simple linear regression analysis. Significant *p*-values (< 0.05) are in bold. Open circles represent men, and close circles represent women. PSQI: Pittsburgh Sleep Quality Index.

Table 3. Association of sleep quantity and quality with platelet parameters (Model 0) adjusted by age (Model 1) and by sex (Model 2).

	Global PSQI score			Total sleep time			Wake after sleep onset			Sleep efficiency		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Platelets (x10³/uL)												
Model 0	0.199	0.040	0.109	0.297	0.088	**0.013**	–0.091	0.008	0.456	0.151	0.023	0.211
Model 1	0.205	0.040	0.110	0.298	0.089	**0.013**	–0.091	0.008	0.459	0.151	0.023	0.214
Model 2	0.121	0.159	0.310	0.144	0.189	0.244	–0.058	0.176	0.604	0.076	0.178	0.501
Mean platelet volume (fL)												
Model 0	–0.278	0.077	**0.024**	–0.264	0.070	**0.027**	0.059	0.004	0.626	–0.137	0.019	0.258
Model 1	–0.252	0.093	**0.044**	–0.277	0.103	**0.020**	0.063	0.030	0.602	–0.141	0.046	0.242
Model 2	–0.242	0.104	0.052	–0.223	0.077	0.092	0.045	0.038	0.712	–0.105	0.047	0.390

β (standardized regression coefficient), R^2, and *p*-value of simple and multiple-regression analysis. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index.

R^2 = 0.070, p = 0.027, Figure 4F). No significant associations of WASO and SE with platelet count and MPV were found (all $p \geq 0.05$, Figures 4C and 4D, and 4G and 4H).

Table 3 shows the association of both subjective and objective sleep quantity and quality with platelet parameters after including age and sex in the statistical models. The above-mentioned findings persisted once age was included in the statistical model, while disappeared when adjusted by sex.

DISCUSSION

The aim of the present study was to elucidate the relationship of sleep quantity and quality with leukocyte and platelet parameters in sedentary middle-aged adults. The main findings of our study indicate that a poor sleep quantity and quality did not influence leukocyte parameters in sedentary middle-aged adults. However, a poor subjective sleep quality was associated with decreased MPV in sedentary middle-aged adults. Decreased MPV in presence of normal platelet count is a characteristic feature of an aged platelet population which may result in an inappropriate functioning and troubled hemostasis. Low MPV along with high platelet count is associated to inflammation [52,53].

Sleep is considered one of the most important components of the regulation and maintenance of numerous physiological functions [54]. Previous studies evidenced that sleep quantity is related to all-cause mortality risk through a 'U' -shaped graph, where the risk of death increases in people with excessive or insufficient sleep durations [31,55–58]. It is important to point out that the National Sleep Foundation issued its recommendations, including 8–10 hours for teenagers (aged 14–17), 7–9 hours for adults (18–64) and 7–8 hours for older adults (65–) [58]. A multitude of biological mechanisms have been proposed as potential explanations for the link between short sleep quantity and the increased morbidity and mortality, mainly from cardiovascular disorders, such as the involvement of the neurovegetative system, endothelial function, metabolic regulation, the hemostatic system, and inflammation [59,60].

Sleep alterations can modify the homeostasis of the immune system - which acts as the main line of defense of the human body against internal and external threats [16,28] - generating changes in the hypothalamic-pituitary-adrenal axis and in the sympathetic nervous system [30]. In turn, the decrease of hormones levels regulated by circadian rhythms during the night (e.g., cortisol and adrenaline) regulates the activity of the immune system [30]. [16]. In the present study, our results did not show any statistically significant association of both subjective and objective poor sleep quantity and quality with count of total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils in middle-aged adults. These results are in accordance with those reported by Feng et al. [43] and Down et al. [44] in two cross-sectional studies, where no association between self-reported short sleep duration and total leukocytes count was found in adult populations. However, they found that longer sleep duration was significantly associated with elevated total leukocyte count [43,44]. Moreover, an observational study in healthy shift workers by Nakao et al. [38] detected no significant difference in total leukocyte count between baseline and after night-shift work.

Nevertheless, the results in our study are not in accordance with those reported by Shattuck et al. [31] and Hu et al. [32] in mediation studies, where significant relationships were found between self-reported sleep duration, total leukocyte count, and mortality risk [31] or cognition [32]. In addition, a cross-sectional study by Pérez de Heredia et al. [33] showed that subjective sleep duration was negatively associated with count of total leukocytes, neutrophils, monocytes and some lymphocyte subsets in adolescents, whereas no association was found between sleep duration and count of eosinophils and basophils. Regarding experimental studies, Born et al. [34] and Ackermann et al. [35] analyzed the effect of acute sleep deprivation on the immune system through polysomnography monitoring in healthy young men. Contradictory results appear when comparing both studies as the first one showed an increase in total leukocyte, lymphocyte, and monocyte count during sleep deprivation, but no significant associations of sleep deprivation with neutrophil, basophil, and eosinophil count [34], whereas the second one found just an increase in total granulocyte count [35].

Changes in environments and lifestyles today, which diverge from those in our ancestral past, are supposed to generate new health problems, highlighting the concept of an evolutionary mismatch [61,62]. Recent evidence suggests that ancestral human sleep was more flexible or segmented - biphasic or even polyphasic pattern - than commonly experienced today by Western populations [63,64]. Interestingly, studies by Samson et al. [63,64] in nonelectric populations in both Malagasy and Hazda showed a segmented sleep pattern, and registered a

lower total sleep quantity and quality, but more stable circadian rhythms than reported in postindustrial Western countries, proposing that flexible sleep-wake patterns may be a feature of human plasticity [63,64]. In contrast, a study by Yetish et al. [65] in three preindustrial societies (Hadza, Tsimane, and San) reported a monophasic sleep pattern, suggesting, therefore, that the disappearance of segmented sleep was not a deleterious development attributable to restricted sleep quantity, but rather a return to a pattern, caused by the electric lights and temperature control. Assuming that sleep plasticity is characteristic of human evolution [31,64], it could be speculated that a shorter sleep quantity or a fragmented sleep pattern could not lead to negative health outcomes as disruptions in immunological homeostasis in a healthy adult population.

The above controversial findings could be potentially explained by diverse reasons. Firstly, population variations in terms of levels of melatonin or cortisol, whose effects on both sleep and the immune system are well known. [31]. Secondly, the inclusion/exclusion criteria of recruited participants may cause these discrepancies. In this sense, participants selected in acute sleep deprivation studies are often habitual 7 to 8 hour sleepers, which would not accurately represent the impact of chronic sleep restriction [66]. Furthermore, the methodology used in the measurement of sleep parameters could also contribute to this discordance. Hence, self-reported measures are subject to recall bias since self-reported short sleepers underestimate how much sleep they truly obtain while long sleepers overestimate their sleep quantity and may reflect extended time in bed rather than actual sleep [66]. In addition, the use of 'invasive' methods (e.g., polysomnography) in sleep deprivation studies could generate some level of stress, and, therefore, leukocyte parameters alterations. On the contrary, the use of alternative procedures (e.g., actigraphy) could show the impact of sleep deprivation on leukocyte parameters in a more precisely way, despite not being the gold standard.

Finally, another factor that could explain these inconsistent results is the circadian nature of the immune function [16,67–69], which mainly responds to two patterns during sleep period: (i) an increase in blood levels of prolactin, leptin, melatonin and growth hormone, with the consequent increase of pro-inflammatory interleukins (IL) such as IL-1, IL-12, tumor necrosis factor alpha, and Th1 cytokines such as interferon gamma, whereas (ii) a decrease of cortisol, epinephrine, and norepinephrine (anti-inflammatory hormones) [70]. Thus, leukocytes show a strong migratory capacity and are constantly recirculating between several tissues and organs through the blood and the lymphatic system. This migration leads to acute changes in the blood count of leukocyte subsets and has a very pronounced circadian component [16]. Consequently, a single blood

sample could be insufficient to assess the impact of sleep quantity and quality on the count of total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils. Moreover, changes in leukocyte subsets could also depend on the duration and type of sleep loss, that could lead to variations as consequence of adaptative mechanisms of compensation, therefore highlighting the importance of taking several samples during the whole day or even different days to evaluate the impact of sleep quantity and quality in the long term [16,71]. For all this, more research is needed to elucidate the link between sleep and leukocyte parameters.

Sleep disturbances could also influence the homeostasis of the hemostatic system [25], one of its main components being blood platelets, which play central role in hemostasis and thrombosis [25,72]. Initial platelet adhesion, activation and aggregation upon tissue injury, stimulates coagulation factors and other mediators to achieve hemostasis [72]. Platelet count and MPV are two parameters routinely evaluated as a part of complete blood count test. Concretely, MPV has been shown to be an indicator of platelet age: younger platelets tend to present higher MPV, while older platelets tend to the opposite, in the absence of other pathologies [52]. This may play a role in the pathophysiology of thrombosis, myocardial infarction or ischemic stroke [37,41]. The relationship of sleep quantity and quality with platelet parameters has not been extensively studied. The present study found that a higher global PSQI score (i.e., worse perceived sleep quality) was associated with decreased MPV. No significant association was found between global PSQI score and platelet count, but it is important to highlight that the participants categorized as "poor" sleepers presented higher platelet count and lower MPV than those registered by "good" sleepers, which is a characteristic feature of a proinflammatory status [52,53].

Regarding the above-mentioned results, previous experimental studies by Born et al. [34] and Liu et al. [39] reported that platelet count was not affected by sleep deprivation in young populations. Moreover, Nakao et al. [38] found no significant difference in neither platelet count or MPV between baseline and after night-shift work in healthy young shift workers. Contrarily, Khosro et al. [36] observed an increase in platelet count following night shift work compared with day shift work. In this line, Everson et al. [73] suggested an increased bone marrow megakaryocyte (i.e., platelet precursor cell) production during sleep loss in rats, explaining that the functional significance of this rise in the megakaryocyte production was at least two-fold: cell engulfment and platelet production, therefore contemplating a change in hematopoiesis that may reflect demands for cell delivery to the circulation and the promotion of thrombocytosis. On the other hand, Bülbül et al. [40] and Sökücü et al. [41] shown that platelet count or MPV were not

different between adult patients with OSA and their healthy counterparts. Moreover, a recent meta-analysis by Qiu et al. [42] established no statistical differences in platelet count between OSA and control groups. Nevertheless, Varol et al. [37] found that MPV was significantly higher in patients with severe OSA, results that were also supported by Gabryelska et al. [25] who pointed out that platelets were found to be excessively activated in patients with OSA.

These uncertain results could be explained by different causes. Firstly, both platelet count and MPV are modified by several biosocial and lifestyle factors (e.g., race, age, gender, smoking, alcohol consumption, physical activity, diet therapy, among others) [40,41]. Indeed, scientific research has reported conflicting results regarding the effects of sex differences or menstrual cycles on platelet function [38]. In this sense, differences between studies that found a significant relationship between sleep and hemostatic variables and those studies that did not find any association may be a matter of sample sizes [74]. So, both previously-mentioned experimental studies [34,39], and the shift work study by Nakao et al. [38] included only around 10 participants, and one of them even only men [34]. Thus, future studies need to be performed on sufficiently large samples to an adequate control of hemostatic activity covariates [74]. Secondly, most of the studies have been carried out in patients with OSA, a common sleep disorder characterized by the repetitive collapse of the upper airway during sleep, leading to and interrupted sleep and a nocturnal hypoxia environment, the latter one being the fact that could promote platelet activation [42]. In the same way, acute sleep deprivation studies are difficult to compare with the much more normal conditions of our study. Lastly, the controversies among studies might be due to the influence of circadian rhythm on the nature of activation of hemostatic processes [38,75]. Therefore, the variability in the timing and methods of blood sample preparation may play a role in the results [40–42].

Regarding objective sleep assessment, our study showed a positive association between TST and platelet count, and a negative association between TST and MPV. Differences between subjective and objective sleep assessments have been previously reported. Concretely, previous studies have shown weak or inconsistent correlations of subjective measures (i.e., PSQI scores) with objective measures (e.g., actigraphy and polysomnography) [76–78]. Indeed, it has been suggested that the PSQI and the accelerometer may measure different attributes of sleep in youth, reporting the inadequate capacity of accelerometry to detect wakefulness (i.e., lying in bed awake but motionless is likely to be coded as sleep) [79]. Due to this limitation, using both complementary assessment methods to obtain detailed information beyond the limited data derived from body movements is

recommended [80]. PSQI may reflect the overall psychological state of the person, rather than actual quantity or quality of sleep [77]. In addition, it is still not well-defined what a "good night' sleep" actually involves in the perception of the sleeper and stand out that many factors play a role when judging sleep quality [81]. However, PSQI validity is further supported by similar differences between groups using PSQI or polysomnographic sleep measures, and has been used in a wide range of population-based and clinical studies [78].

To the best of our knowledge, this is the first study to describe the relationship of both subjectively and objectively measured sleep quantity and quality with leukocyte and platelet parameters in healthy sedentary middle-aged adults. Results of the current study are of great scientific and clinical interest since we highlight the possible role of inadequate sleep on platelet aging and possible subsequent hemostatic system alterations. Taking into account the notable increase in the prevalence of sleep disorders in worldwide population [4–6], it is necessary to establish health promotion and disease prevention strategies including sleep as a key modifiable factor.

Our study, however, has some limitations that should be consider in future studies. The cross-sectional design of the study does not allow to identify any causal association, so well-designed intervention studies are necessary. Participants included in this study were healthy sedentary middle-aged adults and, therefore, the findings cannot be extrapolated to other individuals with different biological characteristics. The extraction of blood samples was carried out in the morning, not being possible to know the behavior of the studied parameters over 24 hours. Future studies should include the collection of several samples throughout the day. This study used accelerometry to assess objective sleep quantity and quality instead of polysomnography (i.e., the gold standard method). Accelerometry could overestimate TST and SE, as well as underestimate WASO in adults [48]. Future research should also include polysomnography to collect data of additional variables related to sleep architecture (e.g., rapid eye movements sleep and non-rapid eye movements sleep).

CONCLUSIONS

According to the results of the present study, sleep quantity and quality were not associated with leukocyte parameters in sedentary middle-aged adults. However, a poor subjective sleep quality was associated with a lower MPV in sedentary middle-aged adults. Thereby, health promotion and disease prevention strategies including sleep as a key modifiable factor in order to achieve an appropriate sleep quality could be considered a potential strategy for an optimal functioning of the hemostatic system.

