The effects of n-3 fatty acids on muscle function in older adults

By

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i. Thesis structure

The current thesis includes five chapters:

A. **Chapter one** includes the introduction and literature review for the whole thesis; the study aims and hypotheses.
B. **Chapter two** includes the effect of long chain *n*-3 fatty acid supplementation on muscle strength in older adults: A systematic review and meta-analysis.
C. **Chapter three** includes the includes the association of n-3 fatty acid intake with muscle mass and strength in older adults: A cross-sectional analysis of the UK biobank data.
D. **Chapter four** includes the comparison of the effects of krill oil and fish oil supplementation on muscle function in older adults: a randomised controlled pilot study.
E. **Chapter five** includes summary, general discussion, limitations of the current thesis and conclusions.

ii. Abstract

This thesis examines the effects of n-3 fatty acid supplementation on muscle health in older adults through a systematic review and meta-analysis, a cross-sectional analysis, and a randomised controlled trial. Sarcopenia, characterised by the progressive decline in muscle mass and strength with ageing, is recognised as a major global health challenge. There is increasing scientific interest in long-chain n-3 polyunsaturated fatty acids (LCn-3 PUFA) as a potential intervention to prevent or treat sarcopenia. This thesis aimed to examine the effects of LCn-3 PUFA supplementation on muscle health in older adults through three studies: 1) a systematic review and meta-analysis, 2) a cross-sectional analysis of the association of dietary n-3 fatty acid intake with muscle outcomes, and 3) a randomised controlled trial (RCT) comparing the effects of krill oil and fish oil supplementation on muscle strength, size and physical function.

Chapter 2 found, in five RCTs (n=488; 348 females, 140 males), no effect of LCn-3 PUFA on grip strength (SMD 0.57, 95% CI [−0.10, 1.25]; p=0.10) with substantial heterogeneity (I^2=90%). Chapter 3 used UK Biobank data from 61,381 (28,187 men, 33,194 women) older adults (≥60 years) and found that higher n-3 intake was associated with higher grip strength in both men (0.114 kg; 95% CI: 0.02 to 0.21) and women (0.115 kg; 95% CI: 0.05 to 0.18), with no associations for muscle mass index. Chapter 4 found that fish oil supplementation improved knee extensor strength by 11.6 Nm (95% CI, 0.5 to 22.7, p=0.01) and handgrip strength by 2.2 kg (95% CI, 0.2 to 4.2, p=0.007), compared to placebo, while both krill and fish oil enhanced gait parameters, for example cadence improved by 5.3 steps/min (95% CI, 1.2 to 9.4, p = 0.00) with fish oil and 4.0 steps/min 95% CI, 0.1 to 8.0, p = 0.01) with krill oil.

In conclusion this thesis provides substantial evidence that marine-derived LCn-3 PUFA supplementation represents an effective and safe intervention for supporting muscle health in older adults.

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vii. Publications and conference presentations

Publications, posters and presentations containing work undertaken in this thesis, publications related/unrelated to the work in this thesis.

- Publication: **The Effect of Long Chain *n*-3 Fatty Acid Supplementation on Muscle Strength in Older Adults: A Systematic Review and Meta-Analysis.** Maha Timraz [1], Ahmad Binmahfoz [1], Terry J Quinn [1], Emilie Combet [2], Stuart R Gray [1]. **(Published in Nutrients in August 2023).**

- Publication: **The association of n-3 fatty acid intake with muscle mass and strength in older adults: A cross-sectional analysis of the UK biobank data.** Maha Timraz [1], Marion Guerrero Wyss [1], Terry J Quinn [1], Emilie Combet [2], Carlos Celis-Morales [3], Stuart R Gray [4] **(Published in Journal Nutrition, Health and Ageing in Jun 2025).**

- Conference presentation at The Nutrition Society. **The Effect of Long Chain *n*-3 Fatty Acid Supplementation on Muscle Strength in Older Adults: A Systematic Review and Meta-Analysis. (From 05-06 December 2023).**

viii. Acknowledgements

I begin by thanking Allah for granting me the wisdom, perseverance, and opportunity to complete this doctoral research.

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I am profoundly grateful to **Professor Emilie Combet** and **Professor Terry Quinn** for graciously serving as co-supervisors. Their willingness to support my research and provide expert guidance throughout my studies has been invaluable. Their contributions have enriched my academic experience immeasurably.

Statistical analysis can be daunting for many researchers, myself included. Fortunately, I had the privilege of working with **Dr Carlos Celis-Morales**, whose statistical expertise and teaching abilities are exceptional. Dr Celis-Morales has a unique gift for making complex statistical concepts accessible and understandable. His patient guidance has been instrumental in strengthening the analytical foundation of this research.

This achievement carries profound personal significance as it fulfils a dream that **my father** held dear before his passing that I would pursue doctoral studies. Though he is no longer here to witness this accomplishment, his aspirations for my education have been a driving force throughout this journey. I am equally grateful to **my mother, sisters and brother**, whose love, patience, and encouragement sustained me through the most challenging periods

of this endeavour. Their unwavering belief in my abilities has been a constant source of strength and motivation.

I am deeply grateful to my **colleagues** within the department who created such a supportive and collaborative research environment. Their readiness to share knowledge, offer assistance with methodological questions, and provide encouragement during challenging phases of the research made this journey far more manageable. The camaraderie and intellectual discussions we shared enriched my understanding and made the PhD experience genuinely enjoyable.

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Finally, I acknowledge my own journey of growth and discovery. The late nights, the data struggles, the moments of doubt, and the eventual breakthroughs have all contributed to both this thesis and my development as a researcher. This work represents not merely an academic milestone, but the fulfilment of a family legacy and a testament to the power of perseverance.

Thank you.

ix. Author's declaration

I declare that the work presented in this thesis is original and has been conducted by me, **Maha Timraz.** I have been fully organisation and execution of the studies, including clinical measurements, laboratory analyses, and data processing, except where explicitly stated otherwise.

I certify that the work reported in this thesis has been performed by **Maha Timraz.**

x. List of abbreviations

AWGS	Asian Working Group for Sarcopenia
ALA	alpha-linolenic acid
ANCOVA	Analysis of covariance
ALM	Appendicular lean mass
ALMI	Appendicular lean mass index
ASM	Appendicular skeletal muscle mass
BMI	Body mass index
BIA	Bioelectrical Impedance Analysis
CENTRAL	Cochrane central register of controlled trails
CRP	C-reactive protein
CI	Confidence Intervals
CNS	Central nervous system
DHA	Docosahexaenoic acids
DHEA	Dehydroepiandrosterone
DXA	Dual-energy X-ray absorptiometry
DNA	Deoxyribonucleic acid
EPA	Eicosapentaenoic acid
EPR	Electron paramagnetic resonance
EWGSOP	European Working Group on Sarcopenia in Older People
EE	Ethyl-esters
FNIH	Foundation for the National Institutes of Health
4E-BP1	4E-binding protein 1
GH	Growth hormone
GRADE	Grading of recommendations assessment, development and evaluation
GLIM	Global Leadership Initiative on Malnutrition
GAITRite	Electronic walkway system
IAPs	Inhibitor of apoptosis proteins
IPAQ	International physical activity questionnaire
IGF-1	Insulin growth factor 1

IL-1	Interleukin 1
IL-6	Interleukin 6
IL-1β	Interleukin-1β
IWGS	International Working Group on Sarcopenia
LCn-3PUFAs	Long-chain n-3 polyunsaturated fatty acids
LBM	Lean body mass
MD	Standardised mean differences
MCID	Minimal clinically important difference
MUFA	Monounsaturated fatty acid
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
mTOR	Mammalian target of rapamycin
mTORC	Mammalian target of rapamycin complex
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2
MVC	Maximum voluntary contraction
MRI	Magnetic Resonance Imaging
1-RM	One-Repetition Maximum
MuRF1	Muscle RING-finger protein-1
NF-dB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NMJ	Neuromuscular junction
RDA	Recommended dietary allowance
RCT	Randomaized controlled trial
retag	Re-esterified triglycerides
RA	Rheumatoid arthritis
RTE	Resistance training exercise
STS	30- Second sit to stand
S6K1	S6 kinase 1
SD	Standard deviation
SDM	standardized mean difference
SDOC	Sarcopenia definitions and outcomes consortium

SPMs	Specialized pro-resolving mediators
SMI	Skeletal muscle mass index
SMM	Skeletal muscle mass
sEMG	Surface electromyography
SPPB	Short physical performance battery
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TSC2	Tuberous sclerosis complex 2
TNFα	Tumour necrosis factor alpha
TUG	Time to stand-up and go
UPS	Ubiquitin-proteasome system
p70S6K	70-kDa ribosomal protein S6 kinase
PUFA	Polyunsaturated fatty acid
PPAR	Peroxisomal proliferating-activated receptor

Chapter 1: Literature Review

Introduction

Sarcopenia is a condition marked by a progressive loss of skeletal muscle mass, diminished muscle strength, and declining physical function. The term was first introduced by Rosenberg in 1989 to describe the natural decline in muscle quantity and strength linked to ageing (Rosenberg, 1989). Although it has long been considered an age-related condition, recent findings suggest that sarcopenia can also occur in younger populations (Zuo *et al.*, 2024). Sarcopenia is a multifactorial condition that results from a combination of factors such as ageing, hormonal alterations, inadequate nutrition, and lack of physical activity (Abate *et al.*, 2007, Ryall *et al.*, 2008). Despite increasing awareness, currently, there is no universally agreed-upon definition or diagnostic criteria for sarcopenia. Various organisations have proposed different definitions. For instance, the European Working Group on Sarcopenia in Older People2 (EWGSOP2) characterises sarcopenia as the presence of low muscle strength, as a key characteristic of sarcopenia, uses detection of low muscle quantity and quality to confirm the sarcopenia diagnosis, and identifies poor physical performance as indicative of severe sarcopenia (Cruz-Jentoft *et al.*, 2010). Meanwhile, the Foundation for the National Institutes of Health (FNIH) Sarcopenia Project defines the condition based on low grip strength combined with reduced appendicular lean mass, adjusted for body mass index (BMI) (Studenski *et al.*, 2014).

As a progressive degenerative disorder, sarcopenia not only impairs physical health and overall quality of life but also imposes a major economic burden on healthcare systems worldwide. Research consistently shows that sarcopenia contributes significantly to healthcare costs across various countries. Furthermore, financial limitations often hinder patients from accessing timely and effective treatments, which may accelerate the progression of the disease. In the U.S., the direct healthcare costs linked to sarcopenia were estimated at $18.5 billion for 2000, accounting for approximately 1.5% of the nation’s total

healthcare spending (Janssen *et al.*, 2004). While in the UK, the excess health and social care costs linked to muscle weakness are estimated at around £2.5 billion annually (Goates *et al.*, 2019, Janssen *et al.*, 2000). The loss of muscle is closely linked to declines in both muscle and metabolic health, including impaired insulin sensitivity, disrupted glucose regulation, reduced strength, and diminished physical performance (Cruz-Jentoft et al., 2019b). Additionally, the progression and severity of muscle deterioration may vary depending on sex and ethnicity (Jeng *et al.*, 2018).

As life expectancy continues to rise in developed nations, it becomes increasingly likely that a large proportion of the ageing population will face a loss of independence and reduced quality of life due to sarcopenia (Cruz-Jentoft et al., 2019b, Beaudart et al., 2015). Indeed, since 1980, the global population aged 65 years and older has nearly tripled, and projections indicate this group will double in population again by 2050 (Mayoclinic, 2022). Presently, it is estimated that about 30% of individuals over the age of 65 and 50% of those over 80 suffer from clinically significant sarcopenia (Goates *et al.*, 2019). This will coincide with an increase in the number of people with sarcopenia and so the development of strategies to prevent and treat sarcopenia is of the utmost importance.

1.1 Diagnosis and classification of sarcopenia

To address the growing recognition of sarcopenia as a clinically relevant condition, several scientific organisations, have published diagnostic guidelines based on measurable components such as muscle mass, muscle strength, and physical performance. The most widely adopted frameworks are those developed by the European Working Group on Sarcopenia in Older People (EWGSOP), the International Working Group on Sarcopenia (IWGS), the Asian Working Group on Sarcopenia (AWGS), the Foundation for the National Institutes of Health (FNIH), and the Sarcopenia Definitions and Outcomes Consortium (SDOC) (Voulgaridou *et al.*, 2024, Bhasin *et al.*, 2020, Chen *et al.*, 2020a). The Diagnostic

definitions and criteria for sarcopenia according to the international working groups are summarized in **Table 1.1**, while the Methods and cut-off points for diagnostic criteria by different working groups are presented in **Table 1.2** (Voulgaridou *et al.*, 2024).

One of the earliest and most influential efforts was led by the EWGSOP, which published its initial consensus definition in (Cruz-Jentoft *et al.*, 2010). According to this framework, sarcopenia is characterized as a syndrome involving progressive and generalized loss of skeletal muscle mass and strength, associated with increased risk of adverse outcomes such as physical disability, poor quality of life, and mortality (Cruz-Jentoft *et al.*, 2010). The EWGSOP recommended that diagnosis should include assessments of low muscle mass (using dual-energy X-ray absorptiometry [DXA] or bioimpedance analysis [BIA]), low muscle strength (typically measured via handgrip strength), and low physical performance (assessed using gait speed or chair rise tests) (Cruz-Jentoft *et al.*, 2010). In line with these diagnostic components, the EWGSOP also introduced a staging system to classify sarcopenia based on severity: Pre-sarcopenia, defined by low muscle mass only, sarcopenia, identified by low muscle mass in combination with either low muscle strength or poor physical performance, severe sarcopenia, characterized by the presence of all three criteria: low muscle mass, low muscle strength, and poor physical performance (Cruz-Jentoft *et al.*, 2010). These guidelines have played a foundational role in shaping subsequent research and clinical approaches to sarcopenia over the past decade, providing a structured and standardized method for identifying individuals at varying levels of risk (Voulgaridou *et al.*, 2024).

In 2019, the (EWGSOP) updated its guidelines to reflect new scientific evidence and improve clinical applicability, resulting in the revised consensus definition known as EWGSOP2 (Cruz-Jentoft et al., 2019b). This updated framework retained the core components of sarcopenia: low muscle mass, reduced strength, and impaired physical function but placed greater emphasis on muscle strength and physical performance as primary diagnostic indicators, suggesting that low muscle mass alone is insufficient for diagnosis (Cruz-Jentoft et al., 2019b). This shift highlights a growing understanding of sarcopenia as a dynamic condition with varying levels of severity, ranging from early-stage to advanced forms of the syndrome. These categories are supported by standardized cut-off points for grip strength, muscle mass, and gait speed (Cruz-Jentoft et al., 2019b, Voulgaridou et al., 2024). While these updates aim to improve clinical consistency, some evidence suggests they may underestimate sarcopenia prevalence, particularly when using handgrip strength alone (Stuck *et al.*, 2021).

The International Working Group on Sarcopenia (IWGS) also contributed early definitions, emphasizing the role of muscle weakness and functional decline in the ageing population (Fielding *et al.*, 2011). The IWGS established a simplified two-component diagnostic approach: sarcopenia is diagnosed when both low appendicular muscle mass and impaired physical performance (Fielding *et al.*, 2011, Voulgaridou *et al.*, 2024) are present. Their framework characterized sarcopenia as a distinct clinical entity that deliberately excludes muscle strength measurements as a core diagnostic component, setting it apart from other consensus definitions such as those proposed by the EWGSOP (Fielding *et al.*, 2011). The IWGS diagnostic process targets patients with obvious functional decline (bedridden, cannot rise from a chair independently, or have slow walking speed), then confirms diagnosis through DXA assessment of appendicular muscle mass combined with gait speed testing

(Fielding *et al.*, 2011). Unlike other frameworks that screen broadly, the IWGS criteria focus specifically on identifying individuals with significant mobility impairments who require immediate clinical attention (Fielding *et al.*, 2011). While the IWGS acknowledged overlaps with related syndromes like frailty, their framework positioned sarcopenia as a unique condition centred around physical function and independence (Fielding *et al.*, 2011).

Parallel efforts to define sarcopenia in the Asian context have led to the development of region-specific diagnostic criteria by the Asian Working Group for Sarcopenia (AWGS). These guidelines emerged in response to observed differences in body composition, disease patterns, and demographic characteristics compared to Western populations. In 2014, the AWGS first proposed diagnostic thresholds identifying sarcopenia through low muscle mass, low muscle strength, and/or impaired physical performance (Chen *et al.*, 2014). These criteria were later revised in 2019 (AWGS2), introducing updated algorithms tailored specifically for community-based and clinical settings (Chen *et al.*, 2020b). The 2019 revision introduced the concept of "possible sarcopenia", defined as low muscle strength with or without poor physical performance, although this classification was recommended primarily for use in community-dwelling older adults rather than in clinical or research environments (Chen *et al.*, 2020b). Furthermore, dual-energy X-ray absorptiometry (DXA) is considered the preferred method for assessing muscle mass in research settings, while bioelectrical impedance analysis (BIA) is recommended for community screening due to its accessibility, affordability, and non-invasive nature (Chen *et al.*, 2014). This staged approach reflects the AWGS's emphasis on early detection and intervention among at-risk individuals outside institutional settings. Notably, both the original and updated AWGS definitions recommend lower cut-off points for muscle mass, strength, and physical function compared to those used by Western consensus groups such as the EWGSOP and IWGS (Cooper *et al.*,

2013, Fielding *et al.*, 2011, Chen *et al.*, 2014, Chen *et al.*, 2020b). These differences are attributed, by the AWGS, to variations in body size and weight between Asian and Caucasian populations.

In North America, the Foundation for the National Institutes of Health (FNIH) Sarcopenia Project conducted large-scale analyses of epidemiological data to define objective thresholds for diagnosing sarcopenia with particular emphasis on the importance of assessing physical performance alongside muscle parameters (Studenski *et al.*, 2014). Their work identified specific cut-points for grip strength and appendicular lean mass adjusted for body mass index (BMI). The FNIH criteria have been widely adopted in population-based research due to their empirical grounding and reproducibility (Studenski *et al.*, 2014). The FNIH recommendations align with those of the (EWGSOP) in their systematic approach to assessment, typically through gait speed, muscle strength (using handgrip strength) and muscle mass, measured via dual-energy X-ray absorptiometry (DXA) (Studenski *et al.*, 2014). However, a key distinction lies in how muscle mass is normalized. Unlike the EWGSOP, AWGS, and IWGS, which generally use appendicular skeletal muscle mass adjusted for height squared (ASM/height^2), the FNIH proposed using appendicular lean mass adjusted for BMI (ALM/BMI) for identifying low muscle mass (Cruz-Jentoft et al., 2019b, Chen et al., 2014, Cooper et al., 2013, Fielding et al., 2011, Cawthon et al., 2020).

These different adjustment approaches reflect important methodological considerations. Adjustment by height-squared (ASM/height^2) is based on allometric scaling principles designed to account for the correlation between muscle mass and height, thereby controlling for body frame size while remaining stable over time as height changes minimally with ageing (Kim et al., 2016, Muraki, 2023). In contrast, adjustment by BMI (ALM/BMI) accounts for both height^2 and total body mass, potentially better capturing overall body composition (Studenski et al., 2014). For example, the FNIH criteria demonstrated stronger

associations with adverse outcomes, including disability and mortality, providing enhanced empirical validation (Studenski et al., 2014), and superior identification of sarcopenic obesity compared to height-adjusted indices (Kim et al., 2017). However, each approach yields different prevalence estimates, and the optimal method may depend on the population and the specific clinical outcome being predicted (Kim et al., 2016).

More recently, the Sarcopenia Definitions and Outcomes Consortium (SDOC) has sought to harmonize existing definitions to enhance clinical applicability across research settings (Bhasin *et al.*, 2020). Unlike earlier frameworks, the SDOC proposed a definition based solely on muscle weakness and slowness, without requiring low muscle mass as a diagnostic criterion (Bhasin *et al.*, 2020). Specifically, sarcopenia is defined by the presence of both low handgrip strength and slow gait speed, with sex-specific cut-off points established to enhance sensitivity and specificity. These criteria were developed because both parameters have been shown to strongly predict adverse outcomes such as disability, falls, hip fractures, and mortality (Bhasin *et al.*, 2020). A key distinction of the SDOC approach is its exclusion of lean body mass (LBM) or appendicular lean mass as a diagnostic component a feature common to all other consensus definitions (e.g., EWGSOP, AWGS, FNIH, IWGS) (Voulgaridou *et al.*, 2024). Instead, the SDOC emphasized functional measures, arguing that slow gait speed is the most reliable indicator of physical decline in community-dwelling older adults (Bhasin *et al.*, 2020). Notably, the SDOC proposed higher cut-off values for grip strength compared to those used by other groups (Bhasin *et al.*, 2020, Cooper *et al.*, 2013). This adjustment reflects an effort to identify more individuals with clinically relevant muscle weakness who may benefit from early intervention, even if they do not meet traditional thresholds for low muscle mass (Cawthon *et al.*, 2020).

Table 1.1 Diagnostic definitions and criteria for sarcopenia according to the international working groups.

Consensus Group	Criteria for Diagnosis	Note
EWGSOP (2010)	- Low muscle mass. - Low muscle strength. - Low physical performance.	Sarcopenia is classified as follows: •Pre-sarcopenia: When only low muscle mass exists. •Sarcopenia: When low muscle mass with low muscle strength or physical performance exists. •Severe sarcopenia: when all three criteria co-exist.
IWGS (2011)	- Low muscle mass. - Low physical performance.	Older adults with both low muscle mass and function should be considered patients with sarcopenia.
FNIH (2014)	-Low muscle mass. - Low muscle strength. - Low physical performance.	Based on a thorough examination of clinically relevant thresholds for weakness and low LBM.
AWGS (2014)	- Low muscle mass. - Low muscle strength. - Low physical performance.	Same as the EWGSOP definition. Cut-off points are used that are specific to elderly Asian people or those descended from Asians.
EWGSOP2 (2019)	- Low muscle mass. - Low muscle quantity or quality. - Low physical performance.	Updated definition of sarcopenia: Sarcopenia is classified as the following criteria: •Probable sarcopenia: When low muscle strength exists •Sarcopenia: When low muscle strength and low muscle quantity and/ or quality •Severe sarcopenia: When all criteria co-exist
AWGS 2 (2020)	-Low muscle strength. - Low muscle mass. - Low physical performance.	Sarcopenia is classified as the following criteria: •Possible sarcopenia: When low muscle strength with or without low physical performance exists. •Sarcopenia: When low muscle mass, low muscle strength, and/or low physical performance exists. •Severe sarcopenia: When all criteria co-exist.
SDOC (2020)	- Low muscle strength. - Low physical performance.	The definition of sarcopenia is: The existence of both slowness and muscle weakness, regardless of lean mass measured by DXA. Low DXA-derived LBM has no consistent link to negative health consequences (falls, mobility, and mortality).

EWGSOP = European Working Group on Sarcopenia in Older People; IWGS = International Working Group on Sarcopenia; FNIH = American Foundation for the National Institutes of Health; LBM = Lean Body Mass; AWGS = Asian Working Group for Sarcopenia; SDOC = Sarcopenia Definition and Outcomes Consortium; DXA = Dual-energy X-ray absorptiometry. Table adapted from (Voulgaridou *et al.*, 2024).

Table 1.2 The Methods and cut off points for diagnostic criteria by different working groups.

Consensus Group	Muscle Mass	Muscle Strength	Physical Performance
EWGSOP (2010)	DXA (ALM/height²): <7.26 kg/m² (male) <5.5 kg/m² (female). BIA: <8.87 kg/m² (male) <6.42 kg/m² (female) CT or MRI or Total/partial body potassium per fat-free soft tissue.	Grip strength: <30 kg (male) <20 kg (female). Knee flexion/extension or Peak expiratory flow.	SPPB: <8 or 4MGS: <0.8 m/s or TUG or SCTP.
IWGS (2011)	DXA (ALM/height²): <7.23 kg/m² (male) <5.67 kg/m² (female).	Not Specified.	4MGS: <1.0 m/s or standing up from a chair.
FNIH (2014)	DXA (ALM/BMI): <0.789 kg/BMI (male) <0.512 kg/BMI (female).	Grip strength: <26 kg (male) <16 kg (female).	4MGS, 6MGS: <0.8 m/s.
AWGS (2014)	DXA (ALM/height²): <7.0 kg/m² (male) <5.4 kg/m² (female) BIA: ≤7.0 kg·m^{-2} (male) <5.7 kg·m^{-2} (female).	Grip strength: <26 kg (male) <18 kg (female).	6MGS: <0.8 m/s.
EWGSOP2 (2019)	DXA (ALM/height²): <7.00 kg/m² (male) <6.00 kg/m² (female) or BIA/CT/MRI.	Grip strength: <27 kg (male) <16 kg (female). Chair stand test >15 s	Gait speed: <0.8 m/s or SPPB: ≤8 or TUG: ≥20 s or 400 m walk: >6 min.
AWGS 2 (2020)	DXA (ASM): <7.0 kg/m² (male) <5.4 kg/m² (female) BIA (ASM): ≤7.0 kg·m^{-2} (male) <5.7 kg·m^{-2} (female).	Grip strength: <28 kg (male) <18 kg (female).	6MGS: <1.0 m/s or 5TSST: >12 s or SPPB: ≤9.
SDOC (2020)	Not Specified.	Grip strength: <35.5 kg (male) <20 kg (female).	Gait speed: <0.8 m/s.

EWGSOP = European Working Group on Sarcopenia in Older People; IWGS = International Working Group on Sarcopenia; FNIH = American Foundation for the National Institutes of Health; AWGS = Asian Working Group for Sarcopenia; SDOC = Sarcopenia Definition and Outcomes Consortium; DXA = Dual-energy X-ray absorptiometry; ALM = Appendicular lean mass; kg = Kilogram; m = Meter; BIA = Bioimpedance analysis; CT = Computed tomography; MRI = Magnetic resonance imaging; BMI = Body mass index; ASM = Appendicular skeletal mass; s = Second; SPPB = Short Physical Performance Battery; 4MGS = 4 m gait speed; TUG = Time Up and Go; SCTP = Stair climb power test; 6MGS = 6 m gait speed; 5TSST = 5 times stand-to-sit test. Table adapted from (Voulgaridou *et al.*, 2024).

Despite these advances, significant challenges remain in achieving universal agreement on sarcopenia diagnosis. Variations persist not only in the components used to define the condition but also in measurement tools (e.g., DXA vs. BIA), reference populations, and threshold values, leading to inconsistencies in treatment strategies (Lampignano *et al.*, 2021). While most working groups include low muscle mass as a core diagnostic criterion, the SDOC and the IWGS deviate from this norm by focusing primarily on strength and performance (Bhasin *et al.*, 2020, Fielding *et al.*, 2011). This divergence highlights ongoing debate about whether sarcopenia should be diagnosed using all three components (muscle mass, strength, and performance) or just two (as in the SDOC and IWGS models). Furthermore, although most current definitions focus on older adults, emerging evidence suggests that sarcopenia can also affect younger populations, particularly those with chronic diseases or undergoing cancer treatment (Voulgaridou *et al.*, 2024). In summary, while progress has been made toward standardizing sarcopenia diagnosis, continued research is essential to identify the most effective and clinically meaningful criteria that can reliably predict adverse health outcomes and guide targeted interventions across diverse age groups and populations.

1.2 Prevalence of sarcopenia

Numerous studies have investigated the prevalence of sarcopenia among older adult populations, revealing considerable variation depending on the diagnostic criteria and measurement methods used (Miller, 2021). The estimated prevalence of sarcopenia across numerous age groups and settings is presented in

Table 1.3 (Miller, 2021). Due to the absence of a universally accepted definition of sarcopenia, however, it remains challenging to determine its exact prevalence. A meta-

analysis of 151 studies conducted across different continents indicates that the prevalence of sarcopenia ranges between 8% and 36% in individuals under 60 years of age, and between 10% and 27% among older adults (Petermann-Rocha *et al.*, 2022a). Among men, the prevalence range fluctuates between 14.3% (60–64 years) to 59.4% (≥75 years), while in women from 20.3% (60–64 years) to 48.3% (≥75 years) (Kirk *et al.*, 2020). The geographical prevalence of sarcopenia has also been estimated and is presented in **Figure 1-1** (Petermann-Rocha *et al.*, 2022a). Based on the AWGS criteria, a Japanese study found that 20.2% of individuals aged 65 and older exhibit low muscle mass, with sarcopenia affecting 9.6% of men and 7.7% of women (Yuki *et al.*, 2015). In addition, a study from mainland China using the AWGS criteria found that sarcopenia affected 6.4% of older men and 11.5% of older women living in rural areas (Wang and Wei, 2024). These figures are notably lower than those reported in some other ethnic groups (Wang and Wei, 2024), suggesting that regional and ethnic factors significantly influence the epidemiology of sarcopenia.

Table 1.3 Prevalence of Sarcopenia in Older Adults. Table adapted from (Miller, 2021).

Population Group	Age Range	Residential Status	Prevalence
Healthy adults	50-70 years	Community-dwelling	6-24%
Adults Community Health Settings	≥65 years	Community-dwelling	33%
Individuals > 80 years	>80 years	Community-dwelling	50%
Frail Individuals	60-90 years (variable)	Mixed (community and institutionalized)	50-70%

Population Group	Age Range	Residential Status	Prevalence
Healthy adults	50-70 years	Community-dwelling	6-24%
Men Long Term Care Settings	≥65 years (mean ~80 yrs)	Institutionalized (nursing homes)	68%
Acutely Hospitalized Older	≥65 years (mean 82-84 yrs)	Hospitalized (acute geriatric wards)	76%

Table 1.3 demonstrates how sarcopenia prevalence varies by age and residential setting. Community-dwelling populations (healthy adults aged 50-70 years, adults >65 years accessing community health settings, and the 'oldest old' >80 years) show higher prevalence with age, from 6-24% in the youngest group to 50% in those over 80 years. Frail individuals (typically aged 60-90 years), residing in either community or institutional settings, show markedly elevated prevalence (50-70%) compared to non-frail community-dwelling adults. The highest prevalence was observed in institutionalized settings: men in long-term care facilities (typically aged ≥65 years with mean age around 80 years) show 68% prevalence, while acutely hospitalized older patients (mean age 82-84 years) admitted to geriatric wards show the highest prevalence at 76%. This gradient reflects the combined effects of advancing age, declining health status, and environmental factors on muscle mass and function (Miller, 2021).

