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THE ROLE OF FATTY ACIDS IN AGING AND LONGEVITY

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ABSTRACT

Demographic evidence show a gradual increase in human longevity. However, the increasing age of the world population is a leading risk factor for several age-related diseases. For this reason, investigating the factors that contribute to a long and healthy life has become the main focus of scientific research and public health system. Among the environmental factors, a healthy diet is an important modifiable factor for sustaining healthy aging phenotypes. Specifically, fatty acids have been examined as implicated in aging process because they have critical role in maintaining cell and tissue homeostasis and affect inflammatory and oxidative processes. In this thesis, we aimed is to provide new evidence for a better understanding of the role of fatty acids in aging process and age-related phenotypes.

In Study I, we described a simple, sensitive, and reliable method for determining blood fatty acid profile. The method was based on transesterification of the fatty acids (direct, acidic transesterification), and subsequently extracting them with n-hexane. The extracted fatty acids were analysed by gas chromatography with flame ionization detection. The method validation showed satisfactory precision and linearity.

In Study II, we compared the blood fatty acid profile of a cohort of LLIs (90-111 years old, n=49) from Sicily to adults (18-64 years old, n=69) and older adults (65-89 years old, n=54) from the same area. In addition, genetic variants in key enzymes related to fatty acid biosynthesis and metabolism were genotyped to investigate a potential genetic predisposition in determining the fatty acid profile. The method described in Study I was employed to determine the fatty acid profile, and genotyping was performed using high-resolution melt analysis. The results showed that blood levels of total polyunsaturated fatty acids (PUFAs) and total trans fatty acids decreased with age, while the levels of saturated fatty acids (SFAs) remained unchanged. Interestingly, distinctively higher circulatory levels of monounsaturated fatty acids (MUFAs) in LLIs compared to adults and older adults were observed. In addition,

among LLIs the rs174537 in the fatty acid desaturase 1/2 (*FADS1/2*) gene was associated with linoleic acid (LA, 18:2n-6) and docosatetraenoic acid (DTA, 22:4n-6) levels, and the rs953413 in the elongase of very long fatty acids 2 (*ELOVL2*) was associated with DTA levels. Further, the rs174579 and rs174626 genotypes in *FADS1/2* significantly affected delta-6 desaturase activity. Overall, the results suggested that LLIs have a different fatty acid profile characterized by high MUFA content, which indicates reduced peroxidation while maintaining membrane fluidity.

In Study III, the effect of n-3 PUFAs on telomere length was assessed meta-analytically. Four databases (PubMed, Web of Sciences, Scopus, and the Cochrane Library) were searched from inception until November 2021. Of 573 records, a total of 5 clinical trials were included for the quantitative meta-analysis, comprising a total of 337 participants. The results revealed an overall beneficial effect of n-3 PUFAs on telomere length (mean difference = 0.16; 95% CI, 0.02 to 0.30; p = 0.02). Despite a limited number of studies, our analysis suggests that n-3 PUFAs may positively affect telomere length.

In study IV, the relationship between MUFA intake and sarcopenia were examined meta-analytically. A literature search was performed in three databases (PubMed, Scopus, and Web of Science) from inception until August 2022. Of 414 records, a total of 12 observational studies were identified. Ten studies were meta-analysed, comprising a total of 3704 participants. The results revealed that MUFA intake is inversely associated with sarcopenia (standardized mean difference = -0.28, 95% CI: -0.46 to -0.11; p <0.01). Overall, the results suggest that lower MUFA intake maybe associated with a higher risk of sarcopenia.

In summary, we presented new evidence on the role of fatty acids in aging and age-related phenotypes. This compiled thesis could contribute substantially to the literature on the importance of fatty acids in healthy aging from novel perspectives.

LIST OF SCIENTIFIC PAPERS

I. Ali, S.; Intrieri, M.; Pisanti, A.; Cardinale, G.; Corbi, G.; Scapagnini, G.; Davinelli, S. **Determination of n-3 index and arachidonic acid/eicosapentaenoic acid ratio in dried blood spot by gas chromatography**. Biotechniques 2022, 73, 25-33, doi:10.2144/btn-2021-0109.

II. Ali, S.; Aiello, A.; Zotti, T.; Accardi, G.; Cardinale, G.; Vito, P.; Calabrò, A.; Ligotti, M.E.; Intrieri, M.; Corbi, G., et al. **Age-associated changes in circulatory fatty acids: new insights on adults and long-lived individuals**. Geroscience 2022, 10.1007/s11357-022-00696-z, doi:10.1007/s11357-022-00696-z.

III. Ali, S.; Scapagnini, G.; Davinelli, S. **Effect of omega-3 fatty acids on the telomere length: A mini meta-analysis of clinical trials**. Biomolecular Concepts 2022, 13, 25-33, doi:10.1515/bmc-2021-0024.

IV. Ali, S.; Corbi G.; Medoro A.; Intrieri M.; Scapagnini G.; Davinelli S. **Relationship between Monounsaturated Fatty Acids and Sarcopenia: A Systematic Review and Meta-Analysis of Observational Studies**. Manuscript.

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List of Abbreviations

ADA	Aggregate desaturase activity
ALA	Alpha-linolenic acid
AD	Alzheimer's disease
ANOVA	Analysis of Variance
AA	Arachidonic acid
AWGS	Asian Working Group for Sarcopenia
BIA	Bioimpedance analysis
BMI	Body mass index
BDHQ	Brief-type self-administered diet history questionnaire
CR	Calorie restriction
CVD	Cardiovascular disease
r2	Coefficient of determination
CHD	Coronary heart disease
COX-2	Cyclooxygenases-2
5-LOX	5-lipoxygenase.
CRP	C-reactive protein
DNL	*De novo* lipogenesis
D5D	Delta-5 desaturase
D6D	Delta-6 desaturase
DASH	Dietary Approaches to Stop Hypertension
DGLA	Dihomo-gamma-linolenic acid

DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DTA	Docosatetraenoic acid
DBS	Dried blood spots
DXA	Dual-energy X-ray absorptiometry
EA	Eicosadienoic acid
EPA	Eicosapentaenoic acid
ELOVL2	Elongase of very long fatty acids 2
EFA	Essential fatty acid
EWGSOP	European Working Group on Sarcopenia
EWGSOP2	Revised EWGSOP
EVOO	Extra virgin olive oil
FADS1/2	Fatty acid desaturase 1/2
FAME	Fatty acid methyl ester
FID	Flame-ionization detector
FFQ	Food frequency questionnaire
GLA	Gamma-linolenic acid
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HWE	Hardy-Weinberg Equilibrium
HEI	Healthy Eating Index
HGS	Handgrip strength
HDL	High-density lipoprotein
HPLC	High-performance liquid chromatography
HRM	High-resolution melting
LOQ	Limit of quantitation
LA	Linoleic acid
LC-MS	Liquid chromatography-mass spectrometry
LLIs	Long-lived individuals
LDL	Low-density lipoprotein
LLOQ	Lower limit of quantitation
MS	Mass spectrometry
MedDiet	Mediterranean diet
MIND	Mediterranean-DASH Diet Intervention for Neurodegenerative Delay
MAF	Minor allele frequency
MI	Myocardial infarction
MC	Myristic acid
NA	Nervonic acid
NOS	Newcastle-Ottawa Scale
NGS	Next Generation Sequencing
Nrf2	Nuclear factor erythroid 2–related factor 2

NMR	Nuclear magnetic resonance
OL	Oleic acid
PA	Palmitic acid
PLA	Palmitoleic acid
PD	Parkinson's disease
PPAR	Peroxisome proliferator-activated receptor
PCR	Polymerase chain reaction
PUFA	Polyunsaturated fatty acid
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
Q-FISH	Quantitative-fluorescent in situ hybridization.
qPCR	Quantitative real-time polymerase chain reaction
RCT	Randomized clinical trial
ROS	Reactive oxygen species
RSD	Relative standard deviation
RF	Response factor
ROBINS-I	Risk Of Bias In Non-randomized Studies - of Interventions
SFA	Saturated fatty acid
SNP	Single nucleotide polymorphism
SIRT1	Sirtuin 1
SPPB	Short physical performance battery
SMD	Standardized mean difference
SFC	Supercritical fluid chromatography
T_a	Annealing temperature
TLC	Thin-layer chromatography
T_m	Melting temperature.
TC	Total cholesterol
TG	Triglycerides
8-f TUG	Eight-foot time up-and-go

1. INTRODUCTION

1.1 Biology of Human Aging and Longevity

Aging is a ubiquitous biological process that affects cells, tissues, organs, and organisms. As people age, the accumulation of detrimental changes at molecular, cellular, and tissue levels leads to a progressive and irreversible decline in normal physiological functions. Consequently, an individual's ability to adapt to stress and to maintain an adequate homeostasis decreases and becomes more vulnerable to the factors that ultimately lead to death [1].

Although aging is programmed and progressive, a person's longevity is thought to be determined by the combined effects of genetics and environmental factors. The main environmental factors that can modulate aging process and longevity include dietary habits, physical activity, cultural and anthropological factors, education, and socioeconomic status [1-4]. The environmental and lifestyle factors also contribute to the types of aging, i.e., successful and unsuccessful aging (**Figure 1**). According to the classic concept of Rowe and Kahn, successful aging is defined as high physical, psychological, and social functioning at old age without major diseases [5, 6]. On the other

hand, unsuccessful aging is characterized by the presence of age-related diseases, low cognitive and physical functional ability, and decreased engagement with life.

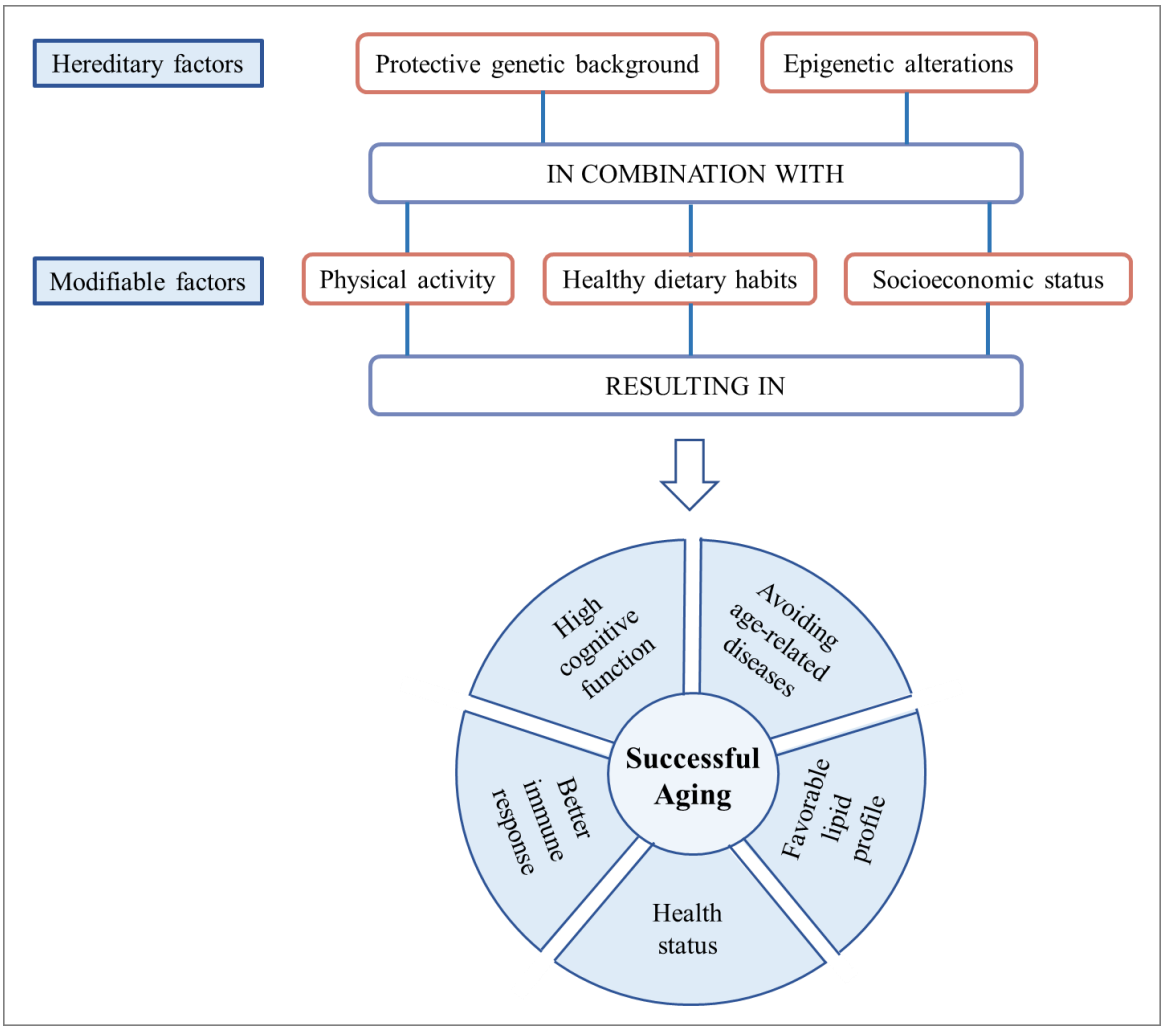


Figure 1. A graphic overview of the factors that contribute to successful aging (adapted from Anna Aiello, 2019).

Phenotypically, aging is characterized by a number of hallmarks that include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, compromised autophagy, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, alteration of intercellular communication, and deregulated nutrient sensing [7]. These hallmarks of aging are also the common determinants of age-related diseases. However, the various age-related diseases in different organs and systems have their own combinations of the hallmarks of aging. At the molecular level, aging is

determined by a number of complex and important pathways, mainly associated with chronic oxidative stress caused by elevated levels of reactive oxygen species (ROS). According to the free radical theory of aging, the progressive and detrimental changes during aging are primarily mediated by the accumulation of ROS-induced damages [8]. Furthermore, the age-related disruption in intracellular redox balance is the primary contributing factor to a chronic low-grade inflammatory status, called “inflammaging”. Inflammaging may result from age-related changes to the immune system, damaged macromolecules and the endogenous host-derived cell debris that accumulate with advancing age. This chronic inflammation negatively impacts physiological functions and contribute to the development of several age-related diseases, including cardiovascular disease (CVD), cancer, type 2 diabetes and other conditions [8].

Over the past decades, demographic evidence has shown a continuing rise in the age at death, which corresponds to a gradual increase in human longevity [9]. From 2015 to 2050, the percentage of the worldwide population aged over 60 years is estimated to double from 12% to 22% [10]. However, the increasing age of the world population is also a leading risk factor of several age-related diseases, including CVD, metabolic diseases, and neurodegenerative disorders [9]. These diseases affect normal life activities and increase the risk of physical disabilities or even death. In other words, the continuous increase of human lifespan does not correspond with the increase of health span. In addition, these diseases place a great burden on the social, economic, and the public health system. Therefore, understanding the factors that contribute to a long and healthy life is of great importance in order to improve the quality of life of older people and to reduce the medical, economic, and social problems associated with advancing years [1, 11]. For this reason, investigating the aging process and age-related diseases has become the main focus of scientific research and public health system.

The mechanisms involved in aging and age-related phenotypes are complex, which makes investigations on aging and the prevention/treatment of age-related diseases challenging. To date,

several strategies and interventions have been proposed to improve health and promote longevity, including calorie restriction (CR), nutritional interventions, and microbiota transplantation. In addition, a number of clinical treatment methods are described to decrease the incidence of age-related diseases, such as senescent cell depletion, stem cell therapy, and antioxidative and anti-inflammatory treatments [8]. Furthermore, new technologies and drug discovery methods are emerging that will further facilitate diagnostic, preventive and treatment strategies of age-related diseases, and consequently promote healthy human longevity. Particularly, the establishment and advancement in genomics, proteomics, and transcriptomics allow researchers to analyse and characterize thousands of epigenetic markers, transcripts, proteins and metabolites, that may help to better understand the overall changes at the molecular level that occur with advancing age [12].

1.2 Long-Lived Individuals as an Ideal Model of Healthy Aging

Long-lived individuals (LLIs) are people living 90 years and more without any major disease and in a relatively good physical and mental state. LLIs are considered an ideal population to study healthy human aging. Contrary to what is seen in normal aging, characterised by the disruption of homeostatic processes that might predispose people to major chronic diseases, LLIs have a lower incidence, or a higher possibility of escaping, the age-related diseases [13-16]. LLIs are divided into low and high performers, based on their ability to avoid or delay major age-related diseases. The high performing LLIs have the capacity to reach their 90s and mostly without chronic diseases. On the other hand, the low-performing LLIs endure the age-related diseases and may need family or nursing facility care [17]. The difference between low and high-performing LLIs is described to be associated with different manifestation of the hallmarks of aging, particularly telomere length and immunosenescence [18].

LLIs are described to have a genetic background that favours a long and healthy life. Additionally, they are equipped with efficient maintenance and repair systems to the different

environmental conditions, and thus a good responsiveness to environmental stressors. Besides, in the past century the overall improvements in the quality of life and dietary patterns, the establishment of a public health system, improvements in hygienic conditions, decreased risks of infection and inflammation, and the development of therapeutic and preventive medicine, are other factors that has contributed to an increased ability to reach 90 years and more [19].

The remarkable resilience of LLIs has attracted the interest of researchers over the past decades, because they represent an informative population for studying genetic and environmental variables involved in extreme longevity. Investigating the positive phenotypes of LLIs could allow us to modify the aging rate by providing important information on how to slow the aging process. Previous studies have shown that some cohorts of Sicilian populations, particularly those living in villages located in the Madonie municipalities, have a high number of LLIs compared to other areas in Sicily and Italy [20]. In 2012, it was demonstrated that this area is characterized by a low mortality rate due to CVDs and cancer. In addition, the LLIs from this area tend to be physically active and have a healthy diet, adherent to the Mediterranean diet (MedDiet). In addition, the Mini Mental State examination of LLIs from this area showed a cognitive impairment from mild to moderate [20]. Therefore, this population is considered ideal for studies aimed at understanding the factors that may determine longevity.

1.3 Effects of Healthy Nutrition on Healthy Aging

Human aging and longevity are shown to be modulated by the interaction between genetic and environmental factors. Among the environmental factors, a healthy diet is an important modifiable factor for sustaining healthy aging phenotypes. A good nutrition can have both short-term and long-term effects on the well-being and can delay and reduce the risk of age-related decline. Indeed, emerging evidence indicate that healthy nutrition not only decrease the risk of functional losses and diseases during aging, but also inhibit progression and advancement of some illnesses [21]. Hence,

the diet and the bioactive compounds present in food represent a good target for interventions. On the contrary, nutritionally unbalanced diets are often related with increased risks of numerous health problems. Eating too few fruits and vegetables, for example, was estimated to be respectively responsible for 5.6 and 7.8 million premature deaths worldwide in a study in 2013 [22]. Healthy aging is also promoted by the cumulative effects of healthy nutrition earlier in life. For example, consuming recommended levels of calcium during childhood and adolescent years, to reach a high peak bone mineral density, is necessary to reduce the incidence of osteoporosis later in life [23, 24]. Higher dietary intakes of flavonoids, for around 2 decades, is shown to be associated with lower risks of Alzheimer's disease (AD) and age-related dementia [25]. Similarly, long-term adherence to MedDiet is linked with a reduced risk of frailty, CVD, and advanced age-related macular degeneration [26-28].

Although the relationship between nutrition and aging is complex and still under investigation, a variety of dietary patterns and indices have been assessed for their role in aging process, including the MedDiet, Okinawan diet, Healthy Eating Index (HEI), the Dietary Approaches to Stop Hypertension (DASH) diet, the Mediterranean-DASH Diet Intervention for Neurodegenerative Delay (MIND) diet, and high-fiber diets. These diets and indices are reported to be linked with prevention of a wide range of age-associated illnesses, including CVD, type 2 diabetes, frailty, osteoporosis, several cancers, age-related cognitive decline and macular degeneration [21]. Additionally, clinical and observational data indicate that specific dietary components are associated with preventing unhealthy aging, such as antioxidant phytochemicals, n-3 polyunsaturated fatty acids (PUFAs), zinc, and vitamins A, B12, C, D, and E [29].

The current available evidence strongly suggests that the adherence to healthy diet help to minimize the inflammaging. Indeed, a healthy nutritional pattern can lower a proinflammatory status, presenting a novel nonpharmacological approach to manage obesity, metabolic syndrome, diabetes and other age-related conditions. Likewise, specific dietary changes, such as CR, elimination of

saturated fatty acids (SFAs) and trans fats, increasing intake of n-3 PUFAs, micronutrients and antioxidants, can help to reduce the inflammaging. Moreover, diets characterized by high quantities of n-3 PUFAs, antioxidant vitamins, and fiber may lower the risk of chronic diseases by regulating oxidative stress and may protect cells from oxidative damage by neutralizing ROS. Similarly, adequate consumption of phytochemicals may confer health benefits, enhancing the maintenance of immune homeostasis, reducing oxidative stress and inflammation, and preventing metabolic disorders. On the contrary, an unhealthy diet, containing an excessive amounts of refined sugars, animal proteins and SFAs, and poor in nutraceuticals, directly contributes to increasing cell damage and inflammation [29].

Overall, the analysis of dietary pattern may provide a meaningful recommendation for healthy aging. Moreover, the synergistic association between dietary components could influence physiological function and health [21]. More recently, with the advancements in the analytical techniques, the study of multiple parameters is more facilitated. The “Omics” platforms have provided unique opportunities to investigate the complex association between nutrition and aging, particularly to inspect the role of dietary components in health maintenance or in disease development. In the following section, we discuss lipidomic analysis and its role in nutrition and aging studies.

1.4 Lipidomic Analysis and its Importance in Nutritional and Aging Research

Lipidomics is an emerging discipline that studies the lipidome, i.e., the total lipid content in cells, organs, or biological systems, as well as their interactions with other lipids, proteins and metabolites. Lipidomic analysis has an interesting application in monitoring metabolic and nutritional status of an individual. It serves as a screening tool for metabolic disorders and to track changes in the lipid profile in nutritional studies [30]. Additionally, it is a effective way to identify potential clinical biomarkers and to determine preventive or therapeutic approaches to manage human diseases [31].

Lipidomics is a subfield of metabolomics that belongs to the final step in the omic cascade (**Figure 2**), starting from genomics, to transcriptomics, proteomics, and finally metabolomics. The metabolomics collects qualitative and quantitative data on the metabolites present in the cell that are essential for the maintenance, growth, and function of the cell. The metabolome comprises amino acids, fatty acids, carbohydrates, vitamins, and lipids, among other compounds. The metabolomics identifies and quantifies specific metabolites, often using sensitive chromatographic methods such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) [32]. Lipidomics was first described in 2003 by Han and Gross, where they reported a method based on mass spectrometry (MS) to identify and quantify the entire lipidome present a cell extract [33]. Before that, the analysis of lipids involved extraction and estimation of single lipid class or species [34]. Therefore, the measurement of the entire lipidome was an important progress over the traditional methods used at that time.

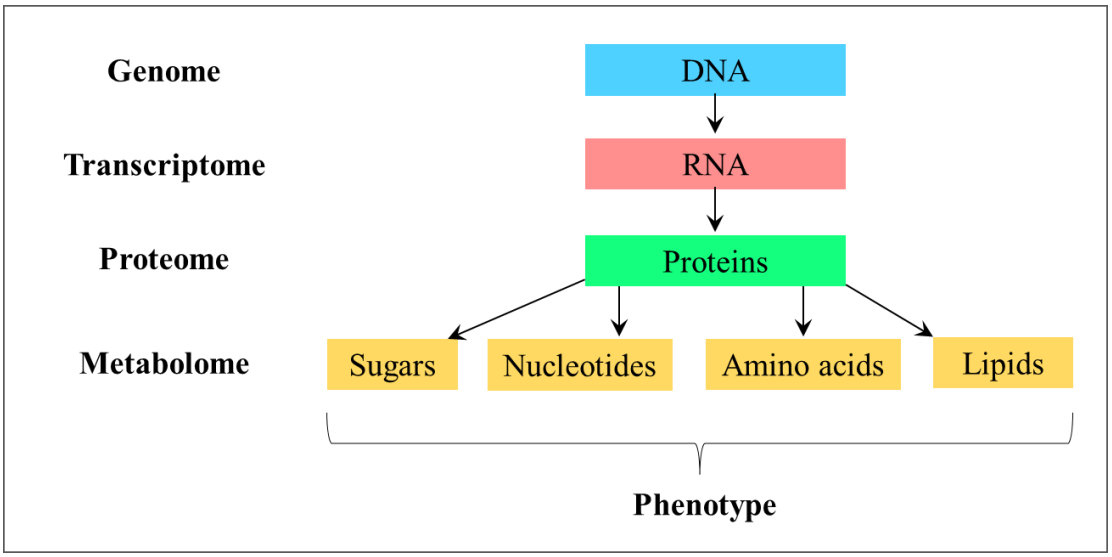


Figure 2. Summary of omics cascade in biological systems.

Lipidomic analysis has attracted considerable attention because lipids are complex and have important biological activities and unique chemical properties. Lipids, also known as fats, are hydrophobic or amphipathic macromolecules. They are essential components of the cell and can

constitute up to 70% of cell's total composition. The Lipid MAPS classification system have classified lipids into eight groups, which are fatty acyls, polyketides, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, and saccharolipids [35]. These compounds play significant roles as building blocks of cellular membranes, and in energy storage and metabolism. In addition, they have an important role in signal transduction by acting as signalling molecules, affecting membrane fluidity, and regulating post-translational modifications [36]. Nevertheless, the dysregulation of lipids is related to a number of health problems, such as CVD, obesity, diabetes, lysosomal disorders, Parkinson's disease (PD) and AD.

Moreover, accumulating evidence from experimental and human studies suggest that lipids have important roles to play in the aging process. Detrimental lipid storage and peroxidation are known to be linked with unhealthy aging. On the other hand, evidence also show that active signalling role of certain lipids may help in the regulation lifespan and longevity [36]. Although the relationship between lipids and aging is complex and not well understood, lipids are considered as a useful target to investigate aging biomarkers. Lipid-related interventions, such as sphingolipid, is reported to modulate lifespan in experimental models [37]. Likewise, specific lipids have been shown to increase or decrease with advancing age. For example, in model organisms phosphatidylcholine and phosphatidylethanolamine decrease with age, while triglycerides (TG) generally increase [38]. Healthy elderly people show higher plasma levels of sphingomyelins and phosphatidylcholines compared to young subjects [39]. Finally, genome-wide association studies have identified several lipid-related genetic variants that are associated with exceptional longevity in humans [40].

Although lipidomics belong metabolomics, the analytical procedures used in lipidomic analysis can be different from the techniques used in metabolomics. Initially, thin-layer chromatography (TLC) and gas chromatography (GC) were mainly used for lipid profiling. Nowadays, lipidome of complex samples can be analysed using GC-MS and LC-MS, high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), and nuclear

magnetic resonance (NMR) [41]. Additionally, with the rapid developments of analytical techniques, lipidomics has been increasingly used in nutritional and aging research to expand our knowledge about the lipidome of long-lived species and centenarians. The study of circulatory lipidome in LLIs and older adults without major age-related diseases is regarded as a useful tool for the identification of aging biomarkers, and to understand physiological mechanisms involved in aging and longevity [36]. Furthermore, the integration of the genomics, proteomics, and metabolomics molecular markers to comprehensively assess biological age and age-related functional decline has become a hot issue of interest for scientific community. In the following sections we will describe the fatty acids, the composition of which potentially linked with aging and longevity. We first describe the main classes of fatty acids, then we describe the role of fatty acids in human aging and age-related phenotypes.

1.5 Fatty Acids

Fatty acids are carboxylic acids with an aliphatic chain. These compounds are classified as SFAs and unsaturated fatty acids, the latter is further divided into MUFAs and PUFAs. Dietary intake is the main source of fatty acids for most mammals, including human. Moreover, several physiologically important fatty acids can be *de novo* synthesized through a series of reactions from metabolites derived from the catabolism of carbohydrates and proteins [42]. In addition, long-chain fatty acids can be synthesized from essential fatty acids through a series of desaturation and elongation reactions. Biologically, fatty acids are esterified with glycerol, phosphoglycerol, and cholesterol forming triacylglycerol, phospholipids, and cholesterol esters, respectively [43].

Fatty acids have important structural and functional roles in human body. The physicochemical properties of these compounds may affect their functions, which ultimately leads to altered functions of the cells and organisms. Fatty acids are important structural components cellular membranes. The level of different fatty acids in cell membrane can affect the fluidity, stability, and function of the membrane. When the membrane level of SFAs is high, the phospholipids form a more

rigid bilayer because SFAs are straight-chained. On the other hand, PUFAs have bents along their chain at the position of double bonds, hence, they cannot align tightly and consequently, they increase the degree of membrane fluidity [44]. Therefore, the importance of controlling and balancing of fatty acids derives from the fact that a balance between the various components regulate fluidity and permeability of the plasma membrane, and thus favouring metabolic alterations within the body. Furthermore, fatty acids are an excellent source of energy through the β-oxidation process, which is mostly used by the heart and the muscle cells. Fatty acids also affect the functions of membrane-proteins, conduct signalling cascades, and act as a precursor to a number of pro and anti-inflammatory mediators [42, 43].

1.5.1 Saturated versus Unsaturated Fatty acids

SFAs are fatty acids with aliphatic carbon chains fully saturated with hydrogen atoms or have only C-C single bond, while unsaturated fatty acids' aliphatic carbon chains contain C=C double bonds. Animal-source foods and tropical oils have a high quantity of SFAs than most plant-source foods. The most common SFA in the diet are palmitic acid (PA, 16:0) and stearic acid (STA, 18:0), which are present in high amounts in palm oil, coconut oil, egg yolk, aged cheese, butter, bacon, and processed meats. SFAs are described to have harmful health effects as they reduce membrane permeability and may cause an increase in low-density lipoprotein (LDL) levels. In fact, excessive SFA intake is correlated with a greater the risk of CVD, metabolic and dyslipidaemia [43, 45].

On the other hand, unsaturated fatty acids are called MUFAs, when they contain only one C=C double bond, and PUFAs, when they contain more than one C=C double bond. The presence of C-C single bonds or C=C double bonds is responsible for the characteristic structural differences in physical and chemical properties of these fatty acids, which consequently influences the structure and function of cellular membranes [43].

1.5.2 Essential versus Nonessential Fatty Acids

Both plant and animal cells can synthesize fatty acids. Animals and human, however, cannot synthesize some of the fatty acids, therefore, they must be obtained from the exogenous dietary sources. These fatty acids are known as essential fatty acids (EFAs) in the human body. Of all the unsaturated fatty acids, two of them are considered to be EFAs: they are linoleic acid (LA,18:2n-6) of the n-6 PUFA series and the alpha-linolenic acid (ALA, 18:3n-3) of the n-3 PUFA series. ALA serves as the precursor for the production of long-chain n-3 PUFAs, such as eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), whereas, LA serves as the precursor of long-chain n-6 PUFA, namely gamma-linolenic acid (GLA,18:3n-6), dihomo-gamma-linolenic acid (DGLA, 20:3n-6), arachidonic acid (AA, 20:4n-6) and docosatetraenoic acid (DTA, 22:4n-6) through a series of desaturation and elongation processes [46, 47]. Other fatty acids, such as SFA and MUFA, are considered nonessential as our body can synthesize them without receiving them directly from the diet [43]. Being nonessential does not mean that they are not important. Our body does require them to function properly; however, we can synthesize them without obtaining them directly from the diet. Therefore, the diet is described to have a limited influence on fatty acid status. Indeed, genetic variants that influence the desaturation and elongation of fatty acids may modulate circulating fatty acid levels. Metabolic pathways, physio-pathological disorders, and gut microbiota are other factors that affect the fatty acid profile [48].

1.5.3 Polyunsaturated Fatty Acids

Based on the location of the C=C double bonds from the methyl terminal position, PUFAs are categorized into n-3 and n-6 PUFAs. The PUFAs that have a double bond between the 6th and 7th carbon position from the methyl terminal end are called n-6 PUFAs, while those with the double bond between the 3rd and 4th carbon are called n-3 PUFAs. Both n-3 and n-6 PUFAs have important structural function and exert receptor-mediated effects through their metabolites. However, they also show great differences in their physical, biochemical, and physiological properties. Some of the

beneficial effects overlap between the n-3 and n-6 PUFAs, while many effects are antagonistic to each other. The n-6 PUFAs can be found in vegetable oils, seeds, meat, and poultry, whereas n-3 PUFAs are found more in fish, marine animals, walnuts, canola oil, and certain plant sources [43].