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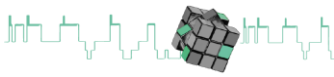

Chapter 5

Is sleep associated with the S-Klotho anti-aging protein in sedentary middle-aged adults? The FIT-AGEING study **(Study 5)**

ABSTRACT

Sleep and Klotho have both been closely related to the aging process, both playing a substantial role in the endocrine and immune systems and, thereby, in oxidative stress and chronic inflammation. However, there are no studies elucidating the relationship between sleep and Klotho. Therefore, this study investigated the association of sleep quantity and quality with the shed form of the α-Klotho gene (S-Klotho plasma levels) in sedentary middle-aged adults.

A total of 74 volunteers (52.7% women; aged 53.7 ± 5.1) were recruited for the present study. Subjective sleep quantity and quality were assessed by the Pittsburgh Sleep Quality Index (PSQI; higher scores indicate worse sleep quality), and objective sleep quantity and quality parameters (total sleep time [TST], wake after sleep onset [WASO], and sleep efficiency [SE]) were determined using a wrist-worn accelerometer over seven consecutive days. S-Klotho plasma levels were measured in the ethylenediaminetetraacetic acid plasma using a solid-phase sandwich enzyme-linked immunosorbent assay. A direct relationship was observed between subjective sleep quality (inverse of PSQI scores) and S-Klotho plasma levels ($p < 0.001$) in sedentary middle-aged adults. Improving sleep quality could be considered an anti-aging therapeutic approach for the prevention, slowing, and even reversal of the physiological decline and degenerative pathologies that are certainly related to the aging process.

Keywords: successful ageing; inflammation; oxidative stress; actigraphy.

INTRODUCTION

The increasing population ageing occurring worldwide, with the subsequent upsurge in vulnerability to morbidity and age-related diseases among adults, has certainly become one of the most significant global clinical and economic burdens for health systems and all aspects of society. According to the Global Burden of Disease Study 2017 [1], 51.3% of all burdens among adults were identified as age-related diseases, mostly including non-communicable diseases such as neoplasms, cardiovascular diseases, chronic respiratory diseases, diabetes and kidney diseases, digestive diseases, and neurological disorders among others [2]. In response to this remarkable demographic transition, the World Health Organization developed a global strategy and action plan on aging and health in 2017 with goals and strategic objectives focusing on health system alignment to the needs of the older population and the enhancement of measurement, monitoring, and research to support healthy ageing [3,4].

In this context, the elucidation of aging mechanisms by advances in medicine and research has led to the emergence of anti-aging medicine, a growing field of research and clinical practice focused on the prevention, slowing, and even reversal of the physiological decline and degenerative pathologies related to the aging process [5–9]. Approaches of anti-aging medicine include, among others, calorie restriction mimetics, hormonal replacement, and gut microbiota and vitamin D interventions [5]. In this field of research, sleep could be considered a substantial key element involved in the restoration and preservation of multiple physiological systems, including the endocrine function and metabolism, immune response, and general brain metabolism [10–12]. Indeed, sleep deprivation has been closely related to energy imbalance [13,14], adverse hormonal changes [15], gut microbiota alterations [16], and vitamin D deficiency [17], all involved in the mechanisms of action of anti-aging medicine interventions. Accordingly, gathered evidence has also widely shown that sleep disturbances certainly lead to a vast number of age-related diseases [18], including obesity [19,20], cardiovascular disease [21], type II diabetes mellitus [22,23], chronic kidney disease [24], and psychiatric disorders [25].

Similarly, Klotho gene family has been established as an "aging-suppressor" factor that accelerates aging when disrupted and extends life span when overexpressed through a wide variety of mechanisms [26–30]. Specifically, evidence suggest that α-Klotho—a single-pass transmembrane glycoprotein encoded by the Klotho gene and mainly expressed in the kidneys and brain choroid plexus—modulates the insulin-like growth factor and Wnt signaling pathways; inhibits oxidative stress; and regulates the metabolism of phosphate, calcium, and vitamin D in humans [28,29]. Within the three identified α-Klotho protein types [31], its secreted (or

soluble) form (S-Klotho)—expressed in blood, plasma, urine, and cerebrospinal fluid—works as a circulating hormone with significant metabolic functions on different tissues and organs, including anti-inflammatory and anti-oxidative stress effects [29,32,33]. Thus, S-Klotho identified in plasma levels, which has been robustly associated with the α-Klotho gene expression [34], may be a powerful biomarker of biological anti-aging and, in turn, a promising therapeutic target for the prevention of aged-related disorders.

Sleep and S-Klotho, therefore, share common underlying mechanisms and physiological pathways through which they are linked to the ageing process in adults, both playing a positive substantial role in the endocrine [32,35–37] and immune systems [38,39] and, thereby, in oxidative stress [33,40] and chronic inflammation [29,41,42]—the leading molecular mechanisms behind all age-related consequences [43–46]. Yet, available evidence on the relationship between sleep and S-Klotho is remarkably limited. A previous study by Pákó et al. [47] found reduced levels of S-Klotho in patients with obstructive sleep apnoea, potentially enhancing the systemic inflammation and endothelial dysfunction associated with this sleep-related breathing disorder. Similarly, another empirical study also concluded that sleep deprivation had an adverse effect on S-Klotho responses to exercise testing in healthy adults [48]. Conversely, results from a recent study on the role of S-Klotho as a potential biomarker of stress exposed that unsatisfactory sleep was positively related to increased S-Klotho levels, although it is worth mentioning that sleep was only subjectively measured [49].

Hence, our study was aimed at elucidating the potential association of subjective (components and global Pittsburgh Sleep Quality Index [PSQI] score) and objective (total sleep time [TST], wake after sleep onset [WASO], and sleep efficiency [SE]) sleep quantity and quality with S-Klotho plasma levels in sedentary middle-aged adults. In accordance with most but not all available evidence, we hypothesized that worse sleep quantity and quality would be significantly associated with decreased plasma levels of S-Klotho.

MATERIALS AND METHODS

Study protocol and participants

A total of 74 healthy sedentary middle-aged volunteers (52.7% women, 53.7 ± 5.1 years old, 26.7 ± 3.8 kg/m^2) were recruited for the FIT-AGEING study [50], an exercise-based randomized controlled trial (clinicaltrial.gov: ID: NCT03334357), approved by the Human Research Ethics Committee of the "Junta de Andalucía" [0838-N-2017]. A detailed explanation of the study methodology can be found elsewhere [50]. The study was in accordance with ethical principles of the Declaration of Helsinki. All participants were given a full explanation of the study, completed a written consent form, and underwent a complete medical and

physical examination prior to their enrolment in the study. Inclusion criteria were: (i) being aged from 40 to 65 years, (ii) having a body mass index (BMI) between 18.5 and 35 kg/m^2, (iii) having a stable weight in the last three months (weight changes < 3 kg), (iv) being a non-smoker, and (v) being sedentary (i.e., self-reported < 150 minutes of moderate-intensity aerobic physical activity throughout the week or < 75 minutes of vigorous-intensity aerobic physical activity throughout the week). Exclusion criteria were: (i) having some acute or chronic illness, (ii) taking some medication, and (iii) being pregnant.

Measurements

Anthropometry and body composition

Body weight and height were measured using an electronic scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany). BMI was calculated as $Weight\ (kg)\ /\ Height^2\ (m^2)$ [51].

A dual-energy X-ray absorptiometry scanner (Hologic, Inc., Bedford, MA, USA) was used to determine the fat mass. The fat mass index (FMI) was calculated as $Fat\ mass\ (kg)\ /\ Height^2\ (m^2)$.

Sleep quantity and quality

Subjective sleep quantity and quality were measured by the PSQI scale [52]. PSQI contains 19 self-rated questions for scoring, combined into 7 components, each of them with a range of 0–3 points: (i) subjective sleep quality, (ii) sleep latency, (iii) sleep duration, (iv) habitual sleep efficiency, (v) sleep disturbances, (vi) use of sleeping medication, and (vii) daytime dysfunction. A global PSQI score is obtained by the sum of 7 components ranging from 0 to 21. Lower scores denote a healthier sleep quality, whereas a global score of more than five indicates poor sleep quality.

Objective characteristics of sleep-wake patterns were assessed with a wrist-worn accelerometer (ActiGraph GT3X+, Pensacola, FL, USA) continuously 24 hours a day for seven consecutive days [50]. Participants received detailed instructions to wear the accelerometer on the non-dominant wrist and to remove it during water activities. They were provided with a seven-day sleep diary to record bed-time, wake up time, and the time they removed the device each day. The accelerometer was initialized to store raw accelerations at a sampling frequency of 100 Hz [53]. Data were processed using ActiLife software (version 6.13.3, ActiGraph, Pensacola, FL, USA). GT3X+ files were subsequently converted to 1-second epoch csv files containing x, y, and z vectors to facilitate raw data processing. These files were processed in R (version 3.1.2, https://www.cran.r-project.org/) using GGIR package (version 1.5-12, https://cran.r-project.org/web/packages/GGIR/). Signal processing included: (i) auto-calibration using local gravity as a reference [54], (ii) detection of sustained abnormal high accelerations, (iii) detection of non-wear time, (iv) calculation of

the Euclidean Norm Minus One (ENMO), (v) calculation of waking and sleeping time by an automatized algorithm [55], and (vi) imputation of abnormal high values and detected non-wear time. The variables analyzed from actigraphy recordings were TST (total amount of time spent in bed minus sleep onset latency), WASO (the sum of wake times from sleep onset to the final awakening), and SE (percentage of sleep time over the bedtime) [56]. Adherence was defined as ≥ 16 hours/day of wear time for at least four of seven possible days of wear (including at least one weekend day).

S-Klotho plasma levels

Blood samples were collected from the antecubital vein applying standard techniques after overnight fasting. The samples were centrifuged and collected at the same time (8:30 AM – 10:00 AM), processed in a controlled-temperature room (22 ± 0.5 °C), and kept in a −80 °C freezer (i.e., 6 months before the analysis). A solid-phase sandwich enzyme-linked immunosorbent assay (Demeditec, Kiel, Germany) was used to measure S-Klotho plasma levels in the ethylenediaminetetraacetic acid plasma, which was previously validated by both the manufacturer (obtaining a sensitivity analysis of 6.15 pg/mL) and our own research group (obtaining intra- and inter-assay coefficients of variation which ranged from ~3% to ~10%). All participants were requested to abstain from caffeine and/or drugs, to eat a standardized dinner before sampling, and to refrain from any physical activity of moderate (24 hours before) and/or vigorous intensity (48 hours before).

Statistical analysis

Normal distribution of the variables was tested through Shapiro-Wilk test and a visual check of histograms, Q-Q, and box plots. Descriptive parameters were reported as mean and standard deviation. Sex differences were determined using independent samples *t*-test.

Simple linear regression models were conducted to examine the association of sleep quantity and quality (global PSQI score, TST, WASO, and SE) with S-Klotho plasma levels. Multiple linear regression models were also performed to test these associations after adjusting by age and by FMI.

Statistical Package for the Social Sciences (SPSS, version 23.0, IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA) was used for the data analyses. *P*-values less than 0.05 were considered statistically significant. GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA) was used to create all the graphical presentations.

RESULTS

Study participants' characteristics can be found in Table 1. Significant differences between sex were observed in height, weight, BMI, subjective sleep quality (PSQI component), habitual sleep efficiency (PSQI component), and TST (all $p \leq 0.033$).

A poor subjective sleep quality (global PSQI score > 5) was identified in 40.3% of the population. Figure 1 reports mean and standard deviation of S-Klotho after categorizing participants in our study as either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. Between these two groups, no statistically significant differences were observed in objective sleep quantity and quality parameters (i.e., TST, WASO, and SE, all $p \geq 0.05$).

Figure 2 shows the association of subjective sleep quantity and quality (components and global PSQI score) with S-Klotho plasma levels. An inverse relationship was observed between global PSQI score and S-Klotho plasma levels ($\beta = -0.438$, $R^2 = 0.192$, $p < 0.001$, Figure 2H), meaning that a higher subjective sleep quality was significantly

Table 1. Descriptive characteristics of participants.

	N	All		N	Men		N	Women	
Age (years)	74	53.66	(5.14)	35	54.39	(5.27)	39	53.01	(5.00)
Geographical origin of the population [n, (%)]	74			35			39		
Spain		74	(100.0)		35	(100.0)		39	(100.0)
Place of residence [n, (%)]	74			35			39		
Urban		63	(85.1)		30	(84.7)		33	(84.6)
Rural		11	(14.9)		5	(15.3)		6	(15.4)
Socio-professional category [n, (%)]	74			35			39		
Technicians and professional intellectual scientists		1	(1.35)		0	(0.00)		1	(2.56)
Technicians and associate professionals		3	(4.05)		1	(2.86)		2	(5.13)
Service and sales workers		4	(5.41)		0	(0.00)		4	(10.26)
Skilled agricultural, forestry and fishery workers		43	(58.11)		23	(65.71)		20	(51.28)
Unemployed		2	(2.70)		2	(5.71)		0	(0.00)
Elementary occupations		16	(21.62)		6	(17.14)		10	(25.64)
Others		5	(6.76)		3	(8.58)		2	(5.13)
Antropometry and body composition									
Height (cm)	74	167.8	(9.81)	35	175.8	(6.48)	39	160.7	(6.10) *
Weight (kg)	74	75.73	(14.98)	35	87.38	(10.95)	39	65.28	(9.32) *
Body mass index (kg/m^2)	74	26.72	(3.76)	35	28.32	(3.61)	39	25.27	(3.31) *
Fat mass index (kg/m^2)	74	10.75	(3.13)	35	10.03	(3.23)	39	11.39	(2.93)
S-Klotho plasma levels (pg/mL)	73	775.3	(363.7)	34	814.1	(452.2)	39	741.4	(265.6)
Sleep quantity and quality									
Subjective sleep quantity and quality									
Subjective sleep quality	67	1.13	(0.82)	31	0.84	(0.78)	36	1.39	(0.77) *
Sleep latency	67	1.07	(0.86)	31	1.03	(0.88)	36	1.11	(0.85)
Sleep duration	67	0.99	(0.77)	31	0.97	(0.66)	36	1.00	(0.86)
Habitual sleep efficiency	67	0.60	(0.95)	31	0.32	(0.75)	36	0.83	(1.06) *
Sleep disturbances	67	1.13	(0.42)	31	1.03	(0.41)	36	1.22	(0.42)
Use of sleeping medication	67	0.31	(0.76)	31	0.19	(0.60)	36	0.42	(0.87)
Daytime dysfunction	67	0.37	(0.55)	31	0.39	(0.50)	36	0.36	(0.59)
Global PSQI score	67	5.61	(3.47)	31	4.77	(3.15)	36	6.33	(3.62)
Objective sleep quantity and quality									
Total sleep time (min)	71	359.9	(48.85)	34	337.9	(46.30)	37	380.1	(42.44) *
Wake after sleep onset (min)	71	63.90	(27.44)	34	65.80	(32.45)	37	62.15	(22.19)
Sleep efficiency (%)	71	85.01	(6.29)	34	83.88	(7.53)	37	86.06	(4.75)

Data are presented as mean (standard deviation). *Significant differences between sexes obtained from an independent samples *t*-test ($p < 0.05$). S-Klotho: secreted Klotho; PSQI: Pittsburgh Sleep Quality Index.

related to increased S-Klotho plasma levels. Furthermore, our results showed inverse associations of subjective sleep quality, sleep latency, habitual sleep efficiency, and sleep disturbance PSQI components with S-Klotho plasma levels ($\beta = -0.368$, $R^2 = 0.135$, $p = 0.002$, Figure 2A; $\beta = -0.519$, $R^2 = 0.269$, $p < 0.001$, Figure 2B; $\beta = -0.277$, $R^2 = 0.077$, $p = 0.024$, Figure 2D; $\beta = -0.407$, $R^2 = 0.165$, $p = 0.001$, Figure 2E, respectively). Therefore, better sleep quality and efficiency, shorter sleep latency, and lower levels of sleep disturbances were all related to higher plasma levels of S-Klotho. We did not observe any significant associations of sleep duration, use of sleeping medication, and daytime dysfunction with the S-Klotho plasma levels (all $p \geq 0.05$, Figures 2C, 2F, and 2G).