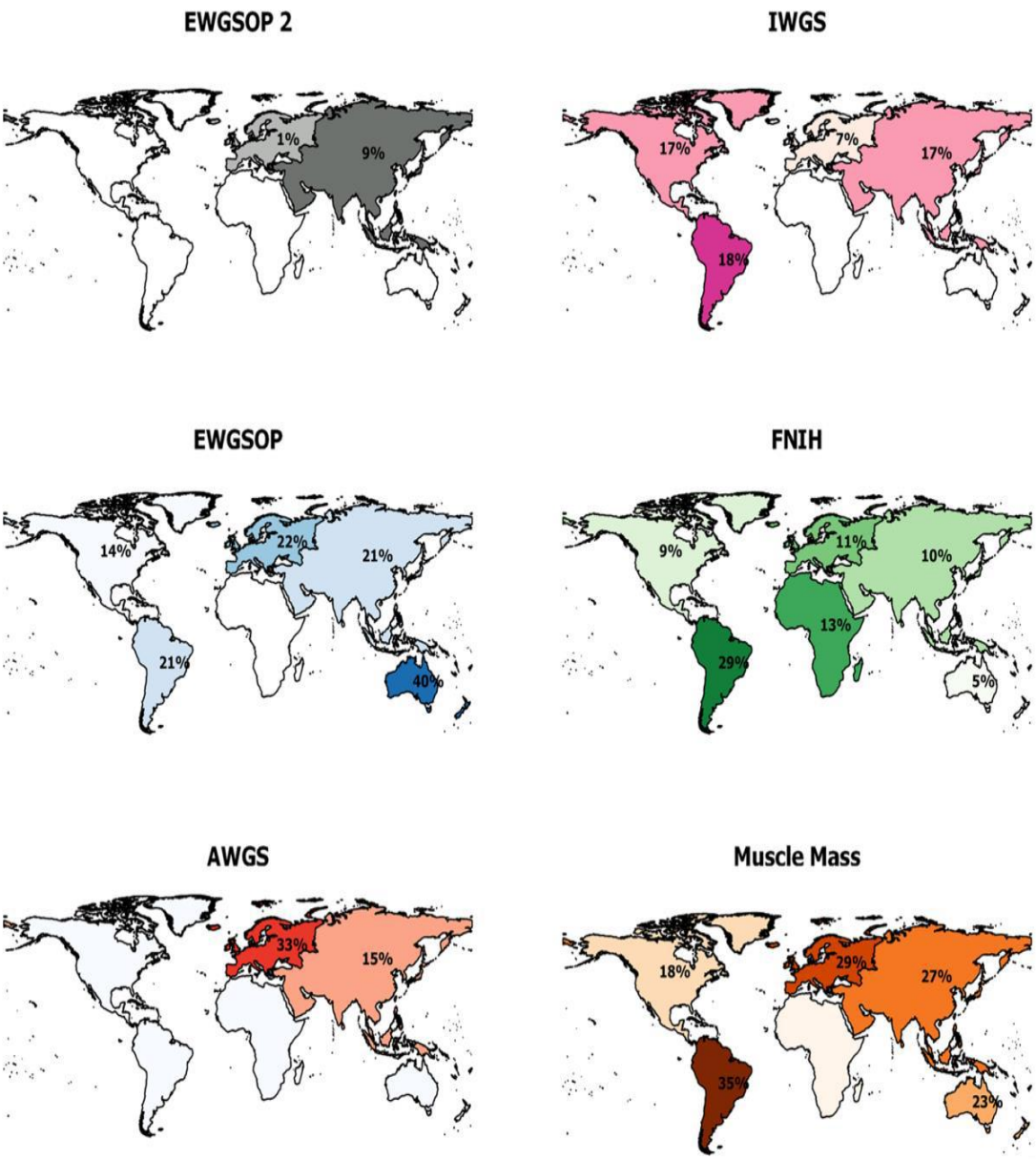

Figure 1-1 The figure is obtained from Petermann-Rocha and colleagues (2022). The figure shows the variation in assessing sarcopenia according the operational measure (S), or definition used. Taking the prevalence of sarcopenia in Asia as an example, it shows the highest prevalence (27%) when Muscle Mass is used as single measure, less prevalence (21%) when EWCSOP definition is used, less prevalence (17%) when the international working group on sarcopenia (IWGS) is used, then 15 % when the Asian Working Group for Sarcopenia (AWGS) is used and 10 % or 9 % when the Foundation for the National Institute of Health (FNIH) or EWCSOP definitions, respectively, are used. Figure adapted from (Petermann-Rocha et al., 2022a).

1.3 Consequences and socioeconomic costs of sarcopenia

Sarcopenia has wide-ranging consequences, affecting not only the health and quality of life of individuals but also the broader healthcare system and society. The condition is strongly associated with physical disability, fall risk and fractures, increased risk of co-morbidities, reduced quality of life, hospitalization, and mortality (Park *et al.*, 2023). There is a clear association between sarcopenia and impaired physical function, loss of functional independence, and increased risk of physical disability (Tanimoto *et al.*, 2012, Guralnik *et al.*, 2000). The progressive decline in muscle mass, strength, and functional capacity contributes to reduced mobility and compromised performance in activities of daily living (Cruz-Jentoft et al., 2019b).

Walking ability is a key factor influencing future disability, mortality, and healthcare expenditures (Hardy *et al.*, 2011) and sarcopenia directly impairs walking ability through reduced muscle strength and function, compromising balance, coordination, and postural stability (Park *et al.*, 2023). Sarcopenia is also strongly linked to an increased risk of falls, which is a major concern due to its association with fractures and functional impairments. Studies show that individuals with sarcopenia have significantly higher odds of falling compared to those without sarcopenia: 4.42 times higher in men (95% CI: 2.08–9.39) and 2.34 times higher in women (95% CI: 1.39–3.94) (Tanimoto *et al.*, 2012). These falls frequently result in fractures and functional impairments, leading to long-term disability and institutionalisation (Hirani et al., 2015, Yeung et al., 2019). Longitudinal evidence indicates that sarcopenia precedes and predicts nursing home admission through progressive

functional decline and loss of independence, rather than being primarily a consequence of institutionalization (Hirani et al., 2017).

Sarcopenia frequently occurs alongside age-related bone loss and commonly coexists with osteoporosis, potentially contributing to an increased risk of fractures. This may occur directly through biological interactions between muscle and bone tissues (Brotto and Johnson, 2014, Cianferotti and Brandi, 2014), or indirectly by increasing the likelihood of falls (Kim *et al.*, 2014a, DiGirolamo *et al.*, 2013). Additionally, several endocrine disorders such as diabetes, hypogonadism, and hypercortisolism as well as conditions like obesity and chronic kidney disease (Kim *et al.*, 2014a), are independently associated with sarcopenia. These associations suggest that sarcopenia may serve as a key underlying mechanism through which chronic diseases result in functional decline and physical disability (Kalyani *et al.*, 2014) (**Figure 1-2 and Figure 1-3**) (Pinedo-Villanueva *et al.*, 2019).

Beyond physical health, sarcopenia negatively impacts psychosocial well-being and overall quality of life. The physical limitations imposed by the condition can reduce social participation, limit engagement in recreational activities, and lead to loss of independence (Cruz-Jentoft et al., 2019b, Morley et al., 2013, Beaudart et al., 2018, Rubenstein, 2006, Ethgen et al., 2017). These effects are frequently accompanied by emotional distress, depression, and social isolation, further diminishing well-being and increasing the need for psychological and social support (Morley *et al.*, 2013, Beaudart *et al.*, 2018, Rubenstein, 2006, Ethgen *et al.*, 2017).

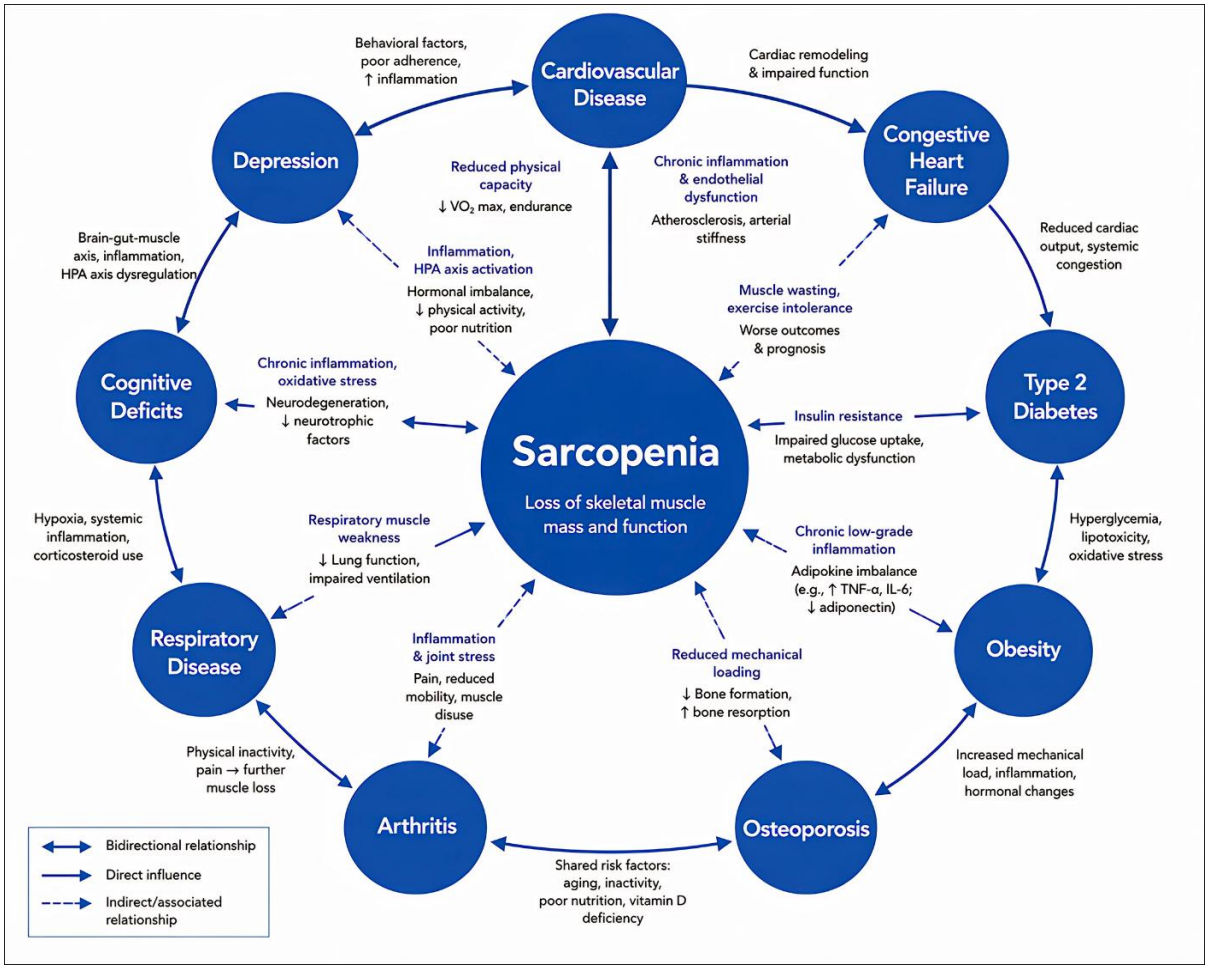

Figure 1-2 Co-Morbidities associated with sarcopenia. Figure adapted from (Miller, 2021).

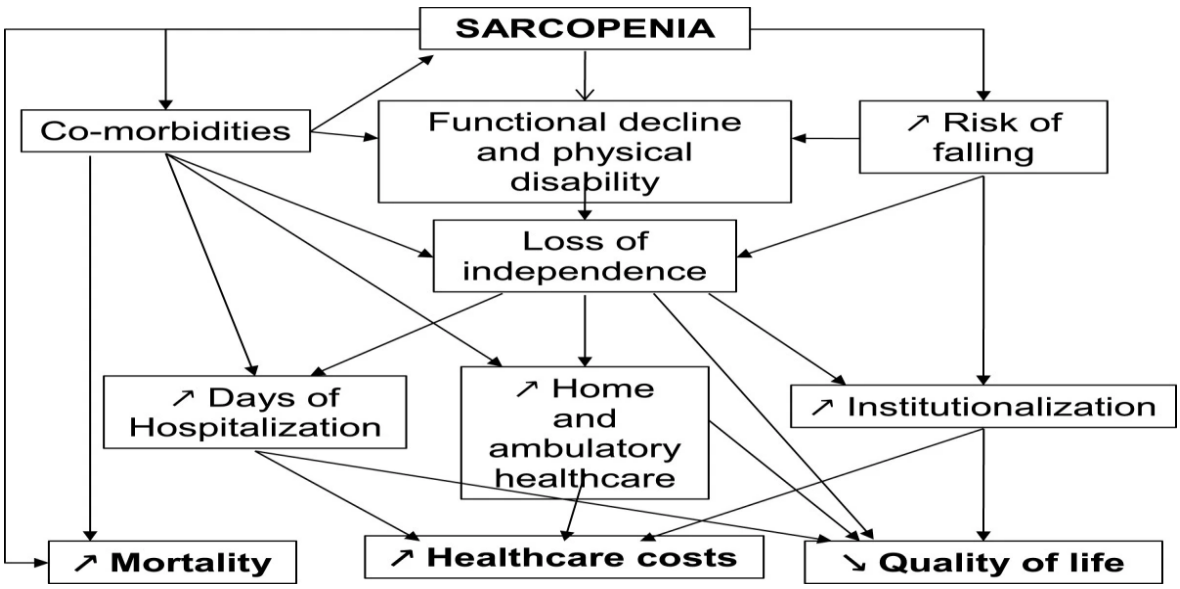

Figure 1-3 The complex burden of sarcopenia on public health. Figure adapted from (Pinedo-Villanueva et al., 2019).

Sarcopenia results in a considerable economic burden on healthcare systems, as both the condition and accompanying muscle weakness contribute significantly to overall healthcare expenditures (Pinedo-Villanueva *et al.*, 2019). Estimating the direct economic burden of sarcopenia remains challenging. However, A U.S.-based study revealed that in the year 2000, sarcopenia was responsible for approximately $18.5 billion in direct healthcare costs, with $10.8 billion attributed to men and $7.7 billion to women. This amount represented 1.5% of the country's total healthcare expenditure for that year. The study also found that each male with sarcopenia incurred an additional $860 in annual healthcare costs, while the corresponding figure for females was $933 per year. The analysis estimated that a 10% reduction in sarcopenia prevalence would save $1.1 billion per year in U.S. healthcare costs (Janssen *et al.*, 2004).

A U.S.-based study by Janssen et al. (2004), using nationally representative survey data (NHANES III and NMCUES), revealed that in the year 2000, sarcopenia was responsible for approximately $18.5 billion in direct healthcare costs (hospitalizations, nursing homes, and home healthcare), with $10.8 billion attributed to men and $7.7 billion to women. This amount represented 1.5% of the country's total healthcare expenditure for that year. Each person with sarcopenia incurred additional annual healthcare costs of $860 (men) or $933 (women). The analysis estimated that a 10% reduction in sarcopenia prevalence would save $1.1 billion per year in U.S. healthcare costs (Janssen et al., 2004, Goates et al., 2019).

Additionally, a cohort study of 422 community-dwelling adults aged 71-80 years, conducted in the UK, found that individuals with muscle weakness, not fully diagnosed sarcopenia, incurred an average annual total cost of £4,592, with the largest contributors being informal

care (38%), secondary inpatient care (23%), and primary care (19%). In comparison, those without muscle weakness had an average annual total cost of £1,885, distributed mainly across informal care (26%), primary care (23%), and formal care (20%) **(Figure 1-4 and Figure 1-5)** (Pinedo-Villanueva *et al.*, 2019). The additional cost associated with muscle weakness was estimated at £2,707 per person per year, of which 46% was attributed to informal care. This translated to an estimated annual excess cost of £2.5 billion for the UK healthcare system (Pinedo-Villanueva *et al.*, 2019).

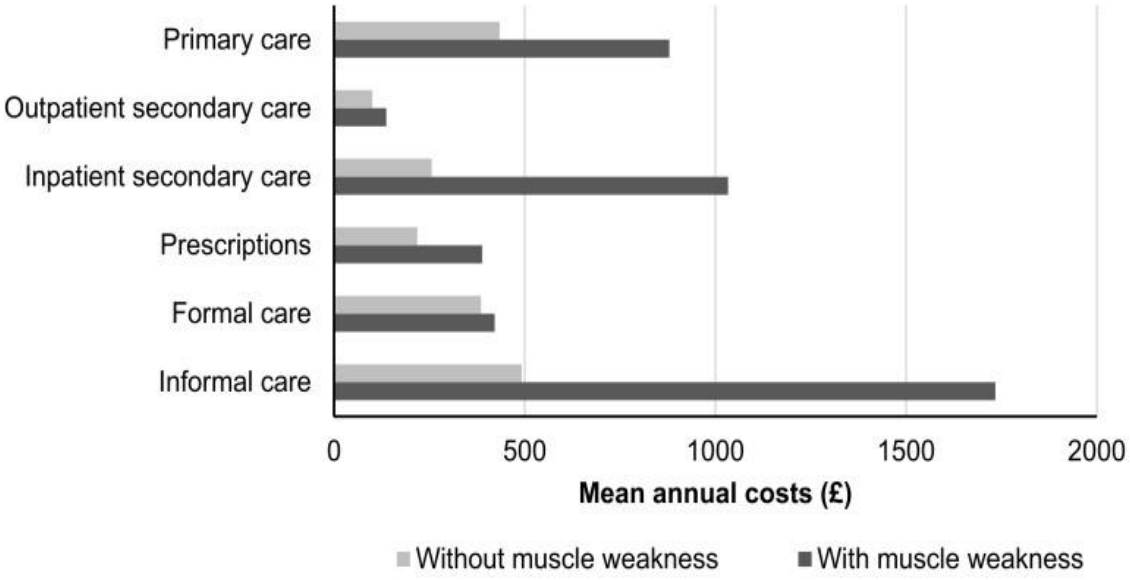

Figure 1-4 Excess annual costs per person for individuals with muscle weakness compared to those without and proportion of costs according to types of health and social care in UK. Muscle weakness was defined using low grip strength (< 26 kg for men, < 16 kg for women). Figure adapted from(Pinedo-Villanueva et al., 2019).

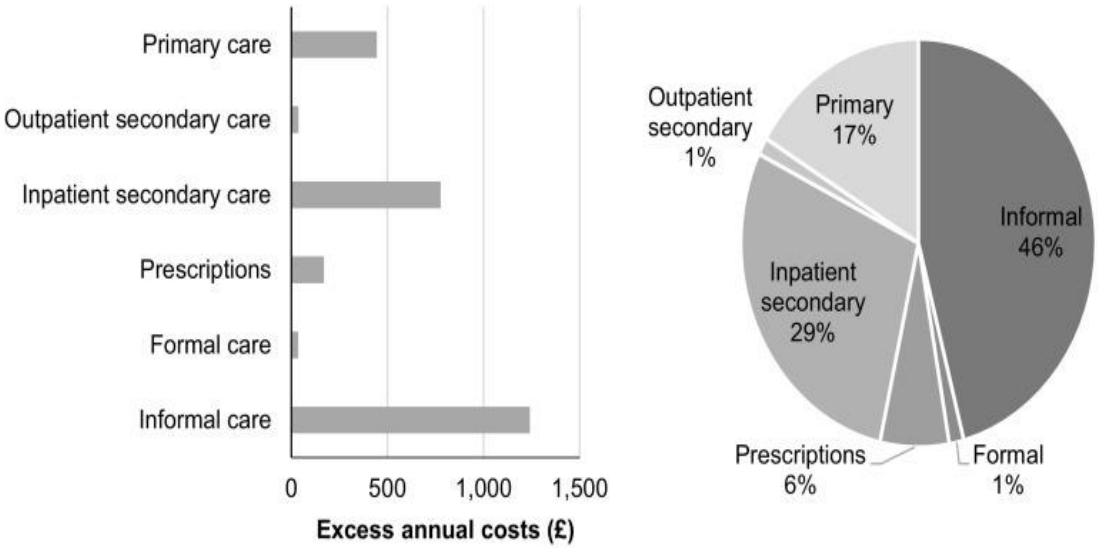

Figure 1-5 Annual costs per person for different uses of health and social care according to muscle strength in UK. Muscle weakness was defined using low grip strength (< 26 kg for men, < 16 kg for women). Figure adapted from (Pinedo-Villanueva et al., 2019).

Given that sarcopenia is a leading cause of dependency among individuals over the age of 70 who do not reside in institutional care settings, it would not be unreasonable to estimate that approximately one-third of the total annual healthcare cost of caring for this population (around £10 billion) is attributable to sarcopenia (Patel *et al.*, 2013). The substantial healthcare costs associated with sarcopenia become even more concerning when viewed against future demographic projections. This economic burden is expected to rise sharply in the coming decades due to projected demographic changes. The old-age dependency ratio, the number of people aged 65 and over/the number aged 15–64, is currently at 29.6% and projected to rise to 51.2% by 2070. This shift implies a move from a current ratio of roughly 10 working-age individuals per 3 older adults to a future ratio of 2 working-age individuals per 1 older adult, amplifying both the healthcare and informal care demands linked to age-related conditions like sarcopenia (COMMISSION, 2018).

1.4 Possible underlying mechanism of sarcopenia

1.4.1 Inactivity

With a large and increasing burden, understanding the causes of sarcopenia is critical for the development of appropriate prevention and treatment strategies. Physical inactivity represents one of the most significant modifiable risk factors for sarcopenia development and progression. The relationship between inactivity and muscle loss is well-established, with disuse atrophy occurring rapidly following periods of reduced physical activity (Aagaard *et al.*, 2010). It is important to highlight that even two weeks of severely restricted physical activity in healthy older adults has been shown to reduce the rate of muscle protein synthesis (MPS) and induce anabolic resistance, resulting in significant loss of lean mass in the legs (Breen *et al.*, 2013). Specifically, when healthy older individuals (average age 72 years) reduced their daily step count by approximately 76%, from habitual levels to just 1413 ± 110 steps per day for 14 days, they experienced a 3.9% reduction in leg fat-free mass, along with a 26% decrease in postprandial MSP rates, increased insulin resistance, and elevated inflammatory markers including TNF-α and C-reactive protein (Breen *et al.*, 2013). Similarly, ten days of bed rest in healthy older adults (mean age 67 ± 5 years) has been shown to reduce the rate of MPS by approximately 30%, resulting in a nearly 1 kg loss of lean mass in the legs and a 15.6% decline in leg strength (Kortebein *et al.*, 2007). This degree of muscle loss is significantly greater than what is observed in younger individuals, among whom similar losses typically require up to 28 days of immobilization (Tanner *et al.*, 2015, Paddon-Jones *et al.*, 2004). The rapid depletion of lean mass during short-term bed rest, often experienced during hospital stays, frequently leads to severe reductions in strength, functional capacity, and independence.

The mechanisms underlying inactivity-induced muscle loss involve multiple pathways. Reduced mechanical loading leads to decreased MPS and increased muscle protein breakdown (MPB) by activating the ubiquitin-proteasome system (Bonaldo and Sandri, 2013). Additionally, physical inactivity contributes to mitochondrial dysfunction and altered muscle fibre type composition, with a shift from type II (fast-twitch) to type I (slow-twitch) fibres (Aagaard *et al.*, 2010).

Recent work by Zhao *et al.* (2024) examined the relationship between physical activity intensity, frequency, duration, and volume and the risk of probable sarcopenia in middle-aged and older adults. The study defined probable sarcopenia based solely on low muscle strength criteria (handgrip strength < 28 kg for men and < 18 kg for women) and low physical performance measures (5-time chair stand test ≥ 12 seconds) without requiring muscle mass measurements, following a modified approach derived from the Asian Working Group for Sarcopenia (AWGS) 2019 consensus guidelines (Zhao *et al.*, 2024). Findings indicate that among older adults (aged ≥ 65 years), engaging in at least one day per week of vigorous-intensity physical activity lasting more than 30 minutes, or accumulating a weekly total of 933 MET-minutes of physical activity, was linked to a 53% lower risk of probable sarcopenia (adjusted OR = 0.47, 95% CI: 0.32, 0.70). This highlights the potential protective role of physical activity in preserving muscle function in older age. In contrast, no significant associations were found between physical activity and probable sarcopenia risk, defined using AWGS 2019 criteria, in middle-aged adults (45–64 years) indicating that the protective benefits of physical activity may be more evident in advanced age (Zhao *et al.*, 2024).

1.4.2 Poor nutrition

Nutritional factors play a fundamental role in the development and progression of sarcopenia. Inadequate nutrient intake, leading to malnutrition, is widely recognized as a significant contributor and older adults often experience nutritional deficiencies across various settings, which further elevates their risk of developing sarcopenia (Miller, 2021). There is considerable overlap between malnutrition and sarcopenia (Petermann-Rocha *et al.*, 2022c, Cruz-Jentoft *et al.*, 2023). A recent meta-analysis combining data from seven studies on hospitalized older adults found that individuals with sarcopenia had a fourfold increased risk of being malnourished, with an odds ratio (OR) of 4.06 (95% CI: 2.43–6.80) (Ligthart-Melis *et al.*, 2020). Recognizing this link, the Global Leadership Initiative on Malnutrition (GLIM) has included low skeletal muscle mass as one of five core diagnostic criteria for malnutrition. The initiative emphasizes the importance of routinely assessing muscle mass as part of a comprehensive nutritional evaluation (Sayer and Cruz-Jentoft, 2022, Barazzoni *et al.*, 2022). While malnutrition and sarcopenia are distinct clinical entities, they share key features, particularly low muscle mass, which is commonly observed in individuals who are undernourished (Sayer and Cruz-Jentoft, 2022). Reduced food intake often leads to insufficient energy consumption, resulting not only in weight loss but also in the depletion of muscle tissue (Lengele *et al.*, 2021). Even in healthy older adults, declines in energy intake can be substantial. For instance, studies have shown that older adults (around 70 years of age) consume approximately 16% to 20% less energy than their younger counterparts (around 26 years of age) (Giezenaar *et al.*, 2016). This age-related decrease in energy intake may contribute significantly to the development of sarcopenia.

Among dietary components, inadequate protein intake is the most extensively studied in relation to sarcopenia (Wiedmer *et al.*, 2021). A systematic review and meta-analysis by

Coelho-Júnior *et al.* (2022) found that older adults with sarcopenia have significantly lower protein intake compared with non-sarcopenic peers (Coelho-Junior *et al.*, 2022). Campbell *et al.* (2008) noted that there is general agreement that moderately increasing daily protein intake beyond 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ may enhance muscle protein anabolism and serve as a strategy to attenuate the age-associated decline in muscle mass (Paddon-Jones *et al.*, 2008). Current UK protein recommendations for all adults, including older adults, are 0.75 g/kg body weight/day (Department of Health, 1991, Morris et al., 2020), with 36-47.5% of older UK adults failing to meet this Reference Nutrient Intake (RNI) (Morris et al., 2020). However, international expert groups recognise higher requirements for older adults, particularly to support muscle health. The PROT-AGE Study Group recommends at least 1.0-1.2 g/kg/day for healthy older adults (>65 years), with higher intakes (1.2-1.5 g/kg/day) for those with illness (Bauer et al., 2013), while ESPEN recommends 1.0-1.2 g/kg/day for healthy older adults and 1.2-1.5 g/kg/day for those malnourished or at risk (Deutz et al., 2014). Recent systematic reviews support these higher recommendations for maintaining muscle mass and function in older adults (Coelho-Junior et al., 2022, Li et al., 2024).

The relationship between inadequate protein consumption and muscle deterioration appears to be particularly pronounced in older adults due to age-related anabolic resistance, where the MPS response to dietary protein becomes blunted compared to younger individuals (Deane et al., 2024) (as discussed further in subsequent sections, page 47-55).

Several studies have shown a strong association between vitamin D deficiency and sarcopenia in older adults, although it is worth noting that there is no universally agreed definition of deficiency. Zhang & Li (2024) noted that research has unequivocally demonstrated that vitamin D receptors are widely present in muscle tissue, and vitamin D directly impacts the differentiation and metabolism of muscle cells to ensure normal muscle

function (Zhang and Li, 2024). According to Remelli *et al.* (2019), the prevalence of vitamin D deficiency is higher among older adults, primarily due to insufficient dietary intake and diminished exposure to ultraviolet radiation. Consequently, vitamin D-deficient older individuals may be at increased risk for sarcopenia (Remelli *et al.*, 2019). In addition to protein and vitamins, deficiency in LCn-3PUFAs has been linked to muscle loss in older adults (Huang *et al.*, 2020) . Similarly, deficiencies in minerals including magnesium, and selenium have been associated with sarcopenia in older adults (Huang *et al.*, 2020, van Dronkelaar *et al.*, 2018, Yang *et al.*, 2022), though effect sizes varied across studies and were not pooled meaning the magnitude of these associations remain unknown. More research, therefore, is needed to confirm these associations.

1.4.3 Muscle protein synthesis/breakdown and anabolic resistance with age

The balance between MPS and MPB determines net muscle protein balance and ultimately muscle mass maintenance. In healthy young adults, MPS and MPB are tightly regulated and remain in equilibrium over 24-hour periods. However, ageing is associated with disruptions in this delicate balance, leading to a gradual shift toward net protein loss, which contributes significantly to the development of sarcopenia (Volpi *et al.*, 2004).

A key feature of ageing muscle is the phenomenon known as anabolic resistance, defined as the reduced ability of muscle to respond to anabolic stimuli such as amino acids and exercise, represents a hallmark of ageing muscle (Cuthbertson *et al.*, 2005). This phenomenon manifests as a blunted MPS response to protein ingestion and resistance exercise in older compared to younger adults. The mechanisms underlying anabolic resistance are multifactorial and include alterations in amino acid transport, reduced mTOR signalling sensitivity, and impaired ribosomal biogenesis (Fry *et al.*, 2011). This is highlighted by work

showing that older adults require higher doses of essential amino acids to achieve the same MPS response as younger individuals. While 10-15 grams of essential amino acids can maximally stimulate MPS in young adults, older adults may require up to 20-25 grams to achieve a similar response (Cuthbertson *et al.*, 2005). This concept is further supported by Moore *et al.* (2015), who demonstrated that older men require approximately 0.40 g/kg body weight of high-quality dietary protein per meal compared to younger men who need only ~0.24 g/kg body weight per meal to maximally stimulate postprandial myofibrillar MPS rates (Moore *et al.*, 2015). This leucine threshold concept has important implications for dietary protein recommendations in older adults, which are 1.0-1.2 g per kg of body weight per day, by emphasizing the need for adequate protein distribution throughout the day to overcome age-related anabolic resistance and effectively stimulate MPS. Supporting this, Houston *et al.* (2008) study found that participants in the highest quintile of protein intake (averaging 1.1 g/kg/day) experienced approximately 40% less lean mass loss over three years compared to those in the lowest quintile, providing observational evidence for the protective role of higher protein intake in preserving muscle mass in older adults. (Houston *et al.*, 2008). The postprandial MPS response is also prolonged in older adults, with peak MPS rates occurring later and returning to baseline more slowly than younger individuals (Koopman *et al.*, 2009). This altered temporal pattern may contribute to developing anabolic resistance and suggests that meal timing and frequency may be important considerations for optimizing muscle protein balance in ageing.

Exercise-induced anabolic resistance is another important consideration. Resistance exercise remains one of the most potent stimuli for MPS, with effects lasting up to three hours post-exercise, promoting muscle growth and hypertrophy (Cuthbertson *et al.*, 2006). However, the magnitude and duration of this response is attenuated in older adults (Kumar *et al.*, 2009),

with longer-term studies demonstrating that women over 75 years show blunted adaptive responses to resistance exercise training compared to younger individuals with respect to increases in muscle volume (+2.5% (old) vs. +6.2% (young)) and strength (+16% (old) vs. +27% (young)), indicating a form of exercise-induced anabolic resistance (Greig *et al.*, 2011). Effective adaptation to resistance training requires two key prerequisites: a sufficient baseline level of muscle mass, including contractile proteins and mitochondrial content (Atherton and Smith, 2012), and regular engagement in physical activity to preserve muscle responsiveness and functional capacity (Breen *et al.*, 2013). The age-related decline in muscle quality and metabolic efficiency further complicates the adaptive response to exercise, suggesting that higher training intensities and longer recovery periods may be necessary to overcome this impairment (Kumar *et al.*, 2009). The magnitude and duration of the response are attenuated in older adults (Kumar *et al.*, 2009).

1.4.4 Molecular regulation of muscle mass and protein synthesis and breakdown during ageing

The molecular mechanisms regulating muscle mass involve complex signalling networks that coordinate anabolic and catabolic processes. The mechanistic target of rapamycin (mTOR) pathway serves as a central regulator of MPS and hypertrophy (Bodine *et al.*, 2001). mTOR operates through two functionally distinct multiprotein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Among these, mTORC1 plays a predominant role in regulating muscle growth and is the primary target of the inhibitor rapamycin, which is widely used in experimental models to study its effects on cellular metabolism (Bodine, 2022). mTORC1 regulates several anabolic processes including protein synthesis, ribosome biogenesis, and mitochondrial biogenesis and catabolic processes such as autophagy (Zoncu *et al.*, 2011). Activation of mTORC1 is primarily mediated by the Akt

signalling pathway, which phosphorylates and inhibits the tuberous sclerosis complex 2 (TSC2) a negative regulator of mTOR. By suppressing TSC2, Akt relieves its inhibitory effect on mTORC1, allowing downstream activation of key regulators of protein translation, including ribosomal S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) (Bodine, 2022). **(Figure 1-6, Figure 1-7 and Figure 1-8)** (Wiedmer, 2021, Garcia-Prat, 2016).