Over the last decades, there has been an increasing interest in researching n-3 PUFAs because of their roles in reducing the risk of numerous diseases. The estimation of n-3 PUFA levels is considered important to determine health status, disease diagnosis, and prevention strategies in human. Particularly, the health benefits of EPA and DHA have been commonly described, as they act as a precursor to a number of anti-inflammatory mediators [49]. These fatty acids play a significant role in human health and considered essential for the heart, brain, eye and joints health. By reducing inflammation, the n-3 PUFAs are associated with a lower risk of heart complications. A meta-analysis study of 14 randomized clinical trials (RCTs), involving 1,35,291 subjects, showed that n-3 PUFA supplementation can reduce the incidence of major adverse cardiovascular events, cardiovascular death, myocardial infarction (MI) in people with coronary heart disease (CHD) [50]. Additionally, higher intake of n-3 PUFAs is associated with a reduced risk of mood and neurodegenerative disorders in the elderly people [51]. On the other hand, increasing dietary intake of the n-6 PUFAs, particularly AA and LA, is described to be involved in inflammatory processes, because they act as a precursor to a number of pro-inflammatory mediators [52]. However, other studies reported that n-6 intake may not be correlated with elevated levels of inflammatory markers. Epidemiological data have also suggested that AA and LA may be related to reduced inflammation [52, 53]. Nevertheless, there is also evidence that a high intake of n-6 PUFAs oppose and inhibits the anti-inflammatory effect of the n-3 PUFAs [52]. Thus, the interaction between n-3 and n-6 PUFAs and their lipid mediators is complex and still not well understood.

When investigating n-3 PUFAs, determining the n-3 index, n-6/n-3 ratio, and AA/EPA ratio provide valuable information on PUFA status and metabolism, nutritional needs, and health outcomes.

N-3 Index

The n-3 index measures the amount of EPA plus DHA in the erythrocyte membranes expressed as the percentage of total fatty acids of erythrocyte membrane. The n-3 index has been shown to be a stable biomarker of dietary intake of long-chain n-3 PUFAs in the body. The n-3 index is used as a guide to evaluate cardiovascular health in observational and clinical studies. This index is inversely associated with depressive symptoms among subjects with elevated oxidative stress biomarkers [54]. The n-3 index also reduce the risk for CHD mortality, and hence it can have significant clinical utility. In addition, cognitive impairment is reported to be correlated with a low n-3 index in the elderly [55]. Clinical studies indicate that a protective level for the n-3 index is around 8%, while an n-3 index level of <4% is associated with the increased risk of several diseases (**Figure 3**) [56].

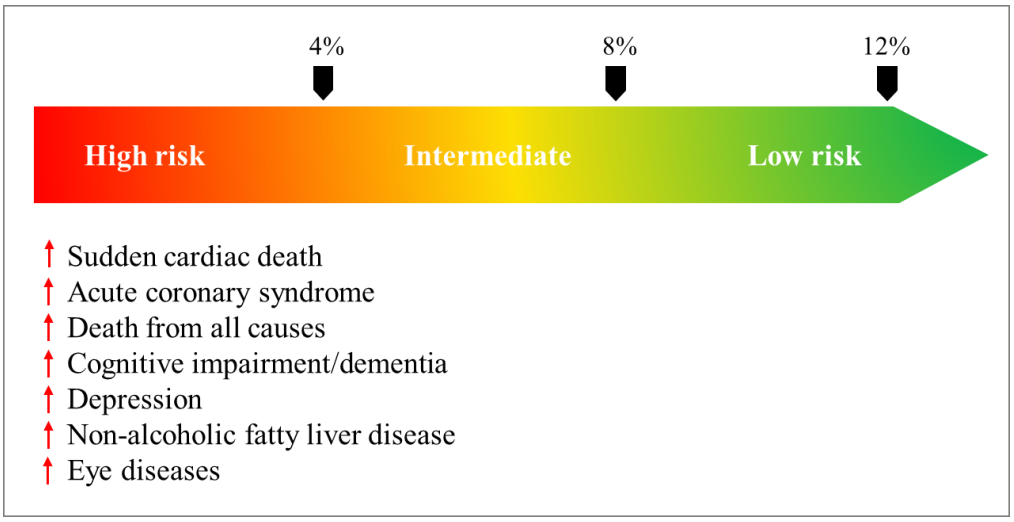


Figure 3. The protective level of n-3 index is around 8%, and a level of <4% is linked with increased risk of diseases (Adapted from Davinelli, 2020).

N-6/n-3 ratio

Both n-3 and n-6 PUFAs compete for the same metabolic enzymes, hence, an imbalance in the n-6/n-3 ratio may lead alter fluidity and function of cellular membrane, and promote an inflammatory state [57]. Therefore, the bioconversion of LA and ALA to their intermediates depends on the ratio of

consumed n-3 and n-6 PUFAs. Evidence suggest that an n-6/n-3 ratio around 1/1 to 5/1 is involved in an improved lipid metabolism, reduced inflammation and oxidative stress, and consequently improved endothelial and cardiovascular function [58, 59]. A diet with high n-3 PUFAs and low n-6/n-3 ratio is reported to prevent adverse health outcomes. For example, among CVD patients, a n-6/n-3 ratio of 4/1 reduced total death by 70%. Likewise, a ratio of 2.5/1 was associated with decreased rectal cell proliferation in colorectal cancer patients, and a ratio of 2-3/1 was related with suppressed inflammation in subjects with rheumatoid arthritis [60]. On the other hand, the n-6/n-3 ratio in the Western diets is approximately 15/1, that is described to promote the pathogenesis of many diseases, such as CVD, cancer, inflammatory and autoimmune diseases [56].

Arachidonic acid/Eicosapentaenoic acid ratio

AA/EPA is a ratio between two molecules that compete for the same enzymes to be converted to bioactive eicosanoids. This ratio shows the level of cellular inflammation in the body because the balance between AA and EPA is important to regulate the synthesis of inflammatory mediators. In addition, AA/EPA ratio is associated with nutritional status and considered to be a reliable indicator of PUFA intake. Although a range of the AA/EPA ratio has not been clearly defined, an AA/EPA ratio of 1.5-3 is considered optimal [56]. A higher AA/EPA ratio corresponds to higher levels of chronic inflammation and enhanced pro-aggregation conditions. It is well established that chronic low-grade inflammation contributes to the development of several age-related diseases. Indeed, the AA/EPA ratio is a valuable predictor of CVD risk. Epidemiological data reveal that a higher AA/EPA ratio is associated with an increased risk of coronary artery disease, acute coronary syndrome, MI, stroke, chronic heart failure [61]. Likewise, clinical data indicate that lowering AA/EPA ratio through EPA supplementation can be effective in primary and secondary prevention of cardiovascular events [61, 62]. In contrast, lower AA/EPA ratio is correlated with decreased risk of coronary artery disease, acute coronary syndrome, MI, stroke, chronic heart failure, peripheral artery disease, and mood disorders [56].

Biosynthesis and Metabolism of Polyunsaturated Fatty Acids

Two different pathways exist for the synthesis of the long chain n-3 and n-6 PUFAs (**Figure 4**). The LA and ALA cannot be synthesized in human body. Once consumed in the diet, LA and ALA are converted to long-chain PUFAs by delta-6 desaturase (D6D) and delta-5 desaturase (D5D) enzymes, and their respective fatty acid elongases. The synthesis of long-chain PUFAs from LA and ALA regulate various homeostatic processes by acting on the synthesis of bioactive signalling lipids called eicosanoids. Those in the n-6 series synthesizes the pro-inflammatory eicosanoids, whereas those in n-3 series induce the synthesis anti-inflammatory eicosanoids [56].

In the n-3 pathway, ALA undergoes a transformation to EPA by cyclooxygenases-2 and 5-lipoxygenase to form 3-series prostaglandins (B3, D3, E3, I3, and thromboxane A3), and 5-series leukotrienes (B5, C5, and D6), respectively. The EPA-derived mediators have important anti-inflammatory action. Next, EPA is elongated through two elongation processes where n-3 DPA is generated. DHA is the latest product of the n-3 pathway. The biosynthesis of DHA from DPA involves elongation and desaturation by D6D, and finally undergoes a beta-oxidation process. DHA can be then metabolized to autacoids such as D-series resolvins and protectins (neuroprotectin D1). The DHA-derived mediators exert powerful anti-inflammatory activities and play an important role in the resolution of inflammation [56].

By an analogous set of reactions catalysed by the same enzymes, LA is converted to GLA through the addition of a double bond by D6D enzyme. By the action of elongase 5 enzyme, GLA is then elongated to form DGLA, the precursor of 1-series prostaglandins. AA is generated from DGLA through a desaturation process by D5D enzyme. AA is widely present in the membranes of red blood cells, making up about 15%. Levels of this fatty acid is important as in some cases to underline the presence of a state of "silent inflammation". AA synthetizes 2-series prostaglandins (A2, E2, I2, and thromboxane A2) by cyclooxygenases-2. Additionally, leukotrienes of the series 4 (B4, C4, and E4) are also synthesized from AA by the action of 5-lipoxygenase. These AA-derived eicosanoids are

involved in several physiological actions, including proinflammation, pro-platelet aggregation, vasoconstriction, and immune response [56].

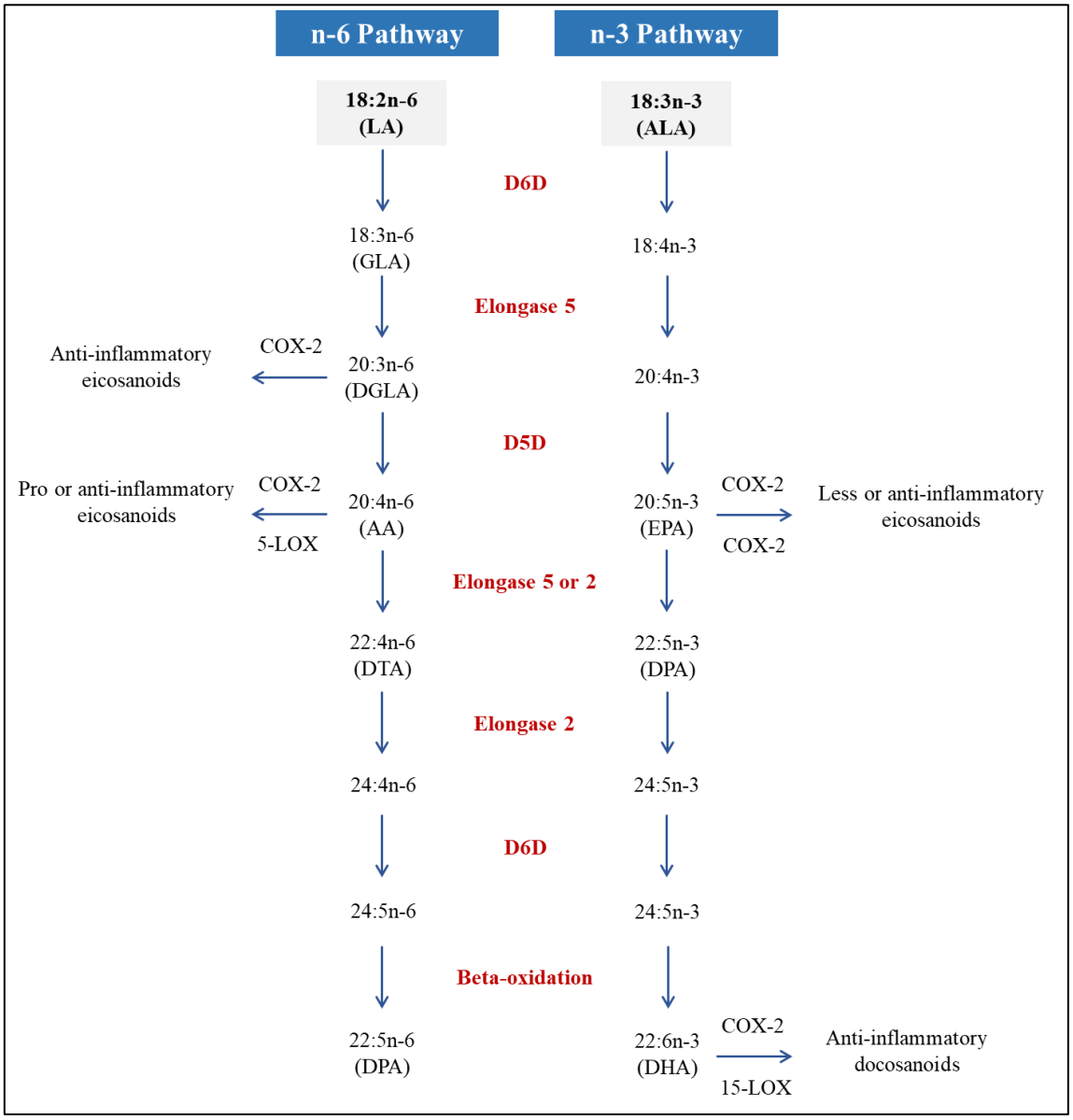


Figure 4. Biosynthesis and metabolism of polyunsaturated fatty acids. LA and ALA are obtained from the diet and converted to long chain fatty acids by a series of desaturation and elongation reactions. LA, linoleic acid; ALA, alpha-linolenic acid; GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid, AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; D6D, delta-6 desaturase; D5D, delta-5 desaturase; COX-2, cyclooxygenases-2; 5-LOX, 5-lipoxygenase.

1.5.4 Monounsaturated Fatty Acids

MUFAs are nonessential fatty acids that can be biosynthesized in the body or obtained from dietary sources. Due to the presence of a C=C bond in the central position of their aliphatic carbon chain, MUFAs enhance the fluidity and permeability of cellular membranes. In addition, MUFAs are less exposed to the oxidative effects of free radicals, hence, low levels of these fatty acids may negatively affect membrane function. Likewise, results of experimental and human nutritional studies indicate that MUFAs has help to impede and reverse SFA induced inflammation [63, 64]. MUFAs are specifically high in the heart healthy MedDiet. MUFA-rich foods mainly include olives and olive oil, rapeseed oil, peanut oil, nuts, seeds, avocados, and some animal-based foods [65]. The most common MUFA is oleic acid (OL, C18:1n-9), which is the highest MUFA provided in the diet (~90% of all MUFA) and constitutes 15% of the fatty acids present in the erythrocyte membrane, followed by palmitoleic acid (PLA, 16:1n-7) and vaccenic acid (18:1n-7) [65].

MUFAs have numerous positive effects on health, particularly as a substitute for dietary SFAs. These fatty acids also have the ability to reduce both plasma LDL cholesterol and TG, and a good efficiency to increase high-density lipoprotein (HDL) cholesterol. A high-MUFA, cholesterol-lowering diet is reported to have beneficial effects on the CVD risk profile compared to a low-fat diet [66]. Beside the cardioprotective effects, MUFAs ameliorate the risk of obesity, insulin resistance and metabolic syndrome, due to their ability to alleviate inflammation, balancing lipid metabolism and preventing fat accumulation [67]. Moreover, high MUFA intakes was inversely related with age-related cognitive decline in elderly people from Southern Italy with a typical MedDiet [68].

1.5.5 Cis-trans Fatty Acids

The unsaturated fatty acids are naturally occurring as cis C-C double bond. However, artificial hydrogenation may generate other forms of trans fatty acids. Trans fatty acids are unsaturated fatty acids with one or more double bond in the trans configuration. Like the essential n-3 and n-6 PUFAs,

trans fatty acids come from diet and cannot be synthesized in human body. A small amount of these fats can be found naturally in meats and dairy products from cows, goat, and other ruminants, as these fatty acids are produced by gut bacteria in the ruminant digestive system [69]. However, around 80-90% of trans fats come from partial hydrogenation of liquid vegetable oils, margarine, fried foods, and bakery products. The most common trans fatty acids are 16:1n-7t (trans isomer of PLA), 18:2n-6t (trans isomer of LA), and elaidic acid (18:1n-9t), which is the trans isomer of OL. Consumption of trans fatty acids has been linked to harmful health effects. Excessive consumption of these fats contributes to an increase in LDL levels, a decrease in HDL levels, and an increased risk of CHD and heart attack [70, 71]. It is reported that trans fatty acids also lead to the progression of atherosclerosis by inducing inflammation and oxidative stress [72]. The American Heart Association, the American Dietetic Association, the Institute of Medicine, US Dietary Guidelines, and the National Cholesterol Education Program Adult Treatment Panel recommend a limited intake of dietary trans fatty acids [73].

1.6 Genetic Variations in Fatty Acid Desaturase and Elongase

Several studies have previously demonstrated that genetic variants in fatty acid desaturase 1/2 (*FADS1/2*) and elongase of very long fatty acids 2 (*ELOVL2*) genes are correlated with circulatory PUFA concentrations in various populations [46, 74]. *FADS1/2* genes encode D5D and D6D, respectively [47]. These enzymes are the main determinants of PUFA levels because they catalyse the formation of long-chain PUFAs from dietary ALA and LA [47]. Likewise, *ELOVL2* encodes one of the key enzymes in the elongation reaction of long-chain PUFA from their precursor [75]. Single nucleotide polymorphisms (SNPs) with the strongest association with PUFA status are rs174537, rs174579, and rs174626 in the *FADS1/2* gene cluster and rs953413 in the *ELOVL2* gene [46, 74, 76]. These SNPs are statistically shown to be correlated either with a variation in the circulatory levels of n-3 and n-6 PUFAs or with a change in desaturase activity (evaluated in terms of product-to-precursor ratio). In addition, a recent study identified an intergenic variant (rs529143) that was observed to

modify the effect of plasma n-3 PUFA and DHA levels on leukocyte telomere length [77]. The rs529143 is located on chromosome 1 in a region that includes multiple phospholipase genes such as *PLA2G2D* and *PLA2G2F* [77]. Together, these studies show that SNPs in genes encoding enzymes related to the metabolism of PUFA contribute to plasma concentrations of fatty acids. The SNPs with an association with fatty acid level and desaturase activities are summarized in **Table 1**.

Table 1. Genetic variants associated with fatty acid status and desaturase activity

Gene	SNP	Genotype	Phenotype	Reference
FADS1/2	rs174579	CC	High desaturase activity High GLA, AA	[74]
		CT TT	Medium/low desaturase activity Low GLA AA	
	rs174626	TT	High desaturase activity High GLA, AA	
		CT CC	Medium/low desaturase activity Low GLA AA	
	rs174537	GG	High AA EPA DHA	[46]
		GT	Medium AA EPA DHA	
		TT	Low AA EPA DHA	
ELOVL2	rs953413	GG	Low EPA/High DHA	
		AG	Medium EPA/Medium DHA	
		AA	High EPA/Low DHA	
Intergenic	rs529143	CC AC AA	Modifies the effect of plasma n-3 PUFAs and DHA on leukocyte telomere length	[77]

SNP, single-nucleotide polymorphism; *FADS1/2*, fatty acid desaturase 1/2; GLA, gamma-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; *ELOVL2*, elongase of very long fatty acids 2; PUFAs, polyunsaturated fatty acids.

1.7 Fatty Acid Profiling

Determination of fatty acid profile is challenging, mainly because of their biological diversity and physicochemical similarity. Although there is no standard protocol, fatty acid analysis should be adjusted for sample collection, storage conditions, extraction and derivatization, instrument setting, and data analysis stages [78].

There are several biological samples to estimate fatty acid status, including adipose tissue, cheek cells, plasma, erythrocytes, and whole blood. Although the adipose tissue is a gold standard to determine long-term dietary changes, it is difficult to obtain and, therefore, adipose tissue is not considered ideal in clinical and population-based studies [79]. Cheek cell sample is simple and sensitive to changes in dietary long-chain PUFAs; however, it is less responsive to dietary changes in SFA content. Therefore, the validity of investigating fatty acid profile in cheek cells remains uncertain in nutritional studies [80]. Another important sample source to estimate fatty acid profile is plasma. A limitation of plasma fatty acid profile is that it reflects short-term dietary fat intake [81]. The erythrocyte membrane preferred as it provides information on the long-term dietary intake of fatty acids and their derived mediators [82]. Furthermore, whole blood is another attractive sample choice commonly used in epidemiological studies, due to its accessibility and minimal sample processing requirements.

The conventional analysis of blood fatty acid in blood requires specific sample preparation steps that need equipment, reagents, and time. As a result, other techniques that use a small sample volume have been developed [83]. Dried blood spots (DBS) is an attractive sample source because some sample preparation steps can be avoided. DBS from fingertip blood has been validated, in research and clinical practice, to effectively determine whole blood fatty acid profile changes associated with lifestyle and dietary factors [84]. In addition, DBS offers a rapid, minimally invasive, and accessible sample collection and can be easily applied to studies of large populations [85]. In fact, the DBS has become a significant sample source used in new-born screening, epidemiological

studies, and drug monitoring. In comparison to whole blood sampling, however, DBS samples have limitations as quantitative results are not possible, except when blood volumes are assessed before spotting. On the other hand, fatty acid contaminants present on items used to spot the blood can be determined, and the high risk of fatty acid loss during storage can be properly managed [78].

Fatty acid separation, identification and quantitation is commonly done by GC on capillary columns, through which individual fatty acids are separated due their different affinity to the liquid at high molecular weight present in the internal wall of the column (stationary phase). However, fatty acids can be analysed only as fatty acid methyl esters (FAMEs), due to their acidity and polarity, which can cause problems for GC columns. For this purpose, fatty acid's bond with glycerine is replaced by a methyl group. This reaction is called transesterification or derivatization [78]. Therefore, fatty acid sample preparation initially involves derivatization into FAMEs, followed by extraction of FAMEs. The derivatization of fatty acids to FAMEs can be achieved utilizing acidic or alkaline because both hydrogen ions and hydroxyl act as catalysts for the reaction. Common methanolic solutions include sulfuric acid, hydrochloric acid, and boron trifluoride-methanol. The method should preferably be simple and rapid and should not produce unwanted structural changes. After derivatization, FAMEs are extracted using a nonpolar solvent, such as hexane [78, 84].

The optimal qualitative and quantitative GC analysis generally presumes a good resolution, high repeatability and reproducibility of retention times, precise and accurate quantification of peak area measurements, and lowest sample degradation [86]. A commonly used detector in GC analysis is a flame-ionization detector (FID) that is easy to manage and offers a wide linear range and rapid response [87]. Under optimized conditions, ordinary GC-FID analysis determines the changes in human tissue fatty acids. Furthermore, by carefully choosing column and instrument conditions, GC-FID allows the estimations of fatty acids and fatty acid-derived lipid mediators from various biological samples to study disease states [56].

Other methods include GC-MS that is a well-established technique for the analysis of fatty acids after the transesterification of all lipids, which provides the information on fatty acid

composition. The LC-MS is another key analytical method for fatty acid characterization. Reversed-phase LC is commonly used as it provides complex separation based on the fatty acid length and also the number and positions of double bond [88]. Furthermore, The HPLC applied for fatty acid separation in relatively short analysis times. HPLC on a stable silver ion column permits the isolation of clean fractions differing in the double number, positions, and cis/trans geometry of the methyl ester derivatives of fatty acids [89].

1.8 The Role of Fatty Acid Status on Aging and Age-Related Phenotypes

In the aging research, there is a persistent and increasing interest in the factors that modulate the aging process. Given the important role of oxidative stress and inflammation in aging, the effects of dietary components with antioxidative and anti-inflammatory properties on aging phenotypes and age-related illnesses have been extensively studied. Among them, fatty acids have been examined as implicated in aging process as their profile is critical in maintaining cell and tissue homeostasis [4]. A number of studies have highlighted the relationship between the fatty acid profile and longevity in human. This association is also demonstrated at the cellular level, where fatty acid composition of cell membranes influences the aging process. Specifically, the protective role of n-3 PUFAs on aging have been extensively investigated and they are implicated as potential lipid biomarkers of aging [4, 90]. In a 22-year study that evaluated healthy and unhealthy aging in older adults, higher intake of EPA from marine sources lowered the risk of unhealthy aging by 24%. However, the intake of marine DHA and plant-derived ALA were not associated with healthy aging in the population [91]. Another study found that regular consumption of n-3 PUFAs from marine dietary sources help to optimize brain aging in the elderly [92]. In addition, previous investigations demonstrate that aging is accompanied by an alteration in the fatty acid profile, and this alteration contributes to the age-related declines [93]. Indeed fatty acid status are not only predict disease risk in the elderly, but can also be helpful in establishing a diagnosis [44].

During the last decades, there has been an increasing interest in the effects of n-3 PUFAs on disease risk and aging. The n-3 PUFAs and their metabolites affect metabolic, inflammatory, and oxidative processes [56]. These fatty acids can attenuate many mechanisms associated with inflammation, including inhibition of pro-inflammatory transcription factors, leucocyte chemotaxis, and eicosanoid production [94]. Likewise, supplementation with n-3 PUFAs ameliorate malondialdehyde, total antioxidant capacity, and glutathione peroxidase activity in different clinical disorders [95]. Moreover, a number of experimental and intervention studies have reported an association between n-3 PUFAs and hallmarks of aging. EPA and DHA are shown to attenuate oxidative stress-induced DNA damage and subsequent cell senescence through upregulation of nuclear factor erythroid 2–related factor 2 (Nrf2)-mediated antioxidant response. It is suggested that n-3 PUFAs may prevent CVD during aging, in part by their genome protective properties [96]. Furthermore, marine n-3 PUFA supplementation is related with alleviating effects on the plasma senescence-associated secretory phenotype components [97]. These fatty acids may also regulate gene expression by modifying epigenetic mechanisms, such as DNA methylation [98]. In AD patients, DHA-rich supplementation was associated with global DNA hypomethylation, which may influence inflammatory and other processes that could lead to AD [99]. Fish oil rich in n-3 PUFAs also has a protective effect on mitochondrial function during aging. These PUFAs can enhance mitochondrial oxidative capacity and increase sarcoplasmic protein synthesis in elderly people [100]. Likewise, the n-3 PUFAs exert neuroprotective actions by delaying the age-related mitochondrial dysfunction in the brain [101]. Furthermore, DHA improve muscle homeostasis delays muscle wasting by inhibiting proteasomal degradation of muscle proteins. Both the ubiquitin–proteasome and the autophagy–lysosome systems are modulated by this fatty acid. This makes DHA a potential therapeutic strategy to manage sarcopenia in older persons [102].

Consequently, n-3 PUFAs directly and indirectly affects the aging process and age-related diseases. In fact, it is well demonstrated that the n-3 PUFAs, particularly EPA and DHA, are associated with decreased risk of CVD, type 2 diabetes, metabolic syndrome, macular degeneration,

breast cancer, and depression in different populations [10, 43, 103-107]. In addition, lower levels of EPA and DHA are linked with an increased risk of cognitive impairment and dementia, making them a potential marker of brain aging [90, 103, 108, 109]. Likewise, the long-chain n-3 PUFA supplementation is associated with an overall improvement in muscle function and may reduce the risk of age-related sarcopenia [110]. Overall, higher levels of n-3 PUFA of erythrocytes are associated with a reduced risk of all-cause mortality [111, 112]. It has also been suggested that circulating n-6 PUFA levels benefit cardiometabolic outcomes, although controversial findings remained [113-116].

Beside PUFAs, the effects of diets rich in MUFAs, such as MedDiet, on aging has also been examined. The MUFA-rich MedDiet is associated with increased longevity and reduced risk of numerous age-related diseases. Extra virgin olive oil (EVOO), the characteristic of MedDiet and particularly rich in MUFA, is also reported to affect the hallmarks of aging. The MedDiet is described to exert a protective effect against genomic instability by preventing DNA damage, enhancing DNA repair, or attenuating telomere shortening [117]. In elderly subjects, MedDiet is shown to protect endothelial cells from oxidative stress, prevent cellular senescence, lower percentage of cell with telomere shortening, and reduce cellular apoptosis [118]. In a meta-analysis of 8 cross-sectional studies comprising a total of 13,733 participants, it was demonstrated that higher adherence to MedDiet is associated with a longer telomere [119]. These effects are described to be due to the anti-inflammatory effects of the MedDiet, but also may be due to direct changes in gene expression or via epigenetic mechanisms. For instance, OL, principal sources of which are vegetable and olive oil, induces a global hypomethylation and consequently an expression pattern that are related with an improvement of the inflammation profile, amelioration of pathways related to atherosclerosis [120].

The MUFA-rich MedDiet mitigates the effects of adverse vascular factors and has a potential to prevent the late-onset AD [121]. In an elderly population of Southern Italy with a typical MedDiet, high MUFA intakes appeared to be protective against age-related cognitive decline [68]. The modulation of proteostasis capacity is one of the mechanisms through which the MedDiet may

prevent neurodegeneration. It is suggested that olive oil participates in regulation of cell proteostasis through activation of the protein deacetylase sirtuin 1 (SIRT1) and enhanced autophagy [122]. In addition, dietary components that reduce, delay, or attenuate the rate of stem cell exhaustion play a role in facilitating healthy aging. In this regard, in an observational study involving 421 very old individuals, higher adherence to the MedDiet was associated with significantly higher endothelial progenitor cells [123]. Similarly, in a crossover trial involving 20 older participants (>65 years), the consumption of a MedDiet was associated with an increased number of circulating endothelial progenitors cells [124].

As such, detailed studies on the effects of fatty acids on aging and age-related phenotypes are well investigated. In addition, a number of clinical studies suggested positive effects of n-3 PUFA supplementation on telomere length, as an important aging hallmark. Nonetheless, the evidence concerning the clinical effect of n-3 PUFAs on telomere length has not been reviewed meta-analytically. Likewise, observational data suggest that MUFA intake promote muscle health in the elderly, however, the effect of MUFAs on sarcopenia, as a geriatric syndrome, is not clear and needs further evaluation. In the following section we briefly describe the possible effects of n-3 PUFA on telomere length, as an aging hallmark. Then, we discuss the effects of MUFAs on sarcopenia, as a geriatric condition.

Effect of Polyunsaturated Fatty Acids on Telomere Length

Among the wide variety of aging hallmarks at the molecular and cellular levels, short telomeres are sufficient to trigger age-related pathologies and decrease lifespan in mice and humans [7, 125, 126]. Telomeres are repetitive sequence of nucleotides at the end of eukaryotic chromosomes. Human telomeres consist of a tract of repeated sequences of DNA (5'-TAGGG-3') and associated protective proteins. Together, telomeric DNA and telomeric proteins maintain the structural integrity of chromosomes, thus keeping genomic stability [127]. At each cycle of cell division, telomeres are

subject to shortening, losing approximately 50 to 100 base pairs per mitotic division in human cells. This telomere attrition can be replenished by an enzyme, telomerase, that adds telomeric repeat sequences to the ends of chromosomes, hence elongating them to compensate for their loss. Additionally, the rate of telomere loss is affected by numerous factors other than the mitotic replication rate [128].

Oxidative stress and low-grade systemic inflammation are thought to be the major contributors to telomere shortening. Due to the high guanine-cytosine content, telomeres are extremely prone to oxidative damage compared to non-telomeric sequences. Likewise, the proinflammatory phenotype that accompanies aging in mammals is also linked to the onset of age-associated diseases and telomere shortening [129, 130]. Furthermore, extensive evidence supports that telomere length is a dynamic trait sensitive to environmental factors. An accelerated telomere shortening has been associated with smoking, air pollution, excessive food intake, and psychological stress. Exposure to these factors may promote telomere attrition by increasing oxidative stress and inflammation [131-133].

Although the association between diet and telomere maintenance is currently under investigation, recent human studies indicate that specific dietary components may be considered a potential nutritional tool for preserving telomere length throughout the lifespan [134, 135]. In particular, a higher intake of antioxidant-rich foods and a greater adherence to an anti-inflammatory diet may play a role in telomere maintenance and influence overall health and longevity [136, 137]. Indeed, current epidemiological and clinical data showed that a higher consumption of vegetables, fruits, nuts, legumes, and seaweed is associated with longer telomere length [138, 139]. These foods provide a range of bioactive compounds affecting endogenous antioxidant response, anti-inflammatory pathways and, at least in part, telomere maintenance [140]. Dietary fiber intake, specifically cereal fiber intake, was positively associated with telomeres. Furthermore, the intake of n-3 PUFA are also suggested to be inversely related to the rate of telomere shortening [141-143]. Nonetheless, no study has reviewed meta-analytically the evidence concerning the clinical effect of

n-3 PUFAs on telomere length, and therefore, the effect of n-3 PUFAs on telomere needs further confirmation.

Effect of Monounsaturated Fatty Acids on Sarcopenia

Sarcopenia is a geriatric disorder characterized by progressive decrease in muscle mass, muscle strength, and physical function [144, 145]. The prevalence of sarcopenia ranges from 10% to 27% in people over 60 years of age [146]. Elderly people suffering from sarcopenia are at high risk of adverse outcomes such as falls, fractures, poor quality of life, physical disability, hospitalization, and mortality [147, 148]. Since the aging population is increasing globally, sarcopenia has become a major public health issue [146]. In the past decade, sarcopenia has become the focus of intense investigations to better understand its pathophysiology and to identify effective preventive and treatment strategies [149, 150].