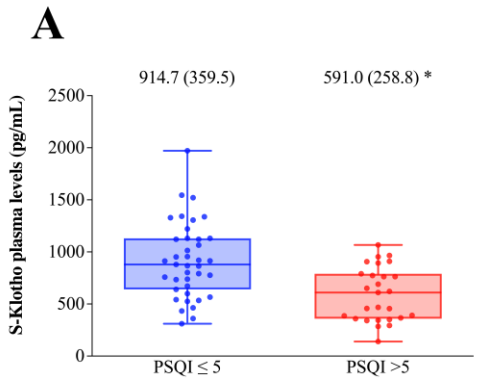

Figure 1. Mean (standard deviation) of S-Klotho plasma levels after categorizing participants as either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. *Significant differences between global PSQI score groups obtained from an independent samples *t*-test ($p < 0.05$). PSQI: Pittsburgh Sleep Quality Index; S-Klotho: secreted Klotho.

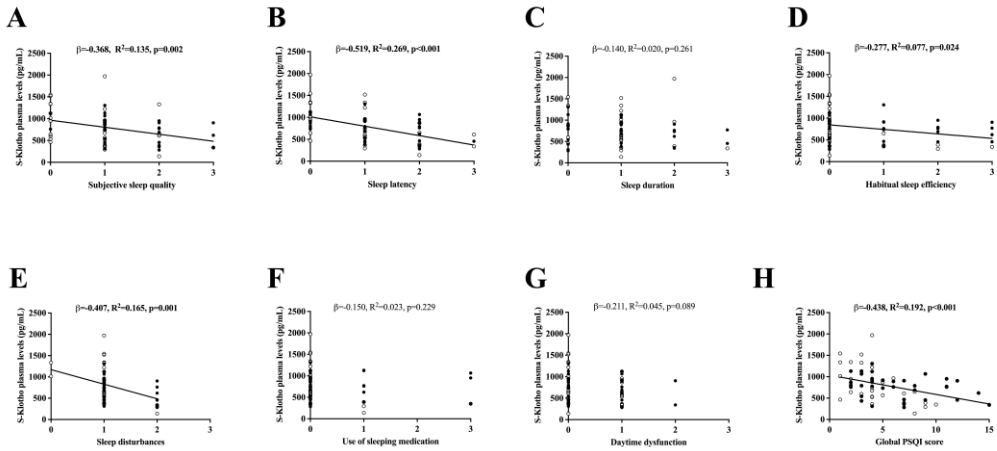

Figure 2. Association of subjective sleep quantity and quality (components and global PSQI score) with S-Klotho plasma levels in sedentary middle-aged adults. β (standardized regression coefficient), R^2, and *p*-value from a simple linear regression analysis. Significant *p*-values (< 0.05) are highlighted in bold. Open circles represent men, close circles represent women, and the straight solid line represents the regression line. PSQI: Pittsburgh Sleep Quality Index; S-Klotho: secreted Klotho.

Table 2 shows the relationship of subjective sleep quantity and quality (components and global PSQI score) with S-Klotho plasma levels adjusted by age and by FMI in the statistical models. All of the above-mentioned findings persisted once age and FMI were included in the statistical models. Furthermore, associations of sleep duration, use of sleeping medication, and daytime dysfunction with S-Klotho plasma levels appeared after adjusting by age.

Figure 3 shows the association of objective sleep quantity and quality with S-Klotho plasma levels. TST, WASO, and SE were not associated with S-Klotho plasma levels (all $p \geq 0.05$, Figures 3A to 3C).

Table 3 shows the relationship of objective sleep quantity and quality with S-Klotho plasma levels adjusted by age and by FMI in the statistical models. TST and SE were negatively associated with S-Klotho plasma levels, while a significant positive association was found between WASO and S-Klotho plasma levels, all associations after including age as covariate.

DISCUSSION

Table 2. Association of subjective sleep quantity and quality (components and global PSQI score) with secreted Klotho plasma levels (Model 0) adjusted by age (Model 1) and by fat mass index (Model 2).

	Subjective sleep quality			Sleep latency			Sleep duration			Habitual sleep efficiency		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Model 0	−0.368	0.135	**0.002**	−0.519	0.269	**<0.001**	−0.140	0.020	0.261	−0.277	0.077	**0.024**
Model 1	−0.275	0.573	**<0.001**	−0.350	0.611	**<0.001**	−0.101	0.509	**<0.001**	−0.209	0.542	**<0.001**
Model 2	−0.349	0.139	**0.009**	−0.505	0.275	**<0.001**	−0.136	0.046	0.228	−0.282	0.107	**0.028**

	Sleep disturbances			Use of sleeping medication			Daytime dysfunction			Global PSQI score		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Model 0	−0.407	0.165	**0.001**	−0.150	0.023	0.229	−0.211	0.045	0.089	−0.438	0.192	**<0.001**
Model 1	−0.125	0.511	**<0.001**	−0.158	0.523	**<0.001**	−0.137	0.517	**<0.001**	−0.304	0.587	**<0.001**
Model 2	−0.400	0.187	**0.001**	−0.143	0.048	0.214	−0.206	0.070	0.103	−0.423	0.204	**0.001**

β (standardized regression coefficient), R^2, and *p*-value of simple and multiple-regression analysis. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index.

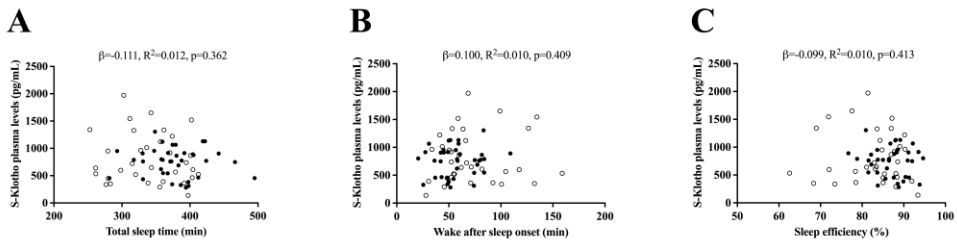

Figure 3. Association of objective sleep quantity and quality with S-Klotho plasma levels in sedentary middle-aged adults. *β* (standardized regression coefficient), R^2, and *p*-value from a simple linear regression analysis. Significant *p*-values (< 0.05) are highlighted in bold. Open circles represent men, close circles represent women, and the straight solid line represents the regression line. S-Klotho: secreted Klotho.

Table 3. Association of objective sleep quantity and quality with secreted Klotho plasma levels (Model 0) adjusted by age (Model 1) and by fat mass index (Model 2).

	Total sleep time			Wake after sleep onset			Sleep efficiency		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Model 0	−0.111	0.012	0.362	0.100	0.010	0.409	−0.099	0.010	0.413
Model 1	−0.057	0.482	**<0.001**	0.108	0.491	**<0.001**	−0.107	0.491	**<0.001**
Model 2	−0.094	0.015	0.597	0.083	0.014	0.633	−0.081	0.013	0.644

β (standardized regression coefficient), R^2, and *p*-value of simple and multiple-regression analysis. Significant *p*-values (< 0.05) are in bold.

Our study sought to elucidate the relationship of sleep quantity and quality with S-Klotho plasma levels in sedentary middle-aged adults. As we expected, the results from the current study indicated that a poor subjective sleep quality (measured by global PSQI score) was related to lower levels of S-Klotho plasma levels in sedentary middle-aged adults. These results therefore have important clinical implications, as improving sleep quality could be considered a novel anti-aging therapeutic approach via increasing S-Klotho plasma levels.

Sleep quantity and quality represent a cornerstone in the maintenance of health and well-being, particularly during the senescence process and thus in the prevention of numerous degenerative chronic diseases [57–62]. Poor sleep quality and both short and long sleep duration have been previously associated with a lifespan reduction due to the associated deleterious effects on health and the higher risk of several diseases [18,63]. Sleep and S-Klotho have both been widely shown to be related to the ageing process [29,32,33,35–42], sharing underlying mechanisms and physiological pathways such as endocrine functions and metabolism, immune response and, consequently, oxidative stress and chronic inflammation. However, the relationship between these two aged-related factors still remains unclear. According to our results, better subjective sleep quality was positively related to enhanced levels of S-Klotho. A previous study by Nakanishi et al. [49], however, conversely found that subjective sleep dissatisfaction was related to higher levels of this anti-aging protein. This inverse association could be explained by a compensatory mechanism triggered to counteract the inflammatory stress-derived environment produced by sleep deprivation [49]. Nevertheless, as previously mentioned, it is also noteworthy that sleep in the study by Nakanishi et al. [49] was solely measured using one item on "relaxation from sleep".

Our results agreed with previous findings [47,48], where sleep deprivation and obstructive sleep apnea—which causes short sleep and sleep fragmentation due to the repetitive collapse of the upper airway during sleep—were related to lower levels of the anti-aging protein. According to the evidence, short sleep and chronic intermittent hypoxia caused by sleep apnea are both closely related

to energy imbalance and obesity [13,14], adverse hormonal changes [15], gut microbiota alterations [16], vitamin D deficiency [17], and thus systemic inflammation and oxidative stress [40–42,47]. These physiological consequences, in turn, may lead to reduced S-Klotho levels, as it is known that factors such as obesity, vitamin D deficiency, and systemic inflammation suppress renal Klotho synthesis [47,64,65]. Subsequently, a reduction in S-Klotho expression may result in endothelial dysfunction, excessive aldosterone production, hypertension, renal structure damage, and functional decline, exacerbating therefore the increased systemic inflammation and oxidative stress found in sleep disturbances such as obstructive sleep apnea and/or sleep curtailment [47,66,67].

Regarding objective sleep assessment, our results indicated that better objective sleep quantity and quality were related to reduced levels of S-Klotho after adjusting by age. Interestingly, we observed different results when the association of sleep quantity and quality with S-Klotho plasma levels was performed considering subjective sleep measured by a PSQI questionnaire versus objective sleep measured with an accelerometer. Differences between subjective and objective sleep measurements methods have been previously reported. In this sense, previous studies have found weak or inconsistent correlations between subjective measures (i.e., PSQI scores) and objective measures (e.g., actigraphy and polysomnography) [68,69]. In this line, a previous study in youth showed that the PSQI and the accelerometer may assess different attributes of sleep, describing the inadequate capacity of accelerometry to detect wakefulness, thus lying in bed awake but motionless is likely to be coded as sleep [70]. As a result of this limitation, it is recommended the use of both complementary measurement methods in order to obtain detailed information beyond the limited data derived from body movements [71]. PSQI may reflect the general psychological state of the person, instead of actual quantity or quality of sleep [72]. In addition, it is still not well-defined what a "good night's sleep" really involves in the sleeper perception and stand out that numerous factors play a role when take sleep quality [72]. Nonetheless, PSQI validity is further supported by similar differences between groups using PSQI or polysomnographic sleep measures, and has been used in a wide range of population-based and clinical studies [69].

To the best of our knowledge, this is the first study describing the relationship of sleep quantity and quality with S-Klotho plasma levels in healthy sedentary middle-aged adults. Our results therefore have robust clinical and research implications, supporting the association of better sleep quality with increased plasma levels of S-Klotho. Considering the increasingly high prevalence of sleep disturbances and its association with age-related disorders [73–79], promising anti-

aging interventions should consider sleep as a modifiable factor for healthy aging. In this regard, the measurement of S-Klotho plasma levels could be used as a marker of a healthier and anti-aging sleep.

However, our study has some limitations that need to be addressed in future studies. Firstly, the cross-sectional study design used does not allow identification of any causal association between the variables included, so well-designed longitudinal studies are needed to robustly analyze and establish causal relationships between sleep and S-Klotho. Secondly, participants included in our sample were healthy sedentary middle-aged adults, such that these findings cannot be extrapolated to other individuals with different biological characteristics. Finally, although we used accelerometry as an objective tool to assess sleep quantity and quality, future studies should include polysomnography, which is the gold-standard method to appropriately assess not only sleep duration and efficiency but also other significant sleep outcomes, such as sleep architecture.

CONCLUSIONS

In accordance with our findings, a poor subjective sleep quality was associated with lower S-Klotho plasma levels in sedentary middle-aged adults. Therefore, improving sleep quality could be considered an anti-aging therapeutic approach for the prevention, slowing, and even reversal of the physiological decline and degenerative pathologies that are certainly related to the aging process.

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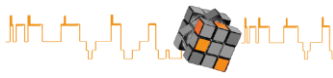

Chapter 6

Role of physical activity and fitness on sleep in sedentary middle-aged adults: The FIT-AGEING study

(Study 6)

ABSTRACT

The relationship of physical activity and fitness with sleep still remains unclear since there is a lack of studies in this field of research using objective measurements of these variables. This study aimed to investigate the association of objectively measured sedentariness, physical activity levels, and physical fitness with sleep quantity and quality in sedentary middle-aged adults.

A total of 74 volunteers (52.7% women; aged 53.7 ± 5.1) were recruited for the present study. Cardiorespiratory fitness was measured through a maximal treadmill test, and muscular strength by extension and flexion peak torque, and by the hand grip test. Physical activity and objective sleep parameters were determined through accelerometry, and subjective sleep by the Pittsburgh Sleep Quality Index (PSQI). Lower levels of overall physical activity, VO_2max, and muscular strength were related to worse subjective sleep quality (all $p \leq 0.048$) in sedentary middle-aged adults. Furthermore, higher levels of sedentariness were related to worse objective sleep quantity ($p = 0.002$) in sedentary middle-aged adults. Reduced sedentariness and increased physical activity and fitness may be a potential prevention and/or treatment pathway to reduce sleep disturbances and, in general, to improve patients physical and psychological health for a successful aging process.