These molecular events are essential for initiating mRNA translation and ribosomal biogenesis, ultimately driving MPS (Bodine, 2022). In skeletal muscle, mTORC1 integrates signals from multiple physiological stimuli that influence muscle mass. It is activated in response to mechanical loading, such as resistance exercise, as well as nutrient availability, including amino acid intake, and exposure to growth factors such as IGF-1 (Bodine, 2022). These inputs collectively support muscle maintenance and adaptation to anabolic challenges. However, ageing has been shown to impair mTOR signalling. Research indicates that older adults exhibit reduced phosphorylation of key downstream targets such as S6K1 and 4E-BP1, which may contribute to anabolic resistance (Paez et al., 2023). This decline in signalling efficiency further exacerbates age-related muscle loss and underscores the importance of interventions aimed at restoring mTORC1 activity in older populations. Recent in vivo studies have identified the regulatory associated protein of mTOR (Raptor) as a key binding partner of mTOR, playing a central role in its signalling function (Bodine, 2022). Raptor interacts with eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and ribosomal S6 kinase 1 (S6K1), making it essential for mTOR-mediated regulation of protein synthesis. Both 4E-BP1 and S6K1 are involved in the control of global translation and ribosomal biogenesis, including the expression of mitochondrial ribosomal proteins. However, evidence suggests that the inhibition of S6K1 either pharmacologically using

rapamycin or genetically in mouse models has minimal impact on these processes in certain tissues such as the liver (Livingstone and Bidinosti, 2012). This has led to increased interest in 4E-BP1, which remains sensitive to mTOR regulation even when S6K1 activity is suppressed. As a result, 4E-BP1 is now considered a more dominant substrate within the mTOR pathway, particularly in relation to mitochondrial energy metabolism (Morita et al., 2013). Recent reviews confirm that changes in net muscle protein balance are primarily driven by alterations in MPS, which are approximately four to five times greater than changes in muscle protein breakdown in response to protein nutrition and resistance exercise (Deane et al., 2024).

The insulin-like growth factor-1 (IGF-1)/Akt pathway represents another critical anabolic signalling cascade that becomes dysregulated with ageing. IGF-1 promotes MPS and hypertrophy through activation of Akt, which subsequently phosphorylates and activates mTOR(Yoshida and Delafontaine, 2020). Age-related declines in IGF-1 levels and reduced Akt signalling sensitivity contribute to impaired anabolic responses in older muscle (Barton *et al.*, 2002). Myostatin, a member of the transforming growth factor-β superfamily, acts as a negative regulator of muscle mass by inhibiting satellite cell activation and MPS (McPherron *et al.*, 1997, Braun and Marks, 2015). Studies have shown that myostatin expression increases with ageing and may contribute to sarcopenic muscle loss. Conversely, inhibition of myostatin signalling has been shown to increase muscle mass and strength in both animal models and human studies (Wagner *et al.*, 2008).

The ubiquitin-proteasome system (UPS) represents the primary pathway for muscle protein degradation. Ageing is associated with increased expression of key ubiquitin ligases,

including MuRF1 and atrogin-1, which target myofibrillar proteins for degradation (Paez et al., 2023). Autophagy, the cellular process responsible for degrading damaged organelles and protein aggregates, also plays important roles in muscle homeostasis. While basal autophagy is essential for maintaining muscle quality, dysregulated autophagy has been implicated in sarcopenic muscle loss (Carnio *et al.*, 2014). Age-related impairments in autophagy may contribute to the accumulation of damaged mitochondria and protein aggregates observed in ageing muscle.

In summary, the molecular regulation of muscle mass during ageing involves the dysregulation of key anabolic and catabolic pathways that collectively contribute to sarcopenic muscle loss. The mTOR pathway, particularly mTORC1, serves as the central hub integrating signals from mechanical loading, nutrient availability, and growth factors to regulate MPS. However, ageing impairs this critical signalling network through reduced phosphorylation of downstream targets such as S6K1 and 4E-BP1, contributing to anabolic resistance. Concurrently, age-related declines in the IGF-1/Akt pathway further compromise anabolic signalling, while increased myostatin expression acts as a brake on muscle growth. On the catabolic side, ageing is characterized by upregulation of the ubiquitin-proteasome system, with increased expression of muscle-specific ubiquitin ligases MuRF1 and atrogin-1 that target myofibrillar proteins for degradation. Additionally, dysregulated autophagy contributes to the accumulation of damaged cellular components, further compromising muscle quality and function. This complex interplay between impaired anabolic signalling and enhanced catabolic processes creates a molecular environment conducive to progressive muscle loss, highlighting the multifaceted nature of sarcopenia and the need for interventions that can simultaneously target multiple pathways to preserve muscle mass and function in older adults.

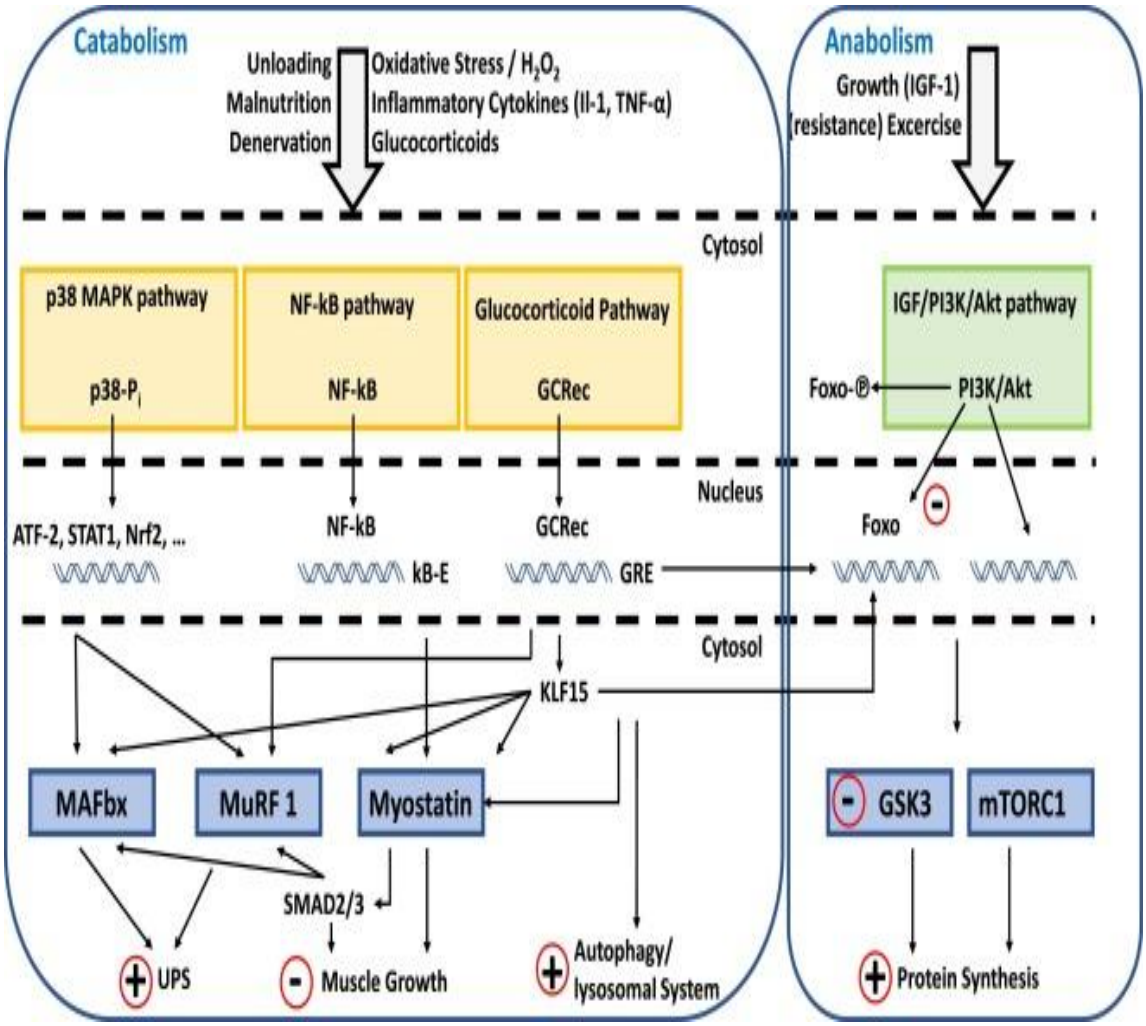

Figure 1-6 Represents the main anabolic signal for muscle protein synthesis, Major signalling pathways influencing synthesis and breakdown of muscle proteins. (Akt: protein kinase B; ATF-2: activating transcription factor 2; FOXO: forkhead box proteins; GCRec: glucocorticoid receptor; GSK3: glycogen synthase kinase 3; GRE: glucocorticoid response element; IGF-1: insulin-like growth factor 1; IL-1: interleukin 1; kB-E: κB response element; KLF15: Krüppel-like factor 15; MAFbx: muscle atrophy F-box; MAPK: mitogen-activated protein kinase; mTORC1: mammalian target of rapamycin complex 1; MuRF1: muscle RING-finger protein-1; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2: nuclear factor E2-related factor 2; p38-P: phosphorylated p38 mitogen-activated protein kinase; PI3K: phosphatidylinositol 3-kinase; TNF-α: tumor necrosis factor alpha; SMAD2/3: SMAD family transcription factors 2 and 3; STAT1: signal transducer and activator of transcription 1; UPS: ubiquitin–proteasome system). Figure adapted from (Wiedmer et al., 2021).

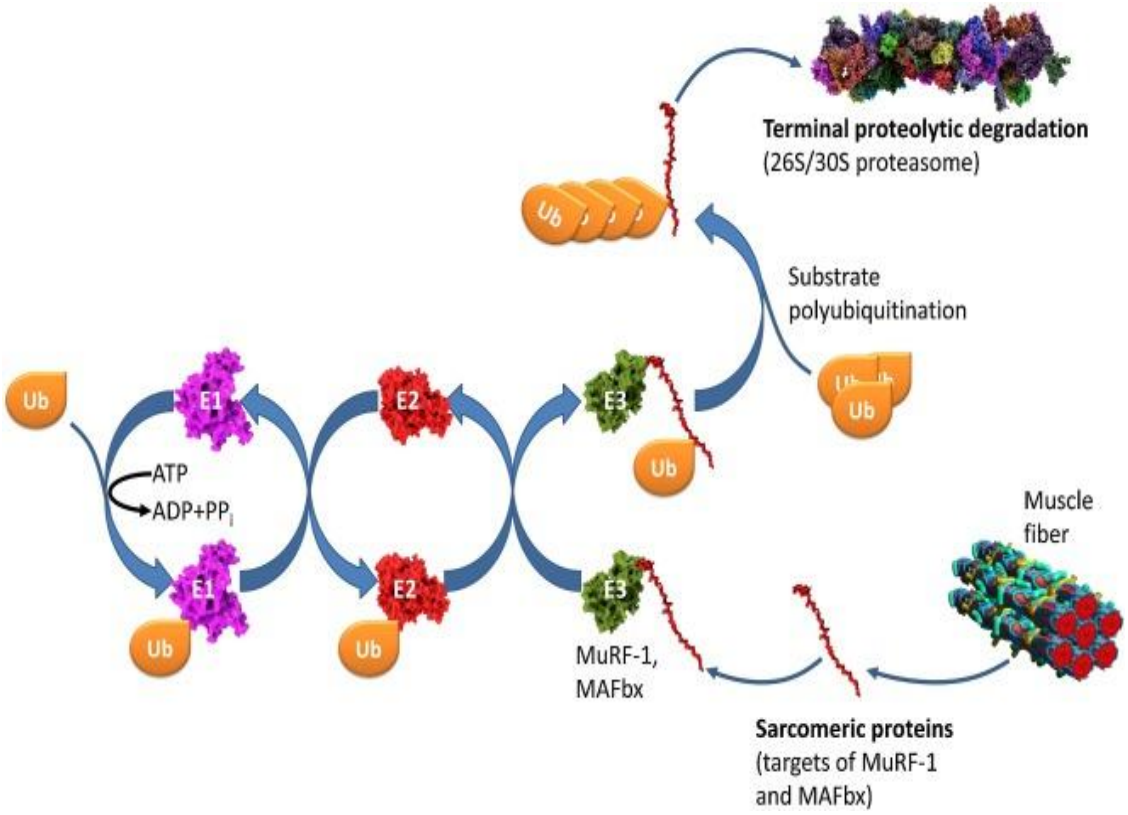

Figure 1-7 Degradation of muscle fibre proteins by the ubiquitin-proteasomal system. The muscle fibers are dissolved to sarcomeric proteins, which can then be recognized by the activated E3 ubiquitin ligases (MuRF-1 or MAFbx). Activation of E3 ubiquitin ligases takes place by binding of ubiquitin (Ub) in a step-wise process via E1 (ubiquitin activating enzyme) and E2 (ubiquitin conjugating enzyme) with the concomitant consumption of ATP. Several molecules of ubiquitin are bound to the substrates in a K48-poly-ubiquitin chain. This poly-ubiquitin chain then binds to the 19S regulator of the 26S/30S proteasome, where the poly-ubiquitin chain is split-off and the substrate protein is 'fed' into the 20S core proteasome where it is degraded, by successive peptide bond cleavages, to short peptides and amino acids. Figure adapted from (Wiedmer et al., 2021).

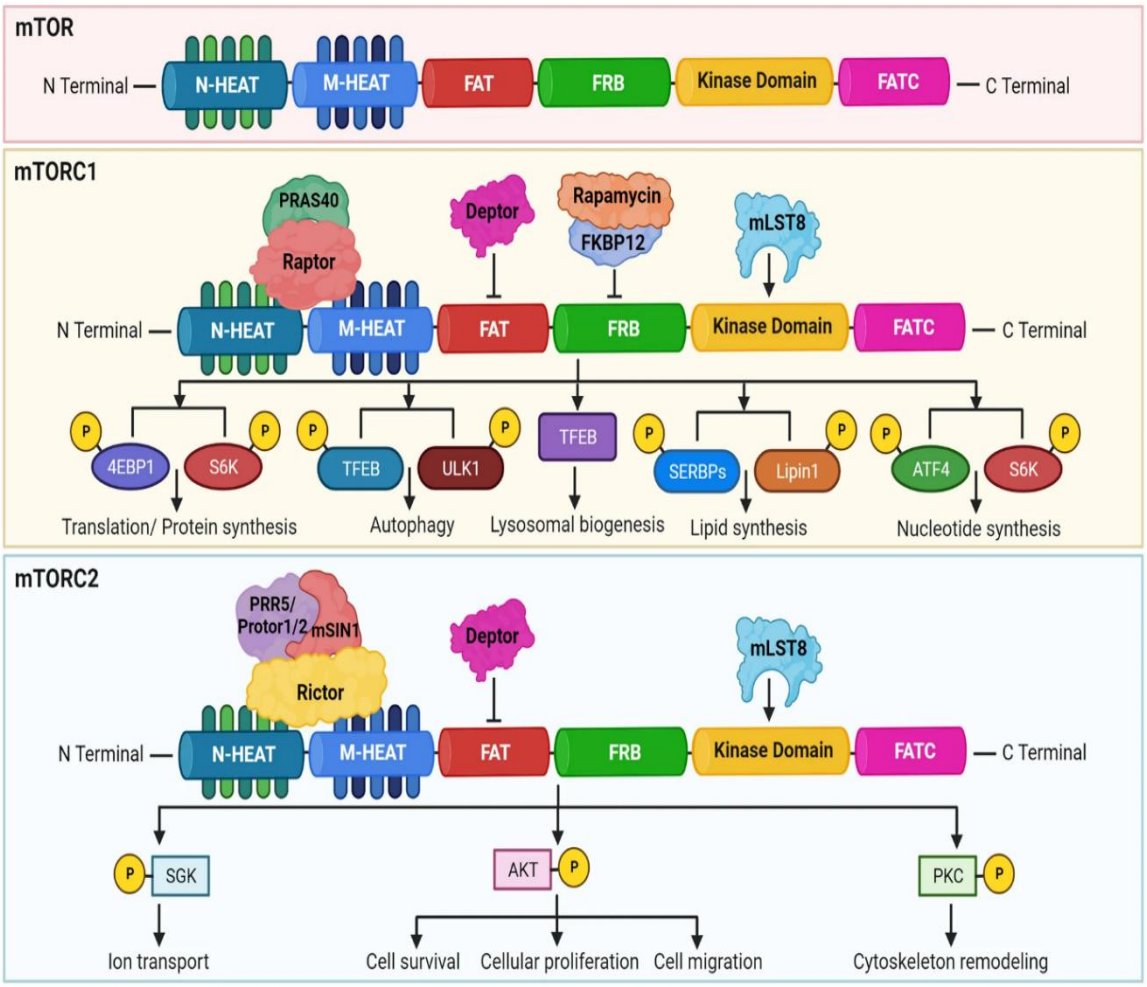

Figure 1-8 The domain structures of mTORC1 and mTORC2, their downstream signalling targets and functional role. N-terminal domain of mTOR possesses tandem HEAT repeats and C-terminal domains composed of FATC, kinase, FRB, and FAT. The mTOR signalling pathway is majorly constituted of two distinctive mTOR complexes named mTORC1 and mTORC2. The mTORC1 is a complex of DEPTOR, Raptor, PRAS40, mLST8, mTOR, and phosphorylate downstream targets to regulate protein synthesis or mRNA translation, lipid synthesis, nucleotide synthesis, lysosomal biogenesis, and autophagy. The mTORC2 is a complex of mTOR, DEPTOR, mSIN1, Rictor, Protor, and mLST8 to regulate cell survival, proliferation, migration, and cytoskeleton remodelling. Created with BioRender.com. Figure adapted from (Garcia-Prat et al., 2016).

1.4.5 Inflammation and its impact on muscle mass and function

Chronic low-grade inflammation during ageing, often referred to as "inflammaging," represents a key contributor to sarcopenia development and progression (Antuña et al., 2022, Liu et al., 2024). Ageing is typically accompanied by a persistent, low-grade elevation in circulating pro-inflammatory cytokines markers, including tumour necrosis factor α (TNFα), interleukin 6 (IL-6), and C-reactive protein (CRP), which negatively impact muscle protein

metabolism through multiple pathways (Liang et al., 2022, Zhang et al., 2024). This phenomenon, commonly referred to as chronic low-grade inflammation, is thought to be partially driven by an increase in senescent cells that exit the cell cycle and adopt a Senescence-Associated Secretory Phenotype which promotes the secretion of various inflammatory cytokines, such as TNFα and IL-6, along with increased activation of the NF-κB signalling pathway, contributing to the systemic inflammatory state observed in ageing tissues (Tchkonia *et al.*, 2013). While ageing itself is considered the primary driver of this inflammatory state, several other factors may contribute to its earlier onset. These include prolonged physical inactivity, insufficient intake of protein, vitamins, and minerals, as well as lifestyle factors such as smoking and psychological stress (Fulop *et al.*, 2017).

Elevated concentrations of pro-inflammatory cytokines and acute-phase proteins in the bloodstream are frequently observed in older adults with sarcopenia. While numerous studies have demonstrated strong associations between these inflammatory markers and increased risks of functional decline and mortality, establishing a direct causal relationship between inflammation and age-related muscle loss remains challenging (Pan et al., 2021). Nonetheless, findings indicate that chronic low-grade inflammation may play a contributory role albeit not fully understood in the development and progression of age-associated skeletal muscle atrophy (Argiles *et al.*, 2015, Beyer *et al.*, 2012). Elevated levels of inflammatory mediators have been linked to accelerated MPB and reduced MPS, through direct catabolic actions and indirect pathways such as suppression of anabolic hormones like growth hormone (Sakuma *et al.*, 2015). Despite this evidence, further research is required to clarify the precise molecular mechanisms by which chronic inflammation influences muscle protein turnover and contributes to sarcopenia.

It is possible that the age-associated reduction in MPS, which contributes to the loss of muscle mass and strength, may be connected to the persistent low-grade inflammation commonly observed with advancing age. However, current evidence regarding the direct causal relationship between this inflammatory state and the decline in MPS remains inconclusive. Recent evidence suggests that chronic low-grade inflammation directly affects MPS through several signalling pathways, with the mTOR pathway potentially inhibited by inflammation (Dalle *et al.*, 2017). In experimental studies using aged rodents, animals displaying markers of systemic inflammation showed completely blunted MPS responses to food intake, while age-matched animals without elevated inflammatory markers maintained normal MPS responses (Balage *et al.*, 2010). Furthermore, when inflammation was pharmacologically reduced using anti-inflammatory medications, like ibuprofen, the normal MPS response was restored (Pan et al., 2021). Similar benefits were observed when antioxidant supplementation was used to reduce inflammatory burden, improving leucine-stimulated MPS in aged muscle tissue (Pan et al., 2021).

However, translating these promising animal findings to human populations has proven challenging (Dalle *et al.*, 2017). Limited human research examining the relationship between systemic inflammation and meal-induced MPS has yielded mixed results (Buffiere *et al.*, 2015, Dideriksen *et al.*, 2016). One study in older adults with elevated C-reactive protein (CRP) levels found no significant differences in MPS responses compared to those with normal CRP levels, though the researchers noted that other inflammatory markers, such as TNF-α or interleukin-1 might be more relevant indicators (Buffiere *et al.*, 2015). Similarly, a short-term intervention using anti-inflammatory medication in older adults with mild systemic inflammation found no effect on MPS responses to protein intake, possibly due to

the brief treatment duration and insufficient reduction in inflammatory markers (Dideriksen *et al.*, 2016).

1.4.6 Sex hormones imbalance

Age-related declines in anabolic hormones represent important contributors to sarcopenic muscle loss, with sex hormone changes being particularly significant. Several sex hormones, including testosterone and oestrogen, are essential in regulating muscle mass and function (Basualto-Alarcon *et al.*, 2014, McDonald *et al.*, 2011).

Testosterone exerts anabolic effects on skeletal muscle by promoting myogenic differentiation, including the maturation of mesenchymal stem cells into muscle fibres, and supporting motor neuron function and nerve regeneration (Hosoi et al., 2024). With advancing age, declining testosterone levels in both men and women contribute to reduced muscle strength and lean body mass. Oestrogen also plays a role in muscle maintenance, through its conversion to testosterone (Messier *et al.*, 2011). In postmenopausal women, accelerated loss of muscle mass and strength, is linked not only to oestrogen deficiency but also to reduced physical activity (Messier *et al.*, 2011).

1.4.7 Cellular senescence and apoptosis in skeletal muscle with ageing

Cellular senescence, characterized by irreversible cell cycle arrest and the acquisition of a pro-inflammatory secretory phenotype, has emerged as a key contributor to age-related muscle deterioration. Recent evidence demonstrates that an accumulation of senescent cells in muscle contributes to the ageing phenotype, creating an unfavourable environment for muscle homeostasis and adaptation (Munoz-Espin and Serrano, 2014, Gorgoulis *et al.*, 2019). Emerging evidence suggests cellular senescence may represent a tractable mediator of skeletal muscle ageing, with researchers demonstrating age-related increases in senescence markers (including P16, P21, and SASP) in both mouse models and human skeletal muscle tissue (Mayoclinic, 2022).

Apoptosis in skeletal muscle ageing is intimately connected to cellular senescence and involves sophisticated regulatory mechanisms. Skeletal muscle cells possess an abundance of inhibitor of apoptosis proteins (IAPs) that bind to the enzymatic site of caspases in their inactive zymogen format (pro-caspases) and prevent their activation (Lalaoui and Vaux, 2018). However, with ageing, IAPs production and signalling pathways become depressed, increasing cellular susceptibility to apoptotic death (Paez et al., 2023). Two primary pathways regulate apoptosis: the extracellular pathway activated by cell death ligands such as TNFα, and the intracellular pathway triggered by mitochondrial dysfunction. Age-related increases in inflammatory cytokines, particularly IL-6 and TNFα, combined with declining mitochondrial oxidative function, can activate both pathways when IAPs levels are reduced (Marzetti *et al.*, 2012).

In adults, the number of muscle cells is largely established, as they do not typically undergo mitosis. However, during skeletal muscle growth and repair, most differentiating myoblasts fuse together to form multinucleated myofibres. A portion of these myoblasts remains undifferentiated; these are referred to as satellite cells or muscle stem cells, and they are located between the sarcolemma (muscle fibre membrane) and the basal lamina (Lalaoui and Vaux, 2018). Ageing significantly increases the susceptibility of skeletal muscle-derived satellite cells to apoptosis, with studies demonstrating this effect across different age groups in animal models (Soendenbroe et al., 2019). In aged muscle, satellite cells that experience prolonged quiescence undergo programmed cellular senescence, an irreversible non-dividing state that handicaps the regenerative capabilities of muscle (Sousa-Victor *et al.*, 2014). This increased apoptotic susceptibility of satellite cells, combined with their reduced proliferative capacity, contributes to the progressive loss of muscle regenerative potential characteristic of sarcopenia (Garcia-Prat *et al.*, 2016, Munoz-Canoves *et al.*, 2020). The interconnection between cellular senescence and apoptosis creates a complex pathophysiological environment in ageing skeletal muscle. In degenerative skeletal muscle states, including advanced age, satellite cell numbers and proliferative potential are significantly compromised. Many undifferentiated myoblasts undergo apoptosis, negatively impacting muscle healing and restoration after injury or prolonged disuse (Vasilikos *et al.*, 2017). Aged muscle significantly reduces regenerative abilities and increases susceptibility to age-related pathologies, with age-related changes in the satellite cell niche components directly impinging on satellite cell function.

Despite these advances in our understanding, limitations remain in translating findings from animal models to human applications. Most evidence supporting the relationship between age and cellular senescence comes from in vitro and animal studies, with limited robust

evidence from in vivo human studies demonstrating a positive relationship between skeletal muscle apoptosis or cellular senescence and chronological age (Vasilikos *et al.*, 2017).

1.4.8 Denervation

Denervation represents one of the most significant underlying mechanisms in the pathogenesis of sarcopenia, characterized by the progressive loss of alpha-motor neurons in the central nervous system (CNS), withdrawal of nerve terminals from the neuromuscular junction (NMJ), and axonal sprouting from neighbouring neurons. This process leads to the formation of enlarged and functionally impaired motor units, which contribute to declines in muscle strength through reduced motor unit firing rates and synchronization, impaired coordination due to altered motor unit recruitment patterns, and diminished overall muscle performance from compromised neuromuscular transmission (Arnold and Clark, 2023).

The NMJ serves as the critical communication interface between the nervous and muscular systems, and its age-related deterioration plays a pivotal role in sarcopenia development (Arnold and Clark, 2023). The structural integrity of the NMJ becomes compromised through multiple pathways, including neuronal degeneration and subsequent muscle fibre denervation, which collectively constitute one of the most important causative factors in sarcopenia (Pannerec *et al.*, 2016). Importantly, research has established that age-related denervation occurs temporally before myofibre atrophy, indicating that neural changes may represent the initiating event in the sarcopenic process rather than a secondary consequence (Arnold and Clark, 2023).

Motor unit number and size undergo significant changes with ageing. Studies using motor unit number estimation techniques have demonstrated substantial motor unit loss beginning in the sixth decade of life, with some individuals losing up to 50% of motor units by age 80 (Piasecki et al., 2016). The loss of motor units occurs preferentially in type II (fast-twitch) motor units, which may explain the preferential loss of fast-twitch muscle fibre observed in ageing. Histological analyses comparing muscle cross section from older and younger individuals demonstrate a significant reduction in both type I and type II muscle fibre, with estimates suggesting a decline of at least 50% by the ninth decade of life (Larsson et al., 2019). These structural changes reflect a long-term neuropathic process that results in the progressive loss of motor neurons. This neuronal degeneration ultimately contributes to the age-related decline in muscle mass and function.

In response to motor unit loss, surviving motor neurons attempt to maintain muscle innervation through compensatory reinnervation processes. This adaptation involves the sprouting of surviving motor fibre to innervate orphaned muscle fibre, resulting in the enlargement of type 1 motor units and helping to preserve some degree of muscle function (Verschueren *et al.*, 2022). However, this compensatory mechanism becomes progressively less efficient with advancing age, and the repeated cycles of denervation and reinnervation eventually lead to significant alterations in fibre-type composition and overall reductions in muscle function (Piasecki *et al.*, 2018).

With older age, a notable reorganization of motor unit fibres occurs, often preceding the phenomenon of fibre type grouping and focal atrophy seen in advanced ageing (Ansved and Larsson, 1990, Ansved *et al.*, 1991, Edstrom and Larsson, 1987). This structural remodelling

is accompanied by an increase in the number of muscle fibres innervated by individual α-motoneurons, alongside a reduction in total muscle fibre count and a decline in the number of large, myelinated nerve fibres within peripheral nerves and ventral roots. These findings are indicative of progressive denervation, likely due to the loss of α-motoneurons, as well as incomplete reinnervation of previously affected muscle fibres (Ansved and Larsson, 1990, Ansved *et al.*, 1991, Edstrom and Larsson, 1987). Recent comprehensive reviews have confirmed these foundational observations using modern electrophysiological and imaging techniques, demonstrating that motor unit loss and impaired reinnervation capacity distinguish sarcopenic from non-sarcopenic ageing, with exercise interventions showing promise in preserving reinnervation capacity (Larsson et al., 2019, Piasecki et al., 2018, Jones et al., 2022).

1.4.9 Disease-related sarcopenia

Muscle loss is not solely a consequence of ageing, it is often accelerated by the presence of chronic diseases such as rheumatoid arthritis (RA), type 2 diabetes, cancer, and chronic organ failure. In these conditions, sarcopenia frequently develops as a secondary effect, driven by systemic inflammation, reduced physical activity, and inadequate nutritional intake. Chronic inflammation promotes catabolic pathways, leading to increased MPB, while disease-related fatigue, pain, or treatment side effects (e.g., chemotherapy) can reduce mobility and appetite, compounding muscle loss (Argiles et al., 2015, Morley et al., 2014b).

In cancer and other severe illnesses, sarcopenia often overlaps with cachexia, a complex metabolic syndrome marked by unintentional weight loss, muscle wasting, and fat loss. Although distinct, these conditions share common features, including systemic inflammation and malnutrition, and may respond to similar therapeutic interventions (Cruz-Jentoft *et al.*,

2010, Nicolini *et al.*, 2013). Obesity can further complicate this picture. In sarcopenic obesity, individuals present with reduced muscle mass and strength alongside increased fat mass, often without noticeable weight loss. Sarcopenic obesity is associated with poor physical function, metabolic disturbances, and a heightened risk of disability and mortality (Batsis *et al.*, 2016). Intramuscular fat infiltration (or "marbling") and shifts in fat distribution particularly increases in visceral and intramuscular fat further reduce muscle quality and exacerbate insulin resistance and cardiovascular risk (Neeland et al., 2015).

1.5 Proposed interventions to prevent or treat sarcopenia

1.5.1 Exercise and protein intake

Exercise, particularly resistance exercise, represents the most effective intervention for preventing and treating sarcopenia, demonstrating consistent benefits in improving skeletal muscle mass, strength, and physical function in older adults (Xiang *et al.*, 2023, Cheng *et al.*, 2024). However, despite its proven efficacy, the implementation of exercise interventions faces significant challenges related to adherence and long-term sustainability in older adults, due to factors such as physical limitations, fear of injury, motivation issues, and poor accessibility (Burton et al., 2015, Hurst et al., 2022). Research consistently indicates a steady decline in physical activity levels with advancing age, with the highest rates of inactivity observed in individuals aged 65 and older (Elgaddal and Kramarow, 2024) with approximately 66% of adults over the age of 75 not engaging in any form of regular physical activity (CDC.gov, 2023).