Besides insufficient protein intake and physical inactivity, accumulating evidence suggests that oxidative stress and inflammation are the main contributors to the complex etiology of sarcopenia [151, 152]. During aging, excessive production of ROS, which controls the redox signal pathway, results in oxidative damage and mitochondrial dysfunction, impaired protein homeostasis, and eventually skeletal muscle dysfunction [153-155]. Age-related impairments in mitochondrial function may also increase oxidative stress and enhance the loss of proteostasis [152]. In addition, chronic low-grade inflammation during aging affects the metabolism of skeletal muscle cells through interactions among various cytokines [156]. Furthermore, inflammation causes oxidative stress and anabolic resistance, leading to the loss of muscle mass, strength, and function [156].

Although the association between diet and sarcopenia is still under investigation, a growing body of evidence suggests that nutrition has an important role in both the prevention and treatment of sarcopenia, in particular, a dietary pattern that ensure an adequate protein intake [157]. In addition, higher intakes of antioxidant nutrients play an important role in the regulation of muscle mass and

function throughout life [158]. Indeed, current observational and clinical data show that increasing fruit and vegetable consumption could be effective tools to prevent or treat sarcopenia, especially by enhancing muscle strength and function [159]. MUFAs have also attracted considerable attention because of their role in health and disease prevention. Detailed studies have shown that olive oil, the main source of MUFA, may attenuate oxidative stress, the main mechanism of sarcopenia pathogenesis [160-162]. Additionally, the MUFA-rich MedDiet reduces inflammation related to aging [163, 164]. Previous observational studies have also evaluated the association between MUFA intake and sarcopenia; however, inconclusive results are reported. The effects on MUFAs on sarcopenia is, therefore, needs further confirmation.

Moving Forward

Previous studies have played a critical role in evidence synthesis and conveyed important and significant information on the role of fatty acids on health and aging process. However, there exists some gaps, some of which could be addressed in this thesis. In order to provide additional evidence from new perspectives, in the following chapters we will examine the age-associated changes in fatty acid profile in a Sicilian cohort and evaluate whether among Sicilian LLIs genetic variants are associated with fatty acid profile. In addition, we will meta-analytically assess the role of n-3 PUFAs and MUFAs on telomere length (an important aging hallmark) and sarcopenia (a geriatric syndrome), respectively.

2. AIMS

The overall aim of this thesis is to provide evidence for a better understanding of the role of fatty acids in aging process and age-related phenotypes. More specifically, the aim of each study is as follows:

I. To describe a sensitive and reliable method based on GC-FID to identify and quantify fatty acids.

II. To evaluate whether a distinctive blood fatty acid profile may be associated with longevity in LLIs from Western Sicily compared to adults and older adults from the same area. Further, to study genetic variants in key enzymes related to fatty acid biosynthesis and metabolism that could be responsible for differences in fatty acid profile between the age groups. Additionally, to investigate whether among LLIs the genetic variants are associated with fatty acid status and desaturase activity.

III. To meta-analytically assess whether supplementation of n-3 PUFA can modulate telomere length in clinical trials.

IV. To meta-analytically evaluate the association between MUFA intake and sarcopenia in observational studies. In addition, to investigate whether this association is influenced by participant characteristics, methods for MUFA assessment, and the diagnostic criteria of sarcopenia.

3. MATERIALS AND METHODS

This chapter is organized as follows: First, in section 3.1. we described data collection, characteristics of study cohort, lipidomic analysis, and genetic analysis that were used in Study I and II. Then, we described search strategy, data extraction, quality assessment, and meta-analysis of Study III and IV in section 3.2. and 3.3., respectively.

3.1 Age-Associated Changes in Circulatory Fatty Acids

3.1.1 Study Design and Participant Characteristics

Subjects participating in the "Discovery of molecular and genetic/epigenetic signatures underlying resistance to age-related diseases and comorbidities (DESIGN, 20157ATSLF)" project were used for the present investigation. Detailed information on participant recruitment have been previously described [20]. A total of 172 subjects from Western Sicily were enrolled. The participants ranged from 18-111 years of age and were recruited at the University of Palermo (Italy) between June 2017 and March 2020. The population was divided into three age groups: adults (69 subjects, age range

18-64 years old), older adults (54 subjects, age range 65-89 years old), and LLIs (49 subjects, age range 90-111 years old). The participants were relatively healthy. Subjects were excluded from the study if they had been diagnosed with chronic and acute diseases, such as neoplastic and autoimmune diseases and severe dementia. The subjects participated voluntarily and written informed consent was obtained from all of them. The study protocol was conducted following the Declaration of Helsinki and its amendments. The Ethics Committee of Palermo University Hospital approved the study (Nutrition and Longevity, No. 032017).

Anthropometric measurements were recorded at the University of Palermo, including body weight and height, which were used to calculate the body mass index (BMI). In addition, data on eating habits were collected by a food frequency questionnaire (FFQ) that has been used in previous studies [165, 166]. Overnight fasting blood samples were collected from all the participants. According to standard procedures, hematochemical and haematological analyses were performed, including serum total cholesterol (TC), TG, HDL, and LDL. The samples were kept at -80°C until the analyses performed in this project.

3.1.2 Fatty Acid Profiling by Gas Chromatography

Method Development and Validation

A GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan), equipped with an SP®-2560 capillary GC column (L x I.D. 100m × 0.25mm × 0.2µm) with a poly (biscyanopropyl siloxane) phase, one FID, an automatic injection system (AOC-20i), and an electronic flow and pressure control system, was used for the GC analysis. The GC column was connected to the injector and the FID. The electronic flow and pressure controller supplied carrier gas (helium) at constant pressure. Samples (1 µl) were introduced by the injector heated to 250°C with a split ratio of 10:0. The initial temperature was 170°C with a 1 min hold followed by 7.00°C/min ramp to 200°C with a 1 min hold and 1.00°C/min ramp to 220°C with a 20 min hold. The FID temperature was 280.0°C with hydrogen, air, and nitrogen

make-up gas flow rates of 40.0 ml/min, 400.0 ml/min, and 30.0 ml/min, respectively. The configuration and condition of this system are described in **Table 2**.

Table 2. The configuration and condition of the gas chromatography system.

Column	Configuration
Gas chromatography column	SP-2560 (100 m x 0.25 mm, df 0.20 µm)
Gas chromatography flow	2.24 ml /min
Autoinjector	
Temperature	250.0°C
Pressure	457.3 kPa
Injection size	1 µl
Total flow	29.6 ml/min
Purge flow	5.0 ml/min
Flame-ionization detector	
Temperature	280.0°C
Hydrogen	40.0 ml/min
Air	400.0 ml/min
Make-up (Nitrogen)	30.0 ml/min
Column oven temperature program	
Initial temperature	170.0°C for 1 min
Ramp number 1	7.00°C/min to 200.0°C for 1 min
Ramp number 2	1.00°C/min to 220.0°C for 20 min

The calibration of the method was produced from average response factor (RF) and linear regression equations. According to the US FDA guideline for biological methods, the procedure was also validated for linearity and precision. Batches of the 22 FAMEs standard mixture were prepared to determine the average RF, the limit of quantitation (LOQ), and linearity range. The average RF was calculated as the average of each calibration factor of the external standard batches. The average RF was then evaluated from the calculated relative standard deviation (RSD). To calculate the RSD,

the standard deviation of the calibration factors was first determined. Then the standard deviation was divided by the mean of the calibration factors to give the RSD. The precision of the method was determined from the calculated RSD. Generally, an RSD of <15% or <20% is utilized as a criterion for accepting the precision of the method. Additionally, the regression analysis formed an association equation between the instrument response (peak area) and the concentration of individual FAMEs in the analyte, generating calibration lines. The linearity of the calibration curve was assessed by the coefficient of determination (r2) that permits the determination of the percent of data nearest to the line, and a value ≥ 0.99 was considered acceptable.

Fatty Acid Extraction

Total fatty acids were extracted from samples of whole blood and analysed using GC-FID, according to the following steps:

1. Initially, 300 ul of whole blood was placed inside a 10 ml headspace vial with a sealing cap, and 2.0 ml of boron trifluoride-methanol (BF3-MeOH) (12% w/v, 1.5 M) (Acros Organics, Geel, Belgium) was added.
2. The vials were sealed and heated at 100°C for 60 mins, using a convectional block heater. This leads to the direct transesterification of the fatty acids in the mixture into FAMEs.
3. After cooling to 25°C, two ml of n-hexane (CARLO ERBA Reagents, S.r.l., France) was added, and the mixture was then vortexed for 1 min, resulting in the formation of a transparent n-hexane top layer containing the FAMEs. This organic layer (the supernatant) was removed into a fresh 2ml glass GC vial.
4. The aliquot was air-dried in the darkness and subsequently redissolved in 400 μl of n-hexane and injected into GC-FID for analysis.

Identification and Quantification of Fatty Acids

Individual FAMEs were identified by the standard mixture (Nu-Chek-Prep, Elysian, MN, U.S.A.), which was dissolved in n-hexane at various concentrations before injection into GC-FID. The retention times of the 22 FAMEs mixture on the GC column were used to identify the fatty acids, as shown in **Table 3**. The retention time used for calibration was representative of five concentrations of the standard. A peak represented each FAME, in which area of the peaks is proportional to the amount of the corresponding fatty acid. The data were analysed using Shimadzu system GC Solutions software, designed for this system. Fatty acid concentration measured in ng/ml was then converted to a percentage of total fatty acids:

Fatty acid % = (peak area corresponding to the fatty acid / sum of all the peak areas corresponding to the total mixture of the fatty acids) × 100

Table 3. Retention time of the fatty acids.

Fatty acid	Retention time (min)	Fatty acid	Retention time (min)
14:0 (MA)	7.536	20:1n-9	14.197
16:0 (PA)	8.791	20:2n-6 (EA)	15.512
16:1n-7t	9.227	20:3n-6 (DGLA)	16.861
16:1n-7 (PLA)	9.450	20:4n-6 (AA)	17.988
18:0 (STA)	10.521	24:0	20.112
18:1t	11.005	20:5n-3 (EPA)	20.543
18:1n-9 (OL)	11.311	24:1n-9 (NA)	21.651
18:2n-6t	11.920	22:4n-6 (DTA)	22.699
18.2n-6 (LA)	12.556	22:5n-6 (n-6 DPA)	23.950
18:3n-3 (ALA)	13.602	22:5n-3 (n-3 DPA)	25.810
18.3n-6 (GLA)	13.879	22:6n-3 (DHA)	27.268

The retention time is representative of five concentrations of the standard used for the calibration. MA, myristic acid; PA, palmitic acid; PLA, palmitoleic acid; STA, stearic acid; OL, oleic acid; LA, linoleic acid; ALA, alpha-linolenic acid; GLA, gamma-linoleic acid; 20:1n-9, eicosenoic acid; EA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; 24:0, lignoceric acid; EPA, eicosapentaenoic acid; NA, nervonic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

The n-3 index, defined as the amount of EPA and DHA content in erythrocytes as a percentage of the total amount of fatty acids, was then calculated. Likewise, the trans-fat index was calculated as the percent of 18:1t and 18:2n-6t trans fatty acids of the total fatty acids. Additionally, the AA/EPA, SFA/MUFA, and n-6/n-3 ratios were determined. Furthermore, desaturase activity was estimated by calculating the ratio of the product fatty acid to precursor fatty acid, as described previously [76, 167]. Four estimates of desaturase activity were studied: D6D activity by dividing the percent composition of GLA by LA, D5D activity by dividing the percent composition of AA by DGLA, and aggregate desaturase activity (ADA), by dividing the percent composition of EPA by ALA in the n-3 pathway, and AA by LA in the n-6 pathway.

3.1.3 Genetic Analysis

Based on previous studies, five SNPs were selected for genotyping: rs174579 (C>T), rs174626 (C>T), rs174537 (G>T), rs953413 (G>A), and rs529143 (A>C) [46, 74]. Three of these SNPs (rs174579, rs174626, and rs174537) are located in the *FADS1/2* gene cluster. The rs953413 is found on the *ELOVL2* gene, while rs529143 is an intergenic polymorphism in a region that includes multiple phospholipase genes such as *PLA2G2D* and *PLA2G2F*.

Genomic DNA extraction

The genomic DNA was extracted from peripheral blood leukocytes and purified using E.Z.N.A.® Blood DNA Mini Kit (Omega Bio-tek, Inc., GA, USA), following the manufacturer's instructions:

1. A volume of 100-200 µl of whole blood was transferred into a sterile microcentrifuge tube and the volume was brought up to 250 µl with 10mM Tris-HCl.
2. Proteinase K Solution (25 µl) and BL Buffer (250 µl) were added. The mixture was vortexed at maximum speed for 15 sec. As RNA-free genomic DNA was required, 5 µl RNase A (50 mg/ml) was also added.
3. The mixture was incubated at 65°C for 10 mins, and vortexed briefly once during incubation.

4. A volume of 260 µl of 100% ethanol was added. The tube was then vortexed at maximum speed for 20 sec, followed by a brief centrifugation to collect any drops from the inside of the lid.
5. The entire sample was transferred to a HiBind® DNA Mini Column that was inserted into a 2 ml Collection Tube. The sample was centrifuged at ≥10,000 x g for 1 min.
6. After the discard of the filtrate and the Collection Tube, the HiBind® DNA Mini Column was inserted into a new 2 ml Collection Tube.
7. A volume of 500 µl of HBC Buffer (diluted with 100% isopropanol before use) was added. The sample was centrifuged at ≥10,000 x g for 1 min. The filtrate was discarded, and the Collection Tube was reused.
8. A volume of 700 µl DNA Wash Buffer (diluted with 100% ethanol before use) was added. The sample was centrifuged at 10,000 x g for 1 min. The filtrate was discarded, and the Collection Tube was reused.
9. Step 8 was repeated for a sec DNA Wash Buffer wash step.
10. The empty HiBind® DNA Mini Column was centrifuged at maximum speed (≥10,000 x g) for 2 mins to dry the column matrix. The column membrane was dried before elution as residual ethanol may interfere with downstream applications.
11. The HiBind® DNA Mini Column was transferred into a nuclease-free 2 ml microcentrifuge tube.
12. Elution Buffer (100-200 µl), heated to 65°C, was added and left at room temperature for 5 mins. The sample was centrifuged at ≥13,000 x g for 1 min.
13. Step 12 was repeated for a sec elution step.
14. The concentration of the extracted DNA was determined using NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., MI, Italy).
15. The eluted DNA was stored at -20°C until further analysis.

High-Resolution Melting Analysis

Genotyping was performed using polymerase chain reaction (PCR)-based high-resolution melting (HRM) assay, which is a post-PCR analysis method that is based on detecting small differences in PCR melting curves [168]. The HRM analysis consisted of the following steps:

1. The reactions were made using white 96-well plates, with 10 μl of the Precision Melt Supermix for HRM analysis (Bio-Rad Laboratories, Inc., USA), 0.6 μl of each forward and reverse primers (10 μM), 3-5 μl of genomic DNA, and nuclease-free water up to 20 μl.
2. The plate was covered with a sealing film and briefly centrifuged.
3. The PCR-HRM was performed with a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc, Singapore). The pairs of primers for amplification of rs174579, rs174626, rs174537, rs953413, and rs529143 and the PCR-HRM conditions are shown in **Table 4**.
4. Data were analysed using Bio-Rad CFX Manager and Bio-Rad Precision Melt Analysis systems designed specifically for HRM analysis.

Table 4. Primer sequences and PCR-HRM conditions

SNP	Primer sequences (5'-3')	T_a (°C)	PCR cycle	T_m (°C)
rs174579	F: TTTCCTTCTCCTCCATCCCT R: GATTGTCATCTGGCACCTTCC	58.5	45	98
rs174626	F: CGGTTAAGCAGAGAGCAGATA R: CTTCCCTGGGTCAAGAAGCT	58.5	45	98
rs174537	F: GGGTGCTTCTGAGGGAGG R: CACCATGTCTGCTGTGTGTC	58.5	45	98
rs953413	F: AAAACGCTAAAGGTCACAAAGC R: TGTTTCTGCCCTTCTTCCAC	58.5	45	98
rs529143	F: CATCCTCATCCTCATTATTATCATTCAA R: CTAAGGTTTGTGCATTTTCCTGGAT	59	39	98

PCR, polymerase chain reaction; HRM, high-resolution melting, SNP, single-nucleotide polymorphism; F, forward; R, reverse; T_a, annealing temperature; T_m, melting temperature.

Sample Preparation for Next Generation Sequencing

To ensure the reliability of the results of PCR-HRM analysis, duplicate samples were used. In addition, sequencing verified genotyped samples (24 samples) were included as quality controls. The sample preparation, which is a method used prepare the target DNA sequence for Next Generation Sequencing (NGS), consisted of the following steps:

1. **DNA extraction** (as described in previous section).
2. **DNA amplification by PCR:**

The DNA segment containing the selected SNPs were amplified by PCR-based protocol with C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Inc, Singapore). The reaction components used for the PCR reaction were assembled on ice (**Table 5**).

Table 5. PCR reaction components and final concentrations

Component	50 µl Reaction	Final Concentration
10 µM Forward Primer	2.5 µl	0.5 µM
10 µM Reverse Primer	2.5 µl	0.5 µM
2X Phusion Master Mix	25 µl	1X
Template DNA	10 µl	50 ng–250 ng
Nuclease-free water	10 µl	

The PCR tubes were then transferred to the PCR machine with the block preheated to 98°C and started thermocycling: The pairs of primers used for amplification of rs174579, rs174626, rs174537, and rs953413, and the thermocycling conditions are shown in **Table 6**.

Table 6. Primer sequences and PCR conditions

SNP	Primer sequences (5'-3')	T_a (°C)	PCR cycle	T_m (°C)
rs174579	F: AAGAATTCTTGCCCCATGAACT R: AACTCGAGTGTAACAACTCTCCTCCCCG	56.5	35	98
rs174626	F: AAGAATTCGGTGTTGGGGTTTGATTGCT R: AACTCGAGTCAACACCCGCTTACCCTAG	56.5	35	98
rs174537	F: AAGAATTCAAGAGGCCATGGGAGAAGAC R: AACTCGAGAGTGACACAGTGCTGCTACT	56.5	35	98
rs953413	F: AAGAATTCCCCCGATGTGAGAAAGGTCA R: AACTCGAGACTCTGTGACGATAAGGACCA	56.5	35	98

PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; F, forward; R, reverse; T_a, annealing temperature; T_m, melting temperature.

3. Gel electrophoresis

The amplified DNA fragments were separated by gel electrophoresis, according to the following steps:

1. A 2% gel was prepared by dissolving 1 g of agarose powder in 50 ml of 1X TBE Buffer in a microwave.
2. Once the agarose is fully dissolved and the solution was cooled to about 55°C, five µL of ethidium bromide was added to the melted agarose. The solution was well mixed by swirling the beaker until the stain was evenly dispersed in the mixture.
3. The solution was then slowly poured into the gel box, which was initially set up. Once the gel solidified, the comb was removed, and the gel was transferred to the casting tray.
4. The DNA ladder was loaded into the first well, then the DNA samples that were pre-mixed with 3 µl loading dye.
5. The gel was run for 40 mins at 80V. Once completed, the gel was visualized under UV light.

4. Gel purification

The purification of PCR products was performed using ISOLATE II PCR and Gel Kit (Bioline Meridian Bioscience, London, UK), according to the manufacturer's instructions, as follows:

1. Gel slice excise and dissolution
   - Using a clean scalpel, the DNA fragment was excised from the gel. The excess agarose was removed, and the weight of the slice was determined and transferred into a clean tube.
   - A volume of 200 µl of Binding Buffer was added per 100 mg of the 2% agarose gel.
   - The sample was incubated at 50°C for 10 mins. The sample was then vortexed briefly every 2-3 mins until the gel slice was completely dissolved.
2. DNA binding
   - An ISOLATE II PCR and Gel Column was placed in a 2 ml Collection Tube and the sample was loaded.
   - The sample was centrifuged for 30 sec at 11,000 x g. The flow-through was discard and the Collection Tube was reused.
3. Washing silica membrane
   - A volume of 700 µl of Wash Buffer was added to the ISOLATE II PCR and Gel Column and centrifuged for 30 sec at 11,000 x g. The flow-through was discarded and the column was placed back into the Collection Tube.
4. Drying the silica membrane
   - After a centrifuge for 1 min at 11,000 x g to remove residual ethanol, the ISOLATE II PCR and Gel Column was placed in a 1.5 ml microcentrifuge tube.
5. DNA elution
   - A volume of 15-30 µl of Elution Buffer was added directly onto the silica membrane.
   - The sample was incubated at room temperature for 1 min, followed by a centrifuge 1 min at 11,000 x g.

After the sample preparation step, the purified PCR products were send abroad for sequencing (CCG - Cologne Center for Genomics, Cologne, Germany). Afterwards, the DNA sequences were analysed using FinchTV (version 1.4) software. An example of the results of DNA sequencing and HRM analyses of rs174537, rs953413, rs174579, and rs174626 are shown in **Figure 5**, **Figure 6**, **Figure 7**, and **Figure 8**, respectively.

3.1.4 Statistical Analysis

Variables were assessed for normality using the Shapiro Wilk's test and skewed data were subsequently log-transformed for all analyses. A two-way Analysis of Variance (ANOVA) test was used to identify whether age, gender, or their interaction affects anthropometric, clinical, and fatty acid measurements. When statistically significant, a post hoc pairwise comparison test using the Bonferroni method was performed. Deviation from Hardy-Weinberg Equilibrium (HWE) was tested for each SNP using a Chi-square test. HWE was determined for all SNPs within the older adults and LLIs. Linear regression models were used to examine the associations between individual blood fatty acids and SNP genotypes among LLIs. These models were adjusted for gender, BMI, and LDL levels. The genotypes of rs174537, rs953413, and rs529143 were coded 0, 1, and 2 reflecting the number of copies of an allele being tested (additive genetic model). A dominant model was used for rs174579, in which we combined heterozygous with minor homozygous subjects. For rs174626, a recessive model was used by combining heterozygous with major homozygous subjects. The analyses were conducted using the R software, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and the interface RStudio version 1.4.1717 (R studio, PBC, Boston, MA, USA). A two-tailed p-value of ≤ 0.05 was considered to be statistically significant.

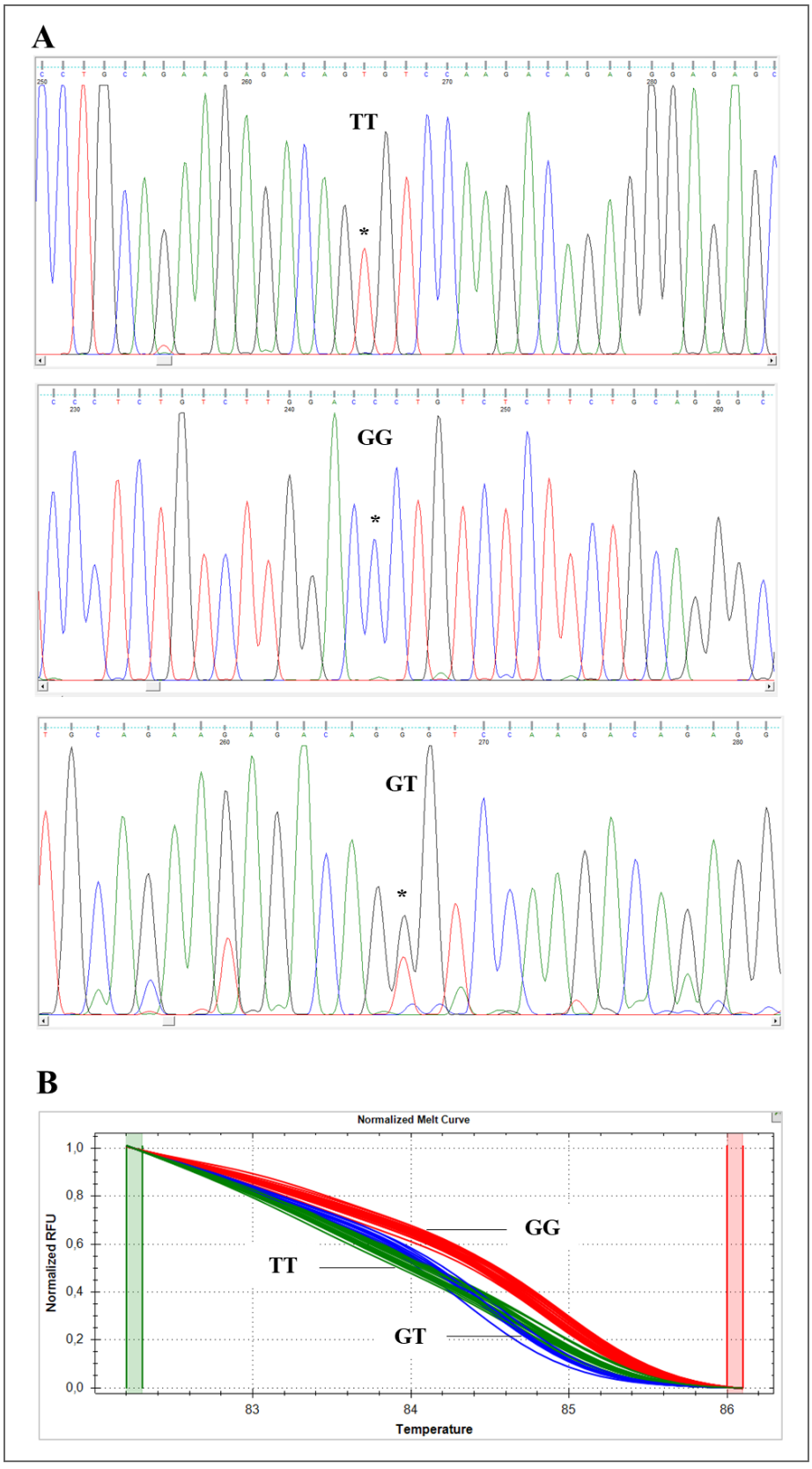


Figure 5. Results of DNA sequencing and high-resolution melting (HRM) analyses. **(A)** sequencing analysis of rs174537 polymorphism (TT, GG, and GT genotypes), **(B)** HRM analysis of rs174537 polymorphism (TT, GG, and GT genotypes).

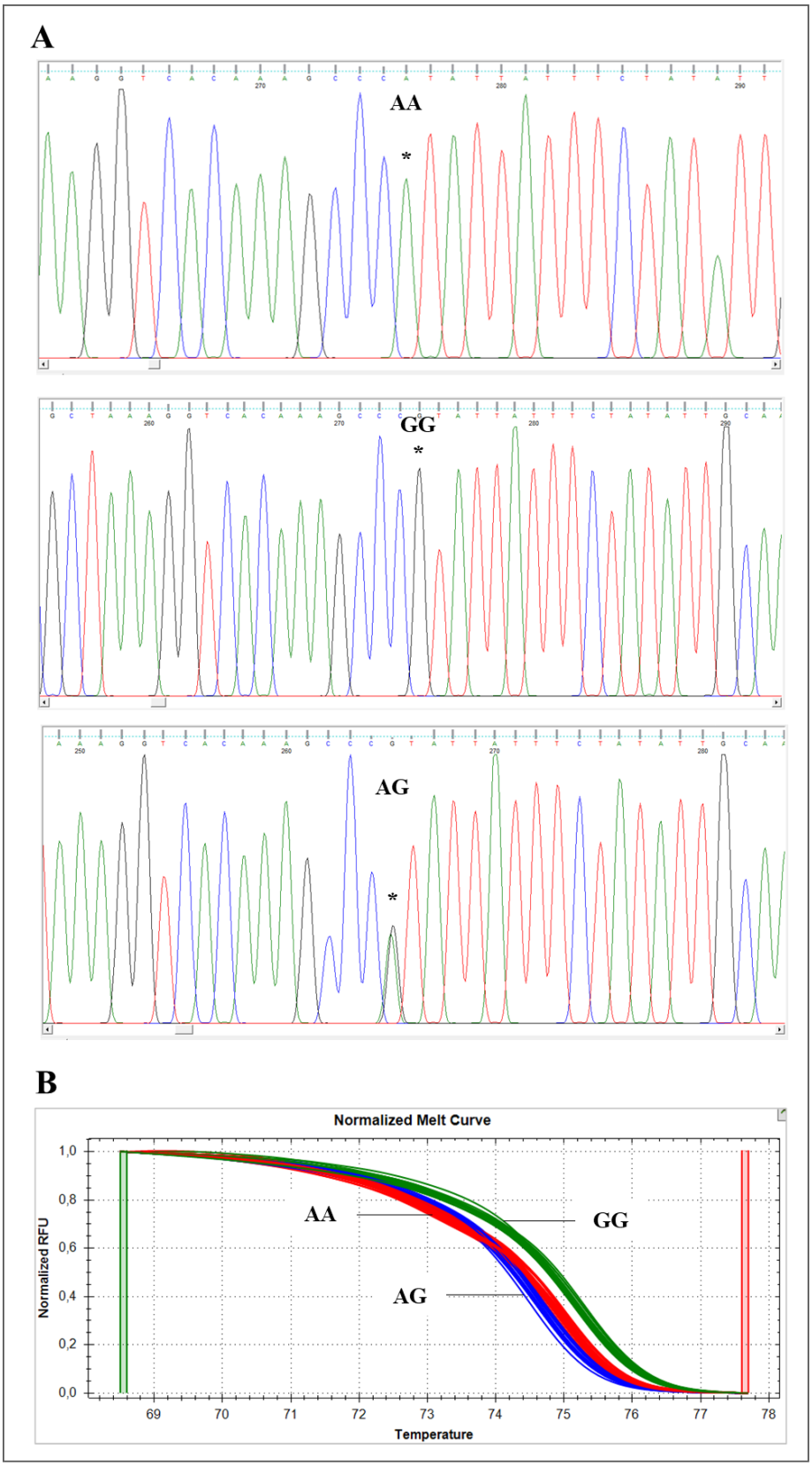


Figure 6. Results of DNA sequencing and high-resolution melting (HRM) analyses. **(A)** Sequencing analysis of rs953413 polymorphism (AA, GG, and AG genotypes), **(B)** HRM analysis of rs953413 polymorphism (AA, GG, and AG genotypes).

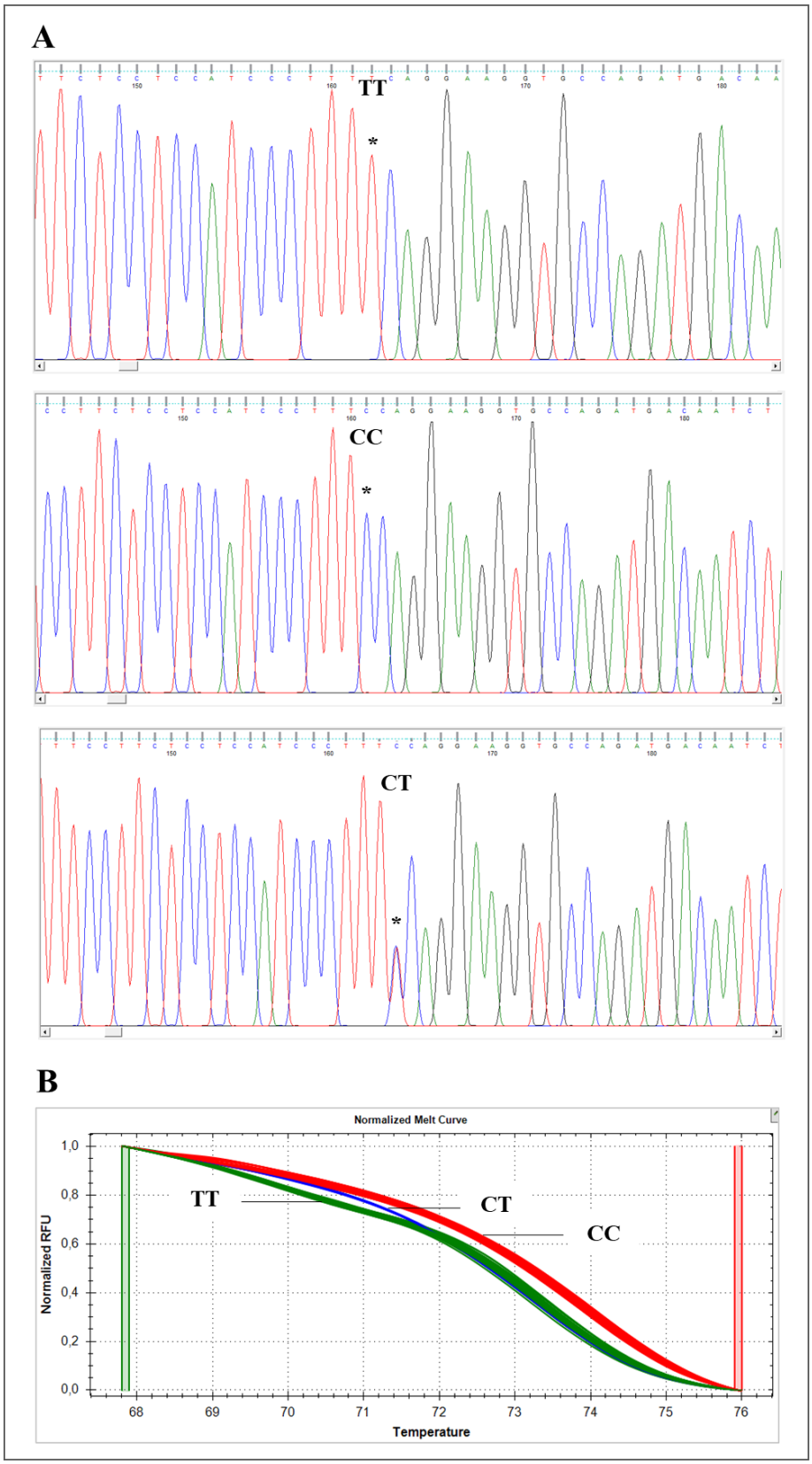


Figure 7. Results of DNA sequencing and high-resolution melting (HRM) analyses. **(A)** Sequencing analysis of rs174579 polymorphism (TT, CC, and CT genotypes), **(B)** HRM analysis of rs174579 polymorphism (TT, CC, and CT genotypes).