Keywords: cardiorespiratory fitness; muscular strength; sedentariness; VO_2max; actigraphy.

INTRODUCTION

Regular physical activity has been widely shown to be a well-established protective factor related to the prevention and management of a vast number of severe pathological conditions [1] such as obesity [2], type II diabetes mellitus [3], life-threating cardiovascular diseases [4], degenerative neurological disorders [5], and other adverse chronic illnesses [6,7]. Programmed physical activity (i.e., exercise) is an effective strategy to fight against several cardiometabolic diseases through physical fitness improvements, since its main components (i.e., cardiorespiratory fitness and muscular strength) are considered powerful predictors of physical and psychological health and all-cause mortality [8–10].

Similarly, sleep is a widely accepted key component of physiological restitution [11,12], essential for mental and physical health, and thus general well-being [13,14]. According to epidemiological studies, the prevalence of sleep disorders in the overall population has dramatically increased in the last decade [15], becoming an economic and clinical burden on the health system, with costs of up to $7494 million per year (Australia, 2004) [16]. Insomnia and obstructive sleep apnoea, the most common sleep disorders with a prevalence of 10–40% and 9–38% in the overall population [17,18], respectively, have been shown to be related to the development and worsening of a wide-range of medical conditions such as obesity [19], type II diabetes mellitus [20], other cardiometabolic alterations [21,22], chronic kidney disease [23], and anxiety and mood disturbances/depression [24], among others.

Research in this field has shown that increased physical activity may be highly effective at improving sleep quantity and quality, and thus an alternative treatment for sleep disorders/disturbances [25–29]. However, at the current time, the evidence is still limited as to whether physical activity, physical fitness, or sedentary behaviour are more strongly associated with sleep quantity and quality. Furthermore, the majority of these studies have assessed physical activity levels using subjective measures (i.e., self-reported questionnaires) [30], instead of objective methods such as accelerometry, which is currently considered the gold-standard [31]. Likewise, although previous studies have reported a positive relationship of cardiorespiratory fitness and muscular strength with sleep quantity and quality [32–35], physical fitness has usually been measured by indirect field test assuming therefore potential bias. Furthermore, although sedentary behaviour, poor physical activity levels, and a decline of physical fitness seem to be indicators for sleep status, to the best of our knowledge, there are no studies investigating the association of sedentariness, physical activity, and physical fitness measured by gold standard methods with sleep quantity and quality in sedentary middle-aged adults.

Therefore, the aim of the present study was to investigate the association of

sedentariness, physical activity levels, and physical fitness with sleep quantity and quality in sedentary middle-aged adults. We hypothesized that high levels of sedentariness, and lower levels of both physical activity and fitness would be positively associated with worse sleep quantity and quality.

MATERIALS AND METHODS

Study protocol and participants

The study population consisted of 74 sedentary middle-aged adults (52.7% women) aged 40–65 years. Participants were enrolled in the FIT-AGEING study [36], an exercise-based randomised controlled trial (clinicaltrial.gov: ID: NCT03334357, registration date: 07/11/2017), approved by the Human Research Ethics Committee of the "Junta de Andalucía" [0838-N-2017]. An extended explanation of the study methodology can be found elsewhere [36]. All participants signed a written informed consent form, and underwent a complete medical and physical examination prior to participation. Inclusion criteria were: (i) to present a body mass index (BMI) between 18.5 and 35 kg/m^2, (ii) to have a stable weight over the previous 3 months, and (iii) to be sedentary (i.e., < 20 minutes of vigorous-intensity physical activity < 3 days/week). Participants with a diagnosis of any physical or psychological disease and/or medical treatment, as well as pregnancy, were excluded from the study. The study complied with the ethical principles described in the Declaration of Helsinki.

Measurements

Anthropometry and body composition

Body weight and height were measured using a seca model 799 electronic scale and stadiometer (seca, Hamburg, Germany). BMI was calculated as $Weight\ (kg)\ /\ Height^2\ (m^2)$ [37].

A dual-energy X-ray absorptiometry scanner (Hologic, Inc., Bedford, MA, USA) was used to determine fat mass. Fat mass index (FMI) was calculated as: $Fat\ mass\ (kg)\ /\ Height^2\ (m^2)$.

Physical activity and sedentary time

Physical activity and sedentary time were determined using a wrist-worn accelerometer (ActiGraph GT3X+, Pensacola, FL, USA) continuously 24 hours a day for 7 consecutive days [36]. Participants were instructed to wear the accelerometer on the non-dominant wrist, and to remove it when swimming or bathing. They were provided with a 7-day sleep diary to register bedtime, wake up time, and the time they removed the device each day. The accelerometer was initialised to store raw accelerations at a sampling frequency of 100 Hz [31]. ActiLife software (version 6.13.3, ActiGraph, Pensacola, FL, USA) was used to download the stored data. GT3X+ files were subsequently converted to 1-second epoch csv files containing x, y and z vectors to facilitate raw data processing. GGIR package

(version 1.5-12, https://cran.r-project.org/web/packages/GGIR/) was selected to process these files in R (version 3.1.2, https://www.cran.r-project.org/). Signal processing included auto-calibration using local gravity as a reference [38], detection of sustained abnormal high accelerations, detection of non-wear time, calculation of the Euclidean Norm Minus One (ENMO), calculation of waking and sleeping time by an automatized algorithm [39], determination of sedentary time, light physical activity (LPA) time, moderate physical activity (MPA) time, vigorous activity (VPA) time, and moderate-vigorous physical activity (MVPA) time using age-specific thresholds for ENMO [40,41], and determination of abnormal high values and detected non-wear time. Only the participants wearing the accelerometers for ≥ 16 hours/day during at least 4 of 7 possible days (including at least 1 weekend day) were included in the analysis.

Cardiorespiratory fitness

Cardiorespiratory fitness was assessed by maximal oxygen uptake (VO_2max) through a maximal treadmill (h/p/cosmos pulsar treadmill, h/p/cosmos sport & medical gmbh, Germany) test using the modified Balke protocol. After a standardized warm-up (i.e., 1 minute at 3 km/h and 2 minutes at 4 km/h) the treadmill was set at a constant speed (5.3 km/h), increasing 1% per minute until volitional exhaustion. The criteria for considering VO_2max were: (i) a respiratory exchange ratio ≥ 1.1, (ii) a plateau in oxygen uptake (VO_2) (change of < 100 mL/min in the last 30 seconds stage), (iii) a heart rate within 10 beats/min of the age-predicted maximal heart rate using an age-based equation (i.e., 209 – 0.73 * age) [42], and (iv) a rating perceived exertion peak greater than 18 in the 6-20 Borg scale [43]. The peak oxygen uptake value was considered when these criteria were not fulfilled [44]. VO_2 and carbon dioxide output (VCO_2) were assessed using a breath-by-breath gas analyzer (CPX Ultima CardiO2, Medical Graphics Corp., St Paul, USA) equipped with an oronasal mask (model 7400, Hans Rudolph Inc., Kansas City, MO, USA) and a prevent metabolic flow sensor (Medgraphics Corp., Minnesota, USA). The gas analyser was calibrated before each maximal test. Heart rate was registered every 5 seconds with a heart rate monitor watch (Polar RS300, Kempele, Finland). Participants were instructed to refrain from caffeine for the previous 24 hours, to fast for 3 hours before the test, and to avoid any moderate and/or vigorous physical activity during the 24 or 48 hours, respectively, prior to the assessment day.

Muscular strength

Knee flexion and extension peak torque were assessed applying an isokinetic strength test using a Gymnex Iso-2 dynamometer (Easytech S.r.l., Italy). Participants were seated for testing in the dynamometer's chair with the backrest angle at 90°, and limbs and hips stabilized with

safety belts. The lateral epicondyle of their knee was aligned with the axis of the dynamometer's resistance lever, and the force pad was placed 3–4 cm above to the medial malleolus. The maximum extension angle was fixed at 170º to avoid knee hyperextension, and the maximum flexion angle was established at 90º. Each participant performed 5 submaximal knee flexions and extensions followed by 3 maximal repetitions, with a resting interval of 60 seconds between submaximal and maximal trials following a previously validated protocol [45]. The flexion and extension peak torque were determined as the single repetition with the highest muscular force output (Nm). Test-retest reliability, calculated using the intra-class correlation coefficient, resulted higher than 0.90 [46].

Handgrip strength was measured using a digital hand dynamometer (T.K.K. 5401 Grip-D; Takei Scientific Instruments Co., Ltd, Tokyo, Japan) with the scores recorded to the nearest 0.1 kg. Participants were asked to perform the test in a standing position with the forearm slightly separated from their torso at the level of the thigh, and to apply the maximum grip strength gradually and continuously for at least 2 seconds. Two repetitions were registered with both right and left hands alternatively, with a resting interval of 60 seconds between trials. The grip span of the dynamometer was fixed at 5.5 cm for men, and an individual hand size adjustment in women was used following a previous study [47].

Muscular strength and VO_2max assessments were determined on a different day (separated by 3-7 days) applying similar preconditions.

Sleep quantity and quality

Subjective sleep quantity and quality were evaluated by the Spanish version of the Pittsburgh Sleep Quality Index (PSQI) scale [48]. This scale consists of 19 self-rated questions grouped into 7 components, each equally scored on a 0–3 scale: (i) subjective sleep quality, (ii) sleep latency, (iii) sleep duration, (iv) habitual sleep efficiency, (v) sleep disturbances, (vi) use of sleeping medication, and (vii) daytime dysfunction [48]. Global PSQI score is obtained by the sum of the 7 components (ranged from 0 to 21). Lower score indicates better sleep quality whereas scores higher than 5 are associated with poor sleep quality.

Objective sleep quantity and quality were determined by accelerometry (see specific details about the procedure above). Total sleep time (TST; total amount of time spent in bed minus sleep onset latency), wake after sleep onset (WASO; the sum of wake times from sleep onset to the final awakening), and sleep efficiency (SE; percentage of sleep time over the bedtime), were calculated from actigraphy recordings [49]. Participants who registered ≥ 16 hours/day of wear time for at least 4 out of 7

days (including 1 weekend day) were included in the final analysis.

Statistical analysis

Shapiro-Wilk test, visual check of histograms, Q-Q, and box plots were used to check the variables' distribution. Descriptive parameters were presented as mean and standard deviation. Sex differences were determined using independent samples *t*-test. Given that no sex interactions were observed, the results for men and women were analysed together.

Simple linear regression models were performed to study the association of sedentariness and physical activity levels (sedentary time, LPA, MPA, VPA, MVPA, overall physical activity) and physical fitness (VO_2max, flexion peak torque, extension peak torque, and hand grip strength) with sleep quantity and quality (global PSQI score, TST, WASO, and SE).

Multiple linear regression models were performed to test these associations after adjusting by age and by FMI.

All analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 23.0, IBM SPSS Statistics, IBM Corp., Armonk, NY, USA) and the level of significance was set at < 0.05. All graphical presentations were created using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Participant's characteristics are shown in Table 1. Significant differences in height, weight, BMI, TST, VO_2max, extension peak torque, flexion peak torque, and hand grip strength were observed between men and women (all $p < 0.001$). A poor subjective sleep quality (global PSQI score > 5) was identified in 40.3% of our cohort. Figure 1 reports mean and standard deviation of physical activity and fitness parameters after categorizing participants in our study as either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. Between these two groups, no statistically significant differences were observed in objective sleep quantity and quality parameters (i.e., TST, WASO, and SE, all $p \geq 0.05$).

Figure 2 shows the association of sedentary and physical activity levels with subjective sleep quantity and quality. We did not observe any significant association of sedentary time, LPA, and VPA with global PSQI score (all $p \geq 0.05$, Figures 2A, 2B, and 2D). MPA, MVPA, and overall physical activity levels were negatively associated with global PSQI score ($\beta = -0.249$, $R^2 = 0.062$, $p = 0.048$, Figure 2C; $\beta = -0.248$, $R^2 = 0.061$, $p = 0.048$, Figure 2E; $\beta = -0.259$, $R^2 = 0.067$, $p = 0.039$, Figure 2F, respectively).

Figure 3 shows the association of sedentariness and physical activity levels with objective sleep quantity and quality. A negative association was observed between sedentary time and TST ($\beta = -0.369$, $R^2 = 0.136$,

p = 0.002, Figure 3A). We did not observe any significant association between sedentary time and WASO or SE (all $p \geq 0.05$, Figures 3B and 3C). No associations of LPA, MPA, VPA, MVPA, and overall physical activity with TST, WASO, and SE were found (all $p \geq 0.05$, Figures 3D to 3R)

Figure 4 shows the association of VO_2max with sleep quantity and quality. Negative associations were found between VO_2max and both TST and SE (β = –0.361, R^2 = 0.131, p = 0.002, Figure 4B; β = –0.258, R^2 = 0.067, p = 0.033, Figure 4D, respectively). Negative associations were found between VO_2max and both TST and SE when VO_2max was expressed relative to body weight (β = –0.309, R^2 = 0.095, p = 0.010, Figure 4F; β = –0.253, R^2 = 0.064, p = 0.037, Figure 4H, respectively). No association was observed between VO_2max and WASO ($p \geq 0.05$, Figure 4C). No association was observed between VO_2max and WASO when VO_2max was expressed relative to body weight ($p \geq 0.05$, Figure 4G). A negative association was observed between VO_2max and global score (β = –0.378, R^2 = 0.143, p = 0.002, Figure 4A). A negative association was observed between VO_2max (expressed relative to body weight)

Table 1. Descriptive characteristics of participants.