Participation in muscle-strengthening exercise is particularly low among older adults

with cross-sectional analysis in Scotland revealing that adherence to muscle-strengthening and balance guidelines significantly decreases with age from 49% among those aged 16-24 to 14% among those aged 75 and over, highlighting these as "forgotten guidelines" in older adult populations (Strain *et al.*, 2016). More recent data from the Health Survey for England 2021 confirms this pattern across the UK, with only 17% of men and 14% of women aged 65 and over meeting both the muscle-strengthening and balance exercise guidelines (NHS Digital, 2023). Sport England's Active Lives Adult Survey 2023-24, the most comprehensive assessment of physical activity in England, shows that while 63.7% of adults achieve the recommended 150 minutes of aerobic activity per week, adherence to muscle- strengthening activities remains substantially lower, with only 36% of men and 29% of women meeting the muscle-strengthening guideline of at least two sessions per week (Sport England, 2025, NHS Digital, 2023). Even among those who are physically active, sustained participation remains challenging, with multiple barriers including lack of familiarity with resistance exercise, concerns about post-exercise discomfort, and inadequate access to supervised programs (Hurst et al., 2022). These barriers create a gap between exercise efficacy and real-world outcomes, further complicated by the need for individualized and supervised programs, which may be difficult to deliver in routine healthcare settings (Picorelli *et al.*, 2014, Valenzuela *et al.*, 2019). Network meta-analyses demonstrate that resistance exercise, either alone or combined with nutritional interventions, represents the most effective approach for improving quality of life and functional outcomes in sarcopenic older adults, underscoring the importance of addressing implementation barriers (Shen et al., 2023).

Protein supplementation alone has shown limited impact on sarcopenia-related outcomes. Systematic reviews indicate that without accompanying exercise, in older adults, protein supplementation does not affect muscle mass, strength, or function (Cheah et al., 2023, Jang

et al., 2023). Additionally, adherence to protein supplementation interventions is low, reported at just 43% due to factors like poor taste, gastrointestinal discomfort, and complex regimens (Kirk et al., 2019). Combining protein supplementation with resistance exercise offers a more effective strategy for managing sarcopenia than either intervention alone, as they work synergistically to improve muscle mass and strength in older adults (Restivo, 2024). This combination supports both the nutritional and mechanical stimuli needed for muscle growth and helps counteract age-related anabolic resistance. However, implementing combined interventions can be challenging due to increased complexity and adherence issues, besides the barriers reported for exercise and protein supplementation alone. Additionally, given that the added benefit of supplementary protein is relatively small compared to the effects of exercise, practitioners should prioritize establishing consistent resistance training routines before focusing on protein supplementation optimization.

1.5.2 Long-chain n-3 polyunsaturated fatty acids (LCn-3PUFAs)

The n-3 fatty acids are a group of unsaturated fats defined by a double bond at the third carbon from the methyl end (Qin *et al.*, 2023). They include both monounsaturated (MUFA) and polyunsaturated (PUFA) forms, classified by chain length with short chain containing 5 or fewer carbon atoms, medium chain 6-12 carbon atoms, long chain 13–21 carbon atoms, and very long-chain with 22 or more carbon atoms (Sinha *et al.*, 2023, Cholewski *et al.*, 2018). The three main n-3 fatty acids are alpha-linolenic acid (ALA; 18:3n-3), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3), each differing in their carbon chain length, degree of unsaturation, and biological functions (Office of Dietary Supplements, 2024). The current thesis primarily focuses on long-chain n-3 (LCn-3PUFAs) (EPA and DHA), which are the primary marine-derived LCn-3PUFAs and well-established effects on muscle health and protein metabolism. (Bird et al., 2021)

(Figure 1-9) (Choi and Calder, 2024). However, Chapter 3 examines total dietary n-3 fatty acid intake, including ALA, as this was n-3 intake data captured in the population-based dietary assessment tools used by the Uk Biobank. The following sections briefly describe each n-3 fatty acid.

1.5.2.1 Alpha-linolenic acid (ALA)

ALA is an 18-carbon essential fatty acid found primarily in plant sources including flaxseed oil, chia seeds, walnuts, canola oil, and soybean oil (Office of Dietary Supplements, 2024). Humans cannot synthesize ALA and must obtain it from diet. While ALA can be converted to EPA and DHA through elongase and desaturase enzymes, this conversion level is limited (8-12% to EPA, <1% to DHA) (Ulven et al., 2011). Beyond serving as a precursor, ALA demonstrates independent cardiovascular benefits through reducing cholesterol, triglycerides, and blood pressure, as well as anti-inflammatory effects via PPAR modulation (Sala-Vila et al., 2022). Recent evidence also suggests cognitive benefits in older adults (Ogawa et al., 2023). For vegetarians and vegans, ALA represents the main dietary n-3 fatty acids source, though the limited conversion to EPA/DHA means direct consumption of these longer-chain fatty acids remains important (Sala-Vila et al., 2022).

1.5.2.2 Eicosapentaenoic acid (EPA)

EPA is a 20-carbon marine-derived n-3 fatty acid found predominantly in fatty fish (mackerel, salmon, herring, sardines), shellfish, and fish oil/krill oil supplements (Office of Dietary Supplements, 2024). EPA exerts potent anti-inflammatory effects by competing with arachidonic acid for membrane incorporation, thereby reducing pro-inflammatory eicosanoid production. Recent research demonstrates that EPA induces anti-inflammatory gene expression in immune cells (downregulating HLA-DRA, CD69, IL2RA) while upregulating antioxidant genes (Bhatt et al., 2025, Reilly et al., 2025). EPA also serves as a precursor to specialized pro-resolving mediators (resolvins) that actively resolve

inflammation, improves endothelial function through enhanced nitric oxide bioavailability, and modulates nuclear transcription factors including NF-κB and PPARs (Mason et al., 2020). The REDUCE-IT trial demonstrated that high-dose EPA supplementation reduced cardiovascular events by 25% through mechanisms extending beyond triglyceride lowering (Bhatt et al., 2019). EPA's anti-inflammatory properties are particularly relevant to counteracting age-related chronic inflammation (inflammaging) that contributes to muscle loss (Jiang et al., 2026).

1.5.2.3 Docosahexaenoic acid (DHA)

DHA is a 22-carbon n-3 fatty acid obtained from fatty fish, fish roe, and algal oil supplements (Office of Dietary Supplements, 2024). DHA is the predominant n-3 fatty acid in the brain and nervous system, comprising over 90% of brain n-3 fatty acids and 10-20% of total brain lipids (Zárate et al., 2017). As a structural membrane component, DHA influences membrane fluidity, receptor function, and signal transduction critical for neurodevelopment, synaptic plasticity, and cognitive function (Ghazal et al., 2025). DHA accumulates most rapidly in the brain from mid-gestation through the first two years of life, supporting neurite outgrowth, synaptogenesis, and neurogenesis. DHA modulates neurotransmitter systems, BDNF expression, and serves as a precursor to neuroprotective specialized pro-resolving mediators (Ghazal et al., 2025). Recent evidence indicates that lysophosphatidylcholine- DHA is the preferred form for blood-brain barrier transport via FATP1 and FABP5 (Chouinard-Watkins et al., 2018). Beyond neural functions, DHA contributes to cardiovascular health and retinal function.

1.5.2.4 Dietary sources and intake recommendations

LCn-3PUFAs, can be found in many natural marine sources such as cold-water fish species, including mackerel, trout, salmon, herring, and sardines, which are rich in these bioactive

lipids and contribute significantly to dietary intake (Choi and Calder, 2024, Rimm et al., 2018). In addition to fish, various seafood organisms serve as valuable reservoirs of LCn-3PUFAs. Shellfish and crustaceans, including krill, shrimp, lobster, cod, and crab, contain moderate levels of these fatty acids, though typically lower than those found in fatty fish (Rimm *et al.*, 2018). Freshwater fish typically contain lower LCn-3PUFA levels compared to their marine counterparts. Beyond traditional marine sources, microalgae present promising sustainable alternatives of LCn-3 PUFA, with species like Nannochloropsis and Schizochytrium demonstrating high EPA and DHA yields (Adarme-Vega *et al.*, 2012, Martins *et al.*, 2013). Biotechnological advances have enabled the development of microbial fermentation systems capable of producing specific LCn-3 PUFAs, offering environmentally sustainable alternatives to conventional fish-derived sources (Qin *et al.*, 2023). Terrestrial sources, including grass-fed meat, dairy products, and eggs from pasture-raised animals, contribute smaller quantities of these essential fatty acids to the human diet.

A comprehensive global review of national and international n-3 fatty acid recommendations identified that the most frequently recommended intakes for adults are 250 mg/day of EPA+DHA combined, with an additional 100-200 mg/day of DHA during pregnancy and lactation (Calder et al., 2025). The European Food Safety Authority (EFSA) recommends 250 mg/day EPA+DHA for maintenance of normal cardiac function in adults, while the Food and Agriculture Organization recommends 250 mg - 2 g/day EPA+DHA based on a joint FAO/WHO expert consultation, with the lower end for general health (FAO, 2010). In the United Kingdom, the Scientific Advisory Committee on Nutrition (SACN) official guidance recommends that adults consume at least 450 mg/day of EPA and DHA, primarily through consumption of at least one portion (approximately 140g) of oily fish per week (Calder et

al., 2025, Lewis et al., 2025). Similarly, the British Heart Foundation recommends approximately 450 mg/day EPA+DHA for cardiovascular health (BHF, 2024).

Despite these recommendations, population intake data reveal substantial gaps between recommended and actual consumption. Analysis of the UK National Diet and Nutrition Survey (NDNS) 2019-2023, published by the Office for Health Improvement and Disparities, indicates that oily fish consumption was well below recommended thresholds across all UK population groups (OHID, 2025, Swan, 2025). Analysis of NDNS data indicates that only approximately 25% of the UK population regularly consumes oily fish, the primary dietary source of EPA and DHA (Derbyshire et al., 2024). A recent analysis of UK Biobank data estimated that the mean Omega-3 Index (a biomarker of EPA+DHA status measured in red blood cells) was 5.58% in UK adults, which falls in the intermediate-risk category for cardiovascular health, below the target level of >8% (Schuchardt et al., 2023). Globally, approximately 76% of the population fails to meet recommended EPA and DHA intake levels, representing a major public health concern (Calder et al., 2025, Lewis et al., 2025). The inadequacy of dietary intake is particularly pronounced in older adults. Among adults aged over 50 years, average EPA and DHA intake is estimated at only 170 mg/day, substantially below the recommended 450 mg/day target (Dideriksen et al., 2016, Richter et al., 2017). This shortfall is even more marked in individuals following plant-based diets, with vegetarians and vegans showing significantly lower EPA and DHA blood concentrations compared to omnivores (Derbyshire et al., 2024). Given the substantial gap between dietary recommendations and actual intakes, n-3 supplements including fish oil, krill oil, and algal oil are commonly used to help meet nutritional requirements (Cholewski et al., 2018). Fish oil supplements remain the most common source, though algal oil provides a sustainable vegetarian/vegan alternative. The inadequacy of current dietary intakes highlights the need for more accessible and sustainable EPA and DHA sources, including

fortified foods and alternative production methods, to support population health (Calder et al., 2025, Lewis et al., 2025).

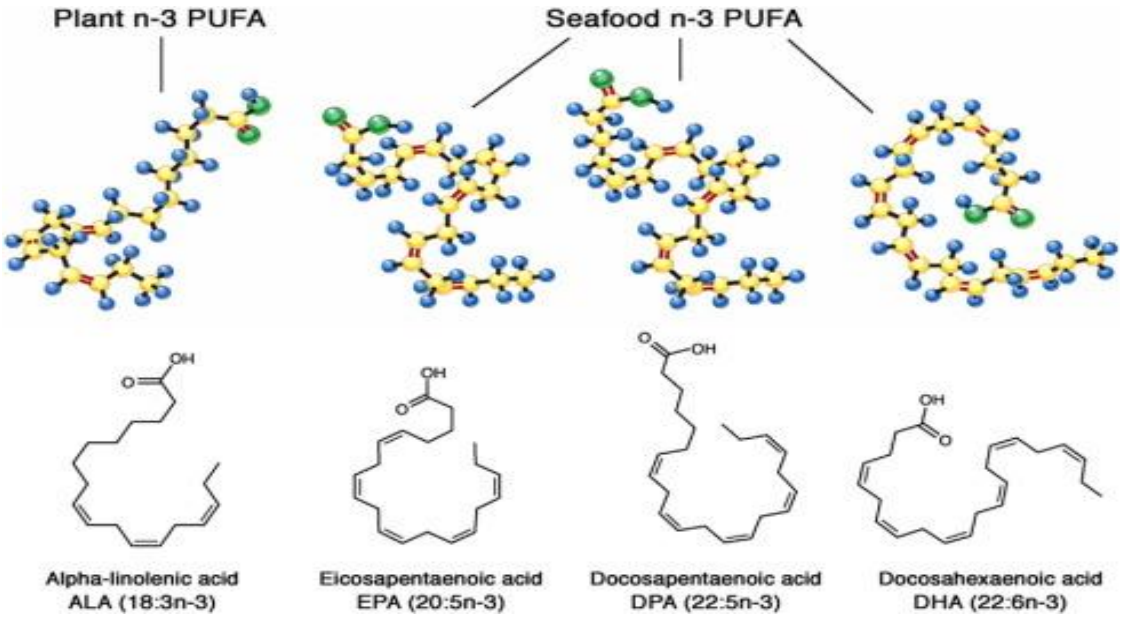

Figure 1-9 Structure of n-3 PUFA

Alpha-linolenic acid is an 18-carbon essential n-3 polyunsaturated fatty acid (PUFA) derived from plant sources. Long-chain n-3 PUFA include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), predominantly derived from seafood consumption, as well as docosapentaenoic acid (DPA) that is contained in smaller amounts in seafood and synthesized endogenously from EPA. The long hydrocarbon backbones, multiple double bonds, and location of the first double bond in the n-3 position result in complex and unique 3-dimensional configurations that contribute to the singular biological properties of these fatty acids. Figure adapted from (Mozaffarian and Wu, 2011).

1.5.3 The biological roles of LCn-3PUFA

The n-3 fatty acids are essential components of cellular membranes, contributing to both structural stability and functional capacity (Harris and Von Schacky, 2004). Alongside n-6 fatty acids, they serve not only as energy sources but also as precursors to bioactive lipid mediators that regulate inflammation and cellular signalling (Patted *et al.*, 2024). However, modern Western diets exhibit a significant imbalance between n-6 and n-3 PUFAs intake, particularly an overconsumption of linoleic acid, resulting in membrane compositions

dominated by n-6 PUFAs (10–20%) and relatively low levels of n-3 PUFAs (2–5%) (Calder, 2017). The membrane incorporation of n-3 PUFAs follows a predictable dose-response relationship, with increased dietary intake directly correlating with enhanced membrane n-3 content (Rees *et al.*, 2006). This biochemical principle underscores the therapeutic potential of targeted nutritional interventions.

EPA and DHA represent the most physiologically active LCn-3 fatty acids (Patted *et al.*, 2024) and are known to reduce inflammation and promote its resolution, suggesting a beneficial role in various therapeutic areas (Calder, 2017). The anti-inflammatory properties of these fatty acids stem from their ability to modulate inflammatory cascade pathways through multiple interconnected mechanisms (Calder, 2017, Azzolino et al., 2024). LCn-3PUFAs influence inflammation by altering the fatty acid composition of cell membranes, which modifies membrane fluidity, cell signalling leading to altered gene expression, and the pattern of lipid mediator production. EPA and DHA can partly inhibit many aspects of inflammation including leucocyte chemotaxis, adhesion molecule expression and leucocyte-endothelial adhesive interactions, and the production of pro-inflammatory eicosanoids like prostaglandins and leukotrienes from arachidonic acid. Additionally, EPA gives rise to eicosanoids that often have lower biological potency than those produced from arachidonic acid, and EPA and DHA give rise to anti-inflammatory and inflammation resolving mediators called resolvins, protectins and maresins. Key mechanisms underlying these anti-inflammatory actions include disruption of lipid rafts, inhibition of activation of nuclear factor κB (reducing expression of inflammatory genes), and activation of the anti-inflammatory transcription factor peroxisome proliferator-activated receptor γ (Calder, 2017).These fatty acids serve as precursors for specialized pro-resolving mediators (SPMs) that actively terminate inflammatory responses and restore tissue homeostasis (Calder, 2017,

Azzolino et al., 2024). LCn-3PUFAs help resolve inflammation through the production of these SPMs, which facilitate tissue repair and inflammation resolution (Blaauw *et al.*, 2024).

1.5.4 Potential role of LCn-3PUFA in sarcopenia

LCn-3PUFAs, particularly EPA and DHA, have gained interest for their therapeutic potential in the treatment and prevention of sarcopenia due to their multifaceted effects (Azzolino *et al.*, 2024, Jannas-Vela *et al.*, 2023). Inflammation plays a detrimental role in sarcopenia pathogenesis (Jannas-Vela *et al.*, 2023) and the anti-inflammatory properties of LCn-3PUFA may translate into meaningful improvements in skeletal muscle mass and functional capacity as elevated levels of cytokines, such as TNF-α and IL-6, accelerate MPB and impairs MPS in ageing populations (Jannas-Vela *et al.*, 2023) **(Figure 1-10)** (Huang *et al.*, 2020)**.** These fatty acids also support mitochondrial biogenesis and function, which is impaired in sarcopenia, contributing to better energy production, reduced oxidative stress, and enhanced muscle performance (Jannas-Vela *et al.*, 2023, Chen *et al.*, 2018).

Their anti-inflammatory properties make them a potential intervention for "inflammaging," the chronic, low-grade inflammation associated with ageing, contributing to muscle loss (Dupont *et al.*, 2019). Furthermore, LCn-3 PUFAs influence gene expression of peroxisomal proliferating-activated receptor (PPAR) (Seo *et al.*, 2005), modulate membrane-bound protein functions, and act as reservoirs for bioactive lipid compounds (Patted *et al.*, 2024). These diverse biological roles reinforce their value in potentially mitigating age-related physiological decline and preventing chronic diseases across various organ systems. This mechanistic foundation has prompted extensive investigation across various research

models, including in vitro cellular studies, animal models, cross-sectional population studies, and acute and chronic human intervention trials (Blaauw *et al.*, 2024, Uchida *et al.*, 2024).

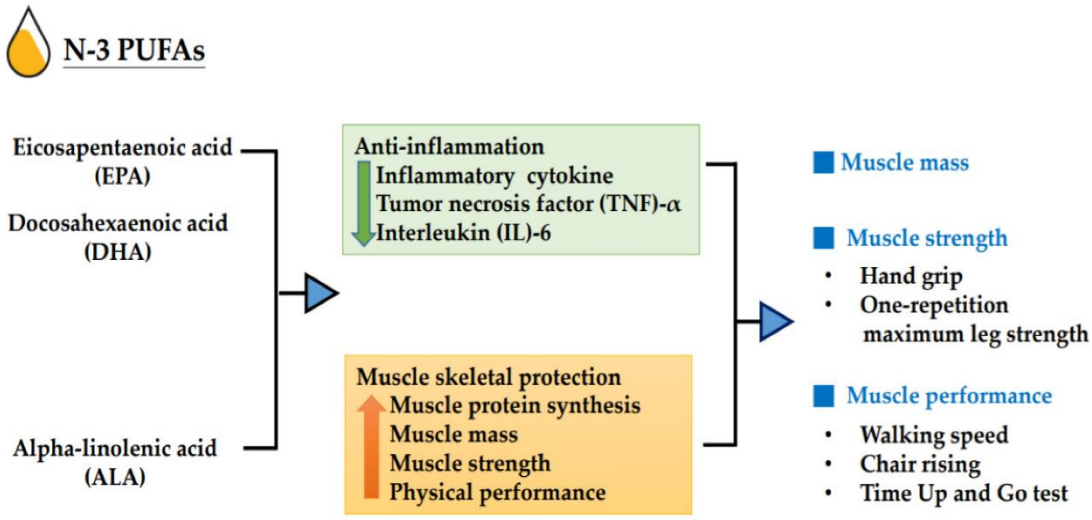

Figure 1-10 Impact of LCn-3 polyunsaturated fatty acids (LCn-3 PUFAs) on muscle mass, muscle strength, and muscle performance. Figure adapted from (Huang et al., 2020).

1.5.5 Animal and cell studies

Cell culture studies have provided mechanistic insights into how LCn-3PUFAs influence muscle protein metabolism and cellular function. Kamolrat and Gray (2013) demonstrated that EPA significantly increased MPS in murine C2C12 myotubes, while both EPA and DHA reduce protein breakdown, providing direct evidence for LCn-3PUFAs effects on muscle protein turnover at the cellular level (Kamolrat and Gray, 2013). Furthermore, Jeromson *et al.* (2018) further revealed that EPA, but not DHA, improved glucose uptake and protein accretion in C2C12 myotubes despite similar incorporation of both fatty acids, with lipidomic analysis revealing that both EPA and DHA led to substantial changes in fatty acid profiles while proteomic analysis showed differential alterations in membrane-associated proteins (Jeromson *et al.*, 2018). More recently, Banic *et al.* (2024) investigated the dose-dependency of a combined EPA:DHA mixture on incorporation, washout, and protein synthesis in C2C12 myotubes, demonstrating that low (12.5 μM EPA + 8.33 μM DHA)

EPA:DHA concentrations increased muscle protein synthesis (MPS) above the insulin/leucine trigger response, with no increase at higher (50 μM EPA + 33.33 μM DHA) concentrations. The authors also noted that EPA and DHA incorporation into muscle tissue is both dose and time-course dependent, with high-dose supplementation (4.4 g EPA + DHA) over 4 weeks achieving similar muscle incorporation as low-dose supplementation (3-3.36 g EPA + DHA) over extended periods (8-12 weeks) (Banic *et al.*, 2024).

LCn-3PUFAs may possess intrinsic anabolic properties that directly stimulate MPS. Two key studies demonstrate these effects in different disease models. Van Norren *et al.* (2009) investigated the effects of a specific dietary combination containing high protein, leucine, and fish oil in tumour-bearing cachectic mice over 18 days. The researchers found that this supplementation significantly improved muscle function (measured by grip strength and rotarod performance), increased daily physical activity, and enhanced muscle mass compared to control diets, demonstrating that LCn-3PUFAs may counteract muscle wasting even in severe catabolic conditions (van Norren *et al.*, 2009). Similarly, a recent systematic review and meta-analysis of severe burn patients demonstrated that n-3 PUFA supplementation significantly reduced the incidence of severe sepsis, septic shock, and multiple organ dysfunction syndrome (RR=0.38, 95% CI [0.19, 0.75], p=0.005), alongside reductions in C-reactive protein levels, suggesting that LCn-3PUFAs may help preserve protein balance during acute catabolic stress by attenuating the inflammatory response (Zhou et al., 2024). Both studies demonstrate that LCn-3PUFAs may maintain protein synthesis and muscle mass even under severe catabolic conditions, suggesting their potential therapeutic value in preventing muscle wasting (Hayashi et al., 1999, van Norren et al., 2009, Zhou et al., 2024). Furthermore, in a study using young, growing steers, diets rich in EPA and DHA, led to a doubling of insulin-stimulated non-oxidative amino acid disposal, a key

marker of whole-body protein synthesis. This was accompanied by enhanced activation of the mTOR-p70s6k signalling pathway in muscle tissue, suggesting that LCn-3PUFAs can stimulate muscle protein synthesis even in healthy, young animals (Gingras *et al.*, 2007). However, recent meta-analyses have yielded conflicting results regarding the direct effects of n-3 PUFA supplementation on muscle protein synthesis rates, with some studies showing no significant effect on MPS in controlled trials (Therdyothin et al., 2025), suggesting that the mechanisms by which LCn-3PUFAs preserve muscle mass during catabolic conditions may be primarily anti-inflammatory and anti-catabolic rather than directly anabolic.

Although findings from animal and cell studies suggest potential benefits of LCn-3PUFA, it is essential to evaluate supporting evidence from human research.

1.5.6 Human epidemiological studies

Large-scale epidemiological studies have provided crucial insights into the association between n-3 fatty acid or oily fish intake and muscle health in older adults. In the Hertfordshire Cohort Study, Robinson *et al.* found that fatty fish intake was the strongest predictor of hand grip strength by analysing 2,983 older adults from Hertfordshire, UK and found that each additional portion of fatty fish consumed per week was associated with a higher grip strength of 0.43 kg in men (95% CI: 0.13-0.74) and 0.48 kg in women (95% CI: 0.24-0.72), after adjusting for height, age, and birth weight (Robinson et al., 2008b). A cross-sectional study in Finland involving 554 women reported that higher dietary intake of n-3 PUFA was positively associated with faster walking speed and greater grip strength in older individuals (Isanejad *et al.*, 2022). A UK biobank study analysed data from over 79,000 adults aged ≥65, and 18802 participants, found that higher plasma levels of n-3 PUFAs, increased

oily fish intake, and fish oil supplementation were associated with a lower prevalence of frailty. In particular, consuming oily fish ≥ 2 times per week was associated with a 41% reduced risk of frailty (Kim *et al.*, 2024). Similarly, a retrospective study from Iceland involving a random sample of 836 adults aged 66 to 96 years found that higher plasma concentrations of LCn-3 PUFAs were positively associated with greater muscle mass measured by computed tomography and increased muscle strength, as determined by maximal knee extension torque. Additionally, nearly half of the participants had repeated assessments of muscle mass and strength over a five-year period. Among this subgroup, those with higher baseline LCn-3 PUFA levels experienced more favourable changes in muscle mass and strength over time (Reinders *et al.*, 2015). These findings suggest that higher intake or circulating levels of LCn-3 PUFAs are linked to better muscle strength and reduced frailty, although causality cannot be inferred.

1.5.7 Acute human supplementation studies

Smith and colleagues investigated the effects of LCn-3PUFA supplementation (4 g/day Lovaza; 1.86 g EPA, 1.50 g DHA) on muscle protein synthesis in both older (65–85 years) and young/middle-aged adults (25–45 years). In older adults, 8 weeks of supplementation did not alter basal muscle protein synthesis but significantly enhanced the MPS response to hyperaminoacidemia-hyperinsulinemia, accompanied by increased mTOR and p70s6k phosphorylation (Smith *et al.*, 2011a). Similar results were observed in younger/middle aged participants, where LCn-3PUFA supplementation led to a 33.9% increase in stimulated MPS, despite no change in basal rates (Smith *et al.*, 2011b).

Lalia and colleagues investigated the influence of LCn-3PUFA supplementation on skeletal muscle protein metabolism and mitochondrial bioenergetics in older adults. The study included 12 young (18-35 years) and 12 older (65-85 years) men and women, with older adults receiving 3.9 g/day of LCn-3PUFA supplementation for 16 weeks. Muscle biopsies were performed to evaluate mitochondrial respiratory capacity and muscle protein synthesis following resistance exercise. The results showed that LCn-3PUFA supplementation significantly reduced mitochondrial oxidant emissions in older adults, though maximal respiration remained lower compared to young adults. Importantly, MPS rates were similar between age groups under both basal and post-exercise conditions, suggesting that LCn-3PUFA supplementation may help preserve the anabolic response to exercise in older adults (Lalia *et al.*, 2017). Furthermore, recent meta-analyses, including one by Therdyothin *et al.* (2025), suggest that while LCn-3PUFA supplementation shows no overall effect on MPS when measured under basal, postprandial, or exercise-stimulated conditions, it may benefit specific groups, particularly older adults with anabolic resistance (Therdyothin *et al.*, 2025).

Acute studies indicate that LCn-3PUFA supplementation enhances the MPS response to nutritional stimuli in older adults, particularly under amino acid and insulin stimulation, suggesting potential benefits for sarcopenia prevention and treatment. However, it is important to note that improvements in acute MPS do not necessarily predict long-term muscle mass or strength outcomes, highlighting the need for longer-term intervention studies to confirm these effects (Mitchell *et al.*, 2014).

1.5.8 Long-term human supplementation studies

Hutchins-Wiese and colleagues conducted a randomized, double-blind pilot study investigating the effects of long-chain polyunsaturated fatty acids on frailty and physical

performance in postmenopausal women. The study included 126 postmenopausal women who received either 2 fish oil capsules (containing 1.2 g EPA and DHA combined) or 2 placebo (olive oil) capsules per day for 6 months. All participants also received calcium and vitamin D supplements. Based on the study results, 6-month fish oil supplementation resulted in a significant increase in walking speed (3.0±16 vs. -3.5±14 m/s, $p = 0.038$) in postmenopausal women. This small change is considered clinically relevant in the context of ageing, as walking speed is a sensitive measure of overall health and function in older adults (Hutchins-Wiese *et al.*, 2013).

Smith *et al.* (2015) conducted a randomized controlled trial involving 60 healthy older adults (aged 60–85 years, both men and women) who received daily supplementation of LCn-3PUFA (1.86 g EPA and 1.50 g DHA) over a six-month period. The intervention resulted in a 3.6% increase in thigh muscle thickness, as measured by imaging techniques, and a 2.3 kg improvement in handgrip strength, indicating enhanced upper body muscular strength. Additionally, participants exhibited significantly greater average isokinetic leg muscle power, reflecting improved lower limb function (Smith *et al.*, 2015). These results are not supported by findings from Logan and colleagues who conducted a randomized controlled trial examining the effects of LCn-3PUFA supplementation on body composition in healthy community-dwelling older females. The study included 24 healthy older women (mean age 64.2 ± 6.8 years) who were supplemented with 3 g/day of fish oil for 12 weeks. Fish oil supplementation had no effect on body composition or muscle strength (Logan and Spriet, 2015).

Alkhedhairi and colleagues conducted a randomized, double-blind controlled trial investigating the effects of krill oil supplementation on skeletal muscle function and size in healthy older adults. The study enrolled 102 men and women aged above 65 years with a BMI less than 35 kg/m², who participated in less than 1 hour per week of structured exercise, between March 2018 and March 2020. Participants were randomized to receive either control or krill oil supplements (4 g/day) for 6 months. A total of 94 participants completed the study (krill group: 26 women and 23 men; placebo group: 27 women and 18 men) with a mean age of 71.2 ± 5.1 years and weight of 71.8 ± 12.3 kg. The 6-month krill oil supplementation intervention resulted in significant increases in knee extensor maximal torque, grip strength, and vastus lateralis muscle thickness relative to control. The treatment effects were 9.3% (95% CI: 2.8, 15.8%) for knee extensor strength, 10.9% (95% CI: 8.3, 13.6%) for grip strength, and 3.5% (95% CI: 2.1, 4.9%) for muscle thickness. Additionally, significant increases in erythrocyte fatty acid profile were observed with EPA increasing by 214% (95% CI: 166, 262%), DHA by 36% (95% CI: 24, 48%), and the omega-3 index by 61% (95% CI: 49, 73%) relative to control (Alkhedhairi *et al.*, 2022).

A large-scale multinational trial, the DO-HEALTH study, examined the effects of vitamin D, LCn-3PUFA supplementation, and home-based exercise on muscle mass and sarcopenia over a 3-year period. This randomized, double-blind, placebo-controlled trial included 2,157 community-dwelling, healthy adults aged 70+ years from 2012 to 2018. Participants were randomized to receive 2000 IU/day vitamin D and/or 1 g/day marine LCn-3PUFA and/or exercise interventions. Among the 1,495 participants who underwent dual energy X-ray absorptiometry (mean age 74.9 years, 63.3% women), the study found that appendicular lean mass index (ALMI) and incidence of sarcopenia (defined as low ALMI according to EWGSOP2 gender-specific cut off definitions: <5.5 kg/m² for women and <7 kg/m² for men)

were not improved by daily 1 g LCn-3PUFA supplementation compared with control over 3 years. However, the authors noted that this population consisted of healthy, physically active older adults with relatively high baseline muscle mass, which may have limited the potential for improvement (Eggimann *et al.*, 2025).

In conclusion, evidence from various study designs suggests that long-term LCn-3PUFA supplementation may improve muscle mass and strength in older adults, but prior to the current thesis this had not been quantified in a systematic review and meta-analysis. Furthermore, while most research to date has focused on fish oil, alternative sources like krill oil though less extensively studied may offer enhanced bioavailability and potentially greater efficacy (Gray and Mittendorfer, 2018).