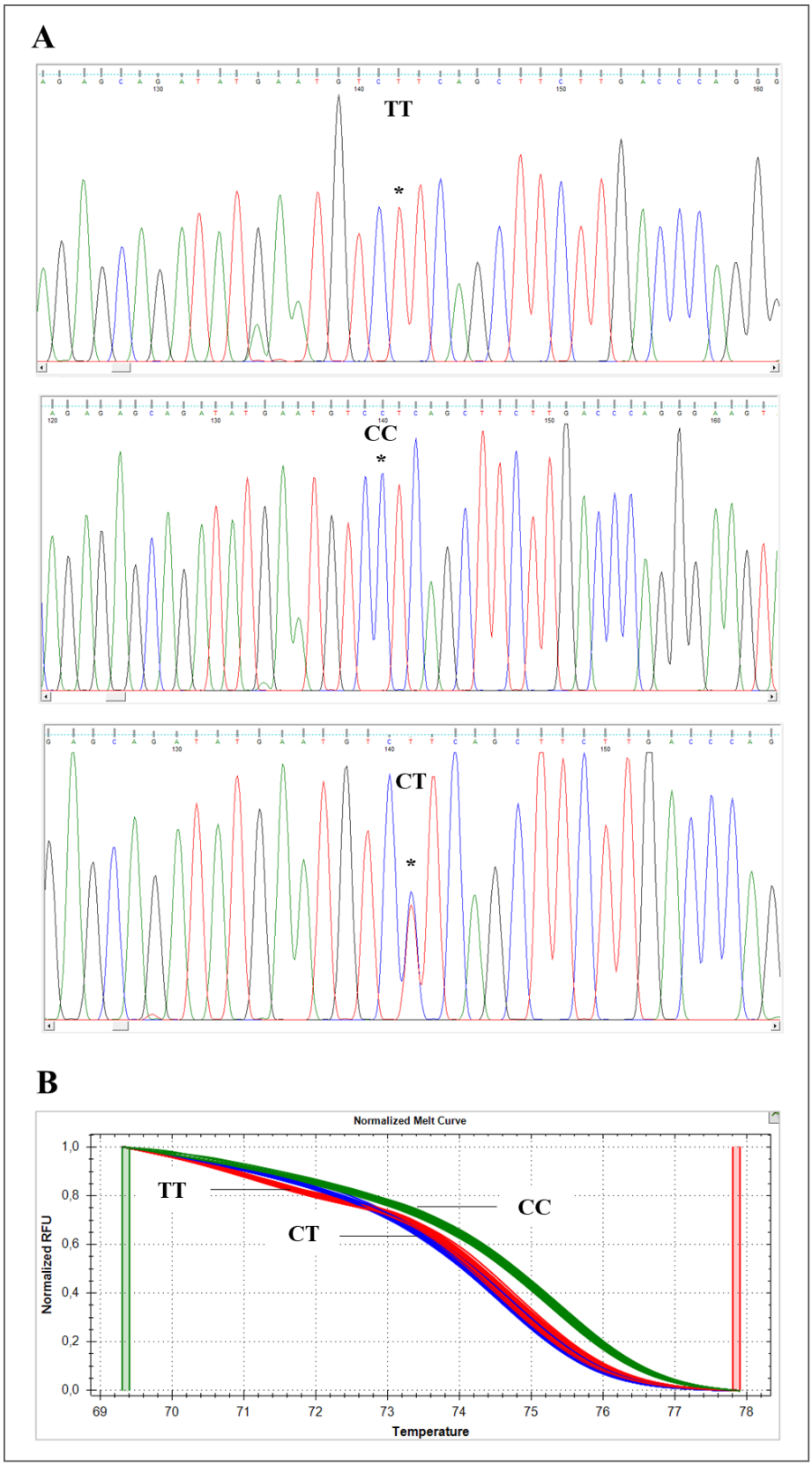


Figure 8. Results of DNA sequencing and high-resolution melting (HRM) analyses. **(A)** Sequencing analysis of rs174626 polymorphism (TT, CC, and CT genotypes), **(B)** HRM genotype analysis of rs174626 polymorphism (TT, CC, and CT genotypes).

3.2 Effect of N-3 Polyunsaturated Fatty Acids on Telomere Length

3.2.1 Search Strategy

The search strategy, screening and selection criteria were developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement [169]. Clinical studies assessing the effect of n-3 PUFAs on telomere length, published up to 15 November 2021, were eligible for this analysis. On the other hand, exclusion criteria involved: studies not published in English language; articles that used secondary data; studies on animal models or *in vitro* experiments; observational studies; studies in individuals younger than 18 years of age.

A comprehensive systematic literature search was conducted in the following databases: PubMed, Web of Sciences, Scopus, and the Cochrane Library. Both controlled vocabulary and free text terms were used for the search. Using Boolean operators, we combined the following terms: "polyunsaturated fatty acids" OR "pufa" OR "unsaturated fatty acid" OR omega-3" OR "n-3" OR "n3" OR "ω-3" "docosahexaenoic acid" OR "DHA" OR "eicosapentaenoic acid" OR "EPA" OR "alpha linolenic acid" OR "ALA" AND "telomere" OR "telomeric" OR "telomere shortening" OR "telomere homeostasis" OR "telomere length" OR "telomere length maintenance" OR "telomere maintenance". Similar queries were used for controlled vocabulary search.

3.2.2 Data Extraction and Quality Assessment

Titles and abstracts obtained from all the databases were reviewed. The removal of duplicate records was conducted with a reference management software (EndNote X8; Clarivate Analytics, Philadelphia, PA, USA). The full texts were then screened, excluding all articles that did not meet the inclusion criteria. Disagreements were resolved by consensus. The following data were extracted and tabulated: author's name, publication year, study country, study design, participant characteristics (sample size, age, gender, duration of intervention, and health status), intervention (type of n-3 PUFA, and dose), outcome assessment for telomere length, fluid analyzed, and results.

To assess the methodological quality and risk of bias of the included RCTs, the Cochrane risk of bias tool was used [170]. The tool evaluates 7 components: (1) sequence generation, (2) allocation sequence concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessment, (5) incomplete outcome data, (6) selective outcome reporting, and (7) other bias. For non-randomized and single-arm clinical trials, Risk Of Bias In Non-randomized Studies - of Interventions (ROBINS-I) was used [171]. This tool assesses 7 components: (1) bias due to confounding, (2) bias in selection of participants into the study, (3) bias in classification of interventions, (4) bias due to deviations from intended interventions, (5) bias due to missing data, (6) bias in measurement of outcomes, and (7) bias in selection of the reported result.

3.2.3 Meta-Analysis

Continuous data were expressed as mean difference with a 95% confidence interval. The summary statistics were the number of participants, the mean change from baseline, and the standard deviation of the mean change. If change from-baseline scores were not provided post-test means and standard deviations were used. The mean difference was used to express the results across studies. The I2 test was used to describe the proportion of the total variation in the study estimates that is due to heterogeneity. The following grades were applied: <25% (very low), from 25 to <50% (low), from 50 to <75% (moderate) and ≥75% (large) [172]. A random effects model was chosen for the meta-analyses because this method of analysis is favored when there is evidence of heterogeneity among studies. To assess whether the pooled estimate was biased by the effect of any particular study, we also carried out a sensitivity analysis, recalculating the pooled estimate. The meta-analysis was conducted using the R Software, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and the interface R-Studio version 1.4.1717 (R studio, PBC, Boston, MA, USA). A *p*-value < 0.05 was considered to be statistically significant.

3.3 Effect of Monounsaturated Fatty Acids on Sarcopenia

3.3.1 Search Strategy

This systematic review and meta-analysis were performed according to the PRISMA statement [169]. Studies were eligible for this study if they met the following criteria: (1) observational studies (cohort, case-control, cross-sectional) that evaluated the association between dietary or circulatory MUFA levels with sarcopenia defined as confirmed/severe sarcopenia, probable sarcopenia, sarcopenic index, or sarcopenia risk score; (2) included adult people (aged 18 years and more); and (3) studies published in English. On the contrary, the exclusion criteria included: (1) RCTs; and (2) studies with secondary data (conference abstracts, meta-analyses, reviews, letters, and case reports). The PICOS criteria (i.e., participants, interventions, comparisons, outcomes, and study design) used to define the research question are shown in **Table 7**.

Table 7. PICOS criteria for inclusion of studies

Parameter	Description
Participants	People aged ≥18 years with and without sarcopenia
Intervention/exposure	Dietary or circulatory MUFA
Comparison	People with vs without sarcopenia
Outcome	Confirmed/severe sarcopenia, probable sarcopenia, sarcopenic index, sarcopenia risk score.
Study design	Observational studies (cohort, cross-sectional, case-control)

MUFA, monounsaturated fatty acids.

A systematic search was performed using three databases: PubMed, Scopus, and Web of Science. The following keywords and Boolean operators were used for the literature search:

("monounsaturated fatty acid" OR "monounsaturated fat" OR "MUFA" OR "palmitoleic acid" OR "oleic acid") AND ("sarcopenia" OR "sarcopenic" OR "muscular atrophy" OR "muscle strength" OR "muscle mass" OR "muscle fatigue" OR "physical performance"). At the same time, similar queries were respectively used for controlled vocabulary search: “monounsaturated fatty acids” [Mesh] AND “sarcopenia” [Mesh], INDEX TERMS “monounsaturated fatty acids” AND “sarcopenia”. The search was conducted from inception to August 2022.

3.3.2 Data Extraction and Quality Assessment

After removing duplicate records with the reference management software EndNote X9 (Clarivate Analytics, Philadelphia, PA, USA), titles and abstracts of retrieved articles were screened for eligibility. If an abstract did not provide enough information for evaluation, the full text was retrieved. Disagreements were resolved by consensus. A data extraction table for the included studies was then developed. The following information was extracted: the first author (along with the year of publication and country of the study), study design, participant characteristics (sample size, age, gender), exposure assessment, outcome measure and sarcopenia definition, and the main findings.

Methodological quality assessments of the included studies were performed using the Newcastle-Ottawa Scale (NOS) for observational studies [173]. The NOS assesses three quality domains (selection, comparability, and outcome) divided into eight specific items. The NOS gives a maximum score of 9 points. Studies with NOS scores of 0-3, 4-6, and 7-9 were considered low, moderate and high quality, respectively [174].

3.3.3 Meta-Analysis

The meta-analysis was conducted using the R Software, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and the interface RStudio version 1.4.1717 (R studio, PBC, Boston, MA, USA). Mean values and standard deviations of MUFA levels were extracted for the meta-analysis. The standardized mean difference (SMD) was used as a summary statistic because the

studies assessed the outcome using different techniques and tools. A *p*-value of less than 0.05 was considered to be statistically significant. Heterogeneity among studies was examined with I^2 statistics [170]. Random-effects models were used for the analyses and the possibility of small study effects was assessed qualitatively by a visual estimate of the funnel plot and quantitatively by calculation of the Egger and Begg's tests [175]. In addition, subgroup analyses were performed that included consideration of sarcopenic obesity, participants' age (<65 and ≥ 65 years to evaluate the aging effect), gender (men, women, and both), sarcopenia classification (confirmed/severe sarcopenia and probable sarcopenia), sarcopenia diagnostic criteria (the European Working Group on Sarcopenia (EWGSOP), [149], EWGSOP2 [150], the Asian Working Group for Sarcopenia (AWGS), and others), MUFA exposure assessment (dietary and circulatory), and MUFA levels unit (percent of total energy, percent of total fatty acids, gm/day, and MUFA/SFA ratio).

4. RESULTS

This section is organized as follows; the results of Study I and Study II are described together in Section 4.1., because the method described in Study I was used for analysis in Study II. Section 4.2 is for Study III, and the last section (4.3) presents the results Study IV.

4.1 Age-Associated Changes in Circulatory Fatty Acids

4.1.1 Characteristics of Study Cohort

Characteristics of the study population are presented in **Table 8**. One hundred and seventy-two healthy subjects with an age range of 18-111 years were included. Fifty-five percent of the study cohort were women. All participants were from Western and South-Western Sicily. The population was divided into three age groups, from adults to LLIs. Subjects in the older adults age class showed an increase in BMI (28.2 ± 4.6 kg/m^2), with no gender effect. Regarding serum lipids, TC was low in LLIs compared to adults and older adults, whereas TG levels were not different among the groups.

HDL levels of LLIs were not significantly different from those observed in adults and older adults. However, LLIs showed lower LDL levels than those observed in adults and older adults. Women had higher HDL levels compared to men. However, there was no effect of gender on the serum levels of TC, TG, and LDL.

Table 8. Characteristics of study participants.

Variable	Adults	Older adults	LLIs	Difference by gender
Age range (y)	18-64	65-89	90-111	
Men/Women	34/35	28/26	15/34	
Weight (kg)	71.67 ± 15.11	74.04 ± 13.35	56.15 ± 13.60 [a, b]	< 0.00001 *
BMI (kg/m^2)	25.17 ± 4.48	28.21 ± 4.60 [c]	25.10 ± 5.43 [d]	0.09
TC (mg/dl)	179.28 ± 35.48	187.47 ± 28.54	163.29 ± 30.59 [e, b]	0.8
HDL (mg/dl)	53.38 ± 14.28	53.94 ± 12.79	49.96 ± 12.78	0.00002 *
LDL (mg/dl)	107.43 ± 27.38	112.23 ± 27.41	89.87 ± 24.98 [f, b]	0.06
TG (mg/dl)	92.03 ± 49.41	111.04 ± 43.92	112.86 ± 49.99	0.9

Data are represented as mean ± SD. [a] $p \leq 0.001$, LLIs vs adults; [b] $p \leq 0.001$, LLIs vs older adults; [c] $p \leq 0.01$, older adults vs adults; [d] $p \leq 0.01$, LLIs vs older adults; [e] $p \leq 0.05$, LLIs vs adults; [f] $p \leq 0.01$, LLIs vs adults. LLIs, long-lived individuals; BMI, body mass index; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride. *significant *p*-value.

4.1.2 Gas Chromatography Method Development and Validation

We used a practical and economical method involving the collection of a low volume of venous whole blood or capillary whole blood from finger prick on a DBS card, which provides compositional data within 1-2 days. The GC analysis could separate 22 fatty acids, including 11 PUFAs of interest in 1 µl of the analyte within 40 mins, indicating that the method is sensitive and satisfactory. Additionally, the n-3 index, AA/EPA, and other index and ratios calculation utilize qualitative fatty acid values; therefore, the analysis is particularly suitable for DBS, as blood volumes are often unknown. As shown in **Figure 9**, the SP-2560 column separated individual FAMEs very well and overlapping was

not observed in the chromatogram. The calibration curves were created from five concentrations of each FAME in the standard mixture. According to US FDA guidelines, the lower limit of quantitation (LLOQ) was identified as the lowest point of the calibration curve, which can be determined with 80-120% accuracy and an imprecision <20% [176]. The standard curves of the PUFAs were linear ($r2 \geq 0.99$), and the RSD was < 20 %.

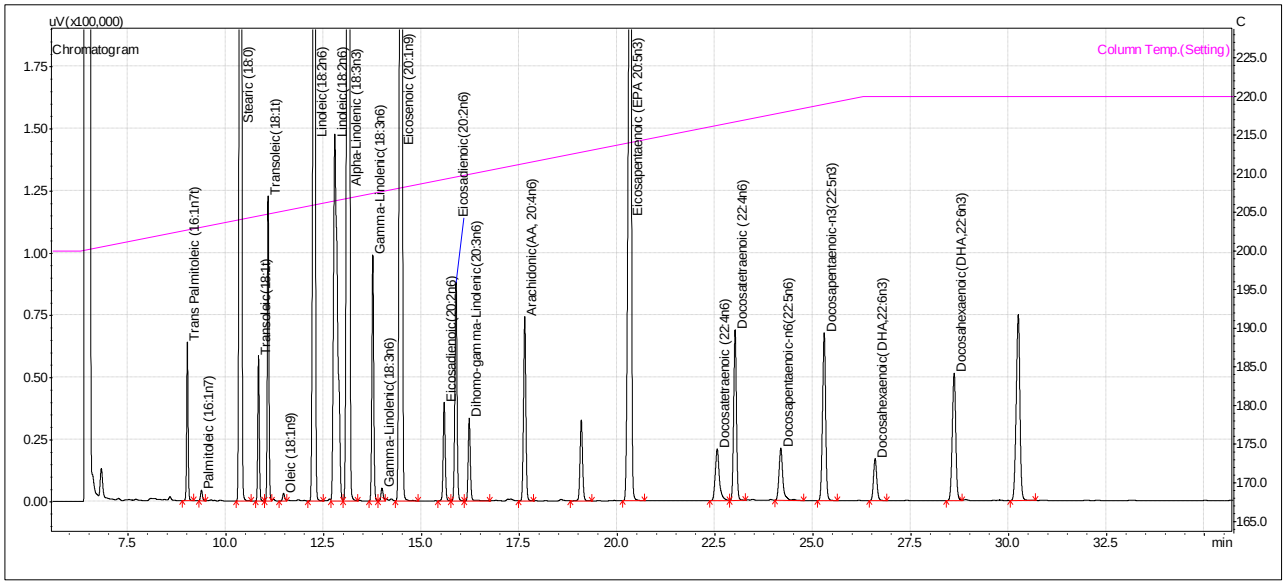


Figure 9. Gas chromatography-flame-ionization detector chromatogram for a standard mixture of fatty acid methyl esters using an SP-2560 column.

4.1.3 Fatty Acid Profile of the Participants

Total blood fatty acid profiles of the population by age group are presented in **Table 9**. **Figure 10** graphically depicts the age-associated changes in the blood levels of total n-3 PUFA, n-6 PUFA, MUFA, SFA and trans fatty acids.

Compared with adults, we found that older adults, but not LLIs, have higher levels of ALA. The values of the DHA ratio in LLIs were similar to adults. However, the DHA value decreased in older adults compared to adults. Although not significantly, a trend towards a reduction in EPA levels with increasing age was also detected. Total n-3 PUFA decreased with age because of overall

decreases in EPA, n-3 DPA, and DHA levels. The reduction in the n-3 index was small but significant in older adults and LLIs with respect to adults.

Total n-6 PUFA decreased with advancing age, primarily due to decreased LA and AA levels. The percentage of LA, the essential n-6 PUFA, significantly differed in LLIs compared to both adults and older adults, with the highest blood levels in adults and the lowest in LLIs. Mean levels of AA (the major n-6 PUFA in erythrocyte membranes) and DTA also decreased in LLIs. However, other LA metabolites, such as DGLA and n-6 DPA, were significantly higher in LLIs than in adults and older adults. No significant difference in AA/EPA and the n-6/n-3 ratio was observed among the groups.

Total SFA compositions remained stable across the lifespan, and there were no gender differences. However, overlapping values and variations in the individual SFA were observed. In particular, the levels of the myristic acid (MC, 14:0) and PA were higher in LLIs compared to adults and older adults, even though the latter was not statistically significant. In contrast, STA and lignoceric acid (24:0) decreased with advancing age.

We also found significant differences in between-group comparisons of total MUFA, with LLIs and adults showing the highest and lowest values, respectively. Consequently, the SFA/MUFA ratio decreased in LLIs compared to adults and older adults. Circulatory levels of the PA metabolite PLA were significantly different among the groups, with the highest values in LLIs compared to adult and older adult subjects. Likewise, OL increased with age, while similar blood levels of eicosenoic acid (20:1n-9) were observed among the study groups.

Regarding trans fatty acids, the fatty acid profile from the LLIs showed a smaller percentage of 18:2n-6t, total trans fatty acids, and trans-fat index compared with both adult and older adult groups. Furthermore, the fatty acid profile from LLIs showed a reduced proportion of elaidic acid compared to adults.

Men and women were significantly different in n-3 DPA, DTA PLA, OL, lignoceric acid, elaidic acid, total MUFA, SFA/MUFA ratio, and trans-fat index. Women had lower n-3 DPA, DTA, lignoceric acid, elaidic acid, trans-fat index, and SFA/MUFA values, while men had lower PLA, OL, and total MUFA percentages. As a result of these differences, we included sex as a covariate in the subsequent analyses. In addition, we observed a significant interaction between age and gender with regard to n-3 DPA, LA, AA, and DTA ($p \leq 0.05$).

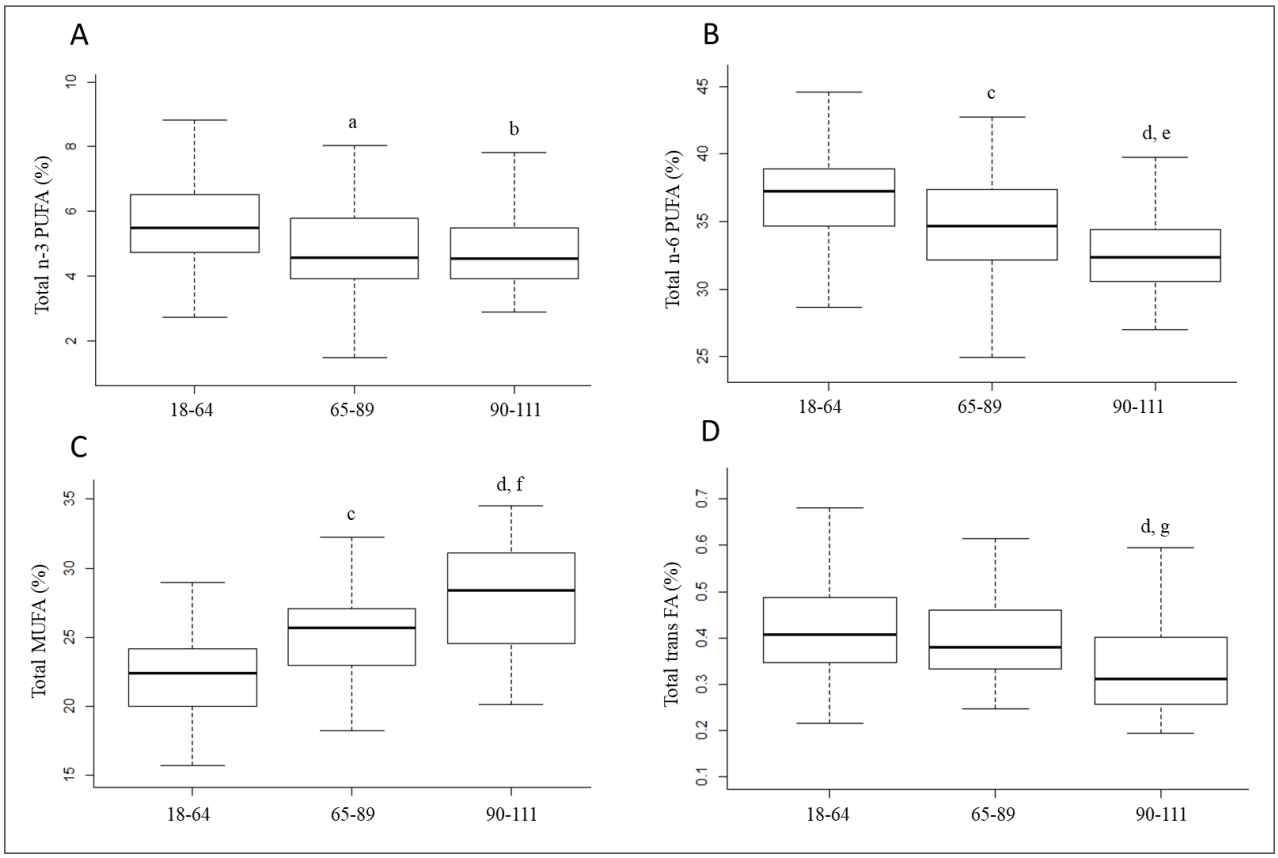


Figure 10. Mean values ± SD of total n-3 PUFA **(A)**, total n-6 PUFA **(B)**, total MUFA **(C)** and total trans fatty acids **(D)**, expressed as % of total fatty acids, in 69 adults (18-64 years); 53 older adults (65-89 years) and 49 LLIs (90-111 years). [a] $p \leq 0.05$, older adults vs adults; [b] $p \leq 0.05$, LLIs vs adults; [c] $p \leq 0.001$, older adults vs adults; [d] $p \leq 0.001$, LLIs vs adults; [e] $p \leq 0.05$, LLIs vs older adults; [f] $p \leq 0.001$, LLIs vs older adults; [g] $p \leq 0.01$, LLIs vs older adults. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; LLIs, long-lived individuals. Fatty acid levels are expressed as percentage of the total fatty acids.

Table 9. Fatty acid status among the study population by age-class

Variable	Adults (n =69)	Older adults (n=54)	LLIs (n = 49)	Difference by gender
ALA (%)	0.32 ± 0.15	0.39 ± 0.15 [a]	0.35 ± 0.13	0.1
EPA (%)	0.79 ± 0.66	0.84 ± 0.76	0.64 ± 0.41	0.6
n-3 DPA (%)	0.95 ± 0.33	0.80 ± 0.30 [a]	0.73 ± 0.25 [b]	0.002 *
DHA (%)	3.71 ± 1.12	3.01 ± 1.19 [a]	3.22 ± 1.08	0.5
Total n-3 PUFA (%)	5.78 ± 1.77	5.03 ± 2.00 [c]	4.94 ± 1.51 [d]	0.3
n-3 index	5.54 ± 1.71	4.76 ± 1.93 [a]	4.70 ± 1.45 [d]	0.2
LA (%)	23.23 ± 4.18	21.77 ± 3.78	19.66 ± 3.57 [b, e]	0.8
GLA (%)	0.28 ± 0.1	0.33 ± 0.12	0.32 ± 0.16	0.9
EA (%)	0.26 ± 0.04	0.26 ± 0.04	0.27 ± 0.04	0.3
DGLA (%)	1.86 ± 0.45	1.90 ± 0.51	2.27 ± 0.59 [b, f]	0.07
AA (%)	10.07 ± 2.27	9.16 ± 2.10	9.08 ± 1.85 [d]	0.6
DTA (%)	1.05 ± 0.56	0.84 ± 0.39	0.82 ± 0.44 [d]	0.01 *
n-6 DPA (%)	0.24 ± 0.10	0.23 ± 0.09	0.32 ± 0.11 [b, g]	0.1
Total n-6 PUFA (%)	36.99 ± 3.35	34.50 ± 4.33 [h]	32.75 ± 3.25 [b, e]	0.6
AA/EPA	18.38 ± 10.02	16.49 ± 8.84	18.36 ± 8.41	0.6
n-6/n-3	6.99 ± 2.17	7.87 ± 3.07	7.19 ± 2.14	0.3
PLA (%)	1.06 ± 0.48	1.64 ± 0.69 [h]	2.09 ± 0.85 [b, e]	0.01 *
OL (%)	19.01 ± 3.00	21.60 ± 3.03 [h]	24.05 ± 3.66 [b, g]	0.02 *
Eicosenoic acid (%)	0.22 ± 0.04	0.23 ± 0.06	0.24 ± 0.05	0.8
NA (%)	1.98 ± 0.77	1.81 ± 0.67	1.52 ± 0.56 [b]	0.2
Total MUFA (%)	22.27 ± 3.05	25.27 ± 3.19 [h]	27.90 ± 3.94 [b, g]	0.01 *
MC (%)	0.62 ± 0.23	0.87 ± 0.36 [h]	0.78 ± 0.28 [i]	0.3
PA (%)	22.59 ± 1.56	23.42 ± 2.24	24.20 ± 1.92 [b]	0.7
STA (%)	10.09 ± 1.87	9.50 ± 1.62	8.49 ± 1.47 [b, f]	0.06
Lignoceric acid (%)	1.13 ± 0.61	0.90 ± 0.39	0.51 ± 0.26 [b, g]	0.002 *
Total SFA (%)	34.44 ± 2.79	34.69 ± 3.41	34.00 ± 2.49	0.06
SFA/MUFA	1.58 ± 0.28	1.40 ± 0.23 [h]	1.25 ± 0.25 [b, f]	0.01 *
16:1n-7t (%)	0.09 ± 0.02	0.10 ± 0.03	0.09 ± 0.03	0.9
Elaidic acid (%)	0.08 ± 0.03	0.09 ± 0.03	0.07 ± 0.02 [g]	0.02 *
18:2n-6t (%)	0.27 ± 0.12	0.22 ± 0.09 [c]	0.18 ± 0.10 [b]	0.1
Total trans fatty acids (%)	0.44 ± 0.13	0.40 ± 0.10	0.34 ± 0.11 [b, f]	0.06
Trans-fat index	0.35 ± 0.13	0.31 ± 0.10	0.25 ± 0.10 [b, f]	0.02 *

Data are represented as mean ± SD. [a] $p \leq 0.01$, older adults vs adults; [b] $p \leq 0.001$, LLIs vs adults; [c] $p \leq 0.05$, older adults vs adults; [d] $p \leq 0.05$, LLIs vs adults; [e] $p \leq 0.05$, LLIs vs older adults; [f] $p \leq 0.01$, LLIs vs older adults; [g] $p \leq 0.001$, LLIs vs older adults; [h] $p \leq 0.001$, older adults vs adults; [i] $p \leq 0.01$, LLIs vs adults. For fatty acid abbreviations see the text. LLIs, long-lived individuals. *significant *p* value. Fatty acid levels are expressed as percentage of the total fatty acids.

We further estimated the D6D, D5D, and ADA desaturase activities reflected in n-3 and n-6 PUFA among the three age classes (see **Table 10**). LLIs had a higher GLA concentration to AA levels than adults, which serves as an indirect marker of D6D enzyme activity. On the other hand, LLIs showed lower D5D enzyme activity than adults and older age groups, as estimated from AA to DGLA concentration levels. No effect of gender was observed.

Table 10. Estimates of D5D and D6D, and ADA desaturase activity reflected in n-3 and n-6 PUFA among the study population by age-class.

Variable	Adults (n =69)	Older adults (n=54)	LLIs (n = 49)	Difference by gender
D6D (GLA/LA)	0.010 ± 0.007	0.015 ± 0.006 [a]	0.020 ± 0.010 [b]	0.7
D5D (AA/DGLA)	5.77 ± 1.95	5.09 ± 1.54	4.28 ± 1.47 [c, b]	0.07
n-3 ADA (EPA/ALA)	3.20 ± 4.09	2.71 ± 3.64	2.00 ± 1.49	0.5
n-6 ADA (AA/LA)	0.46 ± 0.17	0.44 ± 0.15	0.49 ± 0.18	0.8

Data are represented as mean ± SD. [a] $p \leq 0.01$, older adults vs adults; [b] $p \leq 0.001$, LLIs vs adults; [c] $p \leq 0.05$, LLIs vs older adults. LLIs, long-lived individuals; D6D, delta-6 desaturase; D5D, delta-5 desaturase; ADA, aggregate desaturase activity.

The characterisation of the PUFA status in LLIs showed that there were no systematic differences between men and women, except for GLA (see **Table 11**). The average n-3 index was 4.7±1.45. Around 63% of the participants had an n-3 index ranging between 4-8%, while 33% had an n-3 index < 4 %. As for the AA/EPA ratio, the average value was 18.36±8.41. Nearly 40% of the LLIs had an AA/EPA ratio less than 15, while the remaining participants had an AA/EPA ratio higher than 15.

Table 11. Blood polyunsaturated fatty acid status among LLIs.