	N	All		N	Men		N	Women	
Age (years)	74	53.66	(5.14)	35	54.39	(5.27)	39	53.01	(5.00)
Antropometry and body composition									
Height (cm)	74	167.8	(9.81)	35	175.8	(6.48)	39	160.7	(6.10) *
Weight (kg)	74	75.73	(14.98)	35	87.38	(10.95)	39	65.28	(9.32) *
Body mass index (kg/m²)	74	26.72	(3.76)	35	28.32	(3.61)	39	25.27	(3.31) *
Fat mass index (kg/m²)	74	10.75	(3.13)	35	10.03	(3.23)	39	11.39	(2.93)
Sleep quantity and quality									
Global PSQI score	67	5.61	(3.47)	31	4.77	(3.15)	36	6.33	(3.62)
Total sleep time (min)	71	359.9	(48.85)	34	337.9	(46.30)	37	380.1	(42.44) *
Wake after sleep onset (min)	71	63.90	(27.44)	34	65.80	(32.45)	37	62.15	(22.19)
Sleep efficiency (%)	71	85.01	(6.29)	34	83.88	(7.53)	37	86.06	(4.75)
Physical activity									
Sedentary time (min/day)	71	745.9	(84.22)	34	758.3	(78.22)	37	734.4	(88.91)
Light physical activity time (min/day)	71	173.9	(45.14)	34	173.6	(50.13)	37	174.06	(40.71)
Moderate physical activity time (min/day)	71	94.37	(34.84)	34	94.60	(32.81)	37	94.17	(37.07)
Vigorous physical activity time (min/day)	71	1.68	(2.23)	34	2.11	(2.90)	37	1.28	(1.29)
Moderate-vigorous physical activity time (min/day)	71	96.05	(35.38)	34	96.71	(33.40)	37	95.45	(37.56)
Overall physical activity (min/day)	71	269.9	(74.65)	34	270.4	(76.69)	37	270.0	(73.79)
Physical fitness									
VO_2max (mL/min)	71	2339.2	(657.2)	34	2915.4	(373.2)	37	1809.7	(332.5) *
VO_2max (mL/kg/min)	71	30.49	(5.57)	34	33.27	(4.49)	37	27.93	(5.28) *
Extension peak torque (Nm)	71	265.2	(84.82)	33	337.0	(65.97)	38	202.8	(35.40) *
Extension peak torque/Weight (Nm/kg)	71	3.49	(0.74)	33	3.88	(0.74)	38	3.14	(0.56) *
Flexion peak torque (Nm)	71	123.1	(44.35)	33	157.1	(41.97)	38	93.61	(16.62) *
Flexion peak torque/Weight (Nm/kg)	71	1.63	(0.46)	33	1.82	(0.54)	38	1.46	(0.29) *
Hand grip strength (kg)	73	70.98	(23.67)	35	93.08	(12.14)	38	50.62	(8.18) *
Hand grip strength/Weight	73	0.92	(0.21)	35	1.08	(0.17)	38	0.78	(0.12) *

Data are presented as mean (standard deviation). *Significant differences between sexes obtained from an independent samples t-test ($p < 0.05$). PSQI: Pittsburgh Sleep Quality Index; VO_2max: maximal oxygen uptake.

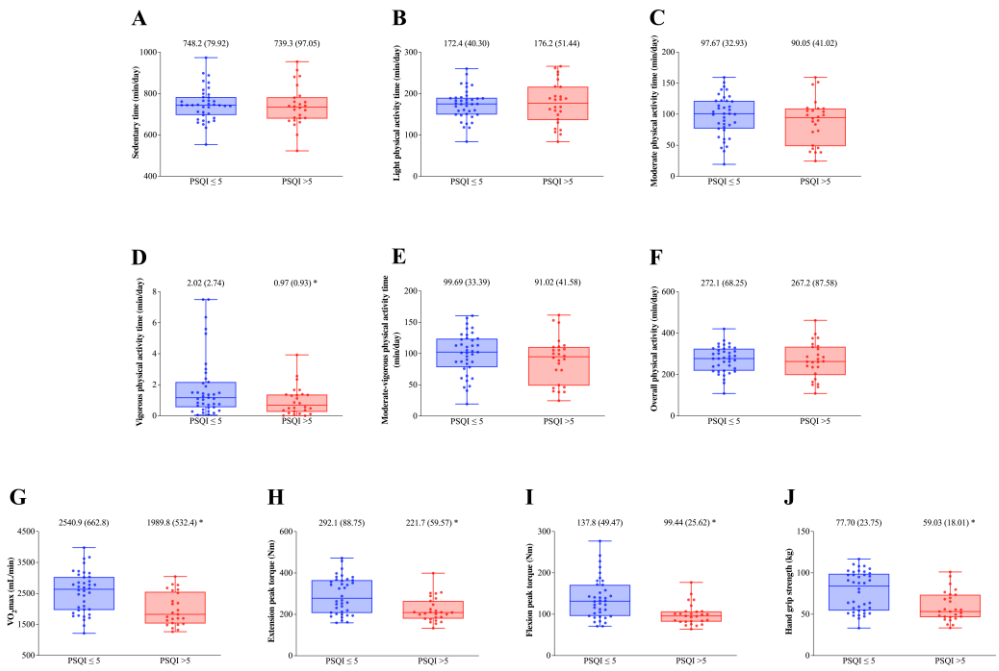

Figure 1. Mean (standard deviation) physical activity and fitness parameters after categorizing participants as either "good" (global PSQI score ≤ 5) or "poor" (global PSQI score > 5) sleepers. *Significant differences between global PSQI score groups obtained from an independent samples *t*-test ($p < 0.05$). PSQI: Pittsburgh Sleep Quality Index; VO_2max: maximal oxygen uptake.

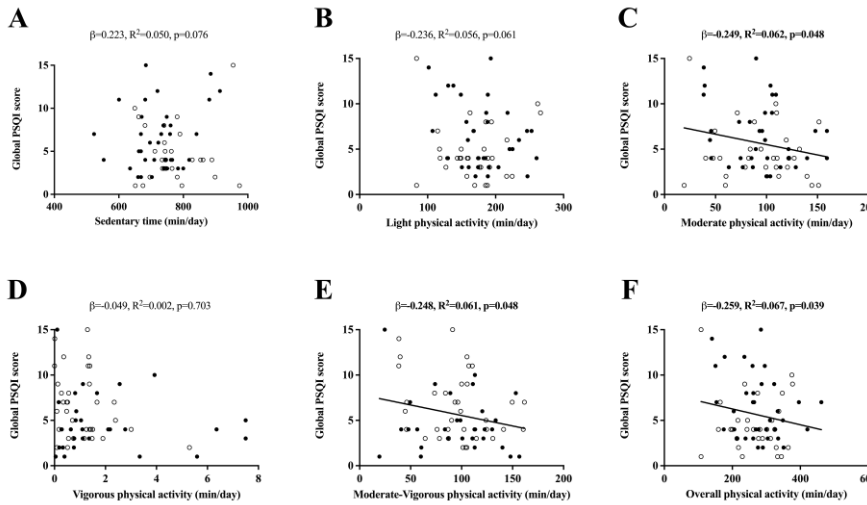

Figure 2. Association of physical activity levels with subjective sleep quantity and quality in sedentary middle-aged adults. β (standardized regression coefficient), R^2, and *p*-value from a simple linear regression analysis. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index.

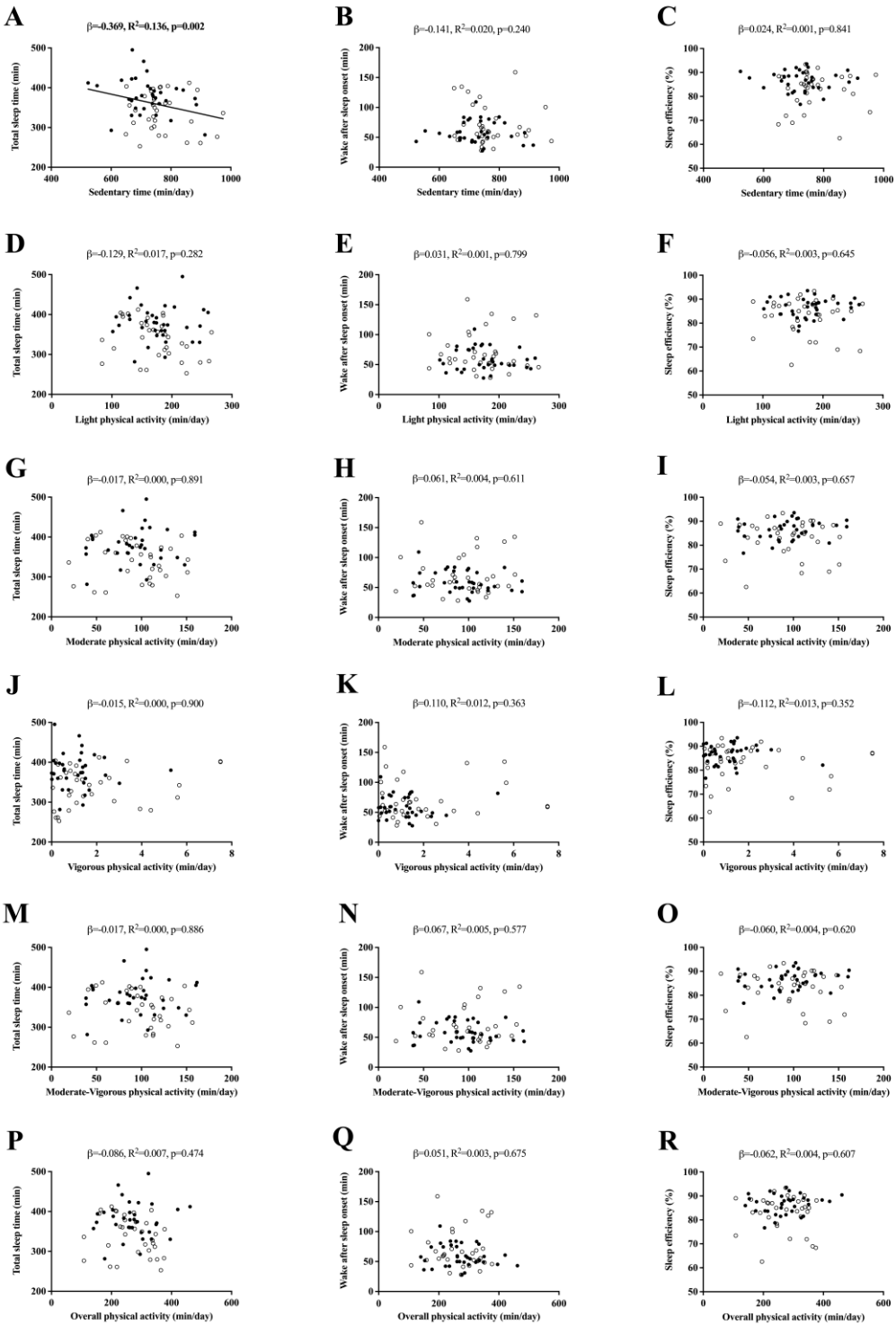

Figure 3. Association of physical activity levels with objective sleep quantity and quality in sedentary middle-aged adults. β (standardized regression coefficient), R^2, and p-value from a simple linear regression analysis. Significant p-values (< 0.05) are in bold.

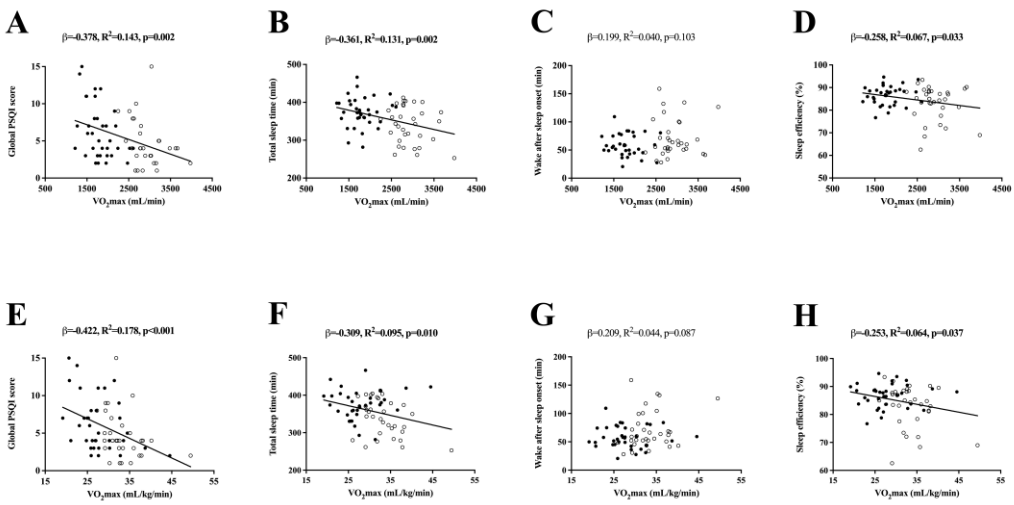

Figure 4. Association of cardiorespiratory fitness with both subjective and objective sleep quantity and quality in sedentary middle-aged adults. β (standardized regression coefficient), R^2, and p-value from a simple linear regression analysis. Significant p-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; VO_2max: maximal oxygen uptake.

and global PSQI score (β = –0.422, R^2 = 0.178, p < 0.001, Figure 4E).

Figure 5 shows the association of muscular strength with subjective sleep quantity and quality. Extension peak torque, flexion peak torque, and hand grip strength were negatively associated with global PSQI score (β = –0.334, R^2 = 0.112, p = 0.007, Figure 5A; β = –0.345, R^2 = 0.119, p = 0.005, Figure 5C; β = –0.375, R^2 = 0.141, p = 0.002, Figure 5E, respectively). Extension peak torque, flexion peak torque, and hand grip strength were negatively associated with global PSQI score when expressed relative to body weight (β = –0.313, R^2 = 0.098, p = 0.011, Figure 5B; β = –0.315, R^2 = 0.099, p = 0.011, Figure 5D; β = –0.366, R^2 = 0.134, p = 0.002, Figure 5F, respectively).

Figure 6 shows the association of muscular strength with objective sleep quantity and quality. Negative associations of extension peak torque, flexion peak torque, and hand grip strength with TST were observed (β = –0.346, R^2 = 0.119, p = 0.004, Figure 6A; β = –0.294, R^2 = 0.087, p = 0.015, Figure 6G; β = –0.413, R^2 = 0.170, p < 0.001, Figure 6M, respectively). Negative associations of extension peak torque and hand grip strength with TST were observed when expressed relative to body weight (β = –0.284, R^2 = 0.081, p = 0.019, Figure 6D; β = –0.413, R^2 = 0.171, p < 0.001, Figure 6P, respectively). No association of flexion peak torque with TST was observed when expressed relative to body weight ($p \geq 0.05$, Figure 6J). No associations of extension peak torque and flexion peak torque with WASO and SE were observed (all $p \geq 0.05$, Figures 6B,

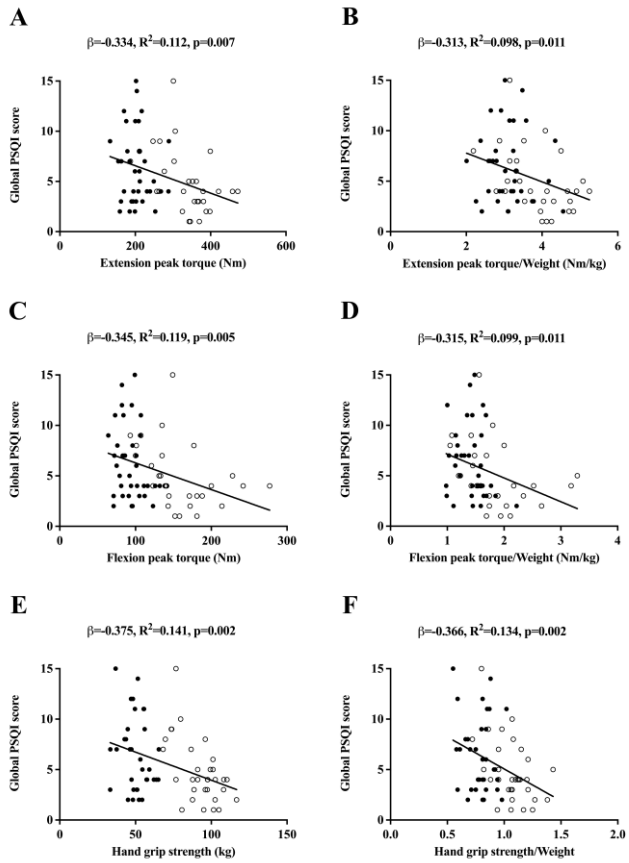

Figure 5. Association of muscular strength with subjective sleep quantity and quality in sedentary middle-aged adults. β (standardized regression coefficient), R^2, and p-value from a simple linear regression analysis. Significant p-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index.