1.5.9 Krill oils vs. fish oils

Krill oil and fish oil differ significantly in their molecular composition, bioavailability, and physiological effects, which has implications for their therapeutic applications in muscle health and sarcopenia prevention. The fundamental difference between krill oil and fish oil lies in the molecular form of their LCn-3 fatty acids. In fish oil, EPA and DHA are primarily stored as triglycerides (TGs), whereas in krill oil, 30-65% of the fatty acids are incorporated into phospholipids (PLs), mainly phosphatidylcholine. Krill oil also contains unique bioactive compounds not found in fish oil, including astaxanthin (a powerful antioxidant that gives krill oil its reddish colour) which provides anti-inflammatory and antioxidant benefits that may help ageing muscle. These structural differences are hypothesized to affect the bioavailability and absorption of LCn-3 PUFAs, as phospholipids comprise the structure of cell membranes and may facilitate the passage of fatty acids through the intestinal wall (Ulven et al., 2015).

Multiple comparative bioavailability studies have investigated the absorption and incorporation of EPA and DHA from krill oil versus fish oil. Schuchardt and colleagues conducted a double-blinded crossover trial using identical doses in humans, comparing three EPA+DHA formulations: re-esterified triglycerides (rTAG), ethyl-esters (EE) from fish oil, and phospholipids from krill oil. Twelve healthy young men received 1680 mg EPA+DHA from each formulation and the highest incorporation of EPA+DHA into plasma phospholipids was observed with krill oil (mean AUC0-72h: 80.03 ± 34.71%*h), followed by fish oil rTAG (59.78 ± 36.75%*h) and EE (47.53 ± 38.42%*h). Although differences did not reach statistical significance due to high variability, a trend toward superior EPA bioavailability was observed with krill oil (Ulven et al., 2015). This requires to be confirmed in larger trials. Ramprasath and colleagues demonstrated that krill oil consumption

significantly increased plasma EPA levels, total n-3 PUFAs, RBC EPA levels, and the omega-3 index to a greater degree compared with fish oil. Notably, the change in omega-3 index after consumption of krill oil was two-fold higher than that observed with fish oil, despite both groups receiving similar amounts of EPA and DHA (600 mg/day total) or placebo control, corn oil in capsule form. This suggests enhanced bioavailability and tissue incorporation of LCn-3 PUFAs from krill oil compared to fish oil (Ramprasath *et al.*, 2013).

However, not all studies have demonstrated superior bioavailability for krill oil. Yurko-Mauro and colleagues conducted a four-week randomized controlled trial comparing fish oil and krill oil, when matched for equal EPA and DHA content. The study found that similar plasma and red blood cell levels of EPA and DHA were achieved with both formulations, demonstrating comparable oral bioavailability regardless of whether the LCn-3 PUFAs were delivered as fish oil or krill oil (Yurko-Mauro *et al.*, 2015). Similarly, Ulven and colleagues found that a significant increase in plasma EPA, DHA, and DPA was observed in subjects supplemented with LCn-3 PUFAs compared with controls, but there were no significant differences in the changes between fish oil and krill oil groups. The researchers concluded that krill oil and fish oil represent comparable dietary sources of LCn-3 PUFAs, even though the EPA + DHA dose in the krill oil was 62.8% of that in the fish oil (Ulven *et al.*, 2011).

Krill oil may offer better bioavailability and greater tissue uptake of LCn-3 PUFAs than fish oil, even at lower doses, potentially improving muscle health. Its unique compounds, like astaxanthin and choline, may provide added benefits. However, higher cost, lower relative LCn-3 PUFA content, and dosing needs should be considered when choosing it for sarcopenia prevention.

From a sustainability perspective, krill oil may represent a more sustainable alternative to traditional fish oil given current marine resource pressures. Antarctic krill (Euphausia superba) is among the most abundant species on Earth with a circumpolar biomass estimated at 300-500 million tonnes (Atkinson et al., 2009), a figure reaffirmed by the 2025 FAO Review of the State of World Marine Fishery Resources (FAO, 2025). The 2025 FAO Review of the State of World Marine Fishery Resources confirmed that all assessed Antarctic fisheries, including krill, are sustainably managed under robust, science-based international regulation (FAO, 2025). This contrasts sharply with global fish stocks, where over 90% are either overfished or maximally exploited (FAO, 2022). The krill fishery operates under stringent CCAMLR (Commission for the Conservation of Antarctic Marine Living Resources) regulations with precautionary catch limits set at approximately 1% of biomass, and major operations have achieved Marine Stewardship Council certification, with 75% of 2023 krill harvest from MSC-certified fisheries (MSC, 2025). However, krill are a keystone species in the Antarctic ecosystem, and concerns exist about localized depletion and climate change impacts, with spatial catch restrictions expiring in November 2024 (Meyer et al., 2025). Nevertheless, exploring krill oil as an n-3 fatty acid source may offer sustainability advantages over fish oil, provided continued rigorous monitoring and adaptive management are maintained.

In summary: Sarcopenia affects 10-27% of older adults globally and represents a major public health challenge with substantial impacts on physical function, quality of life, and healthcare costs (Petermann-Rocha et al., 2022a, Cruz-Jentoft et al., 2019a). The condition arises from multifactorial mechanisms including anabolic resistance, inflammation, hormonal changes, and reduced physical activity (Walston, 2012). While resistance training and protein supplementation are established interventions, their benefits remain limited and may not be feasible for all older individuals (Dennison et al., 2017). LCn-3 PUFAs have emerged as promising candidates based on their anti-inflammatory properties and ability to

enhance muscle protein synthesis (Smith et al., 2011a, Calder, 2015). Animal and acute human studies support beneficial effects on muscle metabolism (Kamolrat and Gray, 2013, Smith et al., 2011b), and observational evidence suggests associations between fish consumption and better muscle function in older adults (Robinson et al., 2008a).

However, longer-term intervention studies have yielded inconsistent results, with some reporting improvements in muscle outcomes (Smith et al., 2015, Alkhedhairi et al., 2022) while others show no effects (Logan and Spriet, 2015, Eggimann et al., 2025). Critical knowledge gaps, therefore, remain. First, no systematic review has specifically quantified the effects of LCn-3 PUFA supplementation on muscle strength in older adults. Second, large-scale analyses of the relationship between dietary n-3 intake and muscle outcomes are limited. Third, while krill oil may offer bioavailability advantages over fish oil (Ulven et al., 2015, Ramprasath et al., 2013), no direct comparison has been conducted for muscle health outcomes. Addressing these gaps is essential for establishing evidence-based recommendations. The present thesis therefore aimed to comprehensively evaluate the potential of LCn-3 PUFA supplementation for supporting muscle health in older adults through three complementary studies.

1.6 Objectives and hypotheses

1) To perform a systematic literature review with the purpose of exploring the impact LCn-3 PUFA, relative to control, oil supplementation on muscle strength, with secondary outcomes of muscle mass and physical function in older individuals under conditions of habitual physical activity/exercise.

The hypothesis is that LCN-3 PUFA supplementation will improve muscle strength in older adults compared to control oil supplementation.

2) To investigate through secondary regression analyses of the data from UK biobank, the association of n-3 fatty acid intake and the n-6/n-3 fatty acid intake ratio with muscle mass and strength in older adults.

The hypothesis is that higher intake of n-3 fatty acids, and a lower n-6/n-3 fatty acid intake ratio, will be positively associated with muscle strength and mass in older adults.

3) To compare the effects of krill oil and fish oil supplementation on muscle function in older adults, in a double-blind randomised-controlled study recruiting healthy older adults of both sexes.

The hypothesis is that 16 weeks supplementation with krill oil or fish oil will lead to comparable improvements in muscle function in both older men and women.

Published work

1- The Effect of Long Chain *n*-3 Fatty Acid Supplementation on Muscle Strength in Older Adults: A Systematic Review and Meta-Analysis.

Timraz M, Binmahfoz A, Quinn TJ, Combet E, Gray SR. The Effect of Long Chain *n*-3 Fatty Acid Supplementation on Muscle Strength in Older Adults: A Systematic Review and Meta-Analysis. *Nutrients*. 2023;15(16):3579. Published 2023 Aug 14. doi:10.3390/nu15163579

2- The association of n-3 fatty acid intake with muscle mass and strength in older adults: A cross-sectional analysis of the UK biobank data.

Timraz M, Wyss MG, Quinn TJ, Combet E, Celis-Morales C, Gray SR. The association of n-3 fatty acid intake with muscle mass and strength in older adults: A cross-sectional analysis of the UK biobank data. *J Nutr Health Aging*. 2025;29(9):100622. doi:10.1016/j.jnha.2025.10062

Chapter 2: The effect of long chain n-3 fatty acid supplementation on muscle strength in older adults: A systematic review and meta-analysis.

Timraz M, Binmahfoz A, Quinn TJ, Combet E, Gray SR. The Effect of Long Chain *n*-3 Fatty Acid Supplementation on Muscle Strength in Older Adults: A Systematic Review and Meta-Analysis. *Nutrients*. 2023;15(16):3579. Published 2023 Aug 14. doi:10.3390/nu1516357

2.1 Abstract

2.1.1 Aims

The main objective of the current study was to perform a systematic literature review with the purpose of exploring the impact of LCn-3 PUFA relative to control oil supplementation on muscle strength, with secondary outcomes of muscle mass and physical function in older individuals under conditions of habitual physical activity/exercise.

2.1.2 Methods

The review protocol was registered with PROSPERO (CRD42021267011) and followed the guidelines outlined in the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement. The search for relevant studies was performed utilizing databases such as PubMed, EMBASE, CINAHL, Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials (CENTRAL) up to June 2023. Randomized controlled trials (RCTs) in older adults comparing the effects of LCn-3 PUFA with a control oil supplement on muscle strength were included. Five studies involving a total of 488 participants (348 females and 140 males) were identified that met the specified inclusion criteria and were included.

2.1.3 Results

Upon analysing the collective data from these studies, it was observed that supplementation with LCn-3 PUFA did not have a significant impact on grip strength (standardized mean difference (SMD) 0.57, 95% confidence interval [−0.10, 1.25]; $p = 0.10$) in comparison to the control group. However, there was a considerable level of heterogeneity among the studies ($I^2 = 91\%$; $p < 0.001$). As secondary outcomes were only measured in a few studies, with

significant heterogeneity in methods, meta-analyses of muscle mass and functional abilities were not performed. Papers with measures of knee extensor muscle mass as an outcome n = 3 found increases with LCn-3 PUFA supplementation, but studies measuring whole body lean/muscle mass n = 2 and functional abilities n = 4 reported mixed results.

2.1.4 Conclusions

Our data indicate that LCn-3 PUFA supplementation has no effect on muscle strength or functional abilities in older adults but may increase muscle mass, although, with only a few studies and considerable heterogeneity, further work is needed to confirm these findings.

2.1.5 Keywords

Muscle strength, omega-3 polyunsaturated fatty acid, muscle mass, older adults.

2.2 Introduction

Muscle mass and function play a vital role in overall health and well-being, although this receives little research focus. Various conditions, such as sarcopenia, cachexia, and muscle disuse atrophy, can lead to muscle loss and deterioration in quality and function (Cruz-Jentoft et al., 2019b). For example, as individuals reach the age of 35–40, muscle strength and mass gradually and progressively decline (McGlory *et al.*, 2019). This process, termed sarcopenia, specifically refers to the age-related decrease in muscle strength and mass and is associated with several negative health outcomes. These include, but are not limited to, a decrease in quality of life, reduced functional capacity of muscles, physical disability, diminished quality of life, increased risk of mortality, and a higher likelihood of falls that may result in hospitalization (O'Loughlin *et al.*, 1993). Individuals with sarcopenia also incur substantially higher health and social care costs, including longer hospital stays and a greater need for residential living facilities (Mijnarends *et al.*, 2016, Bruyere *et al.*, 2019). In the United Kingdom alone, the additional health and social care expenses related to muscle weakness are estimated to reach GBP 2.5 billion per year (Pinedo-Villanueva *et al.*, 2019).

Although the exact prevalence of sarcopenia remains uncertain, a recent meta-analysis involving a large number of individuals (n = 692,056, mean age: 68.5 years) estimated the global prevalence to be between 10% and 27% (Petermann-Rocha *et al.*, 2022a). Furthermore, the proportion of older adults is expected to rise in many countries. For example, in the UK, there were 11.9 million (18% of the population) adults of pensionable age (67 years) in 2020, and this is projected to rise to 13.2 million (19% of the population) in 2030 and 15.2 million (21% of the population) in 2045. Similarly, again in the UK, in 2020, there were 1.7 million (2.5% of the population) adults aged 85 years or over, and this is projected to increase to 3.1 million (4.3% of the population) in 2045 (Statistics, 2023). Given this demographic shift, it is

crucial to prioritize the development of treatments aimed at improving or slowing down the decline in muscle strength and mass among older adults.

Addressing the age-related decline in muscle strength and mass is of paramount importance. Effective medical interventions that can prevent or mitigate the negative consequences of muscle loss are, however, currently lacking (O'Loughlin *et al.*, 1993). As a result, there is a growing interest in exploring alternative approaches to maintain or enhance muscle strength and mass in older adults. Resistance training is the most effective method for increasing muscle strength and mass, even among nonagenarian women (Fiatarone *et al.*, 1990). However, its effectiveness is reduced compared to younger individuals, primarily due to so-called “anabolic resistance” (Greig *et al.*, 2011). On top of that, low rates of participation significantly hinder the effectiveness of resistance exercise interventions targeting muscle strength and mass in older adults (Strain *et al.*, 2016). As a result, these interventions may not be as impactful as desired in terms of public health. To address this issue, modifying dietary habits has been proposed as a potential therapeutic approach to enhance muscle strength and mass in older individuals.

Among these approaches, adjusting protein and fatty acid consumption have been proposed as potential therapeutic strategies (Ter Borg *et al.*, 2019). One promising candidate in this regard is supplementation with the LCn-3 PUFA Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), found in marine sources such as oily fish and krill. There is a mixture of epidemiological, animal, cell culture, and acute human data that indicates the potential beneficial effect of EPA and DHA, but not other n-3 PUFA, such as alpha-linolenic acid (ALA), on muscle. For example, the consumption of fatty fish has a positive correlation with muscle strength among older populations, according to epidemiological data (Robinson et al., 2008b, Gedmantaite et al., 2020). Cell culture and animal studies back up these findings (Kamolrat

and Gray, 2013, Kamolrat *et al.*, 2013). Furthermore, 8 weeks of LCn-3 PUFA supplementation (4 g/day) increased muscle protein synthesis (MPS) during a hyperaminoacidaemic-hyperinsulinaemic clamp in humans (Smith *et al.*, 2011a). However, overall, the current evidence regarding the effects of LCn-3 PUFA on muscle strength and mass in older individuals is limited and inconsistent (Huang *et al.*, 2020, Cornish *et al.*, 2022). Additionally, the level of certainty in the available evidence is low (Huang *et al.*, 2020). Therefore, further research is needed to determine the efficacy of LCn-3 PUFA supplementation in order to address the age-related decline in muscle strength and mass effectively.

A recent scoping systematic review and meta-analysis, which included studies from a wide range of healthy and clinical populations, investigated whether LCn-3 PUFA supplementation increases muscle strength and mass (Bird et al., 2021, Huang et al., 2020). Although studies were small and heterogeneity was high, the results indicated that LCn-3 PUFA supplementation can increase both muscle strength and mass (Bird *et al.*, 2021). There has also been a systematic review and meta-analysis restricted to older people (Huang *et al.*, 2020), which included studies with exercise training alongside LCn-3 PUFA supplementation, which found an increase in muscle mass but not strength. Key studies, however, were unfortunately not included in this latter meta-analysis for muscle strength, and further studies have since been published. There is a clear need, therefore, for a robust and up-to-date meta-analysis investigating the impact LCn-3 PUFA on muscle strength and mass in older people in the absence of exercise training. The latter point is of interest to increase possible public health utility due to the previously mentioned issues with uptake and long-term adherence to exercise.

The objective of this study is to conduct a systematic literature review to investigate whether LCn-3 PUFA supplementation relative to control oil affects muscle strength, with secondary

outcomes of muscle mass and functional performance in older adults under conditions of habitual physical activity/exercise.

2.3 Materials and methods

2.3.1 Data sources and searches

This systematic review adheres to the guidelines outlined in the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement (Appendix 2- A) (Liberati et al., 2009, Page et al., 2021). The study was registered with the International Prospective Register of Systematic Reviews (PROSPERO) in 2021 under the identification number CRD42021267011 (Appendix 2-B). A comprehensive search of the literature was conducted using several databases, including PubMed, EMBASE, CINAHL, Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials (CENTRAL). Search terms are presented in (Appendix 2-C). Additionally, the reference lists of the included articles were examined to identify any additional articles that met the inclusion criteria outlined in the PICOS format described in Error! Not a valid bookmark self-reference.. We restricted our search to papers published in the English language, with no restriction on the publication period. The search was from the dates of inception until June 2023. Two researchers (MT and AB) independently screened the titles, abstracts, and full texts of the identified studies to determine their eligibility. In case of disagreements, a third researcher (SRG) was consulted to resolve conflicts.

Table 2.1 Participant, intervention, comparison, outcome, and study design (PICOS) with inclusion and exclusion criteria to determine study eligibility.

Review Questions	Inclusion criteria	Exclusion criteria
Population (P)	Healthy adults with mean sample age 65 years of age or over.	Participants who were pregnant or had major disease.

Review Questions	Inclusion criteria	Exclusion criteria
Intervention (I)	LCn-3 PUFA supplementation, such as fish oil or krill.	Other interventions, such as exercise or other supplements, included.
Comparator (C)	A placebo oil, such as vegetable oil or olive oil.	No placebo oil.
Outcomes (O)	Measurement of muscle mass and muscle strength, with data on and muscle function also extracted.	
Study design	Randomised control trials (RCTs).	Any other study design.
Other	Published peer-reviewed articles in scientific journals, in an English language, human subjects.	

2.3.2 Selection criteria

The study employed specific criteria for inclusion, namely randomized controlled trials that investigated the effects of LCn-3 PUFA (EPA and DHA) supplementation (with no other changes to habitual diet or physical activity) on muscle strength in older individuals. The mean age of the participants in the sample studies was 65 years and older. The primary focus of the studies was to assess muscle strength as the main outcome, while secondary outcomes encompassed muscle mass and muscle function, including functional tests such as the time-up-and-go test. Studies that did not provide primary data, such as abstracts, meta-analyses, and reviews, were considered ineligible for inclusion**.** **Table 2.1** presents the detailed inclusion and exclusion criteria with respect to this study's PICOS framework.

2.3.3 Data extraction and quality assessment

Data extraction was independently performed by two investigators (MT and AB) using a pre-specified data collection form (Appendix 2-D). The following information was extracted during the data extraction process: authors' names, publication year, study design, sample size,

mean age, gender, population characteristics, duration of follow-up, intervention period, details of exercise, type of LCn-3 PUFA, dosage (in grams per day), and outcomes related to muscle mass, muscle strength, and muscle function. Two investigators (MT and AB) utilized the Cochrane Collaboration risk-of-bias tool (Higgins et al., 2011) to evaluate the quality of evidence. In the event of any discrepancies, a third reviewer (SRG) was involved in the discussion to reach a consensus. The level of bias was categorized as high, low, or unclear based on seven criteria: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other potential sources of bias. The strength of the recommendations and the quality of the evidence were assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) methodology. Judgments were made based on criteria such as the risk of bias, inconsistency, imprecision, indirectness, and publication bias, and the quality of evidence was classified as high, moderate, low, or very low (Appendix 2-E).

Data synthesis and statistical analysis

The meta-analyses were carried out utilizing Review Manager Version 5.3 (RevMan) software. The effect size was estimated as standardized mean differences (SMDs) with 95% confidence intervals (CIs) and employing a random-effects model (Smith *et al.*, 2011a). The mean changes in scores (final–baseline) and SDs were used. When these data were not available, the authors were contacted, and any missing change SDs or median to mean conversions were carried out using methods described previously (Higgins JPT, 2008). Statistical heterogeneity was evaluated by performing a chi-squared test and the calculation of the I^2 statistic.

2.4 Results

2.4.1 Study identification

Our initial search strategy identified 2931 studies, out of which 1882 remained after removing duplicates (**Figure 2-1)** Following the screening of titles and abstracts, 26 full-text studies underwent a thorough review for eligibility, resulting in the inclusion of 5 studies **Table 2.2.** There were 488 participants in these articles (348 females and 140 males).

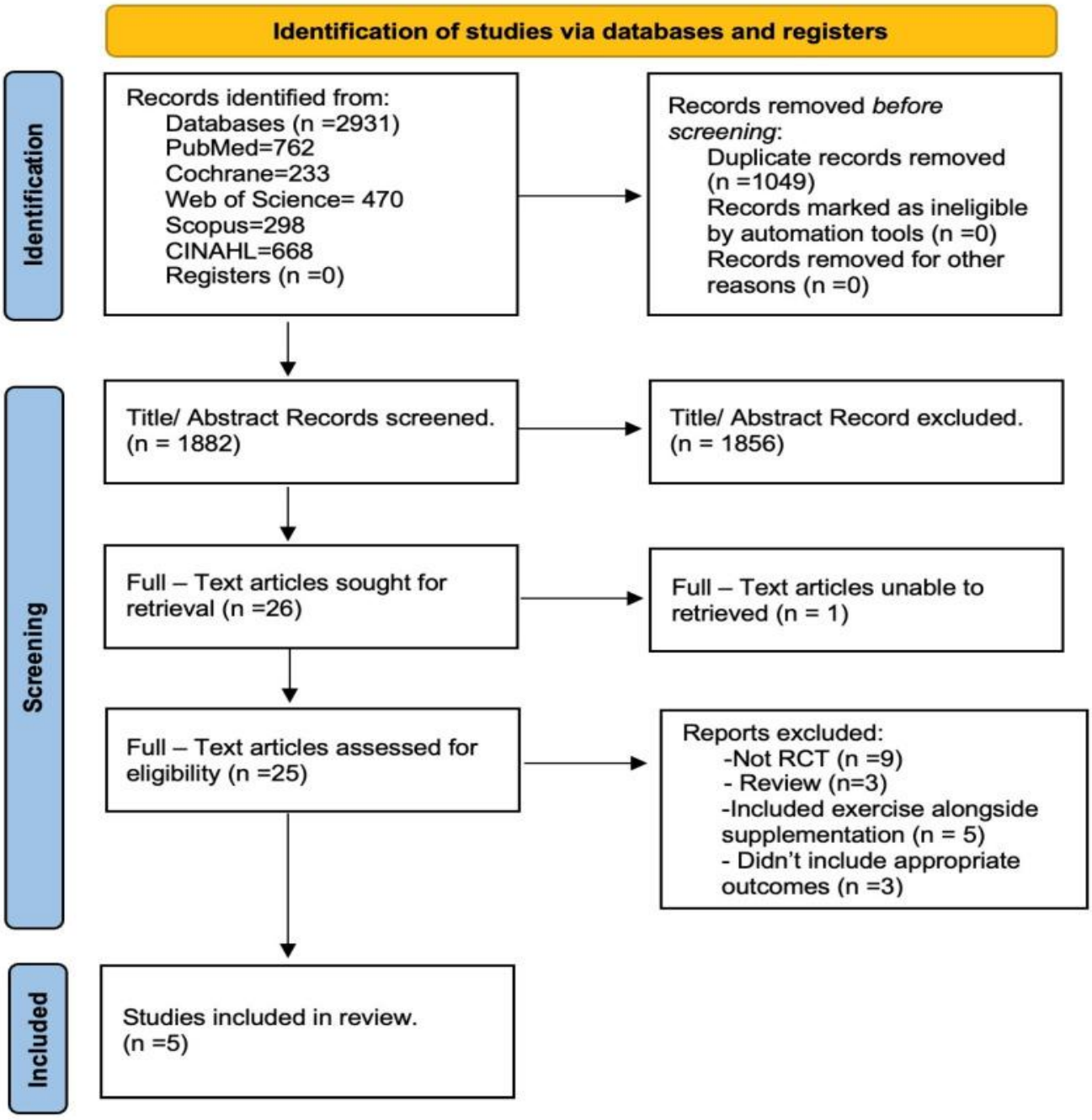

Figure 2-1 Flow diagram illustrating the study selection process according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (Page et al., 2021).

2.4.2 Study characteristics

Table 2.2 Summarises the main characteristics of the five studies that were included in the analysis. Among these studies, two were conducted in the United States, one in the United Kingdom, one in Canada, and one in China. The average age of participants varied between 66 and 75 years old. The duration of interventions was between 12 and 24 weeks. The five RCTs included healthy, community-dwelling older adults. Two of the five studies included only women. The rest included both males and females. The studies included the main outcomes of muscle mass, muscle strength, and muscle function (Smith et al., 2015, Logan and Spriet, 2015, Hutchins-Wiese et al., 2013, Alkhedhairi et al., 2022, Xu et al., 2022).

Table 2.2 Studies investigating the impact of omega-3 supplementation on measures of muscle strength and muscle function in older adults.

Author	Country	Design	Sample	Intervention	Main Effects of LCn-3 PUFA Relative to Placebo
Alkhedhairi *et al.* (Alkhedhairi *et al.*, 2022).	UK	Double-blind	n = 94; n = 53 female, n = 41 male; age = 71 ± 5 years.	(4 g/day; of Krill oil 772 mg/d EPA and 384 mg/day DHA) or placebo (4 g/day mixed vegetable oil) for 6 months.	Increase in grip strength (10.9%), leg strength (9.3%), and vastus lateralis muscle thickness (3.5%) (all $p<0.05$). No effect on the short-performance physical battery test or whole-body bioelectrical impedance measured muscle mass.
Hutchins-Wiese *et al.* (Hutchins-Wiese *et al.*, 2013).	USA	Double-blind	n =all females; 126; age = 75 ± 6 years.	(2 capsules Fish oil, 1.2 g/day EPA and DHA) or placebo (2 capsules olive oil, 1.8 g/day olive oil) for 24 weeks.	No effect on grip strength or repeated chair rise test. Increased walking speed (3%) ($p = 0.038$), ($p<0.05$).
Logan (Logan and Spriet, 2015).	Canada	Single-blind	n = all female; 24; age = 66 ± 1 years.	(5 g/day Fish oil (2 g/day EPA and 1 g/day DHA)) or placebo (3 g/day olive oil) for 12 weeks.	No effect on grip strength or the 30-s sit-to-stand test. Increased whole-body bioelectrical impedance measured lean mass (4%) ($p=0.01$) and TUG test (7%) ($p<0.006$).
Smith *et al.* (Smith *et al.*, 2015).	USA	Double-blind	n = 44; male = 15 and female = 29; age = 69 ± 6 years.	(4 × 1 g pills/day of Fish oil providing 1.86 g/day EPA and 1.5 g/day DHA) or placebo (4 × 1 g pills/day of corn oil) for 6 months.	Increased thigh muscle volume (3.6%), handgrip strength (2.3 kg), and 1-RM muscle strength (4.0%) (all $p<0.05$). Average isokinetic power tended to be increased (5.6%) ($p = 0.075$).
Dengfeng Xu (Xu *et al.*, 2022).	China	Double-blind	n = 200; female, n = 116; male, n = 84; age = 67 ± 5 years.	(4 g/day; 1.34 g of Fish oil/d EPA and 1.07 g/day DHA) or placebo (4 g/day corn oil) for 6 months.	Increased thigh muscle volume (3.66 cm), handgrip strength (4.91 kg), and Timed Up and Go strength (1.85 s) (all $p<0.001$).

2.4.3 Intervention and comparators

The supplementation regimens employed in the studies are outlined in **Table 2.2**. Among the included studies, four of them (Xu et al., 2022, Smith et al., 2015, Logan and Spriet, 2015, Hutchins-Wiese et al., 2013) utilized either fish oil or LCn-3 PUFA derived from fish oil as the supplementation, while one study used krill oil (Alkhedhairi et al., 2022). In terms of comparators, two studies used olive oil (Logan and Spriet, 2015, Hutchins-Wiese et al., 2013), one used mixed vegetable oil (Alkhedhairi et al., 2022), and two used corn oil (Smith et al., 2015, Xu et al., 2022). These details provide an overview of the different supplementation and comparator options employed across the studies.

2.4.4 Risk of bias

An overview of the risk of bias observed in the studies is presented in **Figure 2-2.** Overall, the studies demonstrated a low risk of bias in several domains, including random sequence generation, blinding of participants and personnel, allocation concealment, and selective reporting. Two studies (Logan and Spriet, 2015, Smith et al., 2015) were deemed to have a high risk of bias regarding allocation concealment, while one study (Hutchins-Wiese et al., 2013) had an unclear risk of bias in this domain. In terms of the blinding of outcome assessment, three studies (Logan and Spriet, 2015, Smith et al., 2015, Xu et al., 2022) were classified as having a high risk of bias. One of the studies showed an unclear risk of bias for incomplete data outcomes (Hutchins-Wiese et al., 2013), and five studies had other potential biases (Alkhedhairi et al., 2022, Logan and Spriet, 2015, Hutchins-Wiese et al., 2013, Smith et al., 2015, Xu et al., 2022) **(Figure 2-2 and Figure 2-3).** Studies with a high risk of bias in the domain of "measurement of the outcome" were categorized as such because there was no blinding of investigators. Because it could not be determined whether data were analysed according to a pre-specified plan or registered before recruitment, studies with some

concerns were rated as such in the “selection of reported results” **domain (Figure 2-2 and Figure 2-3).**

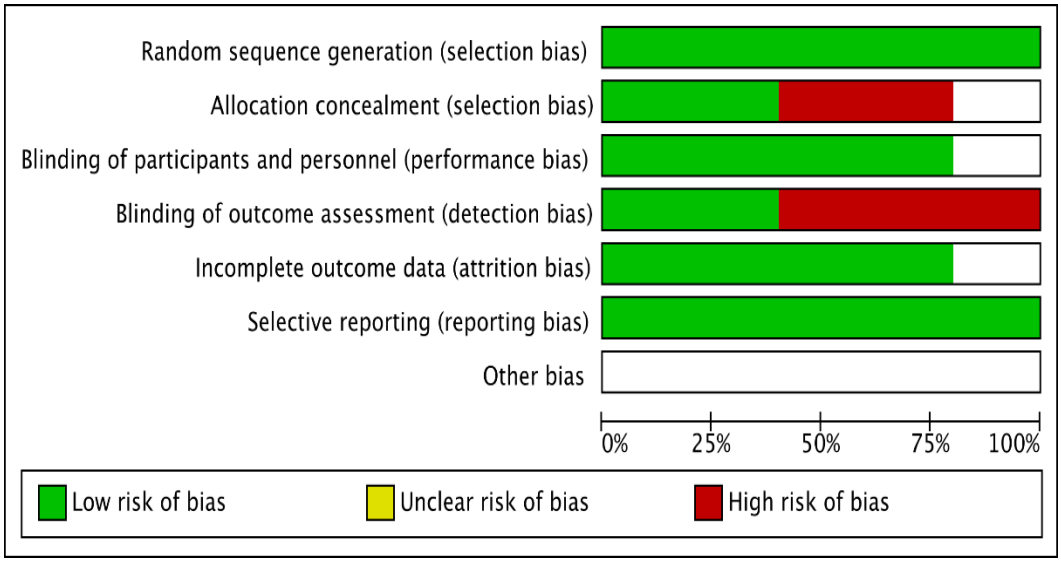

Figure 2-2 Summary of risk-of-bias for all included studies

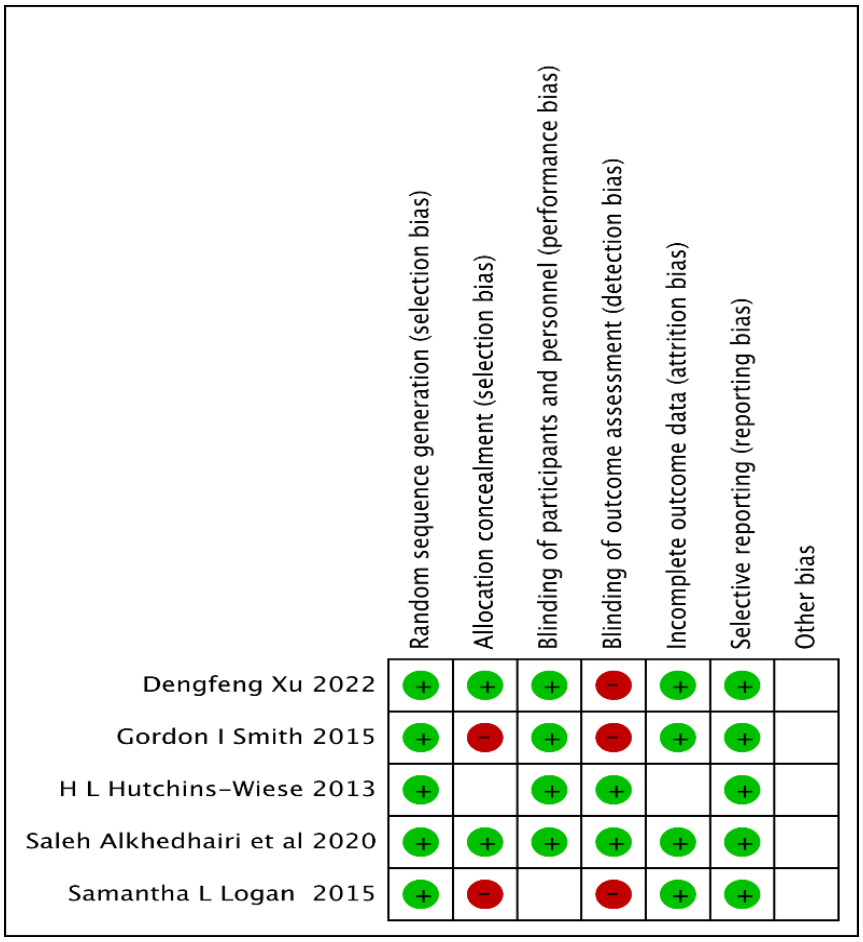

Figure 2-3 Risk of bias assessment for included studies

2.4.5 Effects of LCn-3 PUFA on muscle mass and function

In terms of muscle mass, four studies involving 362 participants (140 males and 222 women) reported the effects of LCn-3 PUFA in older adults compared with a control group (Logan and Spriet, 2015, Smith *et al.*, 2015, Xu *et al.*, 2022, Alkhedhairi *et al.*, 2022). One study reported no significant impact of LCn-3 PUFA on muscle mass, estimated by bioelectrical impedance, but did report an increase in vastus lateralis muscle thickness assessed through ultrasound measurements 3.5% ($p<0.05$) (Alkhedhairi *et al.*, 2022). One study found a 3.6% ($p<0.05$) increase in thigh muscle volume, measured by MRI (Smith *et al.*, 2015), and another reported an increase in whole-body lean mass, measured by bioelectrical impedance 4% ($p<0.01$) (Logan and Spriet, 2015). One study found significant increases in both skeletal muscle mass ($p < 0.001$, interaction time × group effect) and appendicular skeletal muscle mass ($p < 0.001$, interaction time × group effect) (Xu *et al.*, 2022).