	All LLIs (n=49)	Women (n=34)	Men (n=15)
ALA (%)	0.35 ± 0.13	0.37 ± 0.13	0.32 ± 0.12
EPA (%)	0.64 ± 0.41	0.61 ± 0.42	0.59 ± 0.24
n-3 DPA (%)	0.73 ± 0.25	0.75 ± 0.27	0.69 ± 0.19
DHA (%)	3.22 ± 1.08	3.25 ± 1.20	3.15 ± 0.77
LA (%)	19.66 ± 3.57	19.04 ± 3.06	21.08 ± 4.31
GLA (%) *	0.32 ± 0.16	0.30 ± 0.17	0.37 ± 0.14
EA (%)	0.27 ± 0.04	0.27 ± 0.04	0.27 ± 0.05
DGLA (%)	2.27 ± 0.59	2.27 ± 0.62	2.27 ± 0.53
AA (%)	9.08 ± 1.85	9.36 ± 1.89	8.46 ± 1.65
DTA (%)	0.82 ± 0.44	0.84 ± 0.48	0.75 ± 0.35
n-6 DPA (%)	0.32 ± 0.11	0.33 ± 0.10	0.29 ± 0.11
n-3 Index	4.70 ± 1.45	4.77 ± 1.60	4.56 ± 1.05
AA/EPA	18.36 ± 8.41	18.99 ± 8.87	16.93 ± 7.36
n-6/n-3	7.19 ± 2.14	7.12 ± 2.30	7.36 ± 1.77

Data are represented as mean ± SD. LLIs, long-lived individuals. For polyunsaturated fatty acid abbreviations see the text. * $p = 0.03$. Fatty acid levels are expressed as percentage of the total fatty acids.

4.1.4 Genetic Variants and their Association with Fatty Acid Profile

The genotypes in the *FADS1/2* gene cluster (rs174579, rs174626, and rs174537), *ELOVL2* (rs953413), and the intergenic rs529143 were determined in 98% (n = 168) of the participants (see **Table 12**). The genotype distribution for each of the examined SNPs was consistent with HWE ($p>0.05$). The minor allele frequency (MAF) of the SNPs ranged between 22 and 49%. No significant differences in the allelic frequencies were observed between the age groups.

Table 12. Allele and genotype frequency of rs174579, rs174626, rs174537, rs953413, and rs529143 polymorphisms among adults, older adults, and LLIs.

SNP	Gene (locus)	Genotype	Genotype and allele frequency						Chi-square (*p*-value)	
			Adults		Older adults		LLIs		Adults vs LLIs	Older adults vs LLIs
			N	%	N	%	N	%		
rs174579	*FADS1/2* (chr11:61838141)	CC	43	62	33	62	25	55		
		CT	23	34	16	30	19	41		
		TT	3	4	4	8	2	4		
		MAF %		21		23		25	0.30 (0.6)	0.05 (0.8)
rs174626	*FADS1/2* (chr11:61869585)	TT	18	26	9	17	11	24		
		CT	35	51	27	51	23	50		
		CC	16	23	17	32	12	26		
		MAF %		49		42		49	0.06 (0.8)	1.15 (0.3)
rs174537	*FADS1/2* (chr11:61785208)	TT	8	12	5	9	5	11		
		GT	27	39	28	53	23	50		
		GG	34	49	20	38	18	39		
		MAF %		31		36		36	0.4 (0.5)	< 0.01 (0.99)
rs953413	*ELOVL2* (chr6:11012626)	AA	15	22	11	21	8	17		
		AG	32	46	25	47	28	61		
		GG	22	32	17	74	10	22		
		MAF %		45		44		48	0.09 (0.8)	0.12 (0.7)
rs529143	Intergenic (chr1:20125527)	CC	5	7	4	8	6	13		
		AC	31	45	22	41	18	39		
		AA	33	48	27	51	22	48		
		MAF %		30		28		33	0.10 (0.7)	0.25 (0.6)

SNP, single nucleotide polymorphism; LLIs, long-lived individuals; H-W; Hardy-Weinberg equilibrium, FADS, fatty acid desaturase; MAF, minor allele frequency; *ELOVL2*, elongase of very long fatty acids 2. *derived from Pearson's chi-square test for Hardy-Weinberg Equilibrium.

It was investigated whether the polymorphisms examined could alter blood PUFA levels and the desaturase indices in LLIs (see **Table 13** and **Table 14**). A positive correlation was identified between rs174537 genotypes and blood levels of LA, eicosadienoic acid (EA, 20:2n-6), and DTA. The major allele carriers of rs174537 had significantly higher levels of DTA, but lower levels of LA and EA compared with the minor allele carriers. Similarly, the genotypes of rs953413 were associated with the DTA, where the presence of the minor allele (A) was associated with lower percentages of DTA. Regarding rs529143, levels of n-3 PUFA were observed. Although the major allele carriers had higher levels of all the observed n-3 PUFA, no significant correlation was observed.

Table 13. Associations between blood PUFA levels with rs174537, rs953413 and rs529143 genotypes among LLIs.

		Genotypes		R-square (*p*-value)
rs174537	**G/G (n = 18)**	**G/T (n = 23)**	**T/T (n = 5)**	
ALA (%)	0.40 ± 0.11	0.35 ± 0.15	0.33 ± 0.09	0.1 (0.2)
EPA (%)	0.73 ± 0.44	0.64 ± 0.41	0.40 ± 0.24	0.04 (0.3)
n-3 DPA (%)	0.74 ± 0.24	0.69 ± 0.20	0.60 ± 0.15	0.11 (0.1)
DHA (%)	3.20 ± 0.99	3.29 ± 1.19	2.51 ± 0.47	-0.08 (0.8)
LA (%)	19.85 ± 2.93	19.20 ± 2.21	23.44 ± 6.60	0.41 (0.0006) *
GLA (%)	0.34 ± 0.22	0.33 ± 0.11	0.31 ± 0.08	0.11 (0.1)
EA (%)	0.25 ± 0.04	0.28 ± 0.04	0.30 ± 0.08	0.27 (0.01) *
DGLA (%)	2.07 ± 0.52	2.41 ± 0.64	2.60 ± 0.46	0.05 (0.3)
AA (%)	9.48 ± 1.80	8.86 ± 1.85	7.77 ± 1.30	0.03 (0.3)
DTA (%)	0.76 ± 0.28	0.73 ± 0.36	0.67 ± 0.08	0.51 (< 0.0001) *
n-6 DPA (%)	0.30 ± 0.08	0.32 ± 0.16	0.27 ± 0.06	0.15 (0.08)
rs953413	**G/G (n = 10)**	**A/G (n = 28)**	**A/A (n = 8)**	
ALA (%)	0.35 ± 0.13	0.35 ± 0.12	0.43 ± 0.14	0.14 (0.09)
EPA (%)	0.68 ± 0.52	0.65 ± 0.43	0.60 ± 0.16	0.04 (0.3)
n-3 DPA (%)	0.74 ± 0.28	0.69 ± 0.02	0.69 ± 0.11	0.08 (0.2)
DHA (%)	3.16 ± 1.22	3.26 ± 1.09	2.87 ± 0.81	-0.07 (0.7)
LA (%)	19.8 ± 2.39	20.43 ± 3.82	18.27 ± 1.96	0.06 (0.2)
GLA (%)	0.29 ± 0.10	0.36 ± 0.19	0.29 ± 0.07	0.1 (0.2)
EA (%)	0.25 ± 0.03	0.29 ± 0.05	0.25 ± 0.04	-0.01 (0.5)
DGLA (%)	2.03 ± 0.69	2.39 ± 0.55	2.31 ± 0.61	-0.1 (0.9)
AA (%)	9.21 ± 2.30	8.98 ± 1.86	8.67 ± 1.00	-0.002 (0.4)
DTA (%)	0.74 ± 0.31	0.74 ± 0.34	0.71 ± 0.19	0.50 (< 0.0001) *
n-6 DPA (%)	0.29 ± 0.12	0.31 ± 0.09	0.31± 0.08	0.06 (0.2)
rs529143	**A/C + A/A (n = 40)**		**C/C (n = 6)**	
ALA (%)	0.37 ± 0.13		0.36 ± 0.10	0.13 (0.09)
EPA (%)	0.65 ± 0.44		0.61 ± 0.15	0.05 (0.2)
n-3 DPA (%)	0.72 ± 0.22		0.60 ± 0.13	0.15 (0.06)
DHA (%)	3.20 ± 1.12		2.99 ± 0.58	-0.06 (0.7)
Total n-3 PUFA (%)	4.93 ± 1.58		4.57 ± 0.75	-0.04 (0.6)

Data are represented as mean ± SD. LLIs, long-lived individuals. For PUFA abbreviations see the text. *significant *p*-value. Fatty acid levels are expressed as percentage of the total fatty acids.

Additionally, desaturase activities were estimated by calculating the product/precursor ratio as indices to evaluate the potential differences in desaturase activities among the LLIs grouped by rs174579 and rs174626 genotypes. The differences were observed for D6D, D5D, n-3 ADA, and n-6 ADA activities, where the D6D index differed significantly between major and minor allele carriers of both rs174579 and rs174626 after adjustment for covariates (see **Table 14**). The D6D estimated as AA/DGLA ratio was lower in the minor allele carriers compared to the major allele carriers for rs174579. The relationships were contrary for rs174626, where the minor allele carriers had a higher D6D index than major allele carriers. Neither D5D, n-3 ADA, nor n-6 ADA was associated with rs174579 genotypes. Likewise, for rs174626 genotypes, no differences in their PUFA product/precursor ratios were found for D5D, n-3 ADA, and n-6 ADA.

Table 14. Estimates of D5D and D6D, and ADA desaturase activity reflected in n-3 and n-6 PUFA with rs174579 and rs174626 genotypes among LLIs.

	Genotype		**Adjusted R-square (*p*-value)**
rs174579	**C/C (n = 25)**	**C/T + T/T (n = 21)**	
D6D activity (GLA/LA)	0.018 ± 0.008	0.016 ± 0.006	0.18 (0.04) *
D5D activity (AA/DGLA)	4.59 ± 1.51	3.71 ± 1.20	0.09 (0.1)
n-3 ADA activity (EPA/ALA)	2.09 ± 1.38	1.80 ± 1.57	-0.01 (0.5)
n-6 ADA activity (AA/LA)	0.50 ± 0.13	0.43 ± 0.13	0.16 (0.07)
rs174626	**T/T (n = 11)**	**C/C + C/T (n = 35)**	
D6D activity (GLA/LA)	0.018 ± 0.011	0.017 ± 0.006	0.17 (0.04) *
D5D activity (AA/DGLA)	5.12 ± 2.00	3.89 ± 1.08	0.08 (0.2)
n-3 ADA activity (EPA/ALA)	1.66 ± 1.10	2.05 ± 1.56	-0.04 (0.6)
n-6 ADA activity (AA/LA)	0.52 ± 0.15	0.45 ± 0.13	0.14 (0.08)

Data are represented as mean ± SD. LLIs, long-lived individuals. D6D, delta-6-desaturase; D5D, delta-5-desaturase; ADA, aggregate desaturase activity. *significant *p* value.

4.2 Effect of N-3 Polyunsaturated Fatty Acids on Telomere Length

4.2.1 Study Selection and Characteristics

As shown in **Figure 11**, the combined search resulted in 573 published studies from the four databases, among which 313 were duplicates. After evaluation of title and abstract, 241 records were discarded because they did not meet the inclusion criteria. The remaining 19 articles were examined for eligibility assessment through full-text reading. Of these, 14 records did not meet the eligibility criteria. Therefore, a total of 5 studies were included in the final analysis.

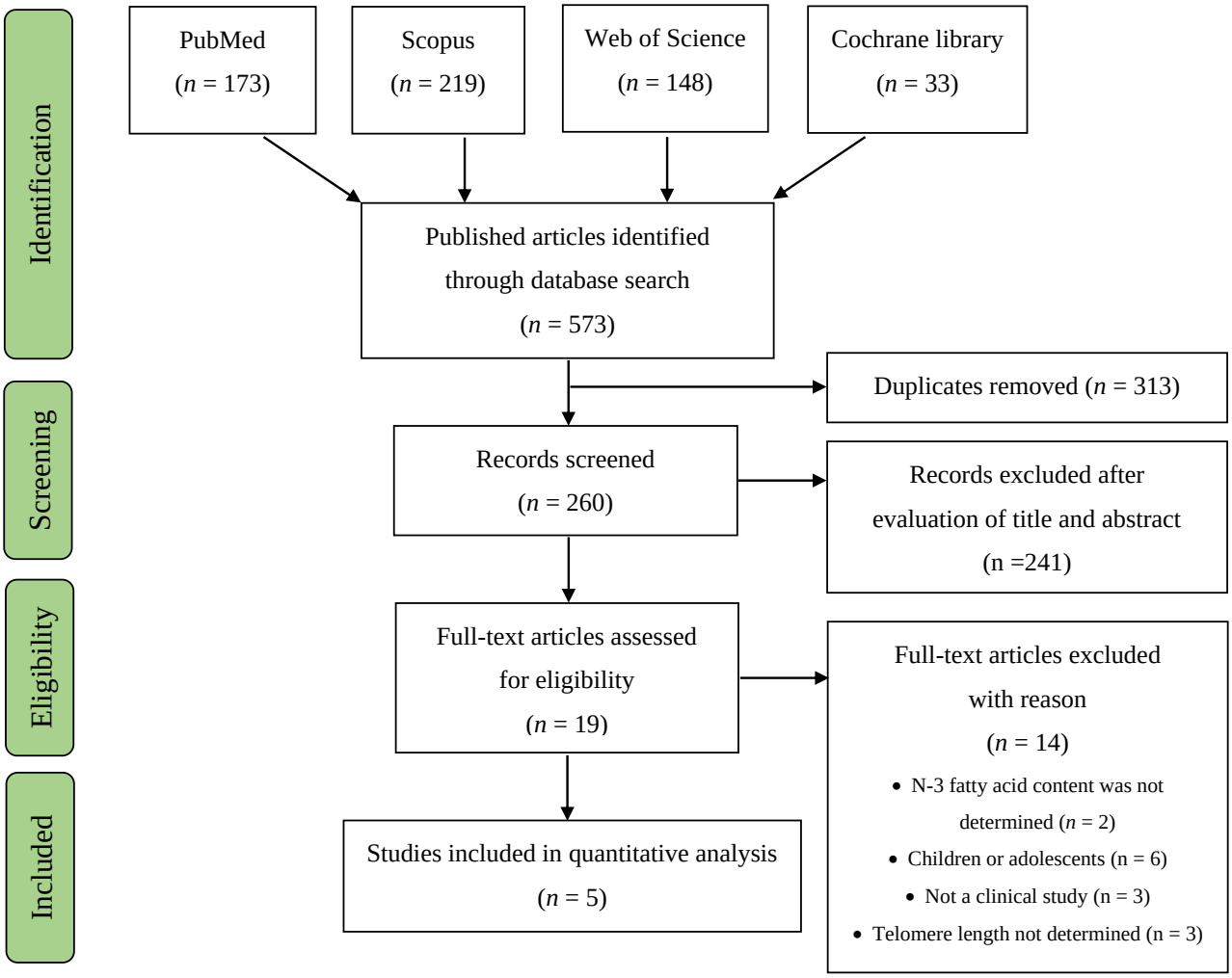


Figure 11. PRISMA flow diagram of the meta-analysis study on the effect of n-3 PUFAs on telomere length.

The 5 included clinical studies were conducted between 2013 and 2019 [142, 177-180]. Three out of the 5 studies selected were RCTs. The sample size ranged from 33 to 106 subjects per study with an average number of 67.4 participants. The clinical trials varied in time duration from 2 to 12 months. The age of all participants varied from 33 to > 65 years. Three studies assessed both men and women, one study assessed only women, and 1 study did not provide information about sex of participants. Quantitative PCR was the preferred method for measuring telomeres and 1 study used quantitative-fluorescent *in situ* hybridization. The characteristics of all the included studies are presented in **Table 15**.

4.2.2 Meta-Analysis

Overall, five clinical trials were meta-analyzed that involved a total of 337 participants. Using a random effect model, the meta-analysis showed a statistically significant effect of n-3 PUFA supplementation on telomere length (mean difference = 0.16; 95% CI, 0.02, 0.30; $p = 0.02$) (**Figure 12A**). However, there was significant evidence of high heterogeneity (I2 = 85%) in the overall analysis. Thus, a sensitivity analysis was conducted by omitting 1 study with no control from the primary analysis and recalculating the effect size. After the sensitivity analysis, the positive effects of n-3 PUFAs on telomere length remained significant (mean difference = 0.21; 95% CI, 0.07, 0.36; $p < 0.01$) (**Figure 12B**). Although the heterogeneity was reduced to I2 = 69%, the grade remained moderate.

Table 15. Characteristics of the included clinical trials in the meta-analysis on the effect of n-3 PUFAs on telomere length.

Study (Author, year, Ref.)	Country	Design	Study characteristics	Intervention	Telomere length assessment method	Fluid analysed	Results
Balcerczyk et al., 2014 **[177]**	Poland	Intervention trial	66 women (age range 35-55 y) Duration: 3 months Condition: healthy	n-3 PUFAs (1350 mg/d)	qPCR (T:S ratio)	Blood	No effect on telomere length.
Barden et al., 2016 **[178]**	Australia	Randomized double-blind placebo-controlled trial	85 subjects (mean age 56.5 ± 1.4 y) (men and women) Duration: 2 months Condition: kidney disease	n-3 PUFAs (4 g/d)	qPCR (kb/genome)	Blood	Significant increase of neutrophil telomere length after correction for neutrophil count ($p = 0.015$).
Kiecolt-Glasera et al., 2013 **[142]**	USA	Randomized double-blind placebo-controlled trial	106 subjects (mean age 50.7 y) (37 men and 69 women) Duration: 4 months Condition: overweight	n-3 PUFAs (2.5 g/d or l.25 g/d)	qPCR (bp)	Blood	Telomere length increased but not significantly
O'Callaghan et al., 2014 **[179]**	Australia	Randomized double-blind controlled pilot study	33 subjects (age > 65 y) Duration: 6 months Condition: mild cognitive impairment	EPA-rich fish oil (1.67 g EPA + 0.16 g DHA/d) or DHA-rich fish oil (1.55 g DHA + 0.40 g EPA/d)	qPCR (kb/genome)	Blood	Significant reduction of telomere shortening ($p < 0.02$)
Tsoukalast et al., 2019 **[180]**	Greece	Intervention trial	47 subjects (mean age 47.1 y) (24 men and 23 women) Duration: 6-12 months Condition: healthy	ALA (370 mg/d); EPA (312,6 mg/d); DHA (154,2 mg/d)	Q-FISH (bp)	Blood	Significant increase in telomere length ($p < 0.05$)

Abbreviations: PUFAs, polyunsaturated fatty acids; qPCR, quantitative real-time polymerase chain reaction; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, alpha-linolenic acid; Q-FISH, quantitative-fluorescent in situ hybridization.

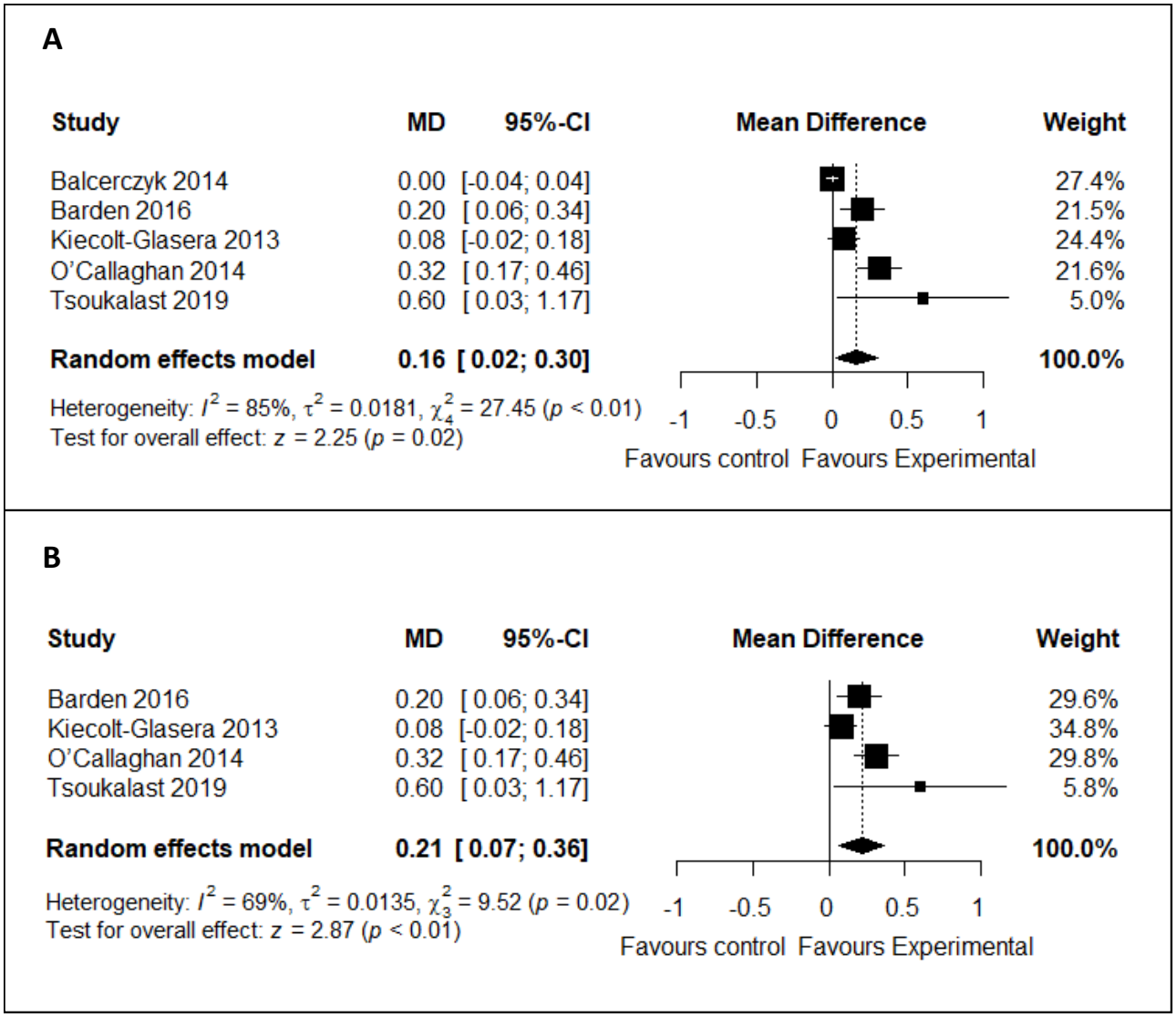


Figure 12. Forest plots showing the effect of n-3 PUFAs on telomere length. **A)** Forest plot of the overall analysis. **B)** Forest plot of the sensitivity analysis.

Furthermore, the publication bias was assessed using funnel plots. As shown in **Figure 13**, the funnel plot analysis revealed that the risk of publication bias was low. Results for the risk of bias are shown in **Table 16** and **Figure 14**. The critical appraisal tools of the intervention trials show good quality in the methodology.

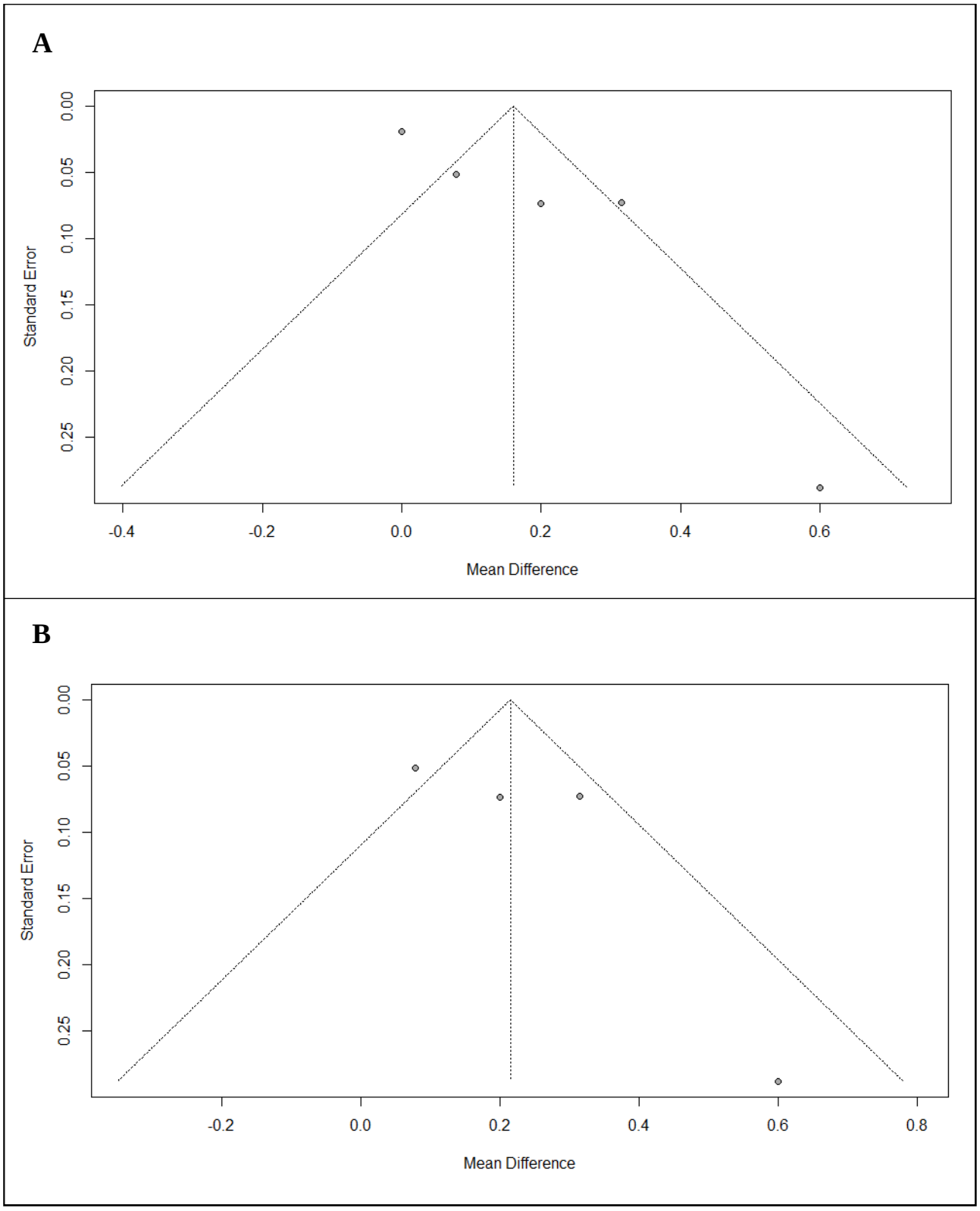
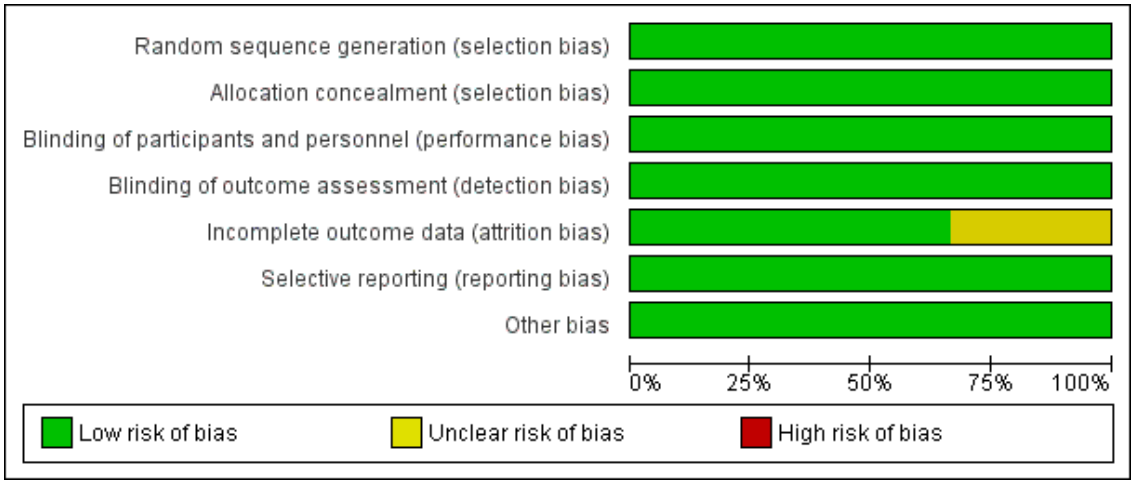


Figure 13. Funnel plots of risk of publication bias of the included studies in the meta-analysis on the effects of n-3 PUFAs on telomere length. **(A)** Overall analysis. **(B)** Sensitivity analysis.

Table 16. Quality assessment of the included interventional trials in the meta-analysis on the effect of n-3 PUFAs on telomere length

Articles	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data (attrition bias)	Selective reporting	Other bias
Barden 2016	Low	Low	Low	Low	Unclear	Low	Low
Kiecolt-Glasera 2013	Low	Low	Low	Low	Low	Low	Low
O'Callaghan 2014	Low	Low	Low	Low	Low	Low	Low
	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result
Balcerczyk 2014	-	-	-	Low	Low	Low	Low
Tsoukalast 2019	Moderate	Low	Low	Low	Low	Low	Low

A



B

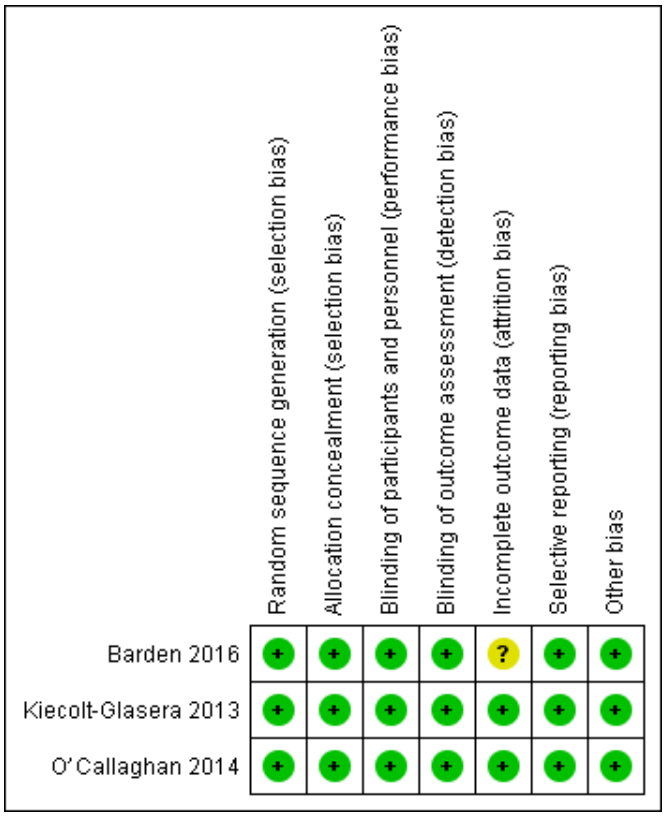


Figure 14. Results for the risk of bias in the meta-analysis on the effect of n-3 PUFAs on telomere length. **(A)** Risk of bias graph. **(B)** Risk of bias summary.

4.3 Effect of Monounsaturated Fatty Acids on Sarcopenia

4.3.1 Study Selection and Characteristics

The PRISMA flow diagram of the systematic review and meta-analysis on the association between MUFA intake and sarcopenia is presented in **Figure 15**. A total of 414 potentially relevant articles (PubMed: 60, Scopus: 300, and Web of Science: 54) were yielded during initial literature searches. After eliminating 112 duplicated articles, 302 articles were screened by titles and abstracts. Eventually, a total of 12 articles were identified to be included in our study [181-192].