6C, 6H, and 6I). No associations of extension peak torque and flexion peak torque with WASO and SE were observed when expressed relative to body weight (all $p \geq 0.05$, Figures 6E, 6F, 6K, and 6L). A negative association of hand grip strength with SE was observed (β = –0.294, R^2 = 0.087, p = 0.013, Figure 6O). A negative association of hand grip strength with SE was observed when expressed relative to body weight (β = –0.346, R^2 = 0.119, p = 0.003, Figure 6R). No association of hand grip strength with WASO was observed ($p \geq 0.05$, Figure 6N). A positive association of hand grip strength with WASO was observed when expressed relative to body weight (β = 0.287, R^2 = 0.083, p = 0.016, Figure 6Q).

Almost all of the above-mentioned findings persisted once age and FMI were included in the statistical models (Tables 2 and 3).

DISCUSSION

Our study was aimed at investigating the association of sedentariness, physical activity levels and fitness with sleep

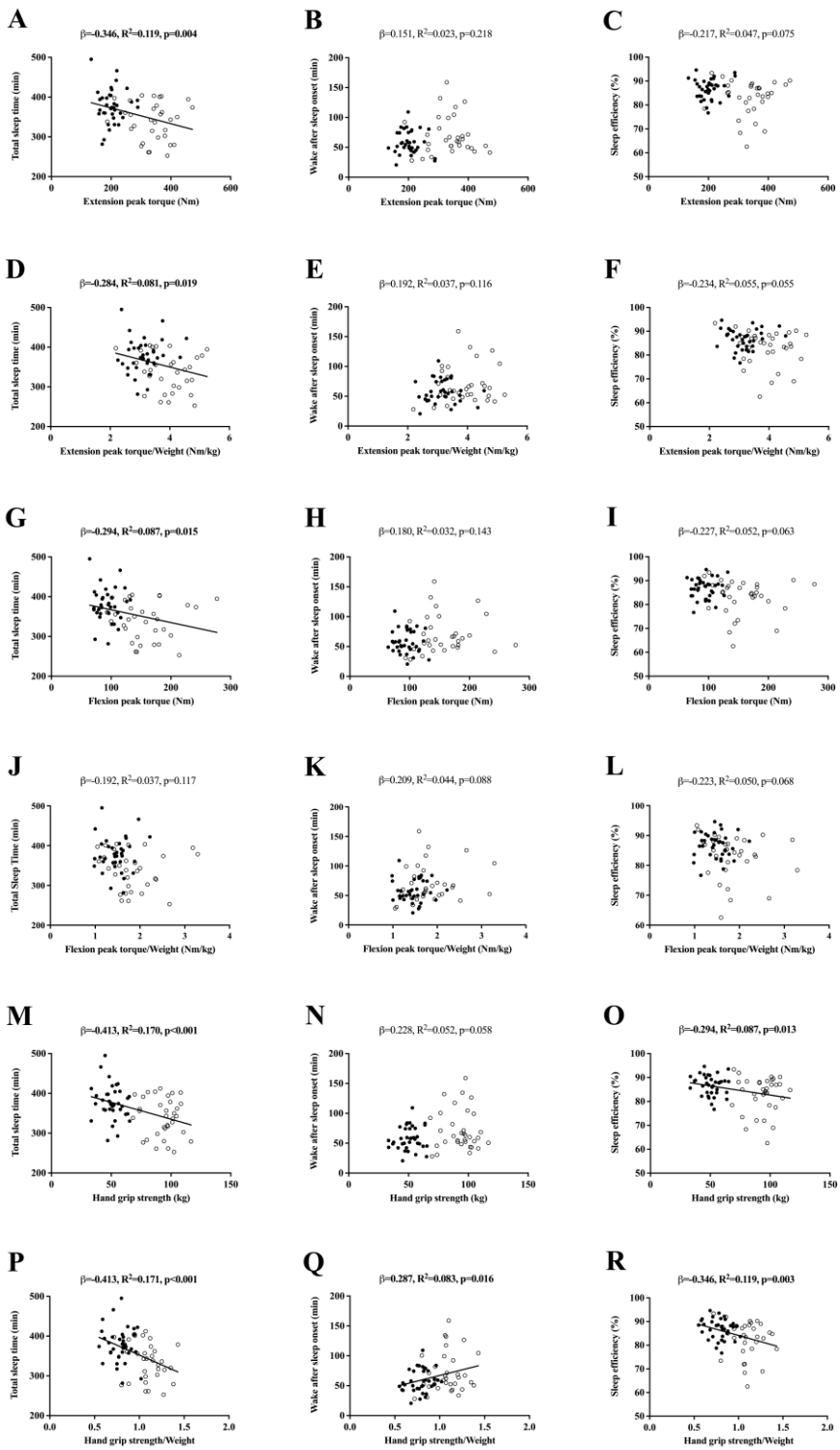

Figure 6. Association of muscular strength with objective sleep quantity and quality in sedentary middle-aged adults. *β* (standardized regression coefficient), R^2, and *p*-value from a simple linear regression analysis. Significant *p*-values (< 0.05) are in bold.

Table 2. Association of physical activity levels with sleep quantity and quality (Model 0) adjusted by age (Model 1) and by fat mass index (Model 2).

	Global PSQI score			Total sleep time			Wake after sleep onset			Sleep efficiency		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
Sedentary time (min/day)												
Model 0	0.223	0.050	0.076	–0.369	0.136	**0.002**	–0.141	0.020	0.240	0.024	0.001	0.841
Model 1	0.228	0.095	**0.048**	–0.368	0.142	**0.006**	–0.141	0.020	0.496	0.024	0.001	0.964
Model 2	0.231	0.073	0.100	–0.351	0.206	**<0.001**	–0.160	0.094	**0.035**	0.044	0.090	**0.041**
Light physical activity (min/day)												
Model 0	–0.236	0.056	0.061	–0.129	0.017	0.282	0.031	0.001	0.799	–0.056	0.003	0.645
Model 1	–0.234	0.098	**0.043**	–0.134	0.024	0.431	0.030	0.001	0.952	–0.055	0.003	0.888
Model 2	–0.232	0.073	0.098	–0.139	0.102	**0.025**	0.039	0.070	0.085	–0.065	0.092	**0.037**
Moderate physical activity (min/day)												
Model 0	–0.249	0.062	**0.048**	–0.017	0.000	0.891	0.061	0.004	0.611	–0.054	0.003	0.657
Model 1	–0.223	0.092	0.053	–0.006	0.007	0.800	0.066	0.005	0.848	–0.058	0.004	0.878
Model 2	–0.236	0.075	0.094	0.006	0.083	0.052	0.041	0.070	0.084	–0.031	0.089	**0.042**
Vigorous physical activity (min/day)												
Model 0	–0.049	0.002	0.703	–0.015	0.000	0.900	0.110	0.012	0.363	–0.112	0.013	0.352
Model 1	–0.021	0.044	0.257	–0.004	0.007	0.801	0.115	0.014	0.629	–0.117	0.014	0.618
Model 2	–0.055	0.023	0.498	–0.039	0.085	0.050	0.131	0.086	**0.048**	–0.137	0.107	**0.022**
Moderate-vigorous physical activity (min/day)												
Model 0	–0.248	0.061	**0.048**	–0.017	0.000	0.886	0.067	0.005	0.577	–0.060	0.004	0.620
Model 1	–0.221	0.091	0.054	–0.006	0.007	0.800	0.072	0.006	0.823	–0.064	0.005	0.855
Model 2	–0.235	0.074	0.095	0.003	0.083	0.052	0.049	0.071	0.082	–0.039	0.089	**0.041**
Total physical activity (min/day)												
Model 0	–0.259	0.067	**0.039**	–0.086	0.007	0.474	0.051	0.003	0.675	–0.062	0.004	0.607
Model 1	–0.244	0.102	**0.037**	–0.084	0.014	0.630	0.051	0.003	0.896	–0.063	0.004	0.859
Model 2	–0.250	0.082	0.074	–0.082	0.090	**0.041**	0.047	0.071	0.083	–0.058	0.091	**0.039**

β (standardized regression coefficient), R^2, and *p*-value of simple and multiple-regression analysis. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index.

Table 3. Association of physical fitness (including cardiorespiratory fitness and muscular strength) with sleep quantity and quality (Model 0) adjusted by age (Model 1) and by fat mass index (Model 2).

	Global PSQI score			Total sleep time			Wake after sleep onset			Sleep efficiency		
	β	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*	*β*	R^2	*p*
VO_2max (mL/min)												
Model 0	–0.378	0.143	**0.002**	–0.361	0.131	**0.002**	0.199	0.040	0.103	–0.258	0.067	**0.033**
Model 1	–0.362	0.163	**0.004**	–0.365	0.132	**0.010**	0.202	0.040	0.261	–0.263	0.069	0.097
Model 3	–0.366	0.148	**0.007**	–0.335	0.171	**0.002**	0.172	0.081	0.063	–0.228	0.118	**0.017**
VO_2max (mL/kg/min)												
Model 0	–0.422	0.178	**<0.001**	–0.309	0.095	**0.010**	0.209	0.044	0.087	–0.253	0.064	**0.037**
Model 1	–0.404	0.193	**0.001**	–0.314	0.097	**0.036**	0.213	0.045	0.226	–0.260	0.067	0.105
Model 3	–0.458	0.183	**0.002**	–0.247	0.107	**0.026**	0.125	0.064	0.117	–0.165	0.087	0.052
Extension peak torque (Nm)												
Model 0	–0.334	0.112	**0.007**	–0.346	0.119	**0.004**	0.151	0.023	0.218	–0.217	0.047	0.075
Model 1	–0.304	0.131	**0.013**	–0.346	0.119	**0.016**	0.167	0.031	0.359	–0.233	0.056	0.155
Model 3	–0.324	0.112	**0.025**	–0.305	0.160	**0.003**	0.108	0.070	0.093	–0.169	0.104	**0.028**
Extension peak torque/Weight (Nm/kg)												
Model 0	–0.313	0.098	**0.011**	–0.284	0.081	**0.019**	0.192	0.037	0.116	–0.234	0.055	0.055
Model 1	–0.283	0.119	**0.020**	–0.284	0.081	0.064	0.210	0.047	0.211	–0.252	0.064	0.115
Model 3	–0.368	0.103	**0.034**	–0.199	0.098	**0.035**	0.085	0.064	0.115	–0.118	0.087	0.053
Flexion peak torque (Nm)												
Model 0	–0.345	0.119	**0.005**	–0.294	0.087	**0.015**	0.180	0.032	0.143	–0.227	0.052	0.063
Model 1	–0.325	0.147	**0.007**	–0.292	0.088	0.051	0.186	0.038	0.282	–0.233	0.057	0.149
Model 3	–0.339	0.119	**0.020**	–0.237	0.122	**0.015**	0.118	0.072	0.088	–0.159	0.100	**0.033**
Flexion peak torque/Weight (Nm/kg)												
Model 0	–0.315	0.099	**0.011**	–0.192	0.037	0.117	0.209	0.044	0.088	–0.223	0.050	0.068
Model 1	–0.302	0.133	**0.012**	–0.190	0.039	0.275	0.212	0.049	0.197	–0.226	0.054	0.166
Model 3	–0.348	0.101	**0.036**	–0.067	0.073	0.084	0.108	0.067	0.104	–0.101	0.084	0.058
Hand grip strength (kg)												
Model 0	–0.375	0.141	**0.002**	–0.413	0.170	**<0.001**	0.228	0.052	0.058	–0.294	0.087	**0.013**
Model 1	–0.350	0.165	**0.003**	–0.408	0.171	**0.002**	0.233	0.054	0.158	–0.301	0.089	**0.044**
Model 3	–0.365	0.142	**0.007**	–0.368	0.210	**<0.001**	0.181	0.096	**0.034**	–0.243	0.140	**0.006**
Hand grip strength/Weight												
Model 0	–0.366	0.134	**0.002**	–0.413	0.171	**<0.001**	0.287	0.083	**0.016**	–0.346	0.119	**0.003**
Model 1	–0.345	0.163	**0.003**	–0.409	0.173	**0.002**	0.291	0.084	0.053	–0.349	0.121	**0.013**
Model 3	–0.425	0.142	**0.008**	–0.370	0.175	**0.002**	0.211	0.095	**0.035**	–0.267	0.133	**0.008**

β (standardized regression coefficient), R^2, and *p*-value of simple and multiple-regression analysis. Significant *p*-values (< 0.05) are in bold. PSQI: Pittsburgh Sleep Quality Index; VO_2max: maximal oxygen

quantity and quality in sedentary middle-aged adults. Results from our study showed that lower levels of overall physical activity, VO_2max, and muscular strength were related to worse subjective sleep quality in sedentary middle-aged adults. Furthermore, higher levels of sedentariness were related to worse objective sleep quantity in sedentary middle-aged adults.