In terms of functional abilities, four studies involving 444 participants (125 male and 319 female) reported the effects of LCn-3 PUFA in older adults (Logan and Spriet, 2015, Hutchins-Wiese *et al.*, 2013, Xu *et al.*, 2022, Alkhedhairi *et al.*, 2022). One study indicated no influence of LCn-3 PUFA on muscle function, as evaluated through the short-performance physical battery test (Alkhedhairi *et al.*, 2022). In one study, no significant effect of LCn-3 PUFA on repeated chair rise performance was observed (Hutchins-Wiese *et al.*, 2013). However, in another study, data demonstrated a 3% increase in walking speed ($p = 0.038$) (Hutchins-Wiese et al., 2013) and an increase in performance of the timed-up-and-go (TUG) test 7% ($p = 0.006$) (Logan and Spriet, 2015). Furthermore, a separate study demonstrated a significant reduction in the TUG time ($p < 0.001$), indicating an improvement in mobility (Xu *et al.*, 2022).

2.4.6 The effects of n-3 fatty acids on hand grip strength—meta-analysis

(Figure 2-4) depicts the combined effects of LCn-3 PUFA supplementation on hand grip strength. Five studies (Logan and Spriet, 2015, Hutchins-Wiese et al., 2013, Smith et al., 2015, Xu et al., 2022, Alkhedhairi et al., 2022) involving 488 participants (n = 140 males, 348 females) were included. The analysis revealed no statistically significant impact of LCn-3 PUFA supplementation (SMD 0.57, [−0.10, 1.25], p = 0.10) on hand grip strength compared to the control group. However, a considerable level of heterogeneity was observed (I^2 = 91%, $p < 0.001$) among the included studies, suggesting variations in the results across the studies. Our GRADE analysis revealed our certainty in these results was very low (Appendix 2-E).

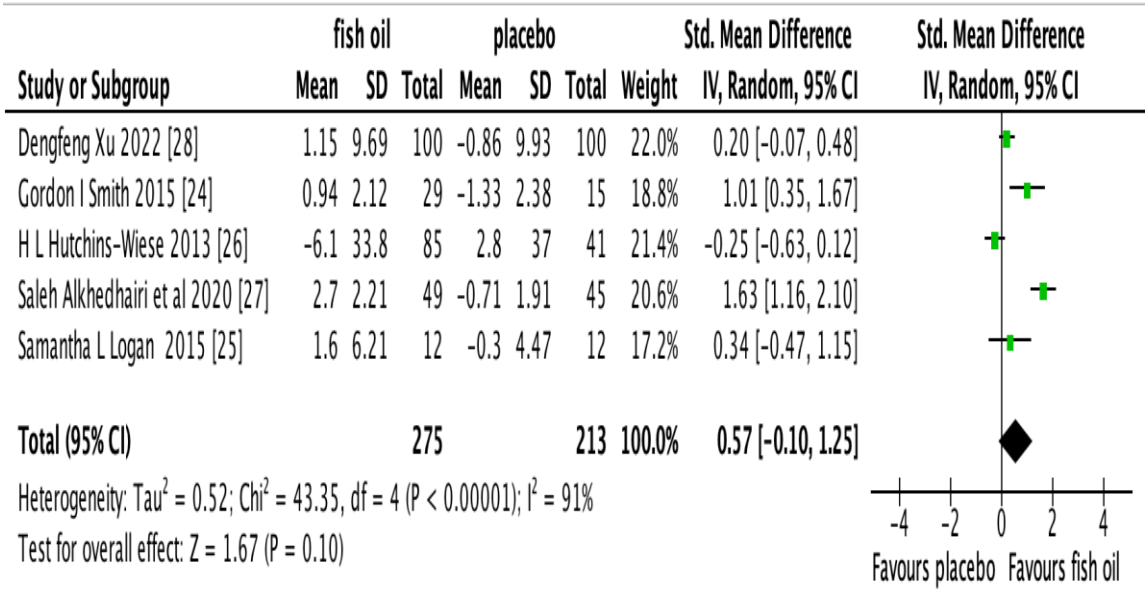

Figure 2-4 Meta-analysis on the effects of n-3 fatty acids on hand grip strength in older adults (Logan and Spriet, 2015, Hutchins-Wiese et al., 2013, Smith et al., 2015, Xu et al., 2022, Alkhedhairi et al., 2022).

2.5 Discussion

In this systematic review, we aimed to investigate the effects of LCn-3 PUFA supplementation on various aspects of muscle health, including muscle strength, muscle mass, and muscle function, in older adults without underlying health conditions, with a meta-analysis performed for the primary outcome of muscle strength. By analysing and synthesizing the findings from five randomized controlled trials that involved a total of 488 healthy older adults, the overall impact of LCn-3 PUFA supplementation on these muscle-related outcomes was assessed. The results of the meta-analysis indicate that LCn-3 PUFA supplementation did not have a significant impact on handgrip strength in healthy older adults. This suggests that LCn-3 PUFA supplementation may not be sufficient to improve handgrip strength in this population. The current data are based on only five studies, and our analysis of statistical heterogeneity indicates that there is considerable heterogeneity, meaning the results of the individual studies are not consistent and should be interpreted with caution. These issues limit our ability to draw firm conclusions, and further work is needed to establish whether LCn-3 PUFA is a potential strategy to counteract the age-related decline in muscle strength. In addition to the meta-analysis, we conducted a narrative synthesis to provide a comprehensive overview of the available evidence. The narrative synthesis revealed that LCn-3 PUFA supplementation may have a positive influence on muscle mass, as there were indications of increased muscle mass in three studies included in our analysis. However, when it came to muscle function, the results were more mixed, and we did not find a clear beneficial effect of LCn-3 PUFA supplementation on muscle function outcomes.

Given the limitations of the current evidence, including the limited data and high heterogeneity among the studies, further research is needed to provide a more definitive understanding of the effects of LCn-3 PUFA supplementation on muscle strength and mass

in older adults. Studies included in the current review had durations of 12 weeks to 6 months and provided doses of ~1–3 g/day LCn-3 PUFA, with insufficient current data to investigate whether dose and/or duration of supplementation can influence the effects of LCn-3 PUFA on muscle. Future studies should aim to address these gaps in the literature. Ultimately, it remains uncertain whether LCn-3 PUFA supplementation can effectively mitigate the age-related decline in muscle strength and mass associated with ageing. Therefore, more robust, well-designed studies are warranted to determine the potential utility of LCn-3 PUFA supplementation as a viable approach to promoting muscle health in older adults without underlying health conditions.

Muscle strength and mass naturally decline as individuals age, typically starting around 35–40 years, and can eventually lead to the development of sarcopenia (Distefano and Goodpaster, 2018). Unfortunately, there are currently no effective and safe medical treatments available for either the prevention or treatment of this condition (Won, 2023). However, emerging research suggests that nutrition may offer a potentially effective approach to delay the age-related decline in muscle mass and function among older individuals (Ter Borg *et al.*, 2019), with LCn-3 PUFA emerging as a strong candidate. The current data indicate that LCn-3 PUFA does not have any significant impact on grip strength in older adults. This finding aligns with previous older meta-analyses that had broader inclusion criteria (Huang *et al.*, 2020, Cornish *et al.*, 2022). For example, in (Huang *et al.*, 2020), studies with and without exercise and/or multi-nutrient supplements were included, with no effect of LCn-3 PUFA on grip strength (on the basis of 3 studies with 97 participants). Similarly, in (Cornish *et al.*, 2022), no effect on grip strength was noted in people 55 years or older (on the basis of 7 studies with 680 participants). In our data, there appears to be a tentative trend suggesting a potential beneficial effect of LCn-3 PUFA on grip strength, and some studies have indicated its potential benefits on lower body muscle strength. This lack of certainty, highlights the need for further large high quality studies in

this area. While grip strength is commonly used in the diagnostic criteria for sarcopenia due to its simplicity (Arnal-Gomez *et al.*, 2021), it can be argued that lower body muscle strength holds greater practical significance for older individuals in terms of maintaining mobility and independent living.

Based on our assessment using the GRADE framework, the level of certainty in the evidence derived from the present study was determined to be low. Therefore, further research is necessary to explore the effects of LCn-3 PUFA on grip strength in older adults. The exact mechanisms through which LCn-3 PUFA might influence muscle strength are not fully understood. However, potential explanations may involve improvements in neuromuscular function, enhanced blood flow for better nutrient delivery, increased mitochondrial content and function, and alterations to the extracellular matrix to facilitate enhanced force transmission (Gray and Mittendorfer, 2018).

Our analysis of the effects of LCn-3 PUFA on muscle function yielded varied results in the narrative synthesis. However, when considering the overall findings, it is suggested that there is no discernible beneficial effect. Our review of the literature on functional abilities had mixed results, with some finding benefits on walking speed (Hutchins-Wiese *et al.*, 2013) and TUG (timed up and go test) (Logan and Spriet, 2015, Xu *et al.*, 2022) , and others finding no effect on chair rise tests (Hutchins-Wiese *et al.*, 2013, Logan and Spriet, 2015) or the short-performance physical battery test (Alkhedhairi *et al.*, 2022). This is perhaps not surprising, as in the studies included in the current analysis, participants were all healthy and so had no functional limitations, a finding exemplified in the recent Do-HEALTH study, which was a 2 × 2 × 2 factorial design study investigating the effects of vitamin D, a home exercise program, and LCn-3 PUFA on a range of clinical outcomes, including the (SPPB) short-performance physical battery test (Bischoff-Ferrari *et al.*, 2020). In this study, involving 2157 older adults who received 3 years of LCn-3 PUFA supplementation, no

significant impact on the short-performance physical battery test score was observed. The participants, however, had baseline scores of 11–12 out of 12 and were highly physically active. Whether LCn-3 PUFA can influence muscle function in older adults with functional limitations remains to be investigated in further studies in people with pre-existing limitations in functional capacity.

When looking at muscle mass as an outcome, our data indicate a beneficial effect of LCn-3 PUFA on muscle mass, which agrees with the previous, more broad meta-analyses on this topic (Smith *et al.*, 2011a, Cornish *et al.*, 2022). In the current review, it was found that MRI-measured thigh muscle volume (Higgins JPT, 2008), ultrasound-measured vastus lateralis muscle thickness (Alkhedhairi *et al.*, 2022), and whole-body bioelectrical impedance-evaluated lean mass, skeletal muscle mass, body fat mass, and fat-free mass (Logan and Spriet, 2015, Xu *et al.*, 2022) , were increased following LCn-3 PUFA supplementation. No effect on whole-body bioelectrical impedance-measured muscle mass was seen in one study, although increases in muscle thickness were seen (Hutchins-Wiese *et al.*, 2013, Alkhedhairi *et al.*, 2022). The potential mechanisms by which LCn-3 PUFA may enhance muscle mass are associated with muscle protein metabolism, specifically the equilibrium between muscle protein synthesis (MPS) and (MPB). To date, there have been no studies that have directly measured (MPB) in response to LCn-3 PUFA supplementation. However, research has demonstrated that LCn-3 PUFA supplementation can elevate (MPS) during a hyperinsulinemic-hyperaminoacidemic clamp, as observed in both young and older adults (Smith *et al.*, 2011a, Smith *et al.*, 2011b). These increases in MPS have been accompanied by elevated levels of signalling proteins (p70s6k and mTOR) associated with translation activation (Stepan *et al.*, 2021, Smith *et al.*, 2011a). These increases in muscle mass may contribute to an increase in muscle strength due to LCn-3 PUFA, but there are also potential mass-independent mechanisms through which strength can be increased. DHA is an essential constituent of membrane phospholipids which are crucial for numerous neural functions

such as receptor affinity and modulation of signal transduction (Salem *et al.*, 2001). In younger adults, there is some evidence that LCn-3 PUFA supplementation can increase surface electromyography (sEMG) levels and muscle power in young male athletes (Lewis *et al.*, 2015). There is less evidence in older adults, but it has been shown that LCn-3 PUFA can increase the M-wave, an indirect measure of fibre membrane excitability, and has shown notable decreases in clinical myopathies (Jammes *et al.*, 2020) in older adults. Other potential mechanisms include improvements in mitochondrial content and function, blood supply and extracellular matrix content, composition and architecture, and the anti-inflammatory effects of LCn-3 PUFA (Gray and Mittendorfer, 2018).

Finally, this systematic review and meta-analysis was published in Nutrients in August 2023 (Timraz et al., 2023). As part of the thesis examination process, we discussed whether subsequently published studies might alter our conclusions. An updated search (August 2023 to January 2025) identified several recent studies, but no new RCTs met our inclusion criteria and this confirms this review remains current and our conclusions robust.

2.6 Conclusions

The findings of our meta-analyses should be interpreted with caution due to several notable limitations. These include the limited number of studies and participants involved, as well as the presence of substantial heterogeneity across the included studies. This makes our conclusions tentative. Supplementation with LCn-3 PUFA seems to have a positive impact on muscle mass; however, its effect on muscle strength and function remains inconclusive. Therefore, additional research is necessary to determine if LCn-3 PUFA supplementation has utility as a strategy for preventing or treating age-related declines in muscle strength, mass, and functional abilities. Additionally, optimal dosing strategies should be investigated.

Chapter 3: The association of n-3 fatty acid intake with muscle mass and strength in older adults: A cross-sectional analysis of the UK biobank data.

Timraz M, Wyss MG, Quinn TJ, Combet E, Celis-Morales C, Gray SR. The association of n-3 fatty acid intake with muscle mass and strength in older adults: A cross-sectional analysis of the UK biobank data. *J Nutr Health Aging*. 2025;29(9):100622. doi:10.1016/j.jnha.2025.100622

3.1 Abstract

3.1.1 Objectives

The main aim was to investigate the association of n-3 fatty acid intake and the n-6/n-3 fatty acid intake ratio with muscle mass and strength in older adults.

3.1.2 Methods

This study included 61,381 individuals (28,187 men and 33,194 women) aged ≥ 60 years. Grip strength and muscle mass index were assessed and n-3 and n-6 fatty acid intake determined. Regression models were adjusted for age, deprivation index, ethnicity, month of assessment, total energy intake, multimorbidity, lifestyle factors and physical activity. A sensitivity analysis was conducted in participants aged ≥ 65 years and in people with sarcopenia.

3.1.3 Results

Data are presented as trend for quintiles from fully adjusted models. Higher n-3 fatty acid intake was associated with a higher grip strength in both men (0.114 kg; 95% CI: 0.02 to 0.21) and women (0.115 kg; 95% CI: 0.05 to 0.18). Similar results were reported for grip strength index, with no associations observed for muscle mass index. No associations were seen in people ≥ 65 years. In people with sarcopenia no associations of n-3 fatty acid intake with grip strength or grip strength index were seen, but a positive association with muscle mass index was noted in men (0.197 kg/m²; 95% CI: 0.05 to 0.33). The n-6/n-3 fatty acid intake ratio was associated with grip strength in women (0.081 kg; 95% CI: −0.16 to 0.00) and with muscle mass index in men (−0.016 kg/m²; 95% CI: −0.02 to 0.00), no other associations were observed. No associations were seen in people with sarcopenia or people ≥ 65 years.

3.1.4 Conclusion

Higher n-3 fatty acid intake, with no consistent association with the n-6/n-3 fatty acid intake, was modestly associated with grip strength, with effects varying by sex and age, suggesting limited benefit for sarcopenia prevention at typical intake levels in older adults.

3.1.5 Keywords

n-3 fatty acids; Muscle strength; Muscle mass; Sarcopenia; Older adults.

3.2 Introduction

The ageing process is a complex and multifaceted phenomenon marked by numerous physiological changes, including a gradual decline in physical capacity. One of the most clinically significant consequences of ageing is the reduction in muscle function that typically begins in mid-adulthood and progresses with advancing age (Rosenberg, 1997). This decline plays a central role in functional impairment, contributing to reduced mobility, poorer physical performance, and a heightened risk of frailty, osteoporosis, and metabolic disorders (Cruz-Jentoft et al., 2019b, Beard et al., 2016). As populations age globally, the health and socioeconomic burden associated with age-related muscle deterioration continues to rise, with excess health and social care costs related to muscle weakness in the UK alone estimated at approximately £2.5 billion per year (Janssen *et al.*, 2000, Goates *et al.*, 2019).

Among these potential strategies, nutritional supplementation with n-3 fatty acids has emerged as a promising avenue (Calder, 2013, Jang *et al.*, 2020, Gielen *et al.*, 2021, Zhang *et al.*, 2021). The primary dietary n-3 fatty acids are (EPA) and (DHA), which are abundant in marine-derived fish oils particularly from oily fish such as salmon, mackerel, sardines, and herring. In contrast, (ALA), found in plant-based sources like walnuts, flaxseeds, and chia seeds, undergoes limited conversion to EPA and DHA via elongation and desaturation (Witard *et al.*, 2020). There is very little evidence supporting a direct effect of ALA on skeletal muscle health (Witard *et al.*, 2020), so most of the research in this area has focussed on EPA and DHA. While these marine-derived n-3 fatty acids have long been studied in relation to cardiovascular health (Cornish *et al.*, 2022, Smith *et al.*, 2011a), recent work has investigated potential roles in muscle health in older adults (Cornish *et al.*, 2022, Huang *et al.*, 2020). For example, fish oil-derived n-3 fatty acids have been shown to enhance muscle protein synthesis during a hyperaminoacidemic-hyperinsulinemic clamp in middle-aged and older adults, with no effect

on basal muscle protein synthesis (Smith *et al.*, 2011a, Smith *et al.*, 2011b, Calder, 2015). Recent meta-analyses have provided early evidence for the benefits of n-3 fatty acid supplementation on muscle outcomes. Huang *et al.* (Huang *et al.*, 2020) conducted a comprehensive meta-analysis of randomized controlled trials and found that n-3 fatty acids supplementation resulted in significant improvements in muscle mass and strength among older people. Similarly, Cornish *et al.* (Cornish *et al.*, 2022) demonstrated that n-3 fatty acid supplementation, particularly when combined with resistance exercise, can increase both muscle strength and mass. Our meta-analysis in chapter 2 supported the beneficial effects of LCn-3 PUFA on muscle mass, with no significant impact on grip strength in older adults. Another meta-analysis by Ma *et al.* (Ma *et al.*, 2021) confirmed these positive effects on muscle function with ageing, while Therdyothin *et al.* provided mechanistic insights into how n-3 fatty acids may counteract sarcopenia (Therdyothin et al., 2023). These findings have been extended in randomised controlled trials, which demonstrated that n-3 fatty acid supplementation (4g/day of fish or krill oil) can increase both muscle strength and mass (Smith *et al.*, 2015, Alkhedhairi *et al.*, 2022). While these results are promising, it remains unclear whether variations in n-3 fatty acids intake within the normal dietary range (1–2 g/day), affects muscle strength and mass (Smith *et al.*, 2015, Rodacki *et al.*, 2012, Pap, 2010).

Limited epidemiological evidence exists examining this relationship. Two epidemiological studies have identified positive correlations between oily fish consumption and grip strength in both men and women (Robinson et al., 2008b, Gedmantaite et al., 2020). Another study examined the association of dietary n-3 fatty acid intake with muscle strength in 18,278 adults, finding that higher n-3 fatty acid intake was associated with lower odds of low hand grip strength. This study relied on a single 24-hour dietary recall, did not investigate muscle mass, and was not restricted to older adults (Shin and Chang, 2023). Another small study of 5,529

participants (2,449 men and 3,080 women) aged 65 and older found that women, but not men, who consumed EPA and DHA at or above the adequate intake level (150 mg/d for women aged 65 to 74 years and 140 mg/d for women aged 75 years), also measured via a single 24-hour dietary recall, had a significantly lower likelihood of having low grip strength compared to those below the adequate intake level, with no measure of muscle mass in this study (Bae *et al.*, 2022).

The dietary intake ratio of n-6 fatty acid to n-3 fatty acid (n-6/n-3 fatty acid intake ratio) may also be critical for muscle health, as both fatty acid families compete for enzymatic pathways and have opposing inflammatory effects (Therdyothin et al., 2023). Western diets typically have elevated n-6/n-3 fatty acid intake ratios (15:1 to 20:1) compared to the recommended 4:1 ratio, promoting a more pro-inflammatory state that may accelerate MPB and inhibit MPS, promoting muscle loss (Therdyothin et al., 2023). While both n-3 and n-6 fatty acid intake and their ratio may influence muscle during ageing, the optimal intake ratio remains unclear (Lee, 2025). A recent cross-sectional study suggested an n-6/n-3 fatty acid intake ratio of 6.8 was optimal for preventing sarcopenia in women, but no cut off value was identified in men (Lee, 2025). Further research supporting this sex difference, reporting stronger associations in women (Yang *et al.*, 2020, Kim and Park, 2023).

Given the uncertainty in the existing literature, the current study aims to quantify the association of dietary n-3 fatty acid intake, and the n-6/n-3 fatty acid intake ratio, with muscle mass and strength in older adults, using data from the UK Biobank study. We hypothesized that higher intake of n-3 fatty acids, and a lower n-6/n-3 fatty acid intake ratio, would be positively associated with muscle strength and mass in older adults.

3.3 Materials and methods

3.3.1 Study design

This cross-sectional study utilised data from the UK Biobank. This study was conducted in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) recommendations (von Elm *et al.*, 2007). From the 502,350 participants recruited into UK Biobank a subsample of 61,381 participants were included, comprising 28,187 men and 33,194 women, in the current analyses. Participants were selected for the main analysis based on the following criteria: age 60 years or older and having complete data for the outcome, predictor, and covariate variables. The primary outcomes measured were grip strength, grip strength index, and muscle mass index. The predictor variable was n-3 fatty acid intake, and the n-6/n-3 fatty acid intake ratio, while the covariates included sociodemographic factors (age, ethnicity/race, Townsend deprivation index), multimorbidity count, total energy intake, physical activity and the month of assessment. Participants completed an electronically signed consent form and a touchscreen questionnaire at the initial assessment. Physical measurements and biological samples (such as blood, urine, and saliva) were also gathered (Sudlow *et al.*, 2015, Petermann-Rocha *et al.*, 2022b). Ethical approval for The UK Biobank was obtained from the Northwest Multi-Centre Research Ethics Committee (Ref: 11/NW/0382) (Liu *et al.*, 2011).

3.3.2 Outcomes assessment

Grip strength was evaluated using a Jamar J00105 hydraulic hand dynamometer, with a single measurement taken for each hand. The average grip strength from both hands was calculated in absolute terms (kg) and as a grip strength index, expressed as (kg) per metre height squared, for subsequent analyses. Muscle mass index was measured by the (Tanita) body composition analyser (Tanita BC418MA, Tokyo, Japan) using bioimpedance, the equations of Janssen *et*

al., were used and muscle mass index, relative to height squared, calculated as kg/m2 (Janssen *et al.*, 2000).

For sensitivity analysis in people with sarcopenia, this was classified in accordance with the revised guidelines of the EWGSOP2, with low grip strength defined as < 27 kg for men and < 16 kg for women. plus, low muscle mass defined as skeletal muscle mass index < 7.0 kg/m² in men and < 5.5 kg/m² in women (Cruz-Jentoft et al., 2019b).

3.3.3 Fatty acid intake

The average daily intake of n-3 and n-6 fatty acids, expressed in grams per day was calculated for 61,653 participants who completed multiple online dietary assessments via the Oxford WebQ, a web-based 24-hour dietary recall tool designed for large-scale population studies (Liu *et al.*, 2011). The number of participants who completed each recall varied: 22,535 completed one recall, 13,785 completed two recalls, 13,057 completed three recalls, 9,853 completed four recalls, and 2,151 completed five recalls. Intake values for each fatty acid were averaged across all completed recalls per participant to estimate habitual intake. The n-6/n-3 fatty acid intake ratio was calculated for each participant by dividing their estimated average daily intake of n-6 fatty acids (g/day) by their corresponding n-3 fatty acid intake (g/day). For the final analysis, 61,381 participants aged ≥ 60 years with complete data on exposure, outcomes, and covariates were included. As a sensitivity analysis, we repeated the analyses restricted to participants aged ≥ 65 years n = 24,711 to examine the robustness of the associations using a different age cut off for definition of older adults.

3.3.4 Covariates assessment

Age was calculated at baseline based on the date of birth recorded during the initial assessment. Sex was self-reported at the baseline. Socioeconomic status, represented by area-based deprivation, was determined using the Townsend score and was derived from the residential postcode (Stand, 1988). The Townsend index is a measure of material deprivation derived from four census indicators: unemployment, lack of car ownership, lack of home ownership, and overcrowded living conditions (Stand, 1988). Ethnicity was self-reported and categorised into groups including White, South Asian, Black, Chinese, or mixed ethnic backgrounds. Height was assessed, with participants not wearing shoes, utilising a wall-mounted SECA 240 stadiometers. Total energy intake was assessed using a web-based 24-hour recall questionnaire and expressed in kcal per day. Physical activity was self-reported via the short-form IPAQ and classified as 'active' (≥ 600 MET-min/week) or 'inactive' based on standard guidelines (M Sjostrom, 2005). Multimorbidity was derived from self-reported long-term conditions. At baseline, participants reported whether they had been diagnosed by a doctor with any of 43 specified long-term conditions. Individuals reporting two or more of these conditions were classified as having multimorbidity (list of conditions are presented in Appendix 3-A).

3.3.5 Statistical analysis

Cohort characteristics are presented as means and standard deviations for continuous variables, and as frequencies and percentages for categorical variables. Multiple linear regression analyses were conducted to investigate the relationship between the intake of n-3 fatty acids with grip strength (absolute and as an index) and muscle mass index. The analyses were conducted in unadjusted (model 0) and adjusted models (model 1, model 2 and model 3). In model 1, we adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle

factors (smoking and alcohol intake). In model 2, we adjusted for all the above confounding factors plus multimorbidity. In model 3, we additionally adjusted for total energy intake and physical activity. Analysis in model 3 was replicated in a sensitivity analysis in people with sarcopenia. Additionally, as the definition of older age varies, 60 or 65 years of age, we examined the association of n-3 fatty intake and the n-6/n-3 fatty acid intake ratio with muscle outcomes in people 65 years or over as a sensitivity analysis in model 3. All analyses were stratified by sex because of the differences in grip strength and muscle mass between men and women. Analysis was performed using STATA MP18 (Stata Crop LLC, College Statin, Texas) and the significance level was at $p < 0.05$.

3.4 Results

From the 502,350 participants recruited to the UK Biobank, this study included individuals aged 60 years or older. Of the total, 217,452 participants met the age criterion, and 211,979 had complete data available for all outcomes. The final analysis included 61,381 individuals (28,187 men and 33,194 women) who meet all inclusion criteria and had complete data for all variables. The mean intake of n-3 fatty acids in all participants was 2.13 g/day and the mean n-6/n-3 fatty acid intake ratio was 5.78. A detailed summary of the participant selection process, including exclusions, is presented in (Appendix 3-B). The cohort characteristics of the included participants categorized based on n-3 fatty acids intake are presented in (Appendix 3-C). Additionally, the characteristics of participants with sarcopenia are presented in (Appendix 3-D).

Table 3.1 The association of n-3 fatty acid intake with hand grip strength and muscle mass in older adult men with physical activity.

Outcomes	Model 0		Model 1		Model 2		Model 3	
Grip Strength (kg)	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**
n-3 <1.15 g/day	Ref.		Ref.		Ref.		Ref.	
n-3 1.15-1.90 g/day	0.359 (-0.005; 0.724)	0.053	0.423 (0.058; 0.788)	0.024	0.454 (0.090; 0.819)	0.014	0.221 (-0.171; 0.614)	0.269
n-3 1.91-2.65 g/day	0.876 (0.507; 1.245)	<0.001	0.963 (0.593; 1.333)	<0.001	0.999 (0.630; 1.368)	<0.001	0.563 (0.155; 0.972)	0.007
n-3 2.66-3.4 g/day	1.175 (0.769; 1.580)	<0.001	1.262 (0.856; 1.669)	<0.001	1.297 (0.891; 1.702)	<0.001	0.707 (0.252; 1.161)	0.002
n-3 >3.40 g/day	0.873 (0.444; 1.302)	<0.001	0.963 (0.533; 1.392)	<0.001	0.998 (0.570; 1.426)	<0.001	0.314 (-0.170; 0.799)	0.204
Trend for quintiles	0.269 (0.188; 0.351)	<0.001	0.285 (0.204; 0.366)	<0.001	0.290 (0.209; 0.371)	<0.001	0.114 (0.018; 0.210)	0.019
Grip Strength Index (kg/m^2)								
n-3 <1.15 g/day	Ref.		Ref.		Ref.		Ref.	
n-3 1.15-1.90 g/day	0.079 (-0.122; 0.281)	0.440	0.111 (-0.090; 0.313)	0.281	0.126 (-0.074; 0.328)	0.218	0.055 (-0.162; 0.272)	0.618
n-3 1.91-2.65 g/day	0.260 (0.056; 0.464)	0.012	0.303 (0.098; 0.508)	0.004	0.321 (0.117; 0.525)	<0.002	0.216 (-0.009; 0.442)	0.061
n-3 2.66-3.4 g/day	0.378 (0.154; 0.603)	<0.001	0.422 (0.197; 0.647)	<0.001	0.439 (0.214; 0.663)	<0.001	0.288 (0.037; 0.540)	0.025
n-3 >3.40 g/day	0.210 (-0.026; 0.447)	0.082	0.254 (0.017; 0.492)	0.036	0.272 (0.352; 0.509)	0.024	0.120 (-0.147; 0.389)	0.377
Trend for quintiles	0.081 (0.036; 0.126)	<0.001	0.089 (0.044; 0.134)	<0.001	0.092 (0.047; 0.136)	<0.001	0.053 (0.000; 0.106)	0.046
Muscle Mass Index (kg/m^2)								
n-3 <1.15 g/day	Ref.		Ref.		Ref.		Ref.	
n-3 1.15-1.90 g/day	0.080 (0.042; 0.117)	<0.001	0.088 (0.050; 0.125)	<0.001	0.084 (0.046; 0.121)	<0.001	0.035 (-0.004; 0.075)	0.082
n-3 1.91-2.65 g/day	0.135 (0.097; 0.173)	<0.001	0.145 (0.107; 0.183)	<0.001	0.140 (0.103; 0.178)	<0.0001	0.037 (-0.003; 0.079)	0.073
n-3 2.66-3.4 g/day	0.174 (0.132; 0.215)	<0.001	0.185 (0.143; 0.226)	<0.001	0.180 (0.139; 0.221)	<0.001	0.042 (-0.003; 0.088)	0.073

n-3 >3.40 g/day	0.188 (0.144; 0.232)	<0.001	0.199 (0.155; 0.243)	<0.001	0.194 (0.151; 0.238)	<0.001	0.050 (-0.001; 0.100)	0.043
Trend for quintiles	0.044 (0.036; 0.052)	<0.001	0.046 (0.038; 0.054)	<0.001	0.045 (0.037; 0.054)	<0.001	0.007 (-0.001; 0.017)	0.115

Results are presented as beta coefficients with corresponding 95% confidence intervals (95% CI) for associations between n-3 fatty acid intake and grip strength or muscle mass. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity. Tests for trend across quintiles reflect the change in grip strength or muscle mass per one-quintile increase in n-3 fatty acid intake.