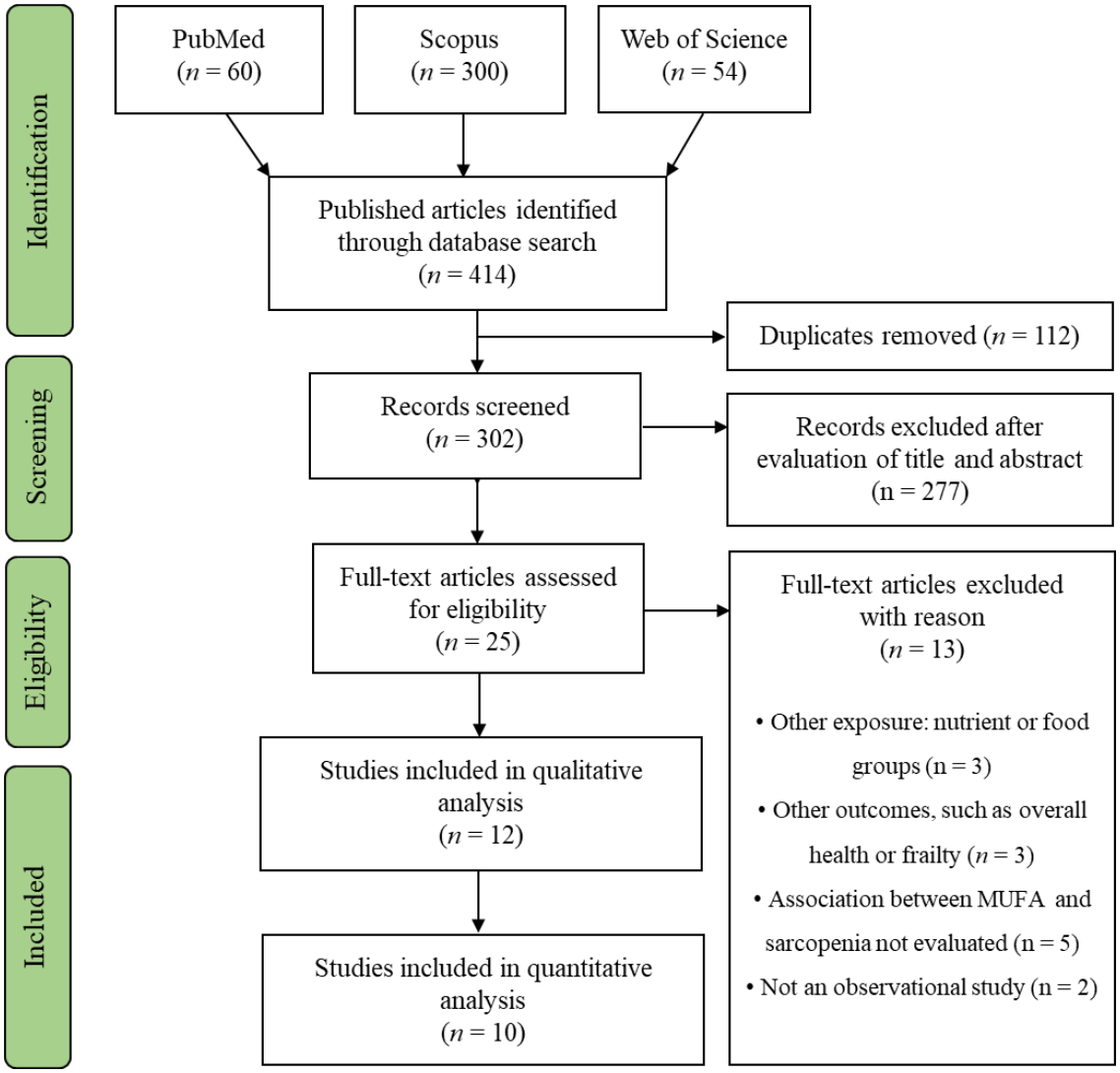


Figure 15. PRISMA flow diagram of the systematic review and meta-analysis on the association between MUFA intake and sarcopenia.

The main characteristics of the included studies are presented in **Table 17**. The studies were published between 2006 and 2021. The countries in which the studies were conducted were Spain [181, 183], Brazil [184, 185], Japan [188, 190], Canada [182], Iran [186], Finland [187], the Netherlands [191], and UK [192], and one study was conducted in multiple European countries [189]. All studies included both men and women, except for one study that included only men [187] and one that included only women [182]. The sample size ranged from 22 to 1535 subjects. The diagnostic criteria for sarcopenia were the EWGSOP [183], the EWGSOP2 [185-187, 189, 191], the AWGS [188, 190], and other diagnostic criteria [181, 182, 184, 192]. Two studies measured circulatory MUFA levels [188, 191], whereas the remaining ten studies estimated dietary intake of MUFA. The dietary MUFA were determined by food-frequency questionnaire (FFQ) [181, 186], 24-hour dietary recall [183, 185], 3-day food diary [187, 192], 7-day food diary [189], dietary recall questionnaires [184], and brief-type self-administered diet history questionnaire (BDHQ) [190].

The quality of the included studies, based on NOS quality assessment, is shown in **Table 18**. One study achieved NOS scores of 6 (moderate quality) [182], and 11 studies was rated as “high” quality (7-9 points) [181, 183-185, 187-190, 192, 193].

4.3.2 Meta-Analysis

Two of the 12 studies were excluded from the meta-analysis because the required pooling data were unavailable [189, 194]. These studies involved a total of 3704 participants. The overall combined SMD showed that the dietary or circulatory MUFA level is inversely associated with the risk of sarcopenia (SMD = -0.28, 95% CI: -0.46 to -0.11; $p < 0.01$) (**Figure 16**). A high level of heterogeneity was found among the studies ($I2 = 79\%$, $p < 0.01$). The funnel plots showed no evidence of publication bias in the included studies (**Figure 17**). Likewise, Egger’s and Begg’s rank tests detected no evidence of publication bias ($p = 0.09$ and $p = 0.7$, respectively).

Table 17. Characteristics of the included studies of the systematic review and meta-analysis on the association between MUFA intake and sarcopenia.

Study (author, year, country, reference)	Design	Participant characteristics	Assessment of MUFA levels	Outcome measure	Sarcopenia definition	Main findings
Abete 2019, Spain **[181]**	Cross-sectional from the PREDIMED-Plus trial	1535 subjects (mean age 65.2 y) 48% women	143-item semi-quantitative FFQ	Skeletal muscle mass index by DXA	Sarcopenic index tertiles (women: T1: <21.0% and T3: ≥22.7; men: T1: <26.3% and T3: ≥28.5%)	No difference in dietary MUFA levels between tertiles of sex-specifict sarcopenic index
Aubertin-Leheudre 2006, Canada **[182]**	Cross-sectional	22 obese postmenopausal women (mean age 66 y)	3-day dietary record	Muscle mass index by DXA	Muscle mass index <14.30 kg fat-free mass/m2	No difference in diettay MUFA levels between sarcopenic-obese and non-sarcopenic-bese subjects
Bibiloni 2018, Spain **[183]**	Cross-sectional	380 subjects (age range 55-80 y) 54% women	24-hour dietary recall	Muscle strength by HGS test and physical performance by 8-f TUG test	EWGSOP	Significant lower MUFA levels in subjects with low HGS compared to those normal HGS (p = 0.001)
De-franca 2020, Brazil **[184]**	Cross-sectional from ISA-Capital 2015 and 2015 ISA-Nutrition studies	218 community-dwelling adults (mean age 63 y) 52% women	Dietary recall questionnaires	Muscle mass by DXA, muscle strength by HGS test, and physical function by 4-m walking test	Muscle mass <0.789 for men and <0.512 for women	No difference in dietary MUFA levels between osteosarcopenic obese and normal subjects

						Results
Dos Reis 2021, Brazil **[185]**	Cross-sectional	125 kidney transplant patients (mean age 48 y) 32% women	24-hour dietary recall	Appendicular muscle mass by BI, muscle strength by HGS test, and physical performance by 4-m walking test	EWGSOP2	Significant lower MUFA levels in sarcopenic subjects compared to non-sarcopenic subjects ($p = 0.005$)
Esmaeily 2021, Iran **[186]**	Cross-sectional	201 community-dwelling older adults (mean age 65.72 y) 77% women	147-item semiquantitative FFQ	Muscle strength by HGS test	Prosarcopenia according to EWGSOP2	Higher ratio of MUFA and PUFA to SFA in subjects with a low prosarcopenia scores compared to high prosarcopenia scores ($p = 0.03$)
Jyvakorpi 2020, Finland **[187]**	Cross-sectional from HBS study	126 men (mean age 87 y)	3-day food diary	Appendicular muscle mass, muscle strength, and physical performance by SPPB	EWGSOP2	Significant negative association between MUFA levels and sarcopenia ($p = 0.01$)
Katoh 2020, Japan **[188]**	Retrospective cross-sectional study	308 cardiovascular patients (mean age 72 y) 43.5% women	Serum MUFA levels	Muscle mass by BI, muscle strength by HGS test, and physical performance by gait speed test	AWGS	Low serum levels of OL ($p = 0.005$) and high serum levels of NA ($p < 0.001$) and erucic acid ($p < 0.001$) in sarcopenic subjecrs compared to non-sarcopenic subjects
Montiel-rojas 2020, European countries **[189]**	Cross-sectional from NU-AGE Cohort	986 older adults (age range 65-79 y) %58 women	7-day food record	Skeletal muscle mass by DXA and muscle strength by HGS test	Sarcopenia risk score according to EWGSOP2	No significant association between MUFA levels and sarcopenia risk score

Otsuka 2021, Japan **[190]**	Cross-sectional from the fifth survey of the ROAD Study	1345 community-dwelling subjects (mean age 71.2 y) 67.5% women	BDHQ	Muscle mass by BI, muscle strength by HGS test, and physical performance by 6-m walking test	AWGS	Significant lower MUFA levels in sarcopenic subjects compared to non-sarcopenic subjects (p = 0.01)
ter Borg 2018, Netherlands **[191]**	Cross-sectional Maastricht Sarcopenia study	227 older adults (age ≥65 y) 52% women	Blood MUFA levels	Skeletal muscle mass by BI, muscle strength by HGS test, and physical performance by gait speed test and chair stand test	EWGSOP	No significant association between MUFA levels and sarcopenia
Verlaan 2017, UK **[192]**	Case-control from PROVIDE study	132 older adults (mean age 71 y) 39% women	3-day food diary	Appendicular muscle mass by DXA, muscle strength by HGS test, and muscle function by SPPB	SPPB score of 4-9, muscle mass index <37% for men and <28% for women, and BMI 20-30 kg/m2	No significant difference in MUFA levels between sarcopenic and non-sarcopenic subjects

FFQ, food frequency questionnaires; DXA, dual-energy X-ray absorptiometry; MUFA, monounsaturated fatty acids; HGS, handgrip strength; 8-f TUG, eight-foot time up-and-go; EWGSOP, the European working group on sarcopenia in older people; BI, bioimpedance; EWGSOP2, revised EWGSOP; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SPPB, short physical performance battery; AWGS, the Asian working group for sarcopenia; OL, oleic acid; NA, nervonic acid, BDHQ, brief-type diet history questionnaire; BMI, body mass index. * the values < 0.789 for men and <0.512 for women were used in the De-franca 2020 as cutpoints for low muscle mass based on the Foundation for the National Institutes of Health Sarcopenia Project.

Table 18. Risk of bias assessment of the included studies of the systematic review and meta-analysis on the association between MUFA intake and sarcopenia

Study	Selection	Comparability	Outcome	Total No. stars	Quality of study
Abete 2019	***	**	**	7	High
Aubertin-Leheudre 2006	***	*	**	6	Moderate
Bibiloni 2018	***	**	**	7	High
De-franca 2020	***	**	**	7	High
Dos Reis 2021	***	**	**	7	High
Esmaeily 2021	***	**	**	7	High
Jyvakorpi 2020	***	**	**	7	High
Katoh 2020	***	**	**	7	High
Montiel-rojas 2020	***	**	**	7	High
Otsuka 2021	***	**	**	7	High
ter Borg 2018	***	**	**	7	High
Verlaan 2017	***	**	***	8	High

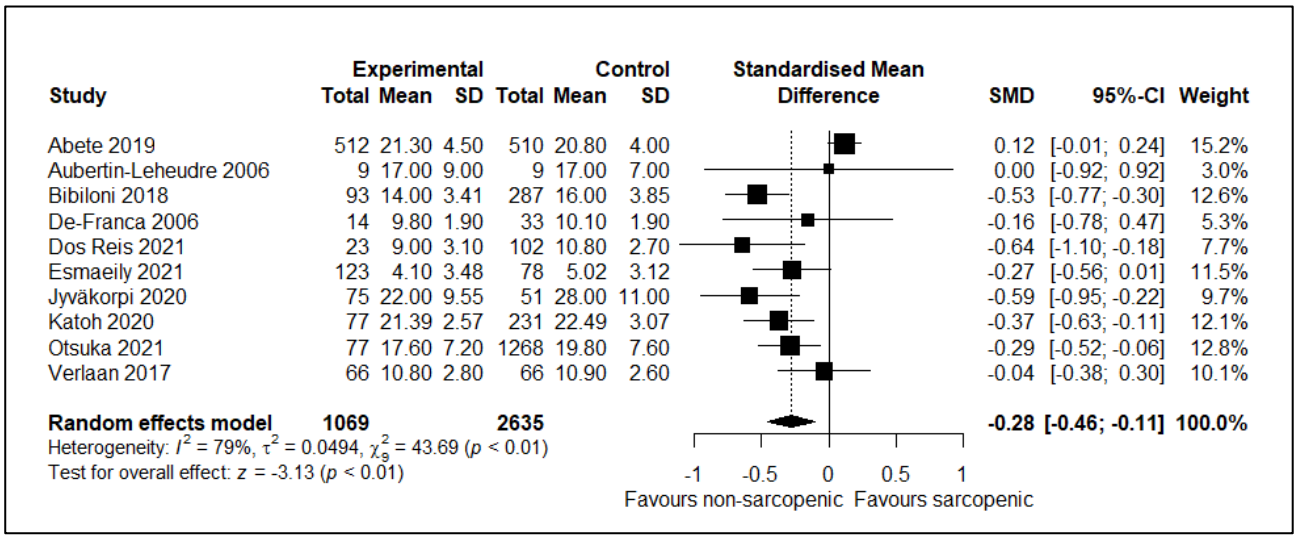


Figure 16. Forest plot showing the effect of dietary or circulatory levels of MUFA on sarcopenia in 10 observational studies.

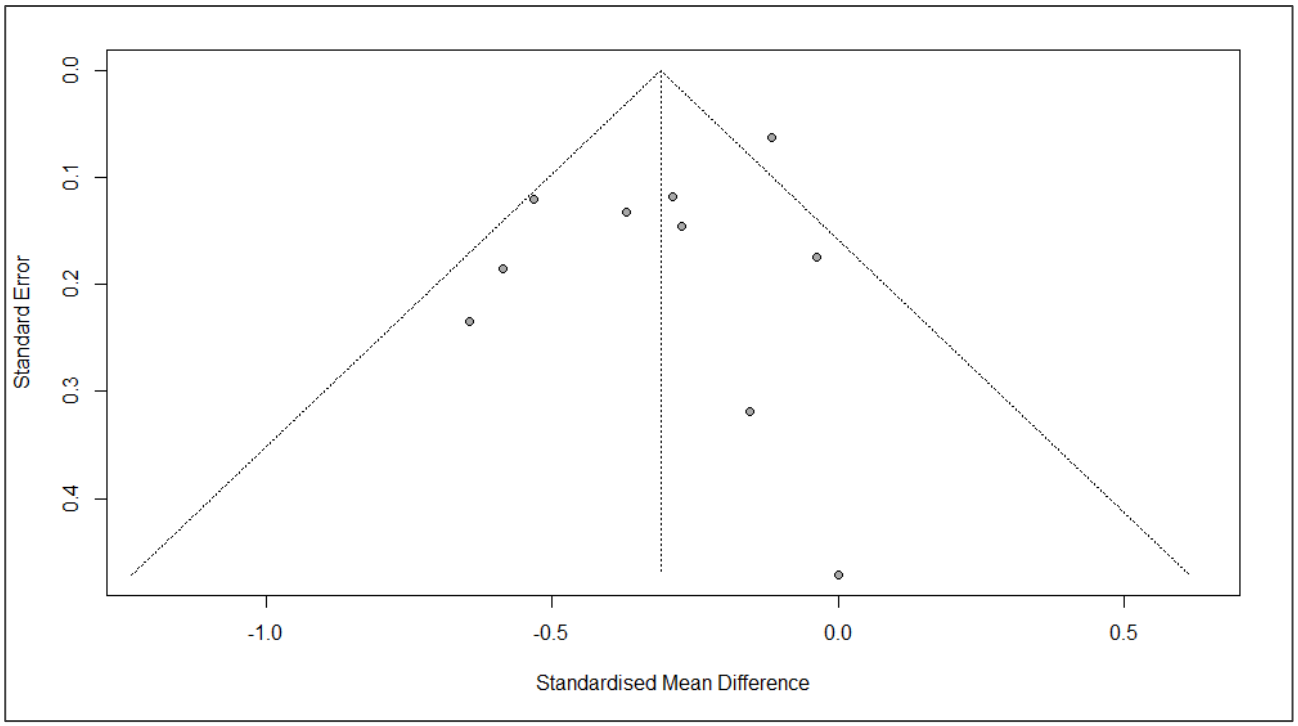


Figure 17. Funnel plot for publication bias of the systematic review and meta-analysis on the association between MUFA intake and sarcopenia.

The results of the subgroup analyses are shown in **Table 19**. The pooled analysis of three studies that involved 1087 subjects with sarcopenic obesity did not show a significant association between MUFA intake and sarcopenic obesity (SMD = 0.11, 95% CI -0.01 to 0.22, $p = 0.08$). Eight studies on 3532 people over 65 years of age confirmed a significant effect of MUFA on sarcopenia (SMD = -0.26, 95% CI: -0.45 to -0.07, $p < 0.01$). The remaining 2 studies involved 172 subjects with an overall mean age less than 65 years, and the pooled analysis showed a negative, although not significant, association between MUFA levels and sarcopenia (SMD = -0.45, 95% CI: -0.92 to 0.02, $p = 0.06$). Likewise, eight studies included both men and women and showed a negative association (SMD = -0.26, 95% CI: -0.45 to -0.07, $p < 0.01$). Regarding the diagnostic criteria of sarcopenia, the significant result existed in EWGSOP2 (SMD = -0.46, 95% CI: -0.71 to -0.21; $p < 0.01$) and AWGS (SMD = -0.33, 95% CI: -0.50 to -0.15; $p < 0.01$), but lost in others (SMD = -0.09, 95% CI: -0.02 to 0.20; $p = 0.12$).

Table 19. Subgroup analyses on the effects of MUFA on sarcopenia

	No. of studies	No. of participants	I2 (%)	SMD (95% CI)	*p*-value
Overall	10	3704	79.0	-0.28 (-0.46, -0.11)	< 0.01
Sarcopenic obesity	3	1087	0.0	0.11 (-0.01, 0.22)	0.08
Age					
≥65 years	8	3532	82.0	-0.26 (-0.45, -0.07)	<0.01
<65 years	2	172	34.0	-0.45 (-0.92, 0.02)	0.06
Gender					
Women	1	18	-	0.00 (-0.92, 0.92)	-
Men	1	126	-	-0.59 (-0.95, -0.22)	-
Women and Men	8	3560	82.0	-0.26 (-0.45, -0.07)	< 0.01
Sarcopenia classification					
Confirmed/severe sarcopenia	7	3028	42.0	-0.26 (-0.41, -0.11)	<0.01
Probable sarcopenia	2	300	46.0	-0.41 (-0.74, -0.08)	0.01
Sarcopenia diagnostic tool					
EWGSOP	1	380	-	-0.53 (-0.77, -0.30)	-
EWGSOP2	3	452	26.0	-0.46 (-0.71, -0.21)	<0.01
AWGS	2	1653	0.0	-0.33 (-0.50, -0.15)	< 0.01
Others	4	1219	0.0	-0.09 (-0.02, 0.20)	0.12
Exposure assessemnt					
Dietary	9	3396	80.0	-0.27 (-0.47, -0.07)	< 0.01
FFQ	2	1223	84.0	-0.06 (-0.44, 0.32)	0.6
Dietary recall	7	2173	34.0	-0.37 (-0.55, -0.19)	< 0.01
Circulatory	1	308	-	-0.37 (-0.63, -0.11)	-
MUFA intake expressed as					
Percent of total energy	6	1832	87.0	-0.29 (-0.57, -0.01)	0.04
Percent of total FA	1	308	-	-0.37 (-0.63, -0.11)	-
Grams/day	3	1488	0.0	-0.28 (-0.48, -0.08)	<0.01
MUFA/SFA ratio	2	327	0.0	-0.34 (-0.57, -0.12)	<0.01

MUFA, monounsaturated fatty acids; SMD, standardized mean difference; EWGSOP, the European Working Group on Sarcopenia in Older People; EWGSOP2, revised EWGSOP; AWGS, the Asian Working Group for Sarcopenia; FFQ, food frequency questionnaires; SFA, saturated fatty acids.

The above findings were also confirmed in dietary MUFA levels (SMD = -0.27, 95% CI: -0.47 to -0.07, p <0.01), considering dietary recall tools (SMD = -0.37, 95% CI: -0.55 to -0.19, p <0.01). Additionally, MUFA levels expressed as a percent of total energy (SMD = -0.29, 95% CI: -0.57 to -0.01, p = 0.04), grams/day (SMD = -0.28, 95% CI: -0.48 to -0.08, p < 0.01), and MUFA/SFA ratio (SMD = -0.34, 95% CI: -0.57 to -0.12, p <0.01) showed significant negative associations between MUFA and sarcopenia.

5. DISCUSSIONS

The work in this thesis has examined the role of fatty acids in aging process. We hypothesized that blood fatty acids, mirroring the whole-body status, could signify physiological decline and better aging. We determined blood fatty acid profile in 69 adults (18-64 years old), 54 older adults (65-89 years old), and 49 LLIs (90-111 years old) from Southern Italy. The results showed that LLI population from this area have a distinctive fatty acid profile, characterized by significantly high MUFA levels. In addition, we found that supplementation of n-3 PUFAs is positively associated with telomere length in clinical trials. Furthermore, we observed that MUFA intake may mediate a negative association with the incidence of sarcopenia. These findings added new evidence in the literature of the important role of fatty acids on aging process and age-related phenotypes.

5.1 General Discussion

5.1.1 Implications

The findings in this thesis largely corroborate previous research of the same topics. However, our studies also highlight the importance of fatty acids in healthy aging from novel perspectives. In Study

I, we applied a method based on GC-FID analysis, the results from which showed a precise and accurate determination of fatty acid profile from low volume blood samples. In Study II, we observationally analysed a cohort of 172 participant aged from 18-111 years to characterize the age-related changes in fatty acid profile and to examine the effect of genetic variants of fatty acid profile. The results showed differences in fatty acids profile among the age groups studied, implying a change in physical properties of the blood cell's membrane with advancing age. In addition, the results suggested the MUFA is potential marker of extreme longevity in this LLI population from Southern Italy. While in Study III and IV we exploited data from RCTs and observational studies to meta-analytically analyse the correlation between n-3 PUFAs and MUFA with telomer length and sarcopenia occurrence, respectively. If no biases are present, then supplementation of n-3 PUFAs is associated with longer telomer, and higher MUFA intakes reduce the risk of sarcopenia. The following discussions might serve as an introduction to the unanswered questions.

5.1.2 Age-Associated Changes in Fatty Acid Profile

Over the past decade, various epidemiological studies on genetic, demographic, and phenotypic characteristics of longevity support that LLIs are an ideal model of healthy human aging [13-15]. Despite decreased physical functioning, LLIs have a high ability to adapt to age-associated challenges, and the majority of them endure or escape diseases that cause death at younger ages [15]. We determined blood fatty acid profile in a cohort of 172 subjects from Southern Italy and assessed the role of gender in determining the blood fatty acid composition. The results showed differences in fatty profile among the different age groups, indicating age-associated changes in physical properties of the blood cell's membrane.

Compared with adults, we found that older adults, but not LLIs, have higher levels of ALA, which is indicative of an accumulation of the precursor of n-3 PUFA metabolic pathways. Despite the high ALA levels, DHA levels were significantly reduced in older adults compared to adults. The reduced

DHA levels among older adults might be explained by the age-associated decline in enzyme activity or decreased absorption of DHA. Despite their age, the LLIs maintained DHA levels similar to adults. Similar data have been reported previously in a population from central Italy, in which higher erythrocyte membrane levels of DHA in centenarians were observed compared with elderly (61 to 99 years old) subjects [195]. Moreover, Puca et al. have examined fatty acid profile of erythrocyte membranes as possible biomarker of longevity by studying another model of successful aging and longevity, i.e., nonagenarian children [4]. They demonstrated a number of modifications of the erythrocyte membrane components including an increase in 3-PUFA compared to the older population. The results of the genetic analysis did not indicate a role of the genetic loci in modulating the lipid composition observed in nonagenarian offspring erythrocyte membranes. In agreement with this, the conversion rate from ALA to EPA and especially to DHA is described to be low [196, 197]. Thus, it is clear that DHA values are lower in older adults than in adults but not in centenarians and that apparently genetics do not play a role. Concerning the possible role of diet to explain the differences between older adults and LLIs, in a survey (Aiello et al., 2021 quoted [20]), no dietary differences was noted between these two populations. Thus, further studies are needed to go insight to this difference between older adults and LLIs.

Regarding EPA, there was a trend in decreasing its amounts with age, but this change did not reach a significant level. Nishihira et al. reported similar results among community-dwelling octogenarians (80 and 94 years old) in Okinawa, in which serum EPA levels did not change significantly with increasing age [198]. In contrast, other studies in different populations have demonstrated that increasing age predicts higher circulatory n-3 PUFA, especially EPA and DHA [199-201]. Increasing EPA and DHA with age in these studies is suggested to be an artifact, resulting from older people consuming more fish and n-3 supplements than younger people [202]. Therefore, it is speculated that aging may not augment n-3 PUFA, but greater n-3 PUFA levels may promote longevity.

There was also a small but significant reduction in the n-3 index in older adults and LLIs compared to adults. The n-3 index is associated with a lower risk of fatal CHD [203]. The cardioprotective target level for the n-3 index is around 8%, and the level associated with the increased risk for CHD death is <4% [204]. A systematic review of healthy adults showed high % EPA + DHA values (> 8 %) in European countries, such as Denmark and Norway, and moderate n-3 index values (> 4-6 %) in Finland and Sweden. In contrast, very low blood levels were observed in Italy (≤ 4%) [205]. The majority of the LLIs in the present study had an average n-3 index between 4 and 8 % (67%, n = 33), while the n-3 index in 33% (n = 16) of LLIs was <4 %. The average moderate value of the n-3 index suggests a decreased risk for chronic diseases among LLIs in this study.

Regarding n-6 PUFA, LA levels were inversely associated with age in our study. A similar trend was seen in other studies, where the plasma LA declined with age [206, 207]. The reason for the inverse relations between age and LA levels is not well understood. One possible explanation would be a change in dietary LA intake [202]. While the increase in LA value was reflected by the level of its D5D metabolite AA, other important LA metabolites, such as DGLA, were higher in LLIs than in adults and older adults. DGLA is a product of GLA and a precursor of AA that acts as a substrate for cyclooxygenases and lipoxygenases to produce anti-inflammatory eicosanoids [208]. AA, the primary n-6 PUFA in the erythrocyte membrane, is a precursor to several pro-inflammatory mediators, such as prostaglandins and leukotrienes. It is either obtained from the diet or endogenously synthesized from LA [209]. Modifying dietary LA does not affect AA circulatory levels, and LA availability is not rate-limiting for AA synthesis [202, 210]. Therefore, metabolic factors might determine membrane AA levels [202]. Our result of a reduced AA level with age is consistent with previous evidence, reporting lower levels of both LA and AA in nonagenarian offspring from Southern Italy than in matched controls [4]. The authors suggested that low levels of the n-6 PUFA may speculate a reduced peroxidability in the cell membrane of nonagenarian offspring.

Surprisingly, our results showed high total MUFA and OL levels among LLIs compared to other age classes. We suppose that the high consumption of the EVOO may have influenced this datum. Indeed, in Sicily, EVOO is the primary source of MUFA [211], and we observed that the frequency of EVOO among LLIs was greater than in adults and older adults. MUFA from olive oil was associated with a significant risk reduction of all-cause mortality, cardiovascular mortality, and cardiovascular events [65]. Higher levels of MUFA indicate maintenance of cell membrane fluidity and resistance toward peroxidation [212]. The high consumption of EVOO (characteristics of the Sicilian diet [211]) also leads to a higher proportion of MUFA in the mitochondrial membrane, which is associated with a better mitochondrial function and a reduction of mitochondrial oxidative stress during aging [213]. Our analysis also showed high PLA levels among LLIs with respect to adults and older adults. This agrees with a recent study by Manca et al. on elderly people from the longevity Blue Zone of Sardinia/Italy, in which elderly people from a high longevity zone of Sardinia/Italy showed higher levels of PLA compared with the elderly from a low longevity zone of the same island [48]. The so-called “Blue Zone” are areas of the world inhabited by exceptionally long-lived populations. The Blue Zone of Sardinia is located in the central-eastern mountain area of the island that displays one of the highest concentrations of LLIs in the world [48]. High levels of circulatory PLA suggest an efficient adipose tissue *de novo* lipogenesis (DNL), which is associated with decreased lipid accumulation and enhanced insulin sensitivity [214, 215]. DNL is also involved in caloric restriction, which extends lifespan and delays age-related dysfunction [216]. In fact, our LLIs showed a better cholesterol profile than that of the other groups. In contrast, we observed small but significantly higher PA levels among LLIs than in adults. PA is PLA precursor, and elevated levels of this SFA exert detrimental effects on brain cells and might increase the risk of neurodegenerative disorders [217]. However, our LLIs do not show any neurodegenerative disorders at the time of the recruitment.

In addition, we observed lower levels of total trans fatty acids and trans-fat index in the blood cell membranes of LLIs compared with adults and older adults. Because elderly people have limited

consumption of foods made with processed sources of trans fatty acids, the age-related changes in dietary habits are possible reasons for this trend.

5.1.3 Gender Differences in Fatty Acid Profile

We further assessed the role of gender in determining the blood fatty acid composition in the population. Gender is considered a possible confounding factor in studies investigating fatty acids, specifically PUFA status, because sex hormones impact enzymes involved in synthesizing long-chain PUFA and fatty acid metabolism [218]. A systematic review of 51 studies in humans showed that plasma values of AA and DHA are significantly lower in men than women, although there were no gender differences in the levels of their precursors, *i.e.*, LA and ALA [218]. In our study population, women had lower proportions of n-3 DPA, DTA, lignoceric acid, elaidic acid, trans-fat index, and SFA/MUFA. In contrast, men had lower PLA, OL, and total MUFA proportions.

5.1.4 Genetic Variants and Fatty Acid Profile

Regarding genetic analyses, previous studies described that people with the T allele of rs174537 polymorphism in *FADS1/2* have lower AA levels and get more benefit from n-3 PUFA than those with the GG allele [46, 219]. The GG genotype of rs174537 is linked with increased PUFA-derived eicosanoid levels that influence downstream inflammatory pathways [220, 221]. The minor allele T of rs174537 is associated with decreased risk of major depressive disorder and breast cancer among women [219, 222]. In addition, the T allele predicts a reduced risk of coronary artery disease [223-225]. The rs174537 also modulates the age-associated changes in serum long-chain PUFA, D5D activity, and oxidative stress [226]. Age-associated increases in AA levels and D5D activity have been observed in GG individuals, whereas the T allele carriers have not [226]. Consistent with previous studies, our results confirmed a significant effect of *FADS1* rs174537 polymorphism on LA, EA, and DTA levels among LLIs [46]. We also observed that GG carriers have higher ALA, EPA,

and AA levels than T allele carriers, but the correlation was insignificant. However, the DHA levels were not linked with rs174537 polymorphism in our study [46].

Besides rs174537, the fatty acid profile is influenced by rs174579 and rs174626 in the *FADS1/2* gene cluster. Previous studies consistently reported that the minor allele carriers for rs174579 and rs174626 polymorphisms have lower levels of AA and a reduced desaturase index [74, 76]. This was also observed in the present study, where minor allele carriers for the examined SNPs showed a reduced D6D index. Therefore, minor allele carriers appear to have a reduced capacity to convert LA into GLA. This result confirms the association between variation in the *FADS1/2* gene cluster and fatty acid metabolism among LLIs. However, we did not observe any effect of rs174579 and rs174626 for D5D, n-3 ADA, and n-6 ADA in our population [74].

Another SNP we examined was rs953413 found in the locus of *ELOVL2* that is involved in the homeostasis of longer chain n-3 PUFA. The minor allele A of rs953413 is described to result in higher EPA but lower DHA levels, whereas the G allele carriers have high DHA levels [227]. The presence of the G allele also shows a protective effect on lipid metabolism and might be an indicator of lower cardiometabolic risk [75, 228]. Although not significant, we observed that AA individuals of this SNP have lower DHA levels. Our result also showed that the minor allele carriers have lower percentages of DTA [46]. However, no relationship between this SNP and EPA level was observed among LLIs. This could be explained by the fact that significant associations are often harder to find with smaller sample sizes [229]. In addition, the genetic background that determine EPA levels in our LLIs population might be different than the populations of previous studies.