Physical activity has been postulated as an effective tool to improve sleep quantity and quality [27], mainly due to its regulatory role on the circadian rhythmicity [50]. Indeed, poor levels of physical activity and a pattern of sedentary behaviour have both been proposed as important risk factors for insomnia and sleep disturbances in adults [30]. In our study, we found that higher levels of overall physical activity were associated with better subjective sleep quality. Moreover, we found that reduced levels of sedentariness were related to improved objective sleep quantity. A systematic review and meta-analysis by Kredlow et al. [27]—which examined the effects of acute and regular exercise on a range of sleep outcomes including 66 studies in the analyses and 2,863 participants of all ages—found small to medium significant beneficial effects of acute and regular exercise on TST, SE, and WASO, and a large positive impact of regular exercise on overall subjective sleep quality. Interestingly, it was reported that the impact of exercise on objective sleep outcomes was higher in young adults. This fact could explain the observed non-significant associations between physical activity and objective sleep outcomes in our study sample (i.e., older adults). In this sense, a recently published original study by Mitchell et al. [51] including 353 women also reported no evidence of a significant relationship of physical activity with sleep quantity and quality both measured by accelerometry. Moreover, Sloan et al. [52] examined the independent, joint, and fully combined associations of objectively measured sedentary behaviour and MVPA with self-reported sleep quality in 757 healthy working adults aged 21–64 years old. They showed that sedentary behaviour and MVPA levels were not significantly associated with sleep quality, which partially concur with the present findings.

Gathered research has well-established that VO_2max is a powerful marker of health and longevity [10]. As the prevalence of sleep disturbances significantly increases in older adults [53], maintaining a correct level of VO_2max while aging may also have beneficial effects on sleep quality, reducing therefore the incidence of sleep disorders in the elderly. Dishman et al. [54] suggested that the maintenance of an appropriated cardiorespiratory fitness during middle-age—when the decline in fitness typically accelerates and an increased risk of developing sleep disturbances appears—may be a protective factor against the onset of sleep complaints. However, there is a reduced

number of available studies measuring VO_2max and sleep quantity and quality parameters using reliable and/or gold-standard methods and specifically including healthy samples, who have not yet suffered the development of degenerative diseases caused by the aging process. Thus, studying the association of VO_2max with sleep quantity and quality in healthy samples using reliable measurements could play an important role in the prevention of the most common sleep disorders, i.e., insomnia and obstructive sleep apnoea. In our study, we found that higher levels of VO_2max were associated with better subjective sleep quality. Our results support those found in previous studies where lower VO_2max was associated to worse sleep quality and insomnia. Strand et al. [32] showed an inverse association between subjectively measured insomnia and VO_2peak, independently to self-reported physical activity. Similarly, Zou et al. [33] also reported that insomnia was related to lower VO_2max in middle-aged men (aged 50–64 years), independently to body composition, living conditions, comorbidities, and lifestyles.

Muscular strength is considered to be a powerful predictor of health and all-cause mortality [9]. According to previous research [55], lower levels of muscular strength may be an important risk factor for poor sleep quality in middle-aged adults, related to severe sleep disorders such as obstructive sleep apnoea, which is characterised by repetitive events of upper airway collapse during sleep due to atony of respiratory muscles. To the best of our knowledge, the association of muscular strength with sleep quantity and quality has not been widely studied in healthy sedentary middle-aged adults using both subjective and objective sleep measurement methods. Furthermore, results found in this field of research are still inconsistent due to several reasons such as different study designs, covariate variables (e.g., age and sex), and different measurements of sleep duration. In our study, our results showed that higher levels of muscular strength were associated with better subjective sleep quality in sedentary middle-aged adults. These results support the findings obtained by previous studies [56] where hand grip strength was associated with subjective sleep quality. Chen et al. [34] found that in older adults (aged 65 years and older) hand grip strength differed between sleep duration groups, observing that short and long sleepers had weaker hand grip strength than the mid-range sleepers. Similarly, Wang et al. [35] studied a middle-aged and older population obtaining that shorter or longer sleep may predict a weaker follow-up grip strength and a faster rate of hand grip strength decline over time compared to intermediate sleep duration. The analysis of these potential differences in muscular strength depending on short, intermediate and long sleep duration was not possible in our study due to the lack of the long sleepers group in our sample.

Regarding the relationship of both VO_2max and muscular strength with objective sleep quantity and quality, contradictory results were observed. Differences between subjective and objective sleep measurements have been previously reported. Indeed, previous works have shown weak or inconsistent correlations of subjective measures (i.e., PSQI scores) with objective measures (e.g., actigraphy and polysomnography) [57–59]. Concretely, a previous study conducted in young adults observed that the PSQI and actigraphy could measure different attributes of sleep, reporting the inadequate capacity of an accelerometer to detect wakefulness, thus lying in bed awake but motionless is likely to be coded as sleep [60]. Due to this limitation, using both complementary assessment methods to obtain detailed information beyond the limited data derived from body movements, is highly recommended [61]. PSQI may reflect the overall psychological state of the person, rather than actual quantity or quality of sleep [58]. Furthermore, it is still not well-defined what a "good night's sleep" actually involves in the perception of the sleeper and stand out that many factors play a role when judging sleep quality [62]. However, PSQI validity is further supported by similar differences between groups using PSQI or polysomnographic sleep measures, and has been used in a wide range of population-based and clinical studies [59].

Our results regarding the impact of physical activity on perceived sleep quality are noteworthy as subjective sleep quality has been related to a vast number of outcomes such as well-being and successful aging, cognitive decline, daytime functioning, and mental health in healthy individuals. Furthermore, poor perceived sleep quality in patients with insomnia has been shown to contribute to the maintenance of sleep disturbances [63] and, therefore, may be an important target in interdisciplinary interventions. Our study has significant implications for research and clinical practice. Consistent with preceding research, we demonstrated that physical activity, cardiorespiratory fitness and muscular strength have a beneficial effect on sleep quality in healthy sedentary middle-aged adults, as well as sedentariness having a negative impact on sleep-related parameters. Thus, increased physical activity and physical fitness may be a potential strategy to prevent and/or treat sleep disturbances. Our inclusion of the gold-standard measure of sedentariness and physical activity provide a more reliable and robust perspective on this field of research.

Our findings, however, must be interpreted with caution as they are limited to the sample included and study design. Firstly, the cross-sectional study design does not allow to establish causal inferences, so future well-designed longitudinal studies are needed in order to clarify causality. Regarding the

sample, our study only included healthy sedentary middle-aged adults, so generalization of results to a wider population may not be possible. Although accelerometry was used as an objective measure of sleep quantity and quality—which has been shown to be as reliable as the gold standard measure for sleep, i.e., polysomnography, when associating physical activity and sleep—it may overestimate TST and SE, as well as underestimate sleep onset latency and WASO in adults [31]. Thus, future research should include, apart from accelerometry as an objective measure of physical activity, polysomnography as the most reliable measure of sleep quantity and quality, also providing data on more specific sleep outcomes such as sleep architecture (i.e., rapid eye movement [REM] sleep stage, and non-REM sleep stages [N1, N2, and N3]).

CONCLUSIONS

In accordance with our findings, lower levels of overall physical activity, VO_2max, and muscular strength were related to worse subjective sleep quality in sedentary middle-aged adults. Furthermore, higher levels of sedentariness were related to worse objective sleep quantity in sedentary middle-aged adults. Reduced sedentariness and increased physical activity and fitness may be a potential prevention and/or treatment pathway to reduce sleep disturbances and, in general, to improve patients physical and psychological health for a successful aging process.

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GENERAL DISCUSSION

AN INTEGRATIVE DISCUSSION OF THE INTERNATIONAL DOCTORAL THESIS

Sleep is a well-known key component of physiological restitution [1,2], essential for mental and physical health, and thus general well-being [3,4], which includes one third part of the life of the human being [5]. In this sense, sleep plays an important role in numerous vital functions as development, energy conservation, brain waste clearance, modulation of immune responses, cognition, performance, vigilance, disease, and psychological state [6].

Current lifestyle choices—mainly characterized by novel technological and sociocultural rhythms—have led to altered sleep patterns which are related to an increased morbidity and mortality risk [7]. The prevalence of sleep disorders in the general population has been substantially increased in the last decade [8–10], becoming on an economic and clinical burden for the health system [11]. Thereby, given that sleep is vital for restoration and preservation of multiple physiological systems, the development of both generalized and personalized sleep promotion strategies to encourage a healthy sleep pattern results crucial in order to avoid significant public health burdens. Thus, the role of sleep on health and aging-related markers needs to be deeply studied.

Previous studies have shown that sleep takes an important role in the modulation of several health and aging-related markers. However, some incongruencies have been found in the scientific literature. Therefore, we investigated the relationships of sleep quantity and quality with body composition (Study 1), energy metabolism (Study 2), cardiometabolic risk (Study 3), hematological parameters (Study 4), S-Klotho anti-aging protein (Study 5), and physical activity and fitness (Study 6) in sedentary middle-aged adults.

Sleep and body composition

Aging is associated with both sleep pattern and body composition changes [12,13], which are linked to several diseases (e.g., coronary heart disease, type 2 diabetes, obesity, sarcopenia, and osteoporosis, among others [14–18]). Previous studies have observed a link of sleep quality with bone mineral density [19], muscle mass [20], and fat mass [21], suggesting that a poor sleep quality (i) deregulates the catabolic/anabolic cycle rising the risk of osteoporosis [22], (ii) favors high insulin resistance increasing the risk of sarcopenia [23], and (iii) induces metabolic and endocrine disturbances augmenting the risk of obesity [24]. However, sleep-related parameters were measured through subjective validated questionnaires instead of by both subjective and objective measurement methods. The results of this International Doctoral Thesis indicate that a poor subjective

sleep quality was negatively associated with bone mineral content, bone mineral density, lean mass, and lean mass index, whereas positively associated with fat mass percentage in sedentary middle-aged adults. Thereby, we suggest that a healthy sleep pattern could take a significant role in the prevention of body composition status alterations (i.e., increased bone mineral density and lean mass, and decreased fat mass).

Sleep and energy metabolism

Sleep pattern variations could be a risk factor for the development of obesity and cardiometabolic diseases [25]. Previous studies have found that poor sleep quantity and quality may decrease basal metabolic rate and basal fat oxidation [26], while sleep deprivation may not affect maximal fat oxidation in young adults [27]. Nevertheless, there is a lack of studies investigating the relationships of both subjective and objective sleep quantity and quality with basal metabolic rate and fuel oxidation in basal conditions and during exercise. Moreover, unhealthy sleep patterns could increase food consumption and, consequently, energy intake [28]. In this line, previous studies have demonstrated that dietary intake could influence basal fat oxidation and maximal fat oxidation [29–31]. Therefore, one of the possible causes of the association of sleep parameters with basal metabolic rate and fuel oxidation could be dietary modifications. However, there is also a lack of evidence testing the mediating role of dietary intake in the association of sleep outcomes with energy metabolism parameters. The results of this International Doctoral Thesis show that a poor subjective sleep quality is associated with lower basal fat oxidation, which is not mediated by dietary intake in sedentary middle-aged adults. Thus, we suggest that improving sleep quality could be considered a potential prevention and/or treatment pathway to reduce metabolism alterations related to lower basal fat oxidation, independently of dietary patterns.

Sleep and cardiometabolic risk

Sleep and cardiometabolic risk share common mechanisms and physiological pathways [32]. Disturbed sleep quantity and quality affect dietary pattern and physical activity levels leading to diverse physiological alterations such as greater oxidative stress, systemic inflammation, endothelial dysfunction, altered hormonal secretion, and sympathetic systemic activation producing a subsequent increase of cardiometabolic risk through hypertension, dyslipidaemia or obesity [33]. Several studies have observed that sleep quantity and quality are closely related to various cardiometabolic risk factors [34–37]. Nonetheless, they have commonly assessed sleep-related parameters by subjective measures and applying different type of analysis in heterogeneous populations obtaining controversial findings [34–37]. The results of this International Doctoral Thesis indicate that a poor subjective sleep quality is

related to higher levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides, and with lower levels of high-density lipoprotein cholesterol in sedentary middle-aged adults. Interestingly, we found that a poor objective sleep quantity is associated with greater waist circumference and higher levels of plasma glucose, but not to other markers of cardiometabolic risk in sedentary middle-aged adults. Therefore, we suggest that a healthy sleep pattern could play an important role in the prevention of abdominal fat accumulation and both glucose and lipid profiles alterations.

Sleep and hematological parameters

Sleep alterations could modify the homeostasis of both immune and hemostatic systems [38–40]. However, controversial findings have been reported in previous scientific researches. While some studies have shown a significant relationship of sleep quantity and quality with leukocytes [41–45] and platelets [40,46,47] parameters, others observed no association with leukocytes [48] or platelets [44,48–52] parameters. Nevertheless, it has been also reported no relationship of short sleep duration with leukocytes but a significant association with long sleep duration [53,54]. The results of this International Doctoral Thesis show that sleep quantity and quality are not associated with leukocyte parameters. However, we showed that a poor subjective sleep quality is associated with a lower mean platelet volume in sedentary middle-aged adults. Thereby, we suggest that health promotion and disease prevention strategies including sleep as a key modifiable factor in order to achieve an appropriate sleep quality could be considered a potential strategy for an optimal functioning of the hemostatic system.

Sleep and the S-Klotho anti-aging protein

Sleep and S-Klotho anti-aging protein are connected by common mechanisms and physiological pathways modulating the ageing process in adults since it has been demonstrated that they have a significant role in the endocrine [55–58] and immune systems [59,60], therefore being involved in oxidative stress [61,62] and chronic inflammation [63–65] processes—the principal molecular mechanisms behind all age-related consequences [66–69]. Up till now, available evidence on the relationship between sleep and S-Klotho is notably limited. A previous study by Pákó et al. [70] showed reduced levels of S-Klotho in patients with obstructive sleep apnoea, potentially enhancing the systemic inflammation and endothelial dysfunction related with this sleep-related breathing disorder. Likewise, another empirical study also reported that sleep deprivation had an adverse effect on S-Klotho responses to exercise testing in healthy adults [71]. Conversely, results from a recent study on the role of S-Klotho as a potential biomarker of stress concluded that unsatisfactory sleep was

positively associated to increased S-Klotho levels, although it should be note that sleep was only subjectively measured [72]. The results of this International Doctoral Thesis indicate that a poor subjective sleep quality is related to lower levels of S-Klotho plasma levels in sedentary middle-aged adults. Thus, we suggest that improving sleep quality could be considered a novel anti-aging therapeutic approach for the prevention, slowing, and even reversal of the physiological decline and degenerative pathologies linked to the aging process.

Physical activity, fitness and sleep

Increased physical activity may be highly effective at improving sleep quantity and quality, being proposed as an alternative treatment for sleep disorders/disturbances [73–77]. Similarly, a positive relationship of cardiorespiratory fitness and muscular strength with sleep have been previously reported [78–81]. Nevertheless, although sedentary behaviour, poor physical activity levels, and a decline of physical fitness appear to be indicators for sleep status, there is a lack of studies in this field of research analysing the relationship of sedentariness, physical activity, and physical fitness measured by gold standard methods with sleep quantity and quality in sedentary middle-aged adults. The results of this International Doctoral Thesis show that lower levels of overall physical activity, VO_2max, and muscular strength are related to worse subjective sleep quality in sedentary middle-aged adults. Moreover, higher levels of sedentariness are associated with worse objective sleep quantity in sedentary middle-aged adults. Therefore, we suggest that reduced sedentariness and increased physical activity and fitness may be a potential prevention and/or treatment pathway to reduce sleep alterations and, at large, to improve patients physical and psychological health for a successful ageing process.