Table 3.2 The association of n-3 fatty acid intake and hand grip strength and muscle mass in older adult women with physical activity.

Outcomes	**Model 0**		**Model 1**		**Model 2**		**Model 3**	
Grip Strength (kg)	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**
n-3 <1.15 g/day	Ref.		Ref.		Ref.		Ref.	
n-3 1.15-1.90 g/day	0.338 (0.148; 0.529)	<0.001	0.366 (0.176; 0.528)	0.024	0.377 (0.189; 0.566)	<0.001	0.209 (-0.006; 0.424)	0.057
n-3 1.91-2.65 g/day	0.592 (0.329; 0.792)	<0.001	0.626 (0.426; 0.825)	<0.001	0.638 (0.440; 0.837)	<0.001	0.373 (0.139; 0.607)	0.002
n-3 2.66-3.4 g/day	0.859 (0.621; 1.097)	<0.001	0.885 (0.647; 1.123)	<0.001	0.893 (0.656; 1.129)	<0.001	0.549 (0.269; 0.829)	<0.001
n-3 >3.40 g/day	0.764 (0.503; 1.025)	<0.001	0.807 (0.546; 1.067)	<0.001	0.812 (0.553; 1.072)	<0.001	0.372 (0.066; 0.678)	0.017
Trend for quintiles	0.215 (0.161; 0.268)	<0.001	0.222 (0.169; 0.275)	<0.001	0.223 (0.170; 0.276)	<0.001	0.115 (0.051; 0.180)	<0.001
Grip Strength Index (kg/m^2)								
n-3 <1.15 g/day	Ref.		Ref.		Ref.		Ref.	
n-3 1.15-1.90 g/day	0.144 (0.030; 0.258)	0.013	0.158 (0.044; 0.273)	0.006	0.165 (0.518; 0.278)	0.004	0.102 (-0.026; 0.232)	0.121
n-3 1.91-2.65 g/day	0.253 (0.133; 0.373)	<0.001	0.271 (0.151; 0.391)	<0.001	0.278 (0.159; 0.397)	<0.001	0.191 (0.050; 0.332)	0.008
n-3 2.66-3.4 g/day	0.389 (0.246; 0.532)	<0.001	0.403 (0.260; 0.546)	<0.001	0.407 (0.265; 0.549)	<0.001	0.288 (0.119; 0.456)	0.001
n-3 >3.40 g/day	0.341 (0.184; 0.498)	<0.001	0.363 (0.207; 0.520)	<0.001	0.367 (0.211; 0.523)	<0.001	0.204 (0.020; 0.388)	0.029
Trend for quintiles	0.097 (0.065; 0.129)	<0.001	0.101 (0.069; 0.133)	<0.001	0.101 (0.069; 0.133)	<0.001	0.063 (0.024; 0.102)	<0.001
Muscle Mass Index (kg/m^2)								
n-3 <1.15 g/day	Ref.		Ref.		Ref.		Ref.	
n-3 1.15-1.90 g/day	0.072 (0.049; 0.096)	<0.001	0.072 (0.048; 0.095)	<0.001	0.071 (0.047; 0.094)	<0.001	0.035 (0.009; 0.062)	0.008
n-3 1.91-2.65 g/day	0.113 (0.088; 0.137)	<0.001	0.112 (0.087; 0.137)	<0.001	0.111 (0.086; 0.136)	<0.001	0.023 (-0.004; 0.052)	0.104
n-3 2.66-3.4 g/day	0.131 (0.101; 0.160)	<0.001	0.130 (0.101; 0.160)	<0.001	0.130 (0.100; 0.159)	<0.001	0.008 (-0.026; 0.042)	0.640

n-3 >3.40 g/day	0.126 (0.094; 0.158)	<0.001	0.125 (0.093; 0.157)	<0.001	0.125 (0.092; 0.157)	<0.001	-0.005 (-0.043; 0.031)	0.759
Trend for quintiles	0.032 (0.025; 0.038)	<0.001	0.031 (0.025; 0.038)	<0.001	0.031 (0.025; 0.038)	<0.001	-0.005 (-0.013; 0.002)	0.148

Results are presented as beta coefficients with corresponding 95% confidence intervals (95% CI) for associations between n-3 fatty acid intake and grip strength or muscle mass. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity. Tests for trend across quintiles reflect the change in grip strength or muscle mass per one-quintile increase in n-3 fatty acid intake.

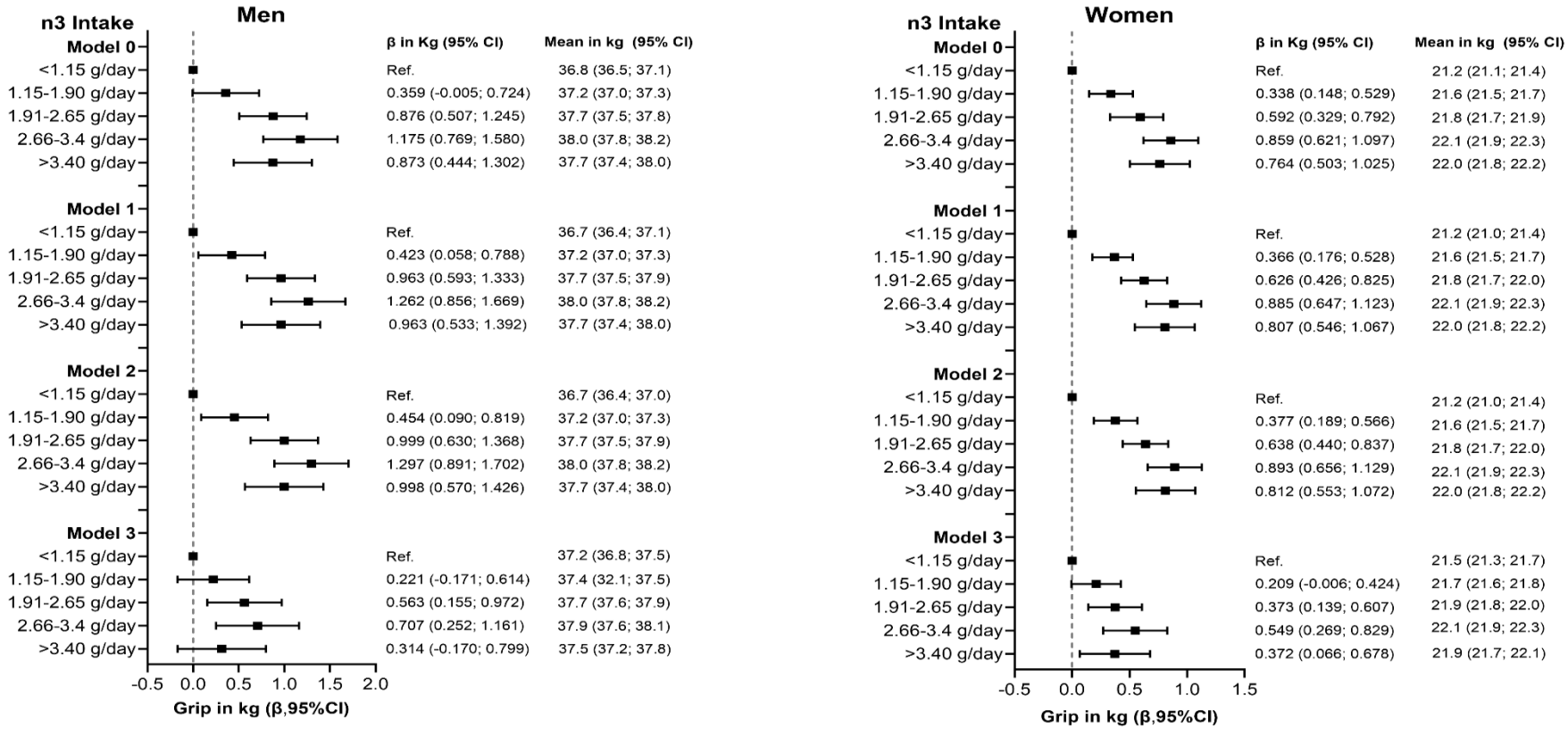

Figure 3-1 Association of n-3 fatty acid intake with handgrip strength.

Data presented as beta coefficients and means with their 95% CI. The reference group was set as the lowest intake of n3 fatty acid (<1.15 g/day). Analysis was unadjusted for Model 0, and Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake), Model 2 additionally was adjusted for multimorbidity, Model 3 further was adjusted for energy intake and physical activity.

3.4.1 Grip strength (kg)

3.4.1.1 n-3 fatty acid intake

The associations between n-3 fatty acid intake grip and strength are shown in **Table 3.1 and Table 3.2 and Figure 3-1.** A significant positive trend was observed across all models, with grip strength increasing across quintiles of n-3 intake in both men and women. The strength of association was consistent from the unadjusted model (Model 0) to the fully adjusted model (Model 2), with beta coefficients ranging from 0.269 to 0.290 in men and 0.215 to 0.223 in women. After further adjustment for energy intake and physical activity (Model 3), the associations were attenuated but remained significant (β = 0.114 in men and 0.115 in women). In absolute terms, grip strength was 1.00 kg higher in men and 0.8 kg higher in women in the highest versus lowest intake quintile. No significant associations were observed among individuals with sarcopenia (Appendix 3-G and Appendix 3-H). When the analysis was restricted to individuals aged 65 and older, no significant associations were observed between n-3 fatty acid intake and grip strength in either men or women, regardless of sarcopenia status (Appendix 3-O and Appendix 3-P).

3.4.1.2 n-6/n-3 fatty acid intake ratio

The associations between the n-6/n-3 fatty acid intake ratio and grip strength are presented in (Appendix 3-I and Appendix 3-J). No significant associations were observed between the n-6/n-3 ratio fatty acid intake ratio and grip strength in men but in women there was a significant negative association (β= –0.081 kg). No significant associations were found among individuals with sarcopenia (Appendix 3-M and Appendix 3-N). In participants aged 65 and older, the n-6/n-3 fatty acid intake ratio was not associated with grip strength in either sex, including those with sarcopenia (Appendix 3-Q and Appendix3-R).

3.4.2 Handgrip strength index

3.4.2.1 n-3 fatty acid intake

Similar patterns were observed when grip strength was expressed as a height-adjusted index (kg/m²), consistent with results based on absolute grip strength **Table 3.1 and Table 3.2 and Figure 3-2.** A significant positive trend across quintiles of n-3 fatty acid intake was found in all models, with beta coefficients for trend ranging from 0.053 to 0.092 kg/m² in men and 0.063 to 0.101 kg/m² in women Table 3.1 and Table 3.2. No associations were observed in individuals with sarcopenia (Appendix 3-G and Appendix 3-H). Among participants aged ≥ 65, no associations were found in either sex, including those with sarcopenia (Appendix 3-O and Appendix 3-P).

3.4.2.2 n-6/n-3 fatty acid intake ratio

The associations between n-6/n-3 fatty acid intake ratio and grip strength index are presented in (Appendix 3-I and Appendix 3-J). There were no associations between n-6/n-3 fatty acid intake ratio and grip strength index in either men or women. Similar, in people with sarcopenia there were no associations in either men or women (Appendix 3-M and Appendix 3-N). In people aged ≥ 65, there were no associations in either men or women, and similar results were observed in people with sarcopenia (Appendix 3-Q and Appendix3-R).

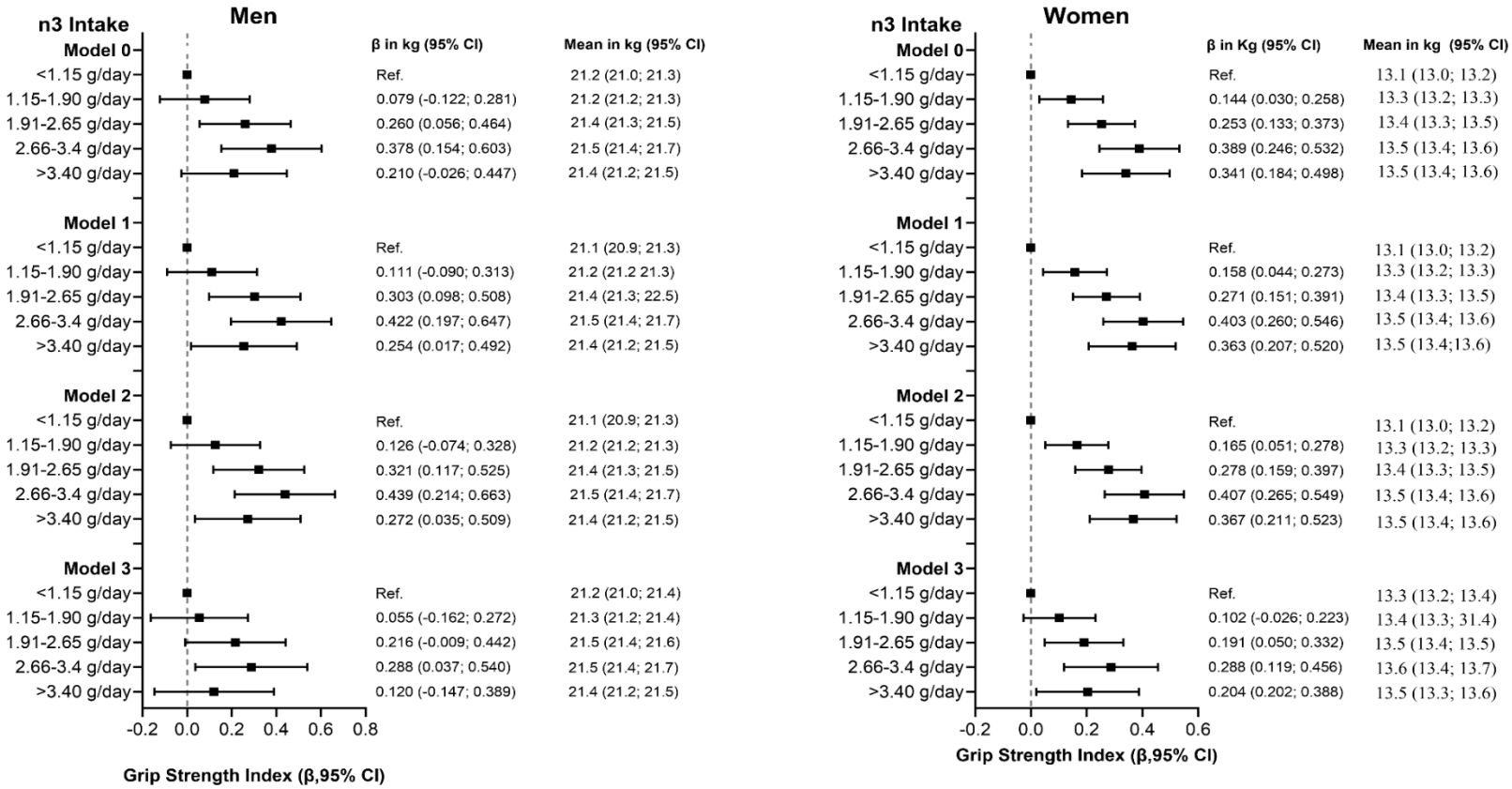

Figure 3-2 Association of n-3 fatty acid intake with handgrip strength index.

Data presented as beta coefficients and means with their 95% CI. The reference group was set as the lowest intake of n3 fatty acid (<1.15 g/day). Analysis was unadjusted for Model 0, and Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake), Model 2 additionally was adjusted for multimorbidity, Model 3 further was adjusted for energy intake and physical activity.

3.4.3 Muscle mass index

3.4.3.1 n-3 fatty acid intake

Results for the muscle mass index are presented in **Table 3.1 and Table 3.2 and Figure 3-3** consistent and significant trend toward higher muscle mass index across increasing quintiles of n-3 fatty acid intake was observed from the unadjusted model (Model 0) through to Model 2 (adjusted for socio-demographic, lifestyle, and health-related covariates), with beta values ranging from 0.044 to 0.046 kg/m² in men and 0.031 to 0.032 kg/m² in women. After full adjustment, including energy intake and physical activity (Model 3), the association was fully attenuated in women (β = –0.005 kg/m²) and men (β = 0.007 kg/m²). Among people with sarcopenia, a positive association was found in men (β = 0.197 kg/m²), with no association in women (β = –0.062 kg/m²) (Appendix 3-G and Appendix 3-H). In participants aged ≥ 65, no associations were observed in either sex, including those with sarcopenia (Appendix 3-O and Appendix 3-P).

3.4.3.2 n-6/n-3 fatty acid intake ratio

No evidence of an association was found between the n-6/n-3 fatty acid intake ratio and muscle mass index in women, but a negative association was seen in men (β = -0.016) (Appendix 3-I and Appendix 3-J). Similarly, no associations were observed among individuals with sarcopenia, either men or women, (Appendix 3-M and Appendix 3-N), or among participants aged ≥ 65 (Appendix 3-Q and Appendix3-R).

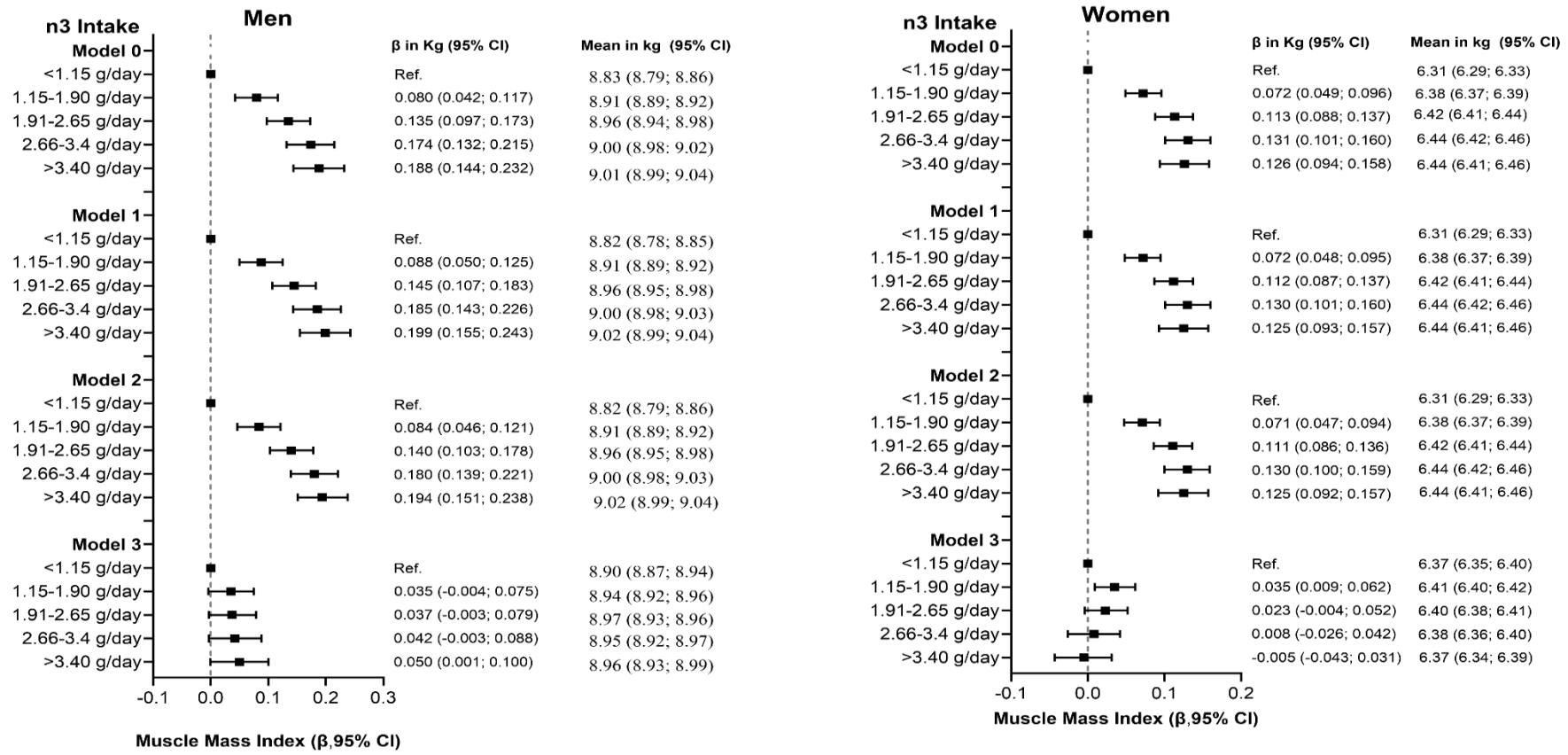

Figure 3-3 Association of n-3 fatty acid intake with muscle mass index.

Data presented as beta coefficients and means with their 95% CI. The reference group was set as the lowest intake of n3 fatty acid (<1.15 g/day). Analysis was unadjusted for Model 0, and Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake), Model 2 additionally was adjusted for multimorbidity, Model 3 further was adjusted for energy intake and physical activity.

3.5 Discussion

This study examined the relationship between n-3 fatty acid intake, and the n-6/n-3 fatty acid intake ratio, and measures of muscle strength and mass in a large sample of 61,381 individuals aged 60 years and older, including a subgroup aged ≥ 65 years. The findings provide valuable insights into the role of both n-3 fatty acid intake and the n-6/n-3 fatty acid intake ratio in muscle health among older adults. A positive association was observed between n-3 fatty acid intake and handgrip strength in both men and women, even after adjusting for key confounders such as socio-demographic, lifestyle, and health-related factors. However, these associations were not present among individuals with sarcopenia. Regarding muscle mass index, n-3 fatty acid intake was not associated with muscle mass in men, or in women, in fully adjusted models, although among those with sarcopenia, a positive association was observed in men. No consistent associations were found between the n-6/n-3 fatty acid intake ratio and muscle mass index or strength, although negative associations were seen with muscle mass index in men and absolute grip strength in women.

There are a few other studies which have examined the relationship between n-3 fatty acid or oily fish intake and muscle strength, with which we can compare our findings. The current data align with findings of Robinson et al, who analysed 2,983 older adults from Hertfordshire, UK and found that each additional portion of fatty fish consumed per week was associated with a higher grip strength of 0.43 kg in men (95% CI: 0.13-0.74) and 0.48 kg in women (95% CI: 0.24-0.72), after adjusting for height, age, and birth weight (Robinson et al., 2008b). Similar results have been found in analysis of the UK Biobank data, where grip strength in both sexes was higher with each

additional portion of oily fish consumed per week (p = 0.0001) (Gedmantaite *et al.*, 2020). While these comparisons are useful, oily fish contains nutrients such as protein and vitamin D and so these data do not isolate the potential role of n-3 fatty acids per se. In contrast, other work in Korea, in people aged 65+, investigated the relationship between the intake of n-3 fatty acids (EPA and DHA) and grip strength finding that in older women, but not men, consuming EPA and DHA at or above an adequate intake had significantly lower odds of low grip strength (OR = 0.777, 95% CI: 0.616-0.979, p = 0.0322), after adjusting for factors like BMI, household income, and smoking status (Shin and Chang, 2023). In contrast, the current study observed a consistent relationship between n-3 fatty acid intake and grip strength in both men and women. Further work in Korea focused on a broader adult population (aged 19+) and included 18,278 participants, finding that higher n-3 fatty acid intake was associated with a reduced odds of low grip strength in both men and women. For men, the odds ratio was 1.42 (95% CI: 1.17-1.72), and for women, it was 1.61 (95% CI: 1.37-1.89), comparing the highest to the lowest quartile of n-3 fatty acid intake (Bae *et al.*, 2022). With the addition of the current data it appears, therefore, that a higher n-3 fatty acid intake is associated with a higher muscle strength in both men and women. The current study extends these findings by demonstrating that there was no clear relationship between n-3 fatty acid intake and grip strength in people with sarcopenia or in those over 65 years of age. This lack of association in those over 65 years and/or those with sarcopenia suggests n-3 fatty acids may help prevent, but not reverse, muscle decline although the magnitude of prevention is likely to be small within the current intake ranges and requires confirmation in appropriately designed trials with no studies currently in people with sarcopenia.

Indeed, the current study and other works suffer the limitation that they are cross sectional in design and so causality cannot be determined, but the associations in the general older adult

population are supported by previous RCTs, using supplementary n-3 fatty acids, in this area. Indeed, a randomized controlled trial, in older adults, showed a 2.3 kg (95% CI: 0.8 to 3.7 kg; $P < 0.01$) increase in handgrip strength after six months of fish oil supplementation (Smith *et al.*, 2015). Similarly, a study reported a 10.9% (95% CI: 8.3 to 13.6%) increase in grip strength in older adults after six months of krill oil supplementation (Alkhedhairi *et al.*, 2022). A meta-analysis further supported these findings, showing significant improvements in grip strength with n-3 fatty acid supplementation among healthy individuals (Ma *et al.*, 2021). There are no RCTs, to our knowledge, of n-3 fatty acid treatment in people with sarcopenia a research gap that needs to be filled. These RCTs all used a daily dose of n-3 fatty acid of around 4g/day, which is far in excess of the dietary intakes of n-3 fatty acid observed in the current study and is also primarily EPA and DHA rather than other n-3 fatty acids. This likely explains the lower effect size seen in the current study cross-sectional data compared to the data from RCTs. Indeed, the minimal clinically important difference (MCID) for grip strength has been estimated to be around 5.0–6.5 kg (Kim *et al.*, 2014b). This suggests that the direct impact of changes in n-3 fatty acid intake, within the normally dietary range, on muscle function is limited, though even small gains may still be relevant for overall health and ageing.

In contrast to the data on grip strength, the evidence for an effect of n-3 fatty acid on muscle mass is more ambiguous. In our study no association was observed between n-3 fatty acid intake and muscle mass index in men or in women. This disagrees with findings from previous research using more robust methodologies to quantify muscle mass, which reported that n-3 fatty acid may improve muscle mass (Therdyothin *et al.*, 2023). For example, a meta-analysis found n-3 fatty acid supplementation positively influenced muscle mass and walking speed in older adults, particularly at doses exceeding 2 g/day (Huang *et al.*, 2020). Another study reported a 3.6% increase in thigh

muscle volume, measured using MRI, after 6 months of fish oil supplementation in older adults (Smith *et al.*, 2015). Another study, despite finding no significant effect of 6 months krill oil supplementation in older adults on muscle mass, measured using bioelectrical impedance analysis (BIA), observed an increase in vastus lateralis muscle thickness, measured via ultrasound measurements (Alkhedhairi *et al.*, 2022). Much of the ambiguity in our findings and the wider literature may be as muscle mass is estimated by BIA. While BIA is commonly used for body composition assessment, it has limitations, including sensitivity to hydration status and potential accuracy issues, which should be considered when interpreting the results (Pinedo-Villanueva *et al.*, 2019). In sensitivity analyses among adults aged ≥ 65 years, no associations were observed between n-3 fatty acid intake and muscle mass in either sex, including individuals with sarcopenia, although as mentioned previously participant numbers were low in these analyses. A positive association was, however, noted in men, but not women, aged over 60 years with sarcopenia, tentatively indicating a potential therapeutic effect in this population. This is worthy of follow up in subsequent appropriately designed trials.

Previous research has indicated the importance of the n-6/n-3 fatty acid intake ratio in chronic disease prevention (Simopoulos, 2002), but its role in sarcopenia is less well explored. In the current study, we found no consistent associations between the n-6/n-3 fatty acid intake ratio and muscle strength or muscle mass in either men or women, although relatively weak negative associations were seen with muscle mass index in men and with grip strength in women. No other associations were observed across both age groups (≥ 60 and ≥ 65 years), including individuals with sarcopenia. These findings are broadly consistent with prior studies that also reported no significant associations between the n-6/n-3 fatty acid intake ratio and muscle health or sarcopenia status (Jang *et al.*, 2020, Zhang *et al.*, 2021, Dupont *et al.*, 2023, Das *et al.*, 2021). Overall, our

results contribute to the growing body of evidence suggesting that the n-6/n-3 fatty acid intake ratio may not have a meaningful impact on muscle health in older adults, regardless of sex, although the associations that were observed may be worthy of further follow up.

The mechanisms by which n-3 fatty acids might influence muscle strength, but not necessarily muscle mass, are multifaceted. For example, n-3 fatty acid, particularly DHA, play a crucial role in supporting neural function by maintaining membrane phospholipid integrity, which is essential for receptor activity and signal transduction (Shin and Chang, 2023, Hayman *et al.*, 2024). Improved muscle power and excitability through increased electromyography and M-wave, a measure of excitability, measurements have been observed in younger adults following n-3 fatty acid supplementation (Lewis *et al.*, 2015, Jammes *et al.*, 2020). Furthermore, after 6 months krill oil supplementation in older adults M-wave was increased by 17.4% in the krill oil group compared to the control group (Alkhedhairi *et al.*, 2022). There is also evidence that n-3 fatty acid may contribute to improved mitochondrial function, enhanced blood flow, and anti-inflammatory effects, all of which could support muscle strength (Gray and Mittendorfer, 2018). On top of this, n-3 fatty acids may help prevent loss of muscle strength due to their anti-inflammatory effects while n-6 fatty acids can potentially promote muscle loss by triggering inflammation. Indeed, metabolites of n-6 fatty acids are linked to increased levels of pro-inflammatory markers such as (IL-1), (IL-6), (TNF), and (CRP) (Zhang *et al.*, 2021, Farvid *et al.*, 2014, DiNicolantonio and O'Keefe, 2018). These markers tend to rise with higher n-6 fatty acid intake and decrease with greater n-3 fatty acid intake, highlighting the potential importance of the balance in n-6 and n-3 fatty acid intake. Despite these differing inflammatory effects, these are not reflected in consistent associations of the n-6/n-3 fatty acid intake ratio with muscle strength or mass in the current data.