Besides *FADS1/2* and *ELOVL2* genes, a recent study on a Singaporean Chinese population showed that an intergenic variant (rs529143) modifies the effects of n-3 PUFA on leukocyte telomere length. Subjects carrying the minor C allele of rs529143 have shorter leukocyte telomere length in the lower tertile of n-3 PUFA and DHA. In comparison, subjects with higher tertile of n-3 PUFA and DHA have longer leukocyte telomere length. Regional genes around rs529143 include multiple

phospholipase genes such as *PLA2G2D* and *PLA2G2F* that contribute to phospholipid metabolism [230]. Although the major allele carriers of our population had higher levels of all the observed n-3 PUFA, no significant correlation was observed.

Our study provides additional evidence that genetic variants in *FADS1/2* and *ELOVL2* are associated with levels of certain PUFA and desaturase activity among LLIs. However, since the allele frequency of these SNPs is similar to those observed in adults and older adults, they might have minimal impact on longevity in a population of LLIs from southern Italy.

5.1.5 Effect of N-3 Polyunsaturated Fatty Acids on Telomere Length

Despite the limited number of clinical studies included in our meta-analysis on the association between n-3 supplementation and telomere length, our results indicate that n-3 PUFAs may have a role in telomere maintenance. Moreover, our results are consistent with previous studies showing that key foods rich in antioxidant and anti-inflammatory components may positively influence telomere attrition [231, 232]. However, most of the trials examined in our analysis used different sample size, treatment durations, and involved a large variety of dosages of n-3 PUFAs. In the RCT conducted by O' Callaghan et al., six months of daily supplementation with two different doses of EPA and DHA (1.67 g EPA + 0.16 g DHA; 1.55 g DHA + 0.40 g EPA) reduced telomere shortening in older adults with mild cognitive impairment when compared to the group supplemented with LA [179]. Although this study has a longer duration than others, the main limitation was the limited sample size (n = 33). In contrast, Kiecolt-Glasera et al. conducted a RCT with a larger sample size (n = 106) and involving overweight subjects. After four months of supplementation with two different doses of n-3 PUFAs (2.5 g/d or l.25 g/d), telomere length increased in the active group, while it tended to decrease in the control group. Likewise, a reduction of F2-isoprostanes, an oxidative stress biomarker, was observed in the supplemented group compared to the placebo [142]. Despite a small number of participants (n = 47), the beneficial effects of n-3 PUFAs on the length of short telomeres was also demonstrated by

Tsoukalast et al. in a cohort of healthy volunteers. They used a nutraceutical supplement with 778,8 mg of EPA + DHA and the supplementation period lasted for 6 to 12 months [180]. In another 2 studies included in this meta-analysis, no statistically significant effect on telomere length was observed after supplementation with n-3 PUFAs [177, 178]. However, Barden et al. obtained a significant result when telomere length was corrected for neutrophil count. This finding was associated with reduced levels of F2-isoprostanes, and it may relate to increased clearance of neutrophils with shorter telomeres from the circulation.

Currently, oxidative stress appears to play a primary role in telomere shortening [233]. The molecular mechanisms underlying the effect of n-3 PUFAs against oxidative stress have been investigated in experimental models and several findings support the involvement of Nrf2 [234, 235]. This transcription factor is known as the master regulator of the antioxidant response and it is responsible for both constitutive and inducible expression of cytoprotective proteins and detoxification enzymes [236]. A recent clinical study in patients with type 2 diabetes established a translational link between DHA and EPA and their antioxidant properties through modulation of Nrf2 [237]. Oxidative stress also represents the most frequent cause of DNA damage at the telomeric level and it is related to telomere shortening/dysfunction [238]. It was reported that n-3 PUFAs may attenuate DNA oxidative damage and protect chromosomal integrity through upregulation of Nrf2 [96]. As mentioned, the n-3 PUFAs may also exert beneficial effects on telomere length through their anti-inflammatory properties. Prospective cohort studies found that higher plasma concentration of n-3 PUFAs was associated with lower levels of proinflammatory markers and reduced attrition of telomere length [53, 141]. Therefore, this anti-inflammatory potential could be a possible mechanism by which n-3 PUFAs exert their effect on telomere maintenance.

5.1.6 Effects of Monounsaturated Fatty Acids on Sarcopenia

Despite the limited number of observational studies included in our systematic review and meta-analysis, the results suggest that MUFA intake is inversely associated with the risk of sarcopenia. In

addition, the corresponding result subsisted in several subgroup analyses that were performed based on population characteristics, MUFA assessment tools, and sarcopenia diagnosis. Among the 10 studies that were meta-analysed, six studies reported a significantly lower MUFA intake in sarcopenic subjects compared to non-sarcopenic ones [183, 185-188, 190]. On the other hand, the remaining four studies did not report any significant difference in MUFA intake between sarcopenic and control participants [181, 182, 184, 192]. Furthermore, two more studies that were included in the review but not in the meta-analysis did not find a significant association between MUFA intake and the risk of sarcopenia [189, 191]. Montiel-rojas 2020 reported that substituting SFAs by MUFAs does not affect sex-specific sarcopenia risk score in 986 older European adults [189]. Similarly, ter Borg 2018 found no significant associations between blood MUFA status and sarcopenia in 227 community-dwelling older adults [191]. Therefore, although MUFA intake showed a negative association with sarcopenia in our meta-analysis, the available data is still insufficient to confirm this association, and further investigations are required.

Our finding that a higher intake of MUFA has may have positive effects on sarcopenia is consistent with previous reports indicating that MUFA may inhibit muscle atrophy by enhancing mitochondrial oxidative ability, protein synthesis, insulin sensitivity, and reducing inflammation [239]. For example, MUFAs have been reported to prevent SFA-induced insulin resistance and exert anti-inflammatory effects in skeletal muscle cells [240-242]. MUFA protects against SFA-induced inflammation and insulin resistance by promoting TG accumulation and mitochondrial beta-oxidation through peroxisome proliferator-activated receptor-alpha (PPAR-α) and protein kinase A-dependent mechanisms [241]. Likewise, OL inhibits SFA-mediated activation of the mammalian target of rapamycin complex 1/p70S6K, leading to a reduction in intracellular inflammatory signalling and cytotoxicity [243]. Additionally, in aging individuals, there is an elevation of pro-inflammatory cytokines, such as tumor necrosis factor-alpha, interleukin 6 and C-reactive protein (CRP), that contribute to low muscle mass and strength [244]. MUFA, such as OL is shown to reduce serum CRP and increase anti-inflammatory gene expression [245, 246]. These anti-inflammatory actions of

MUFA in skeletal muscle cells may contribute to their ability to preserve muscle mass and function [239]. MUFA also prevent muscle atrophy and increase muscle differentiation by enhancing mitochondrial function and reducing mitochondrial ROS [247, 248]. It is demonstrated that an OL-enriched diet may increase muscle protein synthesis, associated with increased expression of genes implicated in stimulating mitochondrial β-oxidation including PPAR-α and PPAR-beta (PPAR-β), as well as carnitine palmitoyl transferase 1 beta [249, 250]. Therefore, MUFA intake, particularly OL, may have the potential as an effective nutrition-based preventive or therapeutic strategy against sarcopenia.

Of the 10 studies included in our meta-analysis, three studies involved subjects with sarcopenic obesity [181, 182, 184]. Sarcopenic obesity is characterized by the coexistence of sarcopenia and obesity, and it is associated with an increased risk of cardiovascular disease and mortality in the aging population [251, 252]. Beside the effects of MUFAs on muscle health, previous studies indicate that MUFAs may accelerate overall lipid oxidation in peroxisome and mitochondria by stimulating peroxisomal and mitochondrial beta-oxidation [241, 253]. Long-chain MUFAs can also improve obesity-related metabolic dysfunction by upregulation of PPAR-gamma (PPAR-γ) and downregulation of inflammatory markers in the adipose tissue [254]. Indeed, the anti-inflammatory effect of MUFA-rich Mediterranean diet is described to decrease the risk of obesity-related metabolic syndrome [63]. Nevertheless, our analysis of three studies involving 1087 participants did not show a significant association between MUFA intake and sarcopenic obesity.

Although the number of studies with sex descriptions is limited, some sex-related genetic differences are described to be associated with the effect of diet on sarcopenia in experimental models [255]. The majority of the studies included in our review were designed for both men and women and sex-specific analyses were not performed. The pooled analysis of these studies showed that MUFA levels are significantly lower in sarcopenic subjects compared to the non-sarcopenic ones. On the other hand, the study by Jyväkorpi et al. examined community-living oldest old men, and Aubertin-

Leheudre et al. studied a group of sarcopenic and non-sarcopenic obese post-menopausal women [182, 256]. Jyväkorpi et al. reported that higher MUFA intake and MUFA/SFA ratio are both associated with lower sarcopenia risk [256]. In contrast, the results of the Aubertin-Leheudre et al. study did not show any significant difference in MUFA intake among the two groups [182]. In this study, however, the non-sarcopenic women had a greater BMI, abdominal fat mass, and visceral fat mass than the sarcopenic women [182]. The BMI and body fat are reported to be associated with sarcopenia [257, 258], which indicates that they should be considered as confounding factors. In addition, Aubertin-Leheudre et al. included 22 women, suggesting that a large sample-sized study may be more reliable to address the correlation [182].

The articles included in our study were published between 2006 and 2021, involving a period with considerable changes in the diagnostic criteria of sarcopenia. Sarcopenia was first described in 1989 as an age-related reduction in muscle mass [144, 145]. In 2000, sarcopenia was defined as two SD below the mean value of appendicular skeletal muscle mass divided by height squared of sex-specific young adult reference value [259]. Other definitions of sarcopenia later emerged that required measurement of a combination of three parameters, which are muscle mass, muscle strength, and physical performance. In 2010, the EWGSOP proposed an operational definition of sarcopenia that added muscle function to previous definitions based on low muscle mass [149]. According to the EWGSOP, the diagnosis of sarcopenia requires the detection of low muscle mass with low muscle function (muscle strength or physical performance). The EWGSOP was then updated in 2019 as EWGSOP2 [150]. The EWGSOP2 used poor muscle strength as the primary parameter of sarcopenia instead of low muscle mass. According to the EWGSOP2, sarcopenia is probable when low muscle strength is detected, sarcopenia diagnosis is confirmed when low muscle quantity and quality are identified, and sarcopenia is severe when low muscle strength, low muscle quantity/quality and poor physical performance are detected altogether. Although the EWGSOP did not advise specific cut-off points, the EWGSOP2 provided clear cut-off points of the parameters used for sarcopenia diagnosis [149, 150]. Furthermore, in 2014, the AWGS was proposed, which has a similar definition as the

EWGSOP and was later revised in 2019 [260]. The AWGS established an EWGSOP-based consensus that denoted cut-off points for diagnostic variables of sarcopenia in Asian populations. When we performed a subgroup analysis by the diagnostic criteria of sarcopenia, the corresponding result existed in EWGSOP2 and AWGS but was lost in the other diagnostic criteria. In addition, we observed a significant decrease in heterogeneity in this subgroup analysis.

5.2 Limitations and Strengths

Limitations

This thesis project has certain limitations. First, the number of LLIs cohort in Study II was relatively small. The detailed information on the dietary quantity of fatty acid intake and physical activities, which might confound data on blood fatty acid levels, were not evaluated. It is also possible that other additional factors, unrelated to fatty acid profile but associated with aging, could affect the examined fatty acids. Therefore, studying new factors that may act as confounders will require further investigations in the future. Furthermore, we used the percentage of product-to-precursor approach to estimate desaturase activity and not their exact concentration. However, previous research has stated that estimates of desaturase activity based on percentage levels of fatty acids are comparable to estimates made using product-to-precursor concentrations [76].

Additionally, due to limited data, a small number of studies were included in the meta-analysis on the association between n-3 PUFA supplementation and telomere length. For this reason, we could not perform a subgroup analysis and, therefore, the effects of dosage, duration, age, and gender on findings remained unclear. Then, the clinical trials assessed different samples sizes, dosages, and clinical conditions, which may have been responsible for the observed heterogeneity.

Finally, when evaluating the effect of MUFA intake on the risk sarcopenia, there was a moderate level of heterogeneity in the meta-analysis, which could be due to the heterogeneity in the methods and diagnostic criteria used to define sarcopenia among the included articles. Second,

previous evidence suggests that there is sex-specific differences in pathophysiological mechanisms and risk factors for sarcopenia [261, 262]. However, many of the included studies did not consider sex differences, and therefore, a subgroup analysis by sex could not be performed in these studies. Another limitation of our study is that due to scarcity of data, we were unable to perform a subgroup analysis based on co-diseases present in the population, which could certainly affect sarcopenia. Finally, there is a limitation of relevant literature, in which 12 studies were identified and only 10 of them could be meta-analysed.

Strengths

This thesis project has several strengths too. First, we studied a small but well-characterised group of healthy LLIs, which may provide a better understanding of the factors implicated in the aging process in the presence of well-preserved physiological functions. Moreover, they represent a population very difficult to recruit both to the time and to the budget expenditure related to the home visit.

Additionally, to our knowledge, we performed the first meta-analysis of observational studies on the relationship between dietary or circulatory MUFA levels and sarcopenia based on a comprehensive literature search. Furthermore, almost all of the included studies were published in recent years, suggesting a potential novel topic in our systematic review and meta-analysis.

Conclusions and Future Perspectives

Our data collected from circulating fatty acids suggests peculiar changes in fatty acid status with advancing age. We observed a reduction in blood levels of total PUFA and trans fatty acids with age, whereas total SFA remained unchanged throughout life. On the other hand, the fatty acid profile of Sicilian LLIs was characterized by distinctively higher circulatory levels of MUFA compared to adults and older adults. This suggests that the circulatory MUFA content may represent a potential marker of longevity in this population. Additionally, our results provide further evidence that SNPs in *FADS1/2* and *ELOVL2* affect PUFA status and desaturase indices among LLIs. However, since the allele and genotypic frequency were similar between the age groups, the examined SNPs might have minimal impact on longevity in this population from southern Italy.

Additionally, although the findings should be interpreted cautiously, our meta-analysis on the association between n-3 PUFA supplementation and telomere length provides preliminary evidence that treatments with n-3 fatty acids may potentially have some clinical efficacy on telomere maintenance. Finally, our systematic review and meta-analysis on the effects of MUFA intake on sarcopenia provide preliminary evidence that dietary MUFA level is different among people with and without sarcopenia. However, current evidence is still insufficient to demonstrate the definite relationship between MUFA intake and sarcopenia. More well-designed prospective cohort studies along with clinical trials are still needed.

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BIBLIOGRAPHY

1. Melzer, D., L.C. Pilling, and L. Ferrucci, *The genetics of human ageing.* Nature Reviews Genetics, 2020. **21**(2): p. 88-101.
2. McLeod, C.B., et al., *Income inequality, household income, and health status in Canada: a prospective cohort study.* Am J Public Health, 2003. **93**(8): p. 1287-93.
3. Shannon, O.M., et al., *Mediterranean diet and the hallmarks of ageing.* European Journal of Clinical Nutrition, 2021. **75**(8): p. 1176-1192.
4. Puca, A.A., et al., *Fatty acid profile of erythrocyte membranes as possible biomarker of longevity.* Rejuvenation Res, 2008. **11**(1): p. 63-72.
5. Rowe, J.W. and R.L. Kahn, *Human aging: usual and successful.* Science, 1987. **237**(4811): p. 143-9.
6. Rowe, J.W. and R.L. Kahn, *Successful Aging1.* The Gerontologist, 1997. **37**(4): p. 433-440.
7. López-Otín, C., et al., *The hallmarks of aging.* Cell, 2013. **153**(6): p. 1194-1217.

8. Guo, J., et al., *Aging and aging-related diseases: from molecular mechanisms to interventions and treatments.* Signal Transduction and Targeted Therapy, 2022. **7**(1): p. 391.
9. Crimmins, E.M. and H. Beltrán-Sánchez, *Mortality and morbidity trends: is there compression of morbidity?* J Gerontol B Psychol Sci Soc Sci, 2011. **66**(1): p. 75-86.
10. Zhu, Y., A. Ferrara, and M.R. Forman, *Omega 3 polyunsaturated fatty acids and healthy ageing.* BMJ (Clinical research ed.), 2018. **363**: p. k4263-k4263.
11. Perls, T. and D. Terry, *Genetics of exceptional longevity.* Exp Gerontol, 2003. **38**(7): p. 725-30.
12. Lorusso, J.S., O.A. Sviderskiy, and V.M. Labunskyy, *Emerging Omics Approaches in Aging Research.* Antioxidants & redox signaling, 2018. **29**(10): p. 985-1002.
13. Brandão, D., et al., *Escaping most common lethal diseases in old age: Morbidity profiles of Portuguese centenarians.* European Geriatric Medicine, 2017. **8**(4): p. 310-314.
14. Jopp, D.S., et al., *Physical, cognitive, social and mental health in near-centenarians and centenarians living in New York City: findings from the Fordham Centenarian Study.* BMC Geriatrics, 2016. **16**(1): p. 1.
15. Sebastiani, P. and T. Perls, *The Genetics of Extreme Longevity: Lessons from the New England Centenarian Study.* Frontiers in Genetics, 2012. **3**.
16. Pareja-Galeano, H., et al., *Serum eicosapentaenoic acid to arachidonic acid ratio is associated with cardio-healthy exceptional longevity.* International Journal of Cardiology, 2015. **184**: p. 655-656.
17. Evert, J., et al., *Morbidity profiles of centenarians: survivors, delayers, and escapers.* J Gerontol A Biol Sci Med Sci, 2003. **58**(3): p. 232-7.
18. Tedone, E., et al., *Telomere length and telomerase activity in T cells are biomarkers of high-performing centenarians.* Aging Cell, 2019. **18**(1): p. e12859.
19. Oeppen, J. and J.W. Vaupel, *Demography. Broken limits to life expectancy.* Science, 2002. **296**(5570): p. 1029-31.

20. Aiello, A., et al., *Age and Gender-related Variations of Molecular and Phenotypic Parameters in A Cohort of Sicilian Population: from Young to Centenarians.* Aging Dis, 2021. **12**(7): p. 1773-1793.

21. Yeung, S.S.Y., M. Kwan, and J. Woo, *Healthy Diet for Healthy Aging.* Nutrients, 2021. **13**(12).

22. Aune, D., et al., *Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies.* Int J Epidemiol, 2017. **46**(3): p. 1029-1056.

23. Weaver, C.M., M. Peacock, and C.C. Johnston, Jr., *Adolescent Nutrition in the Prevention of Postmenopausal Osteoporosis.* The Journal of Clinical Endocrinology & Metabolism, 1999. **84**(6): p. 1839-1843.

24. Teegarden, D., et al., *Previous milk consumption is associated with greater bone density in young women.* The American Journal of Clinical Nutrition, 1999. **69**(5): p. 1014-1017.

25. Shishtar, E., et al., *Long-term dietary flavonoid intake and risk of Alzheimer disease and related dementias in the Framingham Offspring Cohort.* Am J Clin Nutr, 2020. **112**(2): p. 343-353.

26. Veronese, N., et al., *Adherence to a Mediterranean diet is associated with lower incidence of frailty: A longitudinal cohort study.* Clin Nutr, 2018. **37**(5): p. 1492-1497.

27. Bonaccio, M., et al., *High adherence to the Mediterranean diet is associated with cardiovascular protection in higher but not in lower socioeconomic groups: prospective findings from the Moli-sani study.* Int J Epidemiol, 2017. **46**(5): p. 1478-1487.

28. Merle, B.M., et al., *Adherence to a Mediterranean diet, genetic susceptibility, and progression to advanced macular degeneration: a prospective cohort study.* Am J Clin Nutr, 2015. **102**(5): p. 1196-206.

29. Davinelli, S. and G. Scapagnini, *Nutritional biomarkers in aging research*. 2021. p. 295-317.

30. Yang, K. and X. Han, *Lipidomics: Techniques, Applications, and Outcomes Related to Biomedical Sciences.* Trends Biochem Sci, 2016. **41**(11): p. 954-969.

31. Meikle, T.G., et al., *Clinical lipidomics: realizing the potential of lipid profiling.* Journal of Lipid Research, 2021. **62**: p. 100127.

32. Færgestad, E.M., et al., *4.08 - Analysis of Megavariate Data in Functional Genomics*, in *Comprehensive Chemometrics*, S.D. Brown, R. Tauler, and B. Walczak, Editors. 2009, Elsevier: Oxford. p. 221-278.

33. Han, X. and R.W. Gross, *Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics.* J Lipid Res, 2003. **44**(6): p. 1071-9.

34. Pauling, J.K., *Computational Lipidomics*, in *Encyclopedia of Bioinformatics and Computational Biology*, S. Ranganathan, et al., Editors. 2019, Academic Press: Oxford. p. 894-899.

35. Liebisch, G., et al., *Update on LIPID MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures.* J Lipid Res, 2020. **61**(12): p. 1539-1555.

36. Mutlu, A.S., J. Duffy, and M.C. Wang, *Lipid metabolism and lipid signals in aging and longevity.* Dev Cell, 2021. **56**(10): p. 1394-1407.

37. Huang, X., B.R. Withers, and R.C. Dickson, *Sphingolipids and lifespan regulation.* Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 2014. **1841**(5): p. 657-664.

38. Papsdorf, K. and A. Brunet, *Linking Lipid Metabolism to Chromatin Regulation in Aging.* Trends Cell Biol, 2019. **29**(2): p. 97-116.

39. Trabado, S., et al., *The human plasma-metabolome: Reference values in 800 French healthy volunteers; impact of cholesterol, gender and age.* PLOS ONE, 2017. **12**(3): p. e0173615.

40. Johnson, A.A. and A. Stolzing, *The role of lipid metabolism in aging, lifespan regulation, and age-related disease.* Aging Cell, 2019. **18**(6): p. e13048.

41. Holčapek, M., G. Liebisch, and K. Ekroos, *Lipidomic Analysis.* Analytical Chemistry, 2018. **90**(7): p. 4249-4257.

42. Watkins, P.A., *Fatty acids: Metabolism*, in *Encyclopedia of Human Nutrition (Third Edition)*, B. Caballero, Editor. 2013, Academic Press: Waltham. p. 220-230.

43. Hashimoto, M. and S. Hossain, *Fatty Acids: From Membrane Ingredients to Signaling Molecules*. 2018.

44. Katalin, N. and T. Ioana-Daria, *Importance of Fatty Acids in Physiopathology of Human Body*, in *Fatty Acids*, C. Angel, Editor. 2017, IntechOpen: Rijeka. p. Ch. 1.

45. Astrup, A., et al., *Saturated Fats and Health: A Reassessment and Proposal for Food-Based Recommendations: JACC State-of-the-Art Review.* J Am Coll Cardiol, 2020. **76**(7): p. 844-857.

46. Tanaka, T., et al., *Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI Study.* PLoS Genet, 2009. **5**(1): p. e1000338.

47. Tosi, F., et al., *Delta-5 and delta-6 desaturases: crucial enzymes in polyunsaturated fatty acid-related pathways with pleiotropic influences in health and disease.* Adv Exp Med Biol, 2014. **824**: p. 61-81.

48. Manca, C., et al., *Circulating fatty acids and endocannabinoidome-related mediator profiles associated to human longevity.* Geroscience, 2021. **43**(4): p. 1783-1798.

49. Shahidi, F. and P. Ambigaipalan, *Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits.* Annu Rev Food Sci Technol, 2018. **9**: p. 345-381.

50. Shen, S., et al., *Omega-3 Fatty Acid Supplementation and Coronary Heart Disease Risks: A Meta-Analysis of Randomized Controlled Clinical Trials.* Front Nutr, 2022. **9**: p. 809311.

51. Yanai, H., *Effects of N-3 Polyunsaturated Fatty Acids on Dementia.* J Clin Med Res, 2017. **9**(1): p. 1-9.

52. Innes, J.K. and P.C. Calder, *Omega-6 fatty acids and inflammation.* Prostaglandins Leukot Essent Fatty Acids, 2018. **132**: p. 41-48.

53. Ferrucci, L., et al., *Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers.* J Clin Endocrinol Metab, 2006. **91**(2): p. 439-46.

54. Bigornia, S.J., et al., *The Omega-3 Index Is Inversely Associated with Depressive Symptoms among Individuals with Elevated Oxidative Stress Biomarkers.* J Nutr, 2016. **146**(4): p. 758-66.

55. Lukaschek, K., et al., *Cognitive Impairment Is Associated with a Low Omega-3 Index in the Elderly: Results from the KORA-Age Study.* Dement Geriatr Cogn Disord, 2016. **42**(3-4): p. 236-245.

56. Davinelli, S., et al., *Metabolic indices of polyunsaturated fatty acids: current evidence, research controversies, and clinical utility.* Crit Rev Food Sci Nutr, 2021. **61**(2): p. 259-274.

57. Schmitz, G. and J. Ecker, *The opposing effects of n-3 and n-6 fatty acids.* Prog Lipid Res, 2008. **47**(2): p. 147-55.

58. Duan, Y., et al., *n-6:n-3 PUFA ratio is involved in regulating lipid metabolism and inflammation in pigs.* Br J Nutr, 2014. **111**(3): p. 445-51.

59. Yang, L.G., et al., *Low n-6/n-3 PUFA Ratio Improves Lipid Metabolism, Inflammation, Oxidative Stress and Endothelial Function in Rats Using Plant Oils as n-3 Fatty Acid Source.* Lipids, 2016. **51**(1): p. 49-59.

60. Simopoulos, A.P., *The importance of the ratio of omega-6/omega-3 essential fatty acids.* Biomed Pharmacother, 2002. **56**(8): p. 365-79.

61. Nelson, J.R. and S. Raskin, *The eicosapentaenoic acid:arachidonic acid ratio and its clinical utility in cardiovascular disease.* Postgrad Med, 2019. **131**(4): p. 268-277.

62. Tanaka, K., et al., *Reduction in the recurrence of stroke by eicosapentaenoic acid for hypercholesterolemic patients: subanalysis of the JELIS trial.* Stroke, 2008. **39**(7): p. 2052-8.

63. Ravaut, G., et al., *Monounsaturated Fatty Acids in Obesity-Related Inflammation.* Int J Mol Sci, 2020. **22**(1).

64. Lyons, C.L., et al., *Monounsaturated Fatty Acids Impede Inflammation Partially Through Activation of AMPK.* The FASEB Journal, 2016. **30**(S1): p. 296.5-296.5.

65. Schwingshackl, L. and G. Hoffmann, *Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies.* Lipids in Health and Disease, 2014. **13**(1): p. 154.

66. Kris-Etherton, P.M., et al., *High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations.* Am J Clin Nutr, 1999. **70**(6): p. 1009-15.

67. Gillingham, L.G., S. Harris-Janz, and P.J. Jones, *Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors.* Lipids, 2011. **46**(3): p. 209-28.

68. Solfrizzi, V., et al., *High monounsaturated fatty acids intake protects against age-related cognitive decline.* Neurology, 1999. **52**(8): p. 1563-9.

69. Mozaffarian, D., et al., *Trans fatty acids and cardiovascular disease.* N Engl J Med, 2006. **354**(15): p. 1601-13.

70. Katan, M.B., P.L. Zock, and R.P. Mensink, *Trans fatty acids and their effects on lipoproteins in humans.* Annu Rev Nutr, 1995. **15**: p. 473-93.

71. Stender, S. and J. Dyerberg, *Influence of Trans Fatty Acids on Health.* Annals of Nutrition and Metabolism, 2004. **48**(2): p. 61-66.

72. Monguchi, T., et al., *Excessive intake of trans fatty acid accelerates atherosclerosis through promoting inflammation and oxidative stress in a mouse model of hyperlipidemia.* Journal of Cardiology, 2017. **70**(2): p. 121-127.

73. Remig, V., et al., *Trans fats in America: a review of their use, consumption, health implications, and regulation.* J Am Diet Assoc, 2010. **110**(4): p. 585-92.

74. Roke, K., et al., *Variation in the FADS1/2 gene cluster alters plasma n-6 PUFA and is weakly associated with hsCRP levels in healthy young adults.* Prostaglandins Leukot Essent Fatty Acids, 2013. **89**(4): p. 257-63.

75. Fujii, T.M.M., et al., *FADS1 and ELOVL2 polymorphisms reveal associations for differences in lipid metabolism in a cross-sectional population-based survey of Brazilian men and women.* Nutr Res, 2020. **78**: p. 42-49.

76. Merino, D.M., et al., *Polymorphisms in FADS1 and FADS2 alter desaturase activity in young Caucasian and Asian adults.* Mol Genet Metab, 2011. **103**(2): p. 171-8.

77. Chang, X., et al., *Effect of plasma polyunsaturated fatty acid levels on leukocyte telomere lengths in the Singaporean Chinese population.* Nutrition Journal, 2020. **19**(1): p. 119.

78. Ali, S., et al., *Determination of n-3 index and arachidonic acid/eicosapentaenoic acid ratio in dried blood spot by gas chromatography.* Biotechniques, 2022. **73**(1): p. 25-33.

79. Almeida, C.d.G.d., et al., *The fatty acid profile of adipose tissue as a predictor of the ponderal and inflammatory response in adult women six years after bariatric surgery.* Lipids in Health and Disease, 2020. **19**(1): p. 45.

80. Lapillonne, A., et al., *The fatty acid profile of buccal cheek cell phospholipids is a noninvasive marker of long-chain polyunsaturated Fatty Acid status in piglets.* J Nutr, 2002. **132**(8): p. 2319-23.

81. Abdelmagid, S.A., et al., *Comprehensive profiling of plasma fatty acid concentrations in young healthy Canadian adults.* PLoS One, 2015. **10**(2): p. e0116195.

82. Brenna, J.T., et al., *Best practices for the design, laboratory analysis, and reporting of trials involving fatty acids.* Am J Clin Nutr, 2018. **108**(2): p. 211-227.

83. Syrén, M.-L., et al., *Whole blood fatty acid profile of young subjects and adherence to the Mediterranean diet: an observational cohort study.* Lipids in Health and Disease, 2022. **21**(1): p. 23.

84. Metherel, A.H., et al., *Butylated hydroxytoluene can protect polyunsaturated fatty acids in dried blood spots from degradation for up to 8 weeks at room temperature.* Lipids Health Dis, 2013. **12**: p. 22.

85. Marangoni, F., C. Colombo, and C. Galli, *A method for the direct evaluation of the fatty acid status in a drop of blood from a fingertip in humans: applicability to nutritional and epidemiological studies.* Anal Biochem, 2004. **326**(2): p. 267-72.

86. Seppänen-Laakso, T., I. Laakso, and R. Hiltunen, *Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition.* Analytica Chimica Acta, 2002. **465**(1): p. 39-62.

87. Bystrická, Z. and Z. Ďuračková, *Gas chromatography determination of fatty acids in the human erythrocyte membranes - A review.* Prostaglandins Leukot Essent Fatty Acids, 2016. **115**: p. 35-40.

88. Ovčačíková, M., et al., *Retention behavior of lipids in reversed-phase ultrahigh-performance liquid chromatography-electrospray ionization mass spectrometry.* J Chromatogr A, 2016. **1450**: p. 76-85.

89. Christie, W.W., E.Y. Brechany, and K. Stefanov, *Silver ion high-performance liquid chromatography and gas chromatography—mass spectrometry in the analysis of complex fatty acid mixtures: application to marine invertebrates.* Chemistry and Physics of Lipids, 1988. **46**(2): p. 127-135.

90. Cutuli, D., *Functional and Structural Benefits Induced by Omega-3 Polyunsaturated Fatty Acids During Aging.* Curr Neuropharmacol, 2017. **15**(4): p. 534-542.

91. Zhu, Y., A. Ferrara, and M.R. Forman, *Omega 3 polyunsaturated fatty acids and healthy ageing.* BMJ, 2018. **363**: p. k4263.

92. Bianca Velasco, A. and Z.S. Tan, *Chapter 17 - Fatty Acids and the Aging Brain*, in *Omega-3 Fatty Acids in Brain and Neurological Health*, R.R. Watson and F. De Meester, Editors. 2014, Academic Press: Boston. p. 201-219.

93. Ponnappan, U., D.H. Holley, and D.A. Lipschitz, *Effect of age on the fatty acid composition of phospholipids in human lymphocytes.* Experimental Gerontology, 1996. **31**(1): p. 125-133.

94. Calder, P.C., *Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?* Br J Clin Pharmacol, 2013. **75**(3): p. 645-62.

95. Heshmati, J., et al., *Omega-3 fatty acids supplementation and oxidative stress parameters: A systematic review and meta-analysis of clinical trials.* Pharmacol Res, 2019. **149**: p. 104462.

96. Sakai, C., et al., *Fish oil omega-3 polyunsaturated fatty acids attenuate oxidative stress-induced DNA damage in vascular endothelial cells.* PLoS One, 2017. **12**(11): p. e0187934.