GENERAL LIMITATIONS

The findings presented in this International Doctoral Thesis should be considered cautiously since some limitations should be addressed:

- The studies contained in the International Doctoral Thesis present a cross-sectional design, and thus, it is not allowed to establish causal inferences between the variables included. Therefore, well-designed intervention studies are needed to robustly analyze and establish causal relationships.

- The sample in the studies of the present International Doctoral Thesis only included healthy sedentary middle-aged adults (45 to 65 years old). Extrapolation of results to a wider population with different biological characteristics may not be possible. Thereby, it is necessary to evaluate these associations in different populations.

- The sample size of the studies included in the present International Doctoral Thesis was relatively small and future large sample size studies are needed.

- The studies of the present International Doctoral Thesis included accelerometry as an objective measure of sleep quantity and quality. Accelerometry could overestimate total sleep time and sleep efficiency, as well as underestimate wake after sleep onset in adults. Therefore, future research should include polysomnography (i.e., the gold-standard method) to appropriately assess not only sleep duration and efficiency but also other significant sleep outcomes, such as sleep architecture.

- The studies of the present International Doctoral Thesis included Pittsburgh Sleep Quality Index questionnaire as a subjective measure of sleep quantity and quality. Regarding self-reported sleep quality, it is still not well-defined what a "good night's sleep" actually involves in the perception of the sleeper, and numerous factors play a role when judging sleep quality. Thus, more research considering several nights and the inclusion of a subject-specific intercept is necessary to improve the accuracy of perceived sleep quality predictions.

- Some studies of the present International Doctoral Thesis measured blood parameters in a single time point instead of frequent sampling. Future studies should include the collection of

several samples throughout the day in order to understand the behavior of the studied parameters over 24 hours.

- One out of the eight studies of the present International Doctoral Thesis assessed dietary parameters only in a specific timepoint, which do not allow to extrapolate the results to chronic dietary patterns. Moreover, the difficulty of an accurate dietary evaluation with possible underreporting or misclassification should be considered.

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CONCLUDING REMARKS AND FUTURE PERSPECTIVES

GENERAL CONCLUSION

The results of the present International Doctoral Thesis show that subjective sleep quality is strongly related to body composition status (i.e., bone mineral density, lean mass, and fat mass), energy metabolism (i.e., basal fat oxidation), cardiometabolic risk (i.e., plasma lipid profile), hematological parameters (i.e., hemostasis), S-Klotho plasma levels, and sedentariness, physical activity and fitness (i.e., maximal oxygen uptake and muscular strength), all of them widely considered as health and aging-related parameters, in sedentary middle-aged adults. Interestingly, our results show that objective sleep quantity is specifically associated with some altered cardiometabolic risk factors (i.e., greater waist circumference and higher levels of plasma glucose), and increased levels of sedentariness in sedentary middle-aged adults. Thus, these findings suggest that sleep may be a modifiable risk factor of chronic diseases and the aging process.

SPECIFIC CONCLUSIONS

Chapter 1: Sleep and body composition

A poor subjective sleep quality was negatively associated with bone mineral content, bone mineral density, lean mass and lean mass index, whereas positively associated with fat mass percentage in sedentary middle-aged adults. Therefore, a healthy sleep pattern could take a significant role in the prevention of body composition status alterations (i.e., increased bone mineral density and lean mass, and decreased fat mass).

Chapter 2: Sleep and energy metabolism

A poor subjective sleep quality was associated with lower basal fat oxidation in sedentary middle-aged adults. Moreover, the association of global Pittsburgh Sleep Quality Index score with basal fat oxidation was not mediated by dietary intake and Mediterranean diet adherence. Thus, improving sleep quality could be considered a potential prevention and/or treatment pathway to reduce metabolism alterations related to lower basal fat oxidation, independently of dietary patterns.

Chapter 3: Sleep and cardiometabolic risk

A poor subjective sleep quality was related to higher levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides, as well as with lower levels of high-density lipoprotein cholesterol in sedentary middle-aged adults. Interestingly, a poor objective sleep quantity was

associated with greater waist circumference and higher levels of plasma glucose, but not to other markers of cardiometabolic risk in sedentary middle-aged adults. Thereby, a healthy sleep pattern could play an important role in the prevention of abdominal fat accumulation and both glucose and lipid profiles alterations.

Chapter 4: Sleep and hematological parameters

Sleep quantity and quality were not associated with leukocyte parameters. However, a poor subjective sleep quality was associated with a lower mean platelet volume in sedentary middle-aged adults. Therefore, health promotion and disease prevention strategies including sleep as a key modifiable factor in order to achieve an appropriate sleep quality could be considered a potential strategy for an optimal functioning of the hemostatic system.

Chapter 5: Sleep and the S-Klotho anti-aging protein

A poor subjective sleep quality was associated with lower S-Klotho plasma levels in sedentary middle-aged adults. Thereby, improving sleep quality could be considered an anti-aging therapeutic approach for the prevention, slowing, and even reversal of the physiological decline and degenerative pathologies that are certainly related to the aging process.

Chapter 6: Physical activity, fitness and sleep

Lower levels of overall physical activity, VO_2max, and muscular strength were related to worse subjective sleep quality in sedentary middle-aged adults. Furthermore, higher levels of sedentariness were related to worse objective sleep quantity in sedentary middle-aged adults. Therefore, reduced sedentariness and increased physical activity and fitness may be a potential prevention and/or treatment pathway to reduce sleep disturbances and, in general, to improve patients physical and psychological health for a successful aging process.

FUTURE PERSPECTIVES

- Future intervention studies are required to establish whether the previously-reported relationships present causal association in healthy middle-aged adults. Moreover, experimental studies which manipulate sleep under well-controlled lab conditions are needed.

- Future studies evaluating different populations are needed to investigate whether these findings are applicable for individuals with different biological characteristics. Moreover, future large sample size studies in healthy middle-aged adults are needed.

- Future studies measuring sleep parameters by polysomnography, known as the gold standard, are needed. Moreover, studies determining chronotype interactions are also necessary.

- Future studies assessing blood parameters for at least 24 hours to understand the behavior of the studied parameters are required.

- Precision medicine—an emerging approach for disease treatment and prevention based on individual variability in genes, environment, and lifestyle for each person—will allow researchers to accurately predict the treatment and prevention strategies in patients with sleep alterations. However, the role of precision medicine in day-to-day healthcare is relatively limited at this moment.

ANEXES

Papers derived from the International Doctoral Thesis

1 **Mochón-Benguigui S**, Carneiro-Barrera A, Castillo MJ, Amaro-Gahete FJ. Role of physical activity and fitness on sleep in sedentary middle-aged adults: the FIT-AGEING study. *Sci Rep* 2021; **11**: 539.

2 **Mochón-Benguigui S**, Carneiro-Barrera A, Castillo MJ, Amaro-Gahete FJ. Is sleep associated with the S-Klotho anti-aging protein in sedentary middle-aged adults? The FIT-AGEING Study. *Antioxidants* 2020; **9** (8): 738.

3 Jurado-Fasoli L, **Mochon-Benguigui S**, Castillo MJ, Amaro-Gahete FJ. Association between sleep quality and time with energy metabolism in sedentary adults. *Sci Rep* 2020; **10**: 4598.

4 **Mochón-Benguigui S**, Carneiro-Barrera A, Amaro-Gahete FJ, Castillo MJ. Sleep and cardiometabolic risk in sedentary middle-aged adults: The FIT-AGEING study. *In preparation.*

5 **Mochón-Benguigui S**, López-Alarcón R, Carneiro-Barrera A, Amaro-Gahete FJ, Castillo MJ. Is there a relationship of sleep with leukocyte and platelet parameters in sedentary middle-aged adults? The FIT-AGEING study. *In preparation.*

6 **Mochón-Benguigui S**, Jurado-Fasoli L, Amaro-Gahete FJ, Castillo MJ. Associazione tra sonno e composizione corporea in adulti sedentari di mezza età: Studio FIT-AGEING. *In preparation.*

Short Curriculum Vitae

Personal Information

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Education

2011 – 2017	Bachelor's degree in Medicine, University of Granada, Spain.
2018 – 2022	PhD Student in Biomedicine, University of Granada, Spain.

International Internships

2020	Obesity and Lipodystrophy Center, Endocrinology Unit, University Hospital of Pisa, Pisa, Italy. *Prof:* Ferruccio Santini. *Duration:* 3 months.
2017	National Cheng Kung University Hospital, Department of Internal Medicine, Tainan, Taiwan. *Prof:* Hsu Pei-Chun *Duration:* 1 month.
2015 – 2016	Santa Chiara Hospital and Cisanello Hospital, Obstetrics and Gynecology, Pediatric, and Urgency and Emergency Services, Pisa, Italy. *Duration:* 10 months.

Previous and Current Positions

2016 – present	Predoctoral Researcher. Department of Physiology - Faculty of Medicine, and Sport and Health University Research Institute "iMUDS", University of Granada, Spain.
2018 – present	Collaborative teaching to sanitary staff (Simulated Patient). Advanced Multifunctional Centre for Simulation and Technological Innovation "IAVANTE", Progress and Health Foundation - Junta de Andalucía, Granada, Spain.
2019 – 2021	General Practitioner. "Quirón Prevención", Granada, Spain.
2020	Forensic scientist. Institute of Legal Medicine and Forensic Sciences, Jaén, Spain.
2014 – 2017	Internal Student. Department of General Surgery and Medical-Surgical Specialties, Faculty of Medicine, University of Granada, Spain.

Research Experience

2020 – present	Time-Restricted Eating project.
2018 – present	Effect of different electrical parameters in whole-body electro-myo-stimulation on energy expenditure at rest and during exercise project.
2018 – present	BEER-HIIT project: Beer or ethanol effects on the response to high intensity interval training: A controlled study in healthy individuals.
2016 – present	FIT-AGEING project: Exercise training as S-Klotho protein stimulator in sedentary healthy adults.

Publications

1. **Mochón-Benguigui S**, Carneiro-Barrera A, Castillo MJ, Amaro-Gahete FJ. Role of physical activity and fitness on sleep in sedentary middle-aged adults: the FIT-AGEING study. Sci Rep 2021; **11**: 539.
2. **Mochón-Benguigui S**, Carneiro-Barrera A, Castillo MJ, Amaro-Gahete FJ. Is sleep associated with the S-Klotho anti-Aging protein in sedentary middle-aged adults? The FIT-AGEING study. *Antioxidants.* 2020; **9**(8): 738.
3. Jurado-Fasoli L, **Mochon-Benguigui S**, Castillo MJ, Amaro-Gahete FJ. Association between sleep quality and time with energy metabolism in sedentary adults. *Sci Rep*. 2020; **10**: 4598.
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5. **Mochón Benguigui S,** Navarro Freire F. Severe acute pancreatitis of gallblader origin with sequels: pancreatic necrosis, pseudocyst and splenic vein thrombosis. *AMU*. 2016; **4**: 37-41.
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7. **Mochón-Benguigui S**, Carneiro-Barrera A, Amaro-Gahete FJ, Castillo MJ. Sleep and cardiometabolic risk in sedentary middle-aged adults: The FIT-AGEING study. *In preparation.*
8. **Mochón-Benguigui S**, López-Alarcón R, Carneiro-Barrera A, Amaro-Gahete FJ, Castillo MJ. Is there a relationship of sleep with leukocyte and platelet parameters in sedentary middle-aged adults? The FIT-AGEING study. *In preparation.*
9. **Mochón-Benguigui S,** Carneiro-Barrera A, Dote-Montero M, Castillo MJ, Amaro-Gahete FJ. Sleep quality and anabolic/catabolic hormonal profile in sedentary middle-aged adults: The FIT-AGEING study. *In preparation.*
10. Guerrero-Pinzón JJ, Jurado-Fasoli L, Alcantara JMA, García-Buendía G, **Mochón-Benguigui S,** Ramírez-Maldonado M, Ruiz JR. A nutritional intervention for moderate-altitude endurance preparation: a case study. *Under review.*

Congress Communications as First Author

2020	[Role of sleep on insulin sensitivity and cardiometabolic risk factors in healthy adults]. 28th National Congress of the Italian Society of Diabetology, Rimini, Italy.
2020	Impact of sleep on endocrine function in sedentary middle-aged adults: The FIT-AGEING study. IV National Congress of Young Researchers in Biomedicine, Granada, Spain.
2019	[Association of sedentariness and physical activity levels with sleep quality in sedentary middle-aged adults]. II National Congress of Researchers in Training: Promoting interdisciplinarity, Granada, Spain.
2019	Is sleep quality related to the anti-ageing S-Klotho protein in middle-aged sedentary adults? III National Congress of Young Researchers in Biomedicine, Valencia, Spain.
2019	Association between physical fitness and sleep quality in middle-aged sedentary adults. I PTS Researchers Congress, Granada, Spain.
2018	[Effect of moderate alcohol consumption on sleep quality in healthy adults after a high intensity interval training program. Preliminary results of HIIT-BEER project]. VI EXERNET Symposium. Research in Exercise, Health and Wellbeing: "Exercise is Medicine", Pamplona, Spain.
2018	Association between hand grip strength and sleep quality in healthy sedentary adults: preliminary results of FIT-AGING study. I National Congress of Researchers in Training: Promoting interdisciplinarity, Granada, Spain.
2018	[Facial feminization surgery in transgender patient]. IV Biosanitary Research Students Congress, Granada, Spain.
2017	[Autoimmune hepatitis in pediatrics]. IX Update Conference in Pediatrics, Granada, Spain. *Best clinical case award.*
2015	[Severe acute pancreatitis of biliary origin with sequelae]. I IV Biosanitary Research Students Congress, Granada, Spain.

Other Merits

2020 – 2021	Co-supervisor for Undergraduate Thesis. Faculty of Medicine, University of Granada, Spain.
2015 – present	Member of the Organising Committee of 7 Biomedical Research Congresses.
2017	Member of the Organising Committee of I Military Medicine Day, Granada.

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"El agradecimiento es la memoria del corazón."

-Lao Tsé-

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"Health is the greatest possession. Contentment is the greatest treasure. Confidence is the greatest friend."

-Lao Tse-

"La salud es la mayor posesión. La alegría es el mayor tesoro. La confianza es el mayor amigo."

-Lao Tse-

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