97. Chan, J., et al., *Marine n-3 Polyunsaturated Fatty Acids and Cellular Senescence Markers in Incident Kidney Transplant Recipients: The Omega-3 Fatty Acids in Renal Transplantation (ORENTRA) Randomized Clinical Trial.* Kidney Medicine, 2021. **3**(6): p. 1041-1049.

98. Hussey, B., M.R. Lindley, and S.S. Mastana, *Omega 3 fatty acids, inflammation and DNA methylation: an overview.* Clinical Lipidology, 2017. **12**(1): p. 24-32.

99. Karimi, M., et al., *DHA-rich n–3 fatty acid supplementation decreases DNA methylation in blood leukocytes: the OmegAD study.* The American Journal of Clinical Nutrition, 2017. **106**(4): p. 1157-1165.

100. Lalia, A.Z., et al., *Influence of omega-3 fatty acids on skeletal muscle protein metabolism and mitochondrial bioenergetics in older adults.* Aging (Albany NY), 2017. **9**(4): p. 1096-1129.

101. Afshordel, S., et al., *Omega-3 polyunsaturated fatty acids improve mitochondrial dysfunction in brain aging--impact of Bcl-2 and NPD-1 like metabolites.* Prostaglandins Leukot Essent Fatty Acids, 2015. **92**: p. 23-31.

102. Lee, J.H., J.H. Jeon, and M.J. Lee, *Docosahexaenoic Acid, a Potential Treatment for Sarcopenia, Modulates the Ubiquitin-Proteasome and the Autophagy-Lysosome Systems.* Nutrients, 2020. **12**(9).

103. Janssen, C.I. and A.J. Kiliaan, *Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: the influence of LCPUFA on neural development, aging, and neurodegeneration.* Prog Lipid Res, 2014. **53**: p. 1-17.

104. Brostow, D.P., et al., *Omega-3 fatty acids and incident type 2 diabetes: the Singapore Chinese Health Study.* Am J Clin Nutr, 2011. **94**(2): p. 520-6.

105. Zhang, G., et al., *Erythrocyte n-3 fatty acids and metabolic syndrome in middle-aged and older Chinese.* J Clin Endocrinol Metab, 2012. **97**(6): p. E973-7.

106. Zheng, J.S., et al., *Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: meta-analysis of data from 21 independent prospective cohort studies.* Bmj, 2013. **346**: p. f3706.

107. Assies, J., et al., *Plasma and erythrocyte fatty acid patterns in patients with recurrent depression: a matched case-control study.* PLoS One, 2010. **5**(5): p. e10635.

108. Tan, Z.S., et al., *Red blood cell omega-3 fatty acid levels and markers of accelerated brain aging.* Neurology, 2012. **78**(9): p. 658-664.

109. Xie, S.H., et al., *Multi-Omics Interpretation of Anti-Aging Mechanisms for ω-3 Fatty Acids.* Genes (Basel), 2021. **12**(11).

110. Bird, J.K., et al., *The effect of long chain omega-3 polyunsaturated fatty acids on muscle mass and function in sarcopenia: A scoping systematic review and meta-analysis.* Clin Nutr ESPEN, 2021. **46**: p. 73-86.

111. Harris, W.S., et al., *Red blood cell polyunsaturated fatty acids and mortality in the Women's Health Initiative Memory Study.* J Clin Lipidol, 2017. **11**(1): p. 250-259.e5.

112. Chen, G.C., et al., *N-3 long-chain polyunsaturated fatty acids and risk of all-cause mortality among general populations: a meta-analysis.* Sci Rep, 2016. **6**: p. 28165.

113. Wu, J.H., et al., *Circulating omega-6 polyunsaturated fatty acids and total and cause-specific mortality: the Cardiovascular Health Study.* Circulation, 2014. **130**(15): p. 1245-53.

114. Farvid, M.S., et al., *Dietary linoleic acid and risk of coronary heart disease: a systematic review and meta-analysis of prospective cohort studies.* Circulation, 2014. **130**(18): p. 1568-78.

115. McGee, D.L., et al., *Ten-year incidence of coronary heart disease in the Honolulu Heart Program. Relationship to nutrient intake.* Am J Epidemiol, 1984. **119**(5): p. 667-76.

116. Harris, C.P., et al., *Association of Dietary Fatty Acids with Blood Lipids is Modified by Physical Activity in Adolescents: Results from the GINIplus and LISA Birth Cohort Studies.* Nutrients, 2018. **10**(10).

117. Shannon, O.M., et al., *Mediterranean diet and the hallmarks of ageing.* Eur J Clin Nutr, 2021. **75**(8): p. 1176-1192.

118. Marin, C., et al., *Mediterranean diet reduces senescence-associated stress in endothelial cells.* Age (Dordr), 2012. **34**(6): p. 1309-16.

119. Canudas, S., et al., *Mediterranean Diet and Telomere Length: A Systematic Review and Meta-Analysis.* Advances in Nutrition, 2020. **11**(6): p. 1544-1554.

120. Silva-Martínez, G.A., et al., *Arachidonic and oleic acid exert distinct effects on the DNA methylome.* Epigenetics, 2016. **11**(5): p. 321-334.

121. Román, G.C., et al., *Extra-virgin olive oil for potential prevention of Alzheimer disease.* Rev Neurol (Paris), 2019. **175**(10): p. 705-723.

122. Fernández del Río, L., et al., *Olive Oil and the Hallmarks of Aging.* Molecules, 2016. **21**(2): p. 163.

123. Cesari, F., et al., *Aging process, adherence to Mediterranean diet and nutritional status in a large cohort of nonagenarians: Effects on endothelial progenitor cells.* Nutr Metab Cardiovasc Dis, 2018. **28**(1): p. 84-90.

124. Marin, C., et al., *Mediterranean diet reduces endothelial damage and improves the regenerative capacity of endothelium.* Am J Clin Nutr, 2011. **93**(2): p. 267-74.

125. Kong, C.M., X.W. Lee, and X. Wang, *Telomere shortening in human diseases.* Febs j, 2013. **280**(14): p. 3180-93.

126. Whittemore, K., et al., *Telomere shortening rate predicts species life span.* Proc Natl Acad Sci U S A, 2019. **116**(30): p. 15122-15127.

127. Blackburn, E.H., E.S. Epel, and J. Lin, *Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection.* Science, 2015. **350**(6265): p. 1193-8.

128. Allsopp, R.C., et al., *Telomere length predicts replicative capacity of human fibroblasts.* Proc Natl Acad Sci U S A, 1992. **89**(21): p. 10114-8.

129. Zhang, J., et al., *Ageing and the telomere connection: An intimate relationship with inflammation.* Ageing Res Rev, 2016. **25**: p. 55-69.

130. Lin, J. and E. Epel, *Stress and telomere shortening: Insights from cellular mechanisms.* Ageing Res Rev, 2022. **73**: p. 101507.

131. Valdes, A.M., et al., *Obesity, cigarette smoking, and telomere length in women.* Lancet, 2005. **366**(9486): p. 662-4.

132. Zhao, B., et al., *Air pollution and telomere length: a systematic review of 12,058 subjects.* Cardiovasc Diagn Ther, 2018. **8**(4): p. 480-492.

133. Davinelli, S. and I. Vivo, *Lifestyle Choices, Psychological Stress and Their Impact on Ageing: The Role of Telomeres*. 2019. p. 135-148.

134. Davinelli, S., et al., *The potential nutrigeroprotective role of Mediterranean diet and its functional components on telomere length dynamics.* Ageing Res Rev, 2019. **49**: p. 1-10.

135. Ruiz-Narváez, E.A., et al., *Diet and Leukocyte Telomere Length in a Population with Extended Longevity: The Costa Rican Longevity and Healthy Aging Study (CRELES).* Nutrients, 2021. **13**(8).

136. Prasad, K.N., M. Wu, and S.C. Bondy, *Telomere shortening during aging: Attenuation by antioxidants and anti-inflammatory agents.* Mech Ageing Dev, 2017. **164**: p. 61-66.

137. García-Calzón, S., et al., *Dietary inflammatory index and telomere length in subjects with a high cardiovascular disease risk from the PREDIMED-NAVARRA study: cross-sectional and longitudinal analyses over 5 y.* Am J Clin Nutr, 2015. **102**(4): p. 897-904.

138. Tucker, L.A., *Fruit and Vegetable Intake and Telomere Length in a Random Sample of 5448 U.S. Adults.* Nutrients, 2021. **13**(5).

139. Rafie, N., et al., *Dietary patterns, food groups and telomere length: a systematic review of current studies.* Eur J Clin Nutr, 2017. **71**(2): p. 151-158.

140. Alibakhshi, A., et al., *An Update on Phytochemicals in Molecular Target Therapy of Cancer: Potential Inhibitory Effect on Telomerase Activity.* Curr Med Chem, 2016. **23**(22): p. 2380-93.

141. Farzaneh-Far, R., et al., *Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease.* Jama, 2010. **303**(3): p. 250-7.

142. Kiecolt-Glaser, J.K., et al., *Omega-3 fatty acids, oxidative stress, and leukocyte telomere length: A randomized controlled trial.* Brain Behav Immun, 2013. **28**: p. 16-24.

143. Ali, S., G. Scapagnini, and S. Davinelli, *Effect of omega-3 fatty acids on the telomere length: A mini meta-analysis of clinical trials.* Biomol Concepts, 2022. **13**(1): p. 25-33.

144. Rosenberg, I.H., *Summary comments.* The American Journal of Clinical Nutrition, 1989. **50**(5): p. 1231-1233.

145. Rosenberg, I.H., *Sarcopenia: origins and clinical relevance.* J Nutr, 1997. **127**(5 Suppl): p. 990s-991s.

146. Petermann-Rocha, F., et al., *Global prevalence of sarcopenia and severe sarcopenia: a systematic review and meta-analysis.* J Cachexia Sarcopenia Muscle, 2022. **13**(1): p. 86-99.

147. Cruz-Jentoft, A.J. and A.A. Sayer, *Sarcopenia.* Lancet, 2019. **393**(10191): p. 2636-2646.

148. Bruyère, O., et al., *Sarcopenia as a public health problem.* European Geriatric Medicine, 2016. **7**(3): p. 272-275.

149. Cruz-Jentoft, A.J., et al., *Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People.* Age Ageing, 2010. **39**(4): p. 412-23.

150. Cruz-Jentoft, A.J., et al., *Sarcopenia: revised European consensus on definition and diagnosis.* Age Ageing, 2019. **48**(1): p. 16-31.

151. Chen, M., et al., *Skeletal muscle oxidative stress and inflammation in aging: Focus on antioxidant and anti-inflammatory therapy.* Front Cell Dev Biol, 2022. **10**: p. 964130.

152. Coen, P.M., et al., *Mitochondria as a Target for Mitigating Sarcopenia.* Front Physiol, 2018. **9**: p. 1883.

153. Lourenço Dos Santos, S., et al., *Oxidative proteome alterations during skeletal muscle ageing.* Redox Biol, 2015. **5**: p. 267-274.

154. Granic, A., A.A. Sayer, and S.M. Robinson, *Dietary patterns, skeletal muscle health, and sarcopenia in older adults.* Nutrients, 2019. **11**(4).

155. Bellanti, F., A.L. Buglio, and G. Vendemiale, *Chapter 9 - Oxidative stress and sarcopenia*, in *Aging (Second Edition)*, V.R. Preedy and V.B. Patel, Editors. 2020, Academic Press. p. 95-103.

156. Dalle, S., L. Rossmeislova, and K. Koppo, *The Role of Inflammation in Age-Related Sarcopenia.* Frontiers in physiology, 2017. **8**: p. 1045-1045.

157. Beasley, J.M., J.M. Shikany, and C.A. Thomson, *The role of dietary protein intake in the prevention of sarcopenia of aging.* Nutr Clin Pract, 2013. **28**(6): p. 684-90.

158. Ganapathy, A. and J.W. Nieves, *Nutrition and Sarcopenia-What Do We Know?* Nutrients, 2020. **12**(6).

159. Besora-Moreno, M., et al., *Antioxidant-rich foods, antioxidant supplements, and sarcopenia in old-young adults ≥55 years old: A systematic review and meta-analysis of observational studies and randomized controlled trials.* Clinical Nutrition, 2022. **41**(10): p. 2308-2324.

160. Quiles, J.L., et al., *Role of Olive Oil and Monounsaturated Fatty Acids in Mitochondrial Oxidative Stress and Aging.* Nutrition Reviews, 2006. **64**(suppl_4): p. S31-S39.

161. Yazihan, N., et al., *Olive oil attenuates oxidative damage by improving mitochondrial functions in human keratinocytes.* Journal of Functional Foods, 2020. **71**: p. 104008.

162. Musumeci, G., et al., *Effects of dietary extra-virgin olive oil on oxidative stress resulting from exhaustive exercise in rat skeletal muscle: a morphological study.* Acta Histochem, 2014. **116**(1): p. 61-9.

163. Tsigalou, C., et al., *Mediterranean Diet as a Tool to Combat Inflammation and Chronic Diseases. An Overview.* Biomedicines, 2020. **8**(7): p. 201.

164. Sureda, A., et al., *Adherence to the Mediterranean Diet and Inflammatory Markers.* Nutrients, 2018. **10**(1): p. 62.

165. Accardi, G., et al., *The Phenotypic Characterization of the Cammalleri Sisters, an Example of Exceptional Longevity.* Rejuvenation Res, 2020. **23**(6): p. 476-484.

166. Accardi, G., et al., *Nutraceutical effects of table green olives: a pilot study with Nocellara del Belice olives.* Immun Ageing, 2016. **13**: p. 11.

167. Martinelli, N., et al., *FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease.* The American Journal of Clinical Nutrition, 2008. **88**(4): p. 941-949.

168. Reed, G.H. and C.T. Wittwer, *Sensitivity and Specificity of Single-Nucleotide Polymorphism Scanning by High-Resolution Melting Analysis.* Clinical Chemistry, 2004. **50**(10): p. 1748-1754.

169. Moher, D., et al., *Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement.* Syst Rev, 2015. **4**(1): p. 1.

170. Higgins, J.P., et al., *The Cochrane Collaboration's tool for assessing risk of bias in randomised trials.* Bmj, 2011. **343**: p. d5928.

171. Sterne, J.A., et al., *ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions.* BMJ, 2016. **355**: p. i4919.

172. Higgins JPT, T.J., Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors), *Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). Cochrane, 2022. Available from www.training.cochrane.org/handbook.*

173. Stang, A., *Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses.* European Journal of Epidemiology, 2010. **25**(9): p. 603-605.

174. Cui, C.-j., et al., *Tissue Factor-bearing MPs and the risk of venous thrombosis in cancer patients: A meta-analysis.* Scientific Reports, 2018. **8**(1): p. 1675.

175. Egger, M., et al., *Bias in meta-analysis detected by a simple, graphical test.* Bmj, 1997. **315**(7109): p. 629-34.

176. Theodorsson, E., *Validation and verification of measurement methods in clinical chemistry.* Bioanalysis, 2012. **4**(3): p. 305-20.

177. Balcerczyk, A., et al., *Enhanced antioxidant capacity and anti-ageing biomarkers after diet micronutrient supplementation.* Molecules, 2014. **19**(9): p. 14794-808.

178. Barden, A., et al., *n-3 Fatty Acid Supplementation and Leukocyte Telomere Length in Patients with Chronic Kidney Disease.* Nutrients, 2016. **8**(3): p. 175.

179. O'Callaghan, N., et al., *Telomere shortening in elderly individuals with mild cognitive impairment may be attenuated with ω-3 fatty acid supplementation: a randomized controlled pilot study.* Nutrition, 2014. **30**(4): p. 489-91.

180. Tsoukalas, D., et al., *Association of nutraceutical supplements with longer telomere length.* International Journal of Molecular Medicine, 2019. **44**(1): p. 218-226.

181. Abete, I., et al., *Association of lifestyle factors and inflammation with sarcopenic obesity: data from the PREDIMED-Plus trial.* Journal of Cachexia, Sarcopenia and Muscle, 2019. **10**(5): p. 974-984.

182. Aubertin-Leheudre, M., et al., *Effect of sarcopenia on cardiovascular disease risk factors in obese postmenopausal women.* Obesity, 2006. **14**(12): p. 2277-2283.

183. Bibiloni, M.M., et al., *Association between physical condition and body composition, nutrient intake, sociodemographic characteristics, and lifestyle habits in older Spanish adults.* Nutrients, 2018. **10**(11).

184. de França, N.A.G., et al., *Obesity associated with low lean mass and low bone density has higher impact on general health in middle-aged and older adults.* Journal of Obesity, 2020. **2020**.

185. Dos Reis, A.S., et al., *Intake of polyunsaturated fatty acids and ω-3 are protective factors for sarcopenia in kidney transplant patients.* Nutrition, 2021. **81**: p. 110929.

186. Esmaeily, Z., et al., *Association between Healthy Eating Index-2015 scores and probable sarcopenia in community-dwelling Iranian older adults: a cross-sectional study.* J Nutr Sci, 2021. **10**: p. e20.

187. Jyväkorpi, S.K., et al., *Macronutrient composition and sarcopenia in the oldest-old men: The Helsinki Businessmen Study (HBS).* Clin Nutr, 2020. **39**(12): p. 3839-3841.

188. Katoh, A., et al., *Long-chain fatty acids in sarcopenia patients with cardiovascular diseases: importance of n-9 monounsaturated fatty acids.* JCSM Clinical Reports, 2020. **5**(4): p. 121-128.

189. Montiel-rojas, D., et al., *Beneficial role of replacing dietary saturated fatty acids with polyunsaturated fatty acids in the prevention of sarcopenia: Findings from the nu- age cohort.* Nutrients, 2020. **12**(10): p. 1-10.

190. Otsuka, Y., et al., *Dietary Intake of Vitamin E and Fats Associated with Sarcopenia in Community-Dwelling Older Japanese People: A Cross-Sectional Study from the Fifth Survey of the ROAD Study.* Nutrients, 2021. **13**(5).

191. ter Borg, S., et al., *Low Levels of Branched Chain Amino Acids, Eicosapentaenoic Acid and Micronutrients are Associated with Low Muscle Mass, Strength and Function in*

Community-Dwelling Older Adults. Journal of Nutrition Health & Aging, 2019. **23**(1): p. 27-34.

192. Verlaan, S., et al., *Nutritional status, body composition, and quality of life in community-dwelling sarcopenic and non-sarcopenic older adults: A case-control study.* Clin Nutr, 2017. **36**(1): p. 267-274.

193. Esmaeily, Z., et al., *Association between Healthy Eating Index-2015 scores and probable sarcopenia in community-dwelling Iranian older adults: a cross-sectional study.* Journal of Nutritional Science, 2021. **10**.

194. ter Borg, S., et al., *Low Levels of Branched Chain Amino Acids, Eicosapentaenoic Acid and Micronutrients are Associated with Low Muscle Mass, Strength and Function in Community-Dwelling Older Adults.* Journal of Nutrition, Health and Aging, 2019. **23**(1): p. 27-34.

195. Rabini, R.A., et al., *Reduced susceptibility to peroxidation of erythrocyte plasma membranes from centenarians.* Exp Gerontol, 2002. **37**(5): p. 657-63.

196. Plourde, M. and S.C. Cunnane, *Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements.* Appl Physiol Nutr Metab, 2007. **32**(4): p. 619-34.

197. Brenna, J.T., et al., *alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans.* Prostaglandins Leukot Essent Fatty Acids, 2009. **80**(2-3): p. 85-91.

198. Nishihira, J., et al., *Associations between Serum Omega-3 Fatty Acid Levels and Cognitive Functions among Community-Dwelling Octogenarians in Okinawa, Japan: The KOCOA Study.* Journal of Alzheimer's disease : JAD, 2016. **51**(3): p. 857-866.

199. Yanagisawa, N., et al., *Polyunsaturated fatty acid levels of serum and red blood cells in apparently healthy Japanese subjects living in an urban area.* J Atheroscler Thromb, 2010. **17**(3): p. 285-94.

200. Dewailly, E., et al., *n-3 Fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik.* Am J Clin Nutr, 2001. **74**(4): p. 464-73.

201. Dewailly, E., et al., *Cardiovascular disease risk factors and n-3 fatty acid status in the adult population of James Bay Cree.* Am J Clin Nutr, 2002. **76**(1): p. 85-92.

202. Harris, W.S., et al., *Erythrocyte omega-3 fatty acids increase and linoleic acid decreases with age: Observations from 160,000 patients.* Prostaglandins, Leukotrienes and Essential Fatty Acids, 2013. **88**(4): p. 257-263.

203. Harris, W.S., *Omega-3 fatty acids and cardiovascular disease: a case for omega-3 index as a new risk factor.* Pharmacol Res, 2007. **55**(3): p. 217-23.

204. Harris, W.S., L. Del Gobbo, and N.L. Tintle, *The Omega-3 Index and relative risk for coronary heart disease mortality: Estimation from 10 cohort studies.* Atherosclerosis, 2017. **262**: p. 51-54.

205. Stark, K.D., et al., *Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults.* Progress in Lipid Research, 2016. **63**: p. 132-152.

206. de Groot, R.H., et al., *Age dependence of plasma phospholipid fatty acid levels: potential role of linoleic acid in the age-associated increase in docosahexaenoic acid and eicosapentaenoic acid concentrations.* Br J Nutr, 2009. **102**(7): p. 1058-64.

207. Bolton-Smith, C., M. Woodward, and R. Tavendale, *Evidence for age-related differences in the fatty acid composition of human adipose tissue, independent of diet.* Eur J Clin Nutr, 1997. **51**(9): p. 619-24.

208. Innes, J.K. and P.C. Calder, *Omega-6 fatty acids and inflammation.* Prostaglandins, Leukotrienes and Essential Fatty Acids, 2018. **132**: p. 41-48.

209. Hussein, N., et al., *Long-chain conversion of [13C]linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men.* J Lipid Res, 2005. **46**(2): p. 269-80.

210. Rett, B.S. and J. Whelan, *Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: a systematic review.* Nutr Metab (Lond), 2011. **8**: p. 36.

211. Vasto, S., et al., *Mediterranean diet and healthy ageing: a Sicilian perspective.* Gerontology, 2014. **60**(6): p. 508-18.

212. Hulbert, A.J., *Explaining longevity of different animals: is membrane fatty acid composition the missing link?* AGE, 2008. **30**(2): p. 89-97.

213. Quiles, J.L., et al., *Role of Olive Oil and Monounsaturated Fatty Acids in Mitochondrial Oxidative Stress and Aging.* Nutrition Reviews, 2006. **64**(s4): p. S31-S39.

214. Frigolet, M.E. and R. Gutiérrez-Aguilar, *The Role of the Novel Lipokine Palmitoleic Acid in Health and Disease.* Advances in Nutrition, 2017. **8**(1): p. 173S-181S.

215. Cao, H., et al., *Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism.* Cell, 2008. **134**(6): p. 933-44.

216. Bruss, M.D., et al., *Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates.* Am J Physiol Endocrinol Metab, 2010. **298**(1): p. E108-16.

217. Vesga-Jiménez, D.J., et al., *Fatty Acids: An Insight into the Pathogenesis of Neurodegenerative Diseases and Therapeutic Potential.* International Journal of Molecular Sciences, 2022. **23**(5): p. 2577.

218. Lohner, S., et al., *Gender differences in the long-chain polyunsaturated fatty acid status: systematic review of 51 publications.* Ann Nutr Metab, 2013. **62**(2): p. 98-112.

219. Preethika, A., S. Sonkusare, and N. Suchetha Kumari, *Single nucleotide polymorphism of fatty acid desaturase gene and breast cancer risk in estrogen receptor subtype.* Gene, 2022. **823**: p. 146330.

220. Hester, A.G., et al., *Relationship between a common variant in the fatty acid desaturase (FADS) cluster and eicosanoid generation in humans.* J Biol Chem, 2014. **289**(32): p. 22482-9.

221. Waits, C.M.K., et al., *A Pilot Study Assessing the Impact of rs174537 on Circulating Polyunsaturated Fatty Acids and the Inflammatory Response in Patients with Traumatic Brain Injury.* J Neurotrauma, 2020. **37**(17): p. 1880-1891.

222. Cribb, L., et al., *Erythrocyte polyunsaturated fatty acid composition is associated with depression and FADS genotype in Caucasians.* Nutr Neurosci, 2018. **21**(8): p. 589-601.

223. Li, S.W., et al., *FADS gene polymorphisms confer the risk of coronary artery disease in a Chinese Han population through the altered desaturase activities: based on high-resolution melting analysis.* PLoS One, 2013. **8**(1): p. e55869.

224. Kwak, J.H., et al., *FADS gene polymorphisms in Koreans: association with ω6 polyunsaturated fatty acids in serum phospholipids, lipid peroxides, and coronary artery disease.* Atherosclerosis, 2011. **214**(1): p. 94-100.

225. Li, S.W., et al., *Polymorphisms in FADS1 and FADS2 alter plasma fatty acids and desaturase levels in type 2 diabetic patients with coronary artery disease.* J Transl Med, 2016. **14**: p. 79.

226. Hong, S.H., et al., *Association of polymorphisms in FADS gene with age-related changes in serum phospholipid polyunsaturated fatty acids and oxidative stress markers in middle-aged nonobese men.* Clin Interv Aging, 2013. **8**: p. 585-96.

227. Pan, G., et al., *rs953413 Regulates Polyunsaturated Fatty Acid Metabolism by Modulating ELOVL2 Expression.* iScience, 2020. **23**(2): p. 100808.

228. Alsaleh, A., et al., *ELOVL2 gene polymorphisms are associated with increases in plasma eicosapentaenoic and docosahexaenoic acid proportions after fish oil supplement.* Genes Nutr, 2014. **9**(1): p. 362.

229. Hong, E.P. and J.W. Park, *Sample size and statistical power calculation in genetic association studies.* Genomics Inform, 2012. **10**(2): p. 117-22.

230. Chang, X., et al., *Effect of plasma polyunsaturated fatty acid levels on leukocyte telomere lengths in the Singaporean Chinese population.* Nutr J, 2020. **19**(1): p. 119.

231. Freitas-Simoes, T.M., E. Ros, and A. Sala-Vila, *Nutrients, foods, dietary patterns and telomere length: Update of epidemiological studies and randomized trials.* Metabolism, 2016. **65**(4): p. 406-15.

232. Karimi, B., et al., *Foods, Dietary Patterns and Occupational Class and Leukocyte Telomere Length in the Male Population.* Am J Mens Health, 2018. **12**(2): p. 479-492.

233. Coluzzi, E., S. Leone, and A. Sgura, *Oxidative Stress Induces Telomere Dysfunction and Senescence by Replication Fork Arrest.* Cells, 2019. **8**(1).

234. Zgórzyńska, E., et al., *Omega-3 polyunsaturated fatty acids improve the antioxidative defense in rat astrocytes via an Nrf2-dependent mechanism.* Pharmacol Rep, 2017. **69**(5): p. 935-942.

235. Tatsumi, Y., et al., *Omega-3 polyunsaturated fatty acids exert anti-oxidant effects through the nuclear factor (erythroid-derived 2)-related factor 2 pathway in immortalized mouse Schwann cells.* J Diabetes Investig, 2019. **10**(3): p. 602-612.

236. Nguyen, T., P. Nioi, and C.B. Pickett, *The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress.* J Biol Chem, 2009. **284**(20): p. 13291-5.

237. Golpour, P., et al., *Improvement of NRF2 gene expression and antioxidant status in patients with type 2 diabetes mellitus after supplementation with omega-3 polyunsaturated fatty acids: A double-blind randomised placebo-controlled clinical trial.* Diabetes Res Clin Pract, 2020. **162**: p. 108120.

238. Oikawa, S. and S. Kawanishi, *Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening.* FEBS Lett, 1999. **453**(3): p. 365-8.

239. Lipina, C. and H.S. Hundal, *Lipid modulation of skeletal muscle mass and function.* J Cachexia Sarcopenia Muscle, 2017. **8**(2): p. 190-201.

240. Salvadó, L., et al., *Oleate prevents saturated-fatty-acid-induced ER stress, inflammation and insulin resistance in skeletal muscle cells through an AMPK-dependent mechanism.* Diabetologia, 2013. **56**(6): p. 1372-82.

241. Coll, T., et al., *Oleate reverses palmitate-induced insulin resistance and inflammation in skeletal muscle cells.* J Biol Chem, 2008. **283**(17): p. 11107-16.

242. Macrae, K., et al., *Defining the role of DAG, mitochondrial function, and lipid deposition in palmitate-induced proinflammatory signaling and its counter-modulation by palmitoleate.* J Lipid Res, 2013. **54**(9): p. 2366-78.

243. Kwon, B. and H.W. Querfurth, *Palmitate activates mTOR/p70S6K through AMPK inhibition and hypophosphorylation of raptor in skeletal muscle cells: Reversal by oleate is similar to metformin.* Biochimie, 2015. **118**: p. 141-150.

244. Taaffe, D.R., et al., *Cross-sectional and prospective relationships of interleukin-6 and C-reactive protein with physical performance in elderly persons: MacArthur studies of successful aging.* J Gerontol A Biol Sci Med Sci, 2000. **55**(12): p. M709-15.

245. Yoneyama, S., et al., *Dietary intake of fatty acids and serum C-reactive protein in Japanese.* Journal of epidemiology, 2007. **17**(3): p. 86-92.

246. van Dijk, S.J., et al., *A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome.* Am J Clin Nutr, 2009. **90**(6): p. 1656-64.

247. Lee, H., J.Y. Lim, and S.J. Choi, *Oleate Prevents Palmitate-Induced Atrophy via Modulation of Mitochondrial ROS Production in Skeletal Myotubes.* Oxid Med Cell Longev, 2017. **2017**: p. 2739721.

248. Xue, M., et al., *Oleate Ameliorates Palmitate-Induced Impairment of Differentiative Capacity in C2C12 Myoblast Cells.* Stem Cells Dev, 2021. **30**(5): p. 289-300.

249. Muoio, D.M., et al., *Peroxisome proliferator-activated receptor-alpha regulates fatty acid utilization in primary human skeletal muscle cells.* Diabetes, 2002. **51**(4): p. 901-9.

250. Tardif, N., et al., *Oleate-enriched diet improves insulin sensitivity and restores muscle protein synthesis in old rats.* Clin Nutr, 2011. **30**(6): p. 799-806.

251. Atkins, J.L., *Chapter 7 - Effects of Sarcopenic Obesity on Cardiovascular Disease and All-Cause Mortality*, in *Nutrition and Skeletal Muscle*, S. Walrand, Editor. 2019, Academic Press. p. 93-103.

252. Rauen, M., L.C. Bollheimer, and M. Nourbakhsh, *Chapter 10 - Underlying mechanisms of sarcopenic obesity*, in *Sarcopenia*, K. Sakuma, Editor. 2021, Elsevier. p. 231-248.

253. Halvorsen, B., et al., *Effects of long-chain monounsaturated and n-3 fatty acids on fatty acid oxidation and lipid composition in rats.* Ann Nutr Metab, 2001. **45**(1): p. 30-7.

254. Yang, Z.-H., et al., *Dietary supplementation with long-chain monounsaturated fatty acids attenuates obesity-related metabolic dysfunction and increases expression of PPAR gamma in adipose tissue in type 2 diabetic KK-Ay mice.* Nutrition & Metabolism, 2013. **10**(1): p. 16.

255. Kob, R., et al., *Gender-specific differences in the development of sarcopenia in the rodent model of the ageing high-fat rat.* J Cachexia Sarcopenia Muscle, 2015. **6**(2): p. 181-91.

256. Jyvakorpi, S.K., et al., *Macronutrient composition and sarcopenia in the oldest-old men The Helsinki Businessmen Study (HBS).* Clinical Nutrition, 2020. **39**(12): p. 3839-3841.

257. Han, P., et al., *Incidence, Risk Factors, and the Protective Effect of High Body Mass Index against Sarcopenia in Suburb-Dwelling Elderly Chinese Populations.* J Nutr Health Aging, 2016. **20**(10): p. 1056-1060.

258. Cho, Y.J., et al., *Sex- and age-specific effects of energy intake and physical activity on sarcopenia.* Scientific Reports, 2020. **10**(1): p. 9822.

259. Baumgartner, R.N., *Body composition in healthy aging.* Ann N Y Acad Sci, 2000. **904**: p. 437-48.

260. Chen, L.K., et al., *Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia.* J Am Med Dir Assoc, 2014. **15**(2): p. 95-101.

261. Tay, L., et al., *Sex-specific differences in risk factors for sarcopenia amongst community-dwelling older adults.* Age (Dordr), 2015. **37**(6): p. 121.

262. Hwang, J. and S. Park, *Sex Differences of Sarcopenia in an Elderly Asian Population: The Prevalence and Risk Factors.* Int J Environ Res Public Health, 2022. **19**(19).