It is prudent to consider the strengths and limitations of the current study. The large sample size enhances the statistical power of the findings and the inclusion of both men and women provides a comprehensive understanding of sex-specific effects, and the adjustment for various confounding factors strengthens the internal validity of the study. Despite its strengths, the study has notable limitations. Our subgroup analysis in people with sarcopenia and those over 65 years of age must be treated with caution as the participants numbers were, however, low. The use of self-reported dietary data for n-3 and n-6 fatty acid intake may introduce measurement errors, affecting the accuracy of the observed associations (Petermann-Rocha *et al.*, 2022b), and does not allow us to understand the contribution of specific n-3 and n-6 fatty acids. Additionally, the cross-sectional nature of the study design prohibits the establishment of causation and the potential for residual confounding and reverse causation cannot be entirely ruled out. While the study benefits from the large cohort of the UK Biobank, caution is needed when generalizing these findings to broader populations. Although we included adults aged 60 and above, a sensitivity analysis restricted to those aged ≥ 65 showed no major differences in findings, reinforcing the robustness of the results, although in our sensitivity analysis participant numbers were often low. Finally, the characteristics of UK Biobank participants, who are typically healthier and less diverse than the general population, limit the study's external validity (Fry *et al.*, 2017). However, this demographic skew is unlikely to significantly affect the relationships between the variables being studied, meaning the observed associations within the cohort remain robust. The current analysis, although performing stratified analysis by sex, did not formally test for sex differences which may be present. There remains the possibility that current population-based recommendations for n-3 fatty

acid intake may not account for sex-specific physiological differences, suggesting that sex-specific recommendations for n-3 fatty acid intake may need further investigation.

3.6 Conclusion

In summary, the current data demonstrated a relationship between n-3 fatty acid intake and higher grip strength, although the magnitude of association was small, with no clear pattern of associations found with n-6/n-3 fatty acid intake ratio. This suggests that within the typical dietary range, n-3 fatty acid consumption may not offer significant public health benefits for improving muscle strength, and higher dose supplementation strategies with EPA and DHA specifically may be needed to realise the benefits of increased n-3 fatty acid intake.

Chapter 4: A comparison of the effects of krill oil and fish oil supplementation on muscle function in older adults: a randomised controlled pilot study

4.1 Abstract

4.1.1 Aims

This pilot study aimed to compare the effects of krill oil and fish oil supplementation on muscle function in older adults.

4.1.2 Methods

This double-blind, randomised controlled trial included 60 older adults aged ≥ 60 years, who were assigned (1:1:1) to krill oil (4 g/day), fish oil (4 g/day), or placebo (vegetable oil, 4 g/day) for 16 weeks. Supplements were matched with the aim of achieving similar EPA and DHA increases in red blood cells. Assessments of muscle function and body composition were conducted at baseline and 16 weeks.

4.1.3 Results

The study showed that fish oil supplementation significantly improved knee extensor strength by 11.6 Nm (95% CI, 0.5 to 22.7, p = 0.01) and handgrip strength by 2.2 kg (95% CI, 0.2 to 4.2, p = 0.007) compared to placebo. Both krill oil and fish oil enhanced gait parameters: cadence improved by 5.3 steps/min (95% CI, 1.2 to 9.4, p = <0.001) with fish oil and 4.0 steps/min (95% CI, 0.1 to 8.0, p = 0.01) with krill oil, while velocity increased by 0.09 m/s (95% CI, -0.003 to 0.184) with fish oil and 0.066 m/s (95% CI, -0.023 to 0.156) with krill oil. No significant effects were observed in body composition or the 30-second sit-to-stand test.

4.1.4 Conclusion

This double-blind, randomised controlled trial adds to existing evidence that LCn-3 PUFA supplementation is a safe and effective approach to help preserve muscle function and mobility in older adults, with fish oil showing greater benefits for muscle strength measures, while both fish oil and krill oil similarly enhanced gait parameters.

4.1.5 Registration

ClinicalTrials.gov ID: NCT05869526. Date of Registry: 11/05/2023.

4.1.6 Keywords

Muscle strength, LCn-3 PUFA supplementation, muscle mass, older adults.

4.2 Introduction

Preserving skeletal muscle throughout the aging process is fundamental to maintaining independence and quality of life in later years. The progressive deterioration of muscle tissue that occurs with advancing age is associated with substantial functional limitations and elevated risk of adverse health events (Cruz-Jentoft et al., 2019b, Santilli et al., 2014). This condition imposes considerable economic strain on healthcare systems, with annual costs attributed to age-related muscle weakness reaching £2.5 billion in the United Kingdom (Pinedo-Villanueva et al., 2019) and $20 billion in the United States. As demographic shifts continue to increase the proportion of older adults worldwide, with the global share of individuals aged 65 years and over projected to rise from approximately 10% in 2022 to 16% by 2050, the imperative to identify effective interventions has intensified (United Nations, 2022).

To date, pharmaceutical approaches for preventing or reversing age-related muscle deterioration remain unavailable (Cruz-Jentoft et al., 2019b). Progressive resistance training has demonstrated efficacy in enhancing muscle mass and functional capacity across all age groups, including individuals in their tenth decade of life (Fiatarone *et al.*, 1990), however, the adaptive response to mechanical loading is diminished in older versus younger adults, a phenomenon attributed to anabolic resistance (Greig *et al.*, 2011). Moreover, uptake of structured resistance training programs among older populations remains suboptimal (Strain *et al.*, 2016), limiting the population-level impact of exercise-based approaches. These constraints underscore the need for adjunctive strategies that may amplify the muscle-building response to physical training.

Alterations in nutrition have, therefore, been investigated for therapeutic use in the prevention and treatment of sarcopenia. Epidemiological data showed that fatty fish consumption is positively associated with grip strength in older men and women (Robinson et al., 2008b). Expanding on this, in chapter 3, analysis of the UK Biobank data found a positive association between LCn-3 PUFA intake and handgrip strength in older men and women after adjusting for confounding factors (deprivation index, ethnicity, multimorbidity, month of assessment, sex, total energy intake, age, and lifestyle factors). Building on this cross-sectional research, a study found that consumption of 4 g of fish oil-derived LCn-3 PUFA per day for 8 weeks, in healthy older adults, increased the muscle protein synthesis response to an hyperinsulinaemic-euglycaemic clamp (Smith *et al.*, 2011a). Building on this acute data, it has been shown, in a double-blind RCT, that 6 months of LCn-3 PUFA supplementation (n = 29 in LCn-3 PUFA and n = 15 in placebo group), with maintained habitual physical activity levels, increased muscle volume and strength (Smith *et al.*, 2015). Our meta-analysis and systematic review in chapter 2 supported these findings, which indicating that LCn-3 PUFA supplementation significantly improves muscle strength in older adults.

Most studies in this area have focused on fish oils as the source of LCn-3 PUFA. However, krill oil can be considered as an alternative rich source of LCn-3 PUFA. There is a potential for krill oil to be of greater benefit than fish oil, as it contains choline and astaxanthin, which may potentially have beneficial effects on muscle (Moretti *et al.*, 2020, Wong *et al.*, 2020). On top of this, previous supplementation studies have shown comparable increases in plasma EPA and DHA

levels with both krill oil and fish oil, despite the lower EPA and DHA content in krill oil supplements (Ulven *et al.*, 2011). Additionally, when the amounts of EPA and DHA in the supplements were equivalent, krill oil supplementation resulted in a greater increase in the omega-3 index compared to fish oil supplementation (Ramprasath *et al.*, 2013).

A recent randomized controlled trial study investigated the effect of krill oil supplementation on muscle mass and physical function in active older adults (Alkhedhairi *et al.*, 2022). This study found that krill oil supplementation increased muscle mass (measured by vastus lateralis muscle thickness) and strength (measured by knee extensor maximal torque, grip strength) significantly ($p < 0.05$) in healthy older adults (Alkhedhairi *et al.*, 2022). The treatment effects of six months were 9.3% (95%CI: 2.8, 15.8%) in knee extensor maximal torque, 10.9% (95%CI: 8.3, 13.6%) in grip strength and 3.5% (95%CI: 2.1, 4.9%) in vastus lateralis muscle thickness (Alkhedhairi *et al.*, 2022). In the aforementioned fish oil study of Smith et al (Smith *et al.*, 2015), it was shown that 6 months of fish oil supplementation increased thigh muscle volume (treatment effect at 6 month: 3.6%; 95% CI: 0.2%, 7.0%; $P < 0.05$), handgrip strength (2.3 kg; 95% CI: 0.8, 3.7 kg; $P < 0.01$ - ~around 7%), and 1-RM muscle strength (4.0%; 95% CI: 0.8%, 7.3%; $P<0.05$) (Smith *et al.*, 2015). This data comparison indicates that krill oil may have a slightly greater effect than fish oil, although this has not been to be tested in a direct head-to-head study.

Therefore, the current pilot study aimed to directly compare the effects of krill oil and fish oil supplementation on muscle function in older adults.

4.3 Materials and methods

This study is reported following the Consolidated Standards of Reporting Trials (CONSORT) Checklist (Moher et al., 2010).

4.3.1 Trial design

The current study was a double blind randomised controlled trial with older adults (women and men) (≥ 60 years) recruited and randomly assigned, following baseline assessment, stratified by sex to one of three groups: krill oil supplement group (KO), fish oil supplement group (FO) or placebo supplement group (vegetable oil) for the 16-week intervention period. Allocation was carried out in a 1:1:1 ratio with randomisation by an independent colleague using online software (sealedenvelope.com). Blinding was assessed at the end of the intervention by asking participants to guess which supplement they had been assigned to. The trial was registered at clinicaltrials.gov (NCT05869526). Study visits took place at baseline and 16 weeks. The placebo group received 4g/day of vegetable oil supplement capsules. The krill oil group received 4g/day of krill oil supplements, and the fish oil group received 4g/day. Krill oil and fish oil supplements were matched in an attempt to result in similar increases in EPA and DHA in red blood cells, based on previous work (Ulven *et al.*, 2011). The study was approved by the University of Glasgow Medical, Veterinary, Life Sciences College Research Ethics Committee [Project No: 200220105]. The study adhered to the principles of the Declaration of Helsinki, and all participants provided written informed consent.

4.3.2 Participants

Sixty older adult participants were enrolled in this study between May 2023 and March 2024 in Glasgow, UK. They were recruited through older adults' societies, churches, posters, social media platforms such as Facebook, Yammer, and Nextdoor, as well as newspaper/magazine advertisements. The following inclusion/exclusion criteria were used.

Inclusion criteria: Older adults (aged 60 years or older). BMI less than 30kg/m^2.

The age threshold of ≥60 years aligns with the World Health Organization and United Nations definition of older adults established through the UN Decade of Healthy Ageing (2021–2030) (WHO, 2024). This age corresponds with accelerated muscle mass decline (Volpi et al., 2004, Harvard Health Publishing, 2023). The prevalence of sarcopenia increases substantially after age 60, affecting 10–27% of this population globally (Petermann-Rocha et al., 2022a, Larsson et al., 2019), making this threshold appropriate for investigating dietary factors that may preserve muscle health.

Exclusion criteria: Participants diagnosed and being treated for a pre-existing medical condition, cancer, kidney disease, liver disease, diabetes mellitus and cardiovascular disease, uncontrolled hypertension, gait disturbances limiting the ability to perform assessments of muscle function, dementia, taking drugs known to affect muscle (e.g., steroids) were excluded. People with a history of allergy to fish or fish oil and regular consumption of more than 1 portion of oily fish per week or use of fish oil or krill oil supplements were also excluded.

The inclusion/exclusion criteria were evaluated using a health screening questionnaire completed (with the researcher's assistance) (Appendix 4-B) prior to the consent (Appendix 4-C), as well as BMI and blood pressure measurements.

Participants attended study visits prior to beginning supplementation at baseline and 16 weeks (study completion).

4.3.3 Intervention

The supplementation period was 16 weeks, with participants instructed to maintain their normal dietary (other than the supplements) and physical activity habits. The control oil group consumed 4g/day of mixed vegetable oil (a mixture of olive oil (extra virgin, cold pressed), maize oil (refined), palm kernel oil (refined) and medium chain triglycerides, in the ratio 4:4:3:2). The total LCn-3 PUFA content of the control supplement was 4mg/g, with <1mg/g EPA and DHA, although we do not have the fatty acid breakdown in more detail. The fatty acid composition of this mixture was made in order to give a fatty acids ratio similar to the fatty acids in a normal, European diet (Bull et al., 1983). The krill oil group consumed 4g/day krill oil (SuperbaBoostTM) with 193mg/g EPA and 96mg/g DHA with each 1g capsule also containing 79mg choline. The fish oil group consumed 4g/day fish oil with 230mg/g EPA and 120mg/g DHA. The capsules were similar in look and taste **(Figure 4-1).**

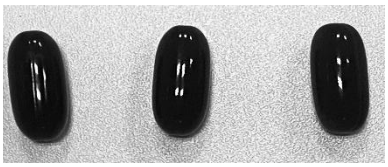

Figure 4-1 Supplements included in the study**.**

4.3.4 Outcome measures

The international physical activity questionnaire used to quantify habitual physical activity (short-form) at baseline (Appendix 4-D). The primary outcome was the change in knee extensor muscle strength from baseline to 16 weeks. Secondary outcomes were the change in handgrip strength, muscle thickness, STS test, walking test, blood lipids, and erythrocyte fatty acid profiles and body composition. The international physical activity questionnaire (IPAQ) used to quantify habitual physical activity (short-form) at baseline and 16 weeks. Measurements were made by the lead researcher (MT), who was masked to group allocation, at baseline and 16 weeks (study completion), unless otherwise stated. Capsule count at the end of the study used to assess compliance.

4.3.4.1 Muscle strength

The muscle strength of the knee extensor maximal torque during a maximal voluntary contraction (MVC) was measured using an isometric dynamometer with force recorded via a load cell (Biometrics Ltd, Newport, UK). Participants were positioned and strapped in a chair with their legs fixed at a 90° angle. A strap was placed around the right ankle connected to a force transducer. Participants were asked to perform a minimum of three contractions for 5 seconds as hard as

possible with a minimum of 1 minute rest between each attempt. If the 3rd attempt was >10% higher than the 2nd attempt, indicative of a learning effect, then further attempts were made. The highest recorded value was used for analysis. Grip strength was measured using a Jamar dynamometer, with participants asked to perform 3 contractions from each hand. Participants were seated with their elbow positioned at a 90° angle, supporting the arm while gripping the dynamometer with maximal effort. The average value from both hands was used in the analysis.

4.3.4.2 Muscle size

An ultrasound imaging device (Echoblaster 128 Ext; Telemed Ltd, Vilnius, Lithuania) was used to assess muscle thickness of the right vastus lateralis (VL) muscle with images collected at the midpoint of the thigh (50% of the difference between the trochanterion and tibiale laterale) with the participants in a supine position.

4.3.4.3 Functional abilities

The 4m walk test were carried out using the Gaitrite® electronic walkway system (CIR Systems Inc., Clifton, NJ, USA), a 4.6-meter-long and 61-cm-wide mat embedded with numerous sensors and connected to a computer for data collection. This system allows characterisation of gait with the parameters: cadence, velocity, step length, stride length, stance time, and swing time, which are recorded. Participants were instructed to walk across the mat at their own pace and then return to the starting point at the beginning of the mat. A 30-second sit-to-stand test (STS) was also carried out on a chair, with participants asked to sit in a chair with their arms crossed over their chest and rise to a full stand position, then sit back down again and repeat this as many repetitions

as they could within the 30-second time period. The number of full repetitions was recorded and used for analysis.

4.3.4.4 Body composition

Total fat and lean mass were measured using the Tanita TBF-300 bioelectrical impedance scale to quantify fat mass and fat free mass. Participants were asked to remove all metal objects and shoes before measurement.

4.3.4.5 Blood sample

A venous blood sample was collected from an antecubital vein by a butterfly needle and collected at baseline, and week 16. Blood samples were stored at −80 °C and shipped for analysis by an external company (Omegaquant) for erythrocyte fatty acid profile analysis. Samples were analysed using a GC-2030 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) with an SP-2560 fused silica capillary column (100 m × 0.25 mm ID).

Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of RBC (GLC OQ-A, NuCheck Prep, Elysian, MN) which was also used to construct individual fatty acid calibration curves. The following 24 fatty acids (by class) were identified: saturated (14:0, 16:0, 18:0, 20:0, 22:0 24:0); cis monounsaturated (16:1, 18:1, 20:1, 24:1); trans (16:1, 18:1∗, 18:2∗ - see below for more details); cis n-6 polyunsaturated (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5); cis

n-3 polyunsaturated (18:3, 20:5, 22:5, 22:6). Fatty acid composition was expressed as a percent of total identified fatty acids. The omega-3 index is defined as the sum of 20:5n-3 (EPA) and 22:6n-3 (DHA) adjusted by a regression equation (r = 0.97) to predict the omega-3 index in the RBC. Omega-6: Omega-3 ratio is calculated by dividing the sum of seven omega-6 fatty acids by the sum of four omega-3 fatty acids in whole blood. The Trans Fat Index is the percent of 18:1 and 18:2 trans fatty acids of total fatty acids in red blood cell membranes.

∗The chromatographic conditions used in this study were sufficient to isolate the C16:1 trans isomers and the C18:2 Δ 9t-12c, 9t-12t, and 9c-12t isomers; the latter is reported as C18:2n6t. However, each individual C18:1 trans molecular species (i.e., C18:1 Δ 6 thru Δ 13) could not be separated but appeared as two blended peaks that eluted just before oleic acid. The areas of these two peaks were summed and referred to a C18:1 trans.

4.3.4.6 Nutritional intake

Habitual diet characterized by using a modified version of the food frequency questionnaire (EPIC food frequency questionnaire) at baseline, and study end (Appendix 4-G). For this study, the EPIC FFQ was modified to assess dietary intake over the previous month (4 weeks) rather than the standard 12-month recall period. Physical activity was assessed using IPAQ. Total physical activity was expressed as MET-minutes per week, calculated by multiplying minutes per week of walking, moderate, and vigorous activity by their corresponding metabolic equivalent values (3.3, 4.0, and 8.0 METs, respectively) and summing the results.

4.3.7 Sample size

The current study is a pilot study to provide data to support (or refute) a larger trial comparing krill oil and fish oil, with the primary outcome of muscle strength (knee extensor maximal torque). With 16 participants in each group, the study was able to detect a difference of 1SD (~15Nm) in knee extensor maximal torque. To account for dropout, the aim was to recruit 20 participants to each group. To fully power such a study, based on knee extensor maximal torque with an estimated minimally clinically important difference (MCID) of between 4 and 6% (Ruhdorfer et al., 2015), with an SD of 9% we would require a sample size of 50 participants per group (80% power at $P < 0.05$).

4.3.8 Statistical analysis

Statistical analysis was performed blinded to treatment allocation using SPSS 29.0.1.0, analysis of covariance (one-way ANCOVA) statistical test was conducted to evaluate differences using the 16-week post intervention outcomes measured as the dependent variable, supplement group as the independent variable and the baseline outcomes measured as a covariate. Where a significant effect was noted in the ANCOVA post-hoc pairwise comparisons were carried out to compare individual groups at 16 weeks. All data were tested for normality and skewness before selecting the appropriate test. $p < 0.05$ was considered statistically significant. Analysis was performed as intention to treat.

4.4 Results

4.4.1 Participant characteristics

A total of 60 men and women were enrolled in the study (NCT05869526) between May 2023 and March 2024 **(Figure 4-2).** After randomisation, 7 participants withdrew from the study. At follow-up, 53 participants (krill oil group; 12 women and 8 men, fish oil group; 8 women and 9 men, and placebo group; 8 women and 8 men) completed the study and were included in the analysis. Adherence to the supplementation protocol was confirmed via leftover pill counts, with average adherence of 91% in the fish oil group, 88% in the krill oil group, and 81% in the control group. Additionally, only 5 participants correctly guessed which supplement they had been assigned to, suggesting a successful blinding procedure. Participant characteristics are presented in **Table 4.1.** There were no major differences between groups at baseline seen for any of the outcome variables measured.

Table 4.1 Baseline demographic characteristics of the participants in control, krill oil groups and fish oil groups at baseline.

	Krill Oil Group (n=20)	Fish Oil Group (n=17)	Control Oil Group (n=16)
Age	66 (3.69)	70 (7.57)	68 (8)
Sex	Male (n=8) Female (n=12)	Male (n=9) Female (n=8)	Male (n=8) Female (n=8)
Height (cm)	169 (10.49)	168 (10.08)	168 (9.45)
Systolic BP (mmHg)	128 (11.27)	126 (13.30)	123 (12.22)
Diastolic BP (mmHg)	77 (8.62)	73.64 (6.77)	72.37 (8.14)
Weight (kg)	68.84 (12.47)	71.04 (14.18)	66.28 (12.44)
a. BMI (kg/m2)	24.03 (2.29)	24.77 (2.68)	23.23 (3.52)
Fat Mass (kg)	18.63 (4.99)	19.20 (5.63)	17.46 (6.95)
Fat Percentage	27.50 (7.20)	27.32 (7.14)	25.91 (8.11)

Fat Free Mass (kg)	50.21 (12.23)	51.83 (12.83)	48.81 (9.33)
Muscle Thickness (mm)	19.75 (3.53)	19.82 (4.51)	19.30 (4.29)
Torque (Nm)	108.76 (28.19)	114.58 (43.15)	101.88 (38.67)
Grip Strength (kg)	26.9 (7.88)	30.96 (10.56)	29.26 (8.69)
b. STS (n)	14.45 (1.84)	15.41 (2.37)	14.93 (3.04)

* Baseline demographics characteristics Data are presented as mean (SD).

a. BMI: Body Mass Index.

b. STS: 30-second sit -to -stand test.

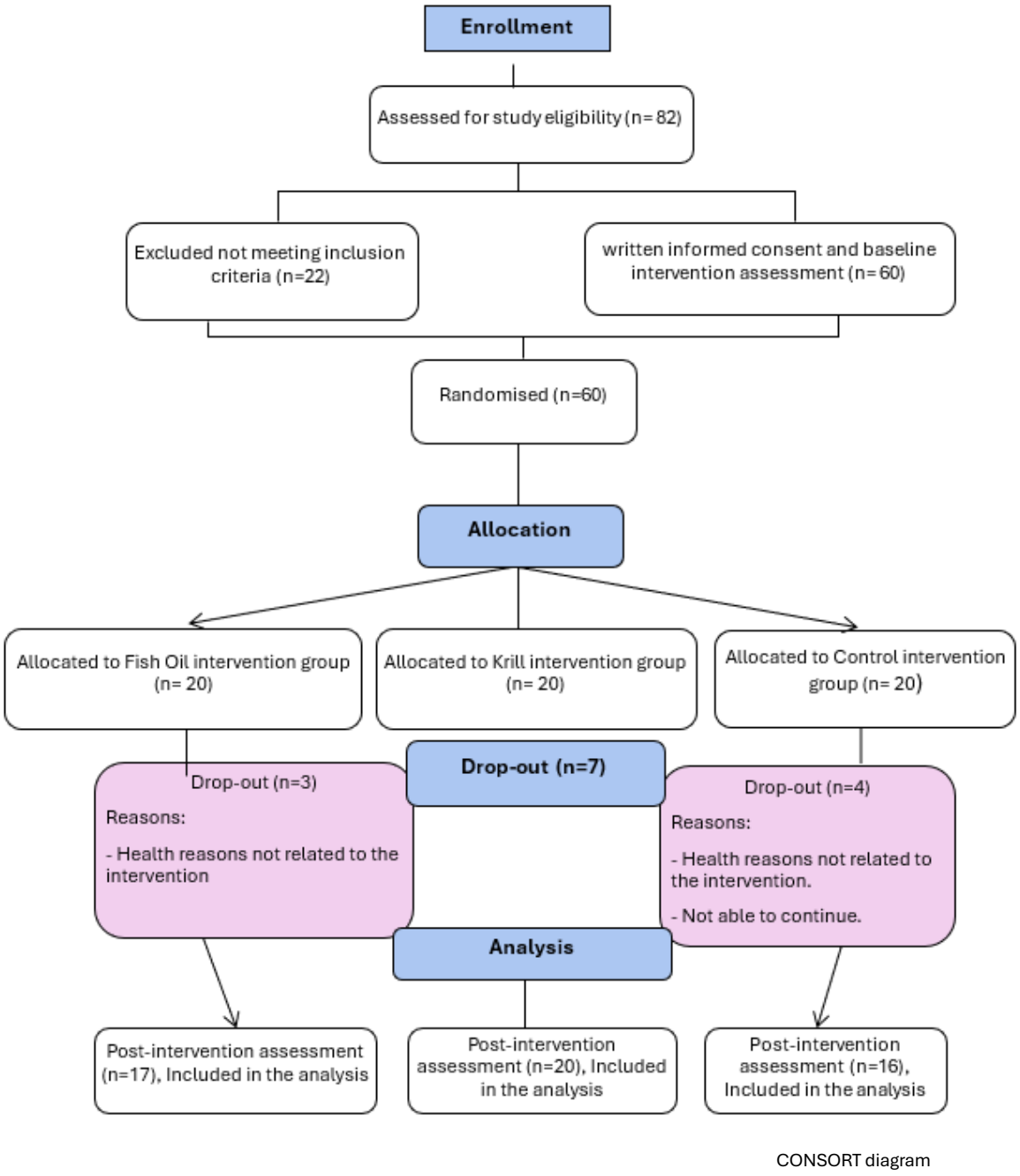

Figure 4-2 Flow diagram illustrating the participant progress through the phases of the study, (participant recruitment, randomisation, dropout, and analysis).

4.4.2 Nutritional intake

Nutritional intake data are presented in **Table 4.2.** No main effect was seen between the three intervention groups in the ANCOVA ($p > 0.05$). Similarly, physical activity did not differ significantly between groups at 16 weeks (ANCOVA $p = 0.696$).

4.4.3 Blood results

The erythrocyte fatty acid composition data are presented in **Table 4.3.** In the ANCOVA, significant main effects ($p < 0.001$), were observed for EPA, DHA, DPA, the omega-3 index, Linoelaidic Acid, alpha-Linolenic Acid, Eicosadienoic Acid, Dihomo-g-linolenic Acid, Arachidonic Acid, Docosatetraenoic Acid, Docosapentaenoic Acid, the Omega 6:3 Ratio, the AA: EPA Ratio, total omega-3 and total omega-6. No differences in other fatty acids were noted.

Relative to control, at 16 weeks, EPA was 2.03% (95% CI, 1.26 to 2.79%, p = <0.001) and 1.94 % (95% CI, 1.22 to 2.67%, p = <0.001) higher in the fish oil and the krill oil groups, respectively. No differences between the fish oil and krill oil groups were noted. Relative to control, at 16 weeks DPA was 0.67% (95% CI, 0.34 to 0.99%, p = <0.001) and 0.47 % (95% CI, 0.16 to 0.79%, p = <0.001) higher in the fish oil and in the krill oil groups, respectively. No differences between the fish oil and krill oil groups were noted. Relative to control, at 16 weeks, DHA was 0.95 % (95% CI, 0.18 to 1.71%, p = <0.001) and 1.34 % (95% CI, 0.62 to 2.05 %, p = <0.001) higher in the fish oil and the krill oil groups, respectively. No differences between the fish oil and krill oil groups were noted. Relative to control, at 16 weeks, the omega-3 index was 2.53 % (95% CI, 1.23 to

3.83%, p = <0.001) and 3.24 % (95% CI, 2.03 to 4.44 %, p = <0.001) higher in the fish oil and the krill oil groups, respectively. No differences between the fish oil and krill oil groups were noted.

Relative to the control group, at 16 weeks, total omega-3 was higher in the krill and fish oil group. On the other hand, at 16 weeks, relative to the control group alpha-linolenic acid, Dihomog-linolenic acid, Docosapentaenoic-n6 acid, Docosatetraenoic acid, Omega 6-3 ratio, and total omega-6 acid were lower in the krill and fish oil groups. No differences between the fish oil and krill oil groups were noted, except for Arachidonic Acid, which was 1.10% (95% CI, 0.30 to 2.00%, p = <0.001) lower in the krill relative to the fish oil group.

Table 4.2 Dietary intake and physical activity in control, krill oil and fish oil groups at baseline, and after 16 weeks.

Outcomes Variables	Krill Oil Group (n=20)			Fish Oil Group (n=17)			Control Oil Group (n=16)			b. Sig (ANCOVA)
	Pre	Post	a. Mean Difference and 95%CI	Pre	Post	a. Mean Difference and 95%CI	Pre	Post	a. Mean Difference and 95%CI	
Energy (kcal/day)	2020 (940.48)	1708 (569.49)	312 (45.41 to 578.48)	1621 (526.03)	1408 (537.83)	213 (-63.37 to 489.68)	2298 (798.05)	1945 (795.34)	353 (-70.24 to 777.37)	.705
Carb (g/day)	247.21 (130.07)	203 (77.88)	-44 (-84.58 to -4.07.)	182 (51.77)	153 (60.96)	-28 (-54.04 to -3.23)	256 (94.40)	223 (78.95)	-34 (-69.72 to 2.11)	.285
Protein (g/day)	84 (26.26)	76 (21.26)	-7 (-17.89 to 3.18)	70 (20.05)	63 (20.25)	-7 (-17.57 to 3.56)	92 (28.15)	84 (31.89)	-8 (-18.64 to 2.76)	.588
Fat (g/day)	79 (42.15)	67 (26.66)	-12 (-23.86 to -0.15)	65 (25.52)	59 (25.33)	-6 (-15.80 to 3.08)	102 (45.73)	81 (46.58)	-21 (-41.12 to -0.86)	.998
Fish intake (g/day)	51 (29.42)	61 (40.21)	10 (-9.11 to 29.55)	45 (35.78)	54 (41.20)	8 (-7.58 to 25.21)	51 (26.66)	55 (42.47)	4 (-12.51 to 21.97)	.896
Total IPAQ (MET-min/week)	4017.8 (2716.37)	4451.95 (3429.30)	434.15 (-1051.19 to 1919.49)	4638.70 (4376.23)	4157.118 (3052.81)	-481.58 (-1991.22 to 1028.04)	3272.25 (2004.35)	3295.438 (3519.96)	23.18 (-1971.96 to 2018.33)	0.696

Differences in dietary intake and physical activity data at baseline and 16 weeks. Data are presented as mean (SD). * Denotes a significant difference from control ($p<0.05$).

a. Adjusted mean difference for the post-outcome variable when controlling the pre-outcome variable.

b. Significant difference for the ANCOVA.

Table 4.3 Fatty acid composition in control, krill oil and fish oil groups at baseline, and after 16 weeks.

Outcomes Variables	Krill Oil Group (n=20)			Fish Oil Group (n=17)			Control Oil Group (n=16)			b. Sig (ANCOVA)
	Pre	Post	a. Mean Difference and 95%CI	Pre	Post	a. Mean Difference and 95%CI	Pre	Post	a. Mean Difference and 95%CI	
EPA (%)	1.09% (0.38%)	3.00*% (1.05%)	1.91% (1.42 to 2.41%)	1.40% (0.46%)	2.96*% (0.99%)	1.56% (1.11 to 2.02 %)	1.05% (0.34%)	1.06% (0.35%)	0.01% (-0.13 to 0.14%)	<0.001
DPA (%)	3.08% (0.36%)	3.64*% (0.52%)	0.57% (0.33 to 0.80 %)	3.10% (0.26%)	3.86*% (0.47%)	0.76% (0.56 to 0.96 %)	2.98% (0.39%)	3.09% (0.30%)	0.11% (-0.01 to 0.24%)	<0.001
DHA (%)	6.01% (1.01%)	7.49*% (1.08%)	1.48% (0.93 to 2.02%)	6.63% (1.49%)	7.51*% (1.12%)	0.88% (0.34 to 1.42 %)	5.67% (1.39%)	5.92% (1.33%)	0.25% (-0.11 to 0.60%)	<0.001
Omega3 Index (%)	7.10% (1.33%)	10.49*% (1.92%)	3.39% (2.50 to 4.28%)	8.03% (1.84%)	10.47*% (1.90%)	2.44% (1.60 to 3.29%)	6.72% (1.67%)	6.98% (1.56%)	0.25% (-0.22 to 0.72%)	<0.001
Myristic Acid (%)	0.30% (0.00%)	0.31% (0.00%)	0.01% (-0.03 to 0.04%)	0.29% (0.00%)	0.33% (0.00%)	0.04% (-0.04 to 0.05%)	0.32% (0.00%)	0.33% (0.00%)	0.01% (-0.04 to 0.05%)	0.595
Palmitic Acid (%)	21.09% (0.00%)	21.70% (0.01%)	0.61% (0.17% to 1.05%)	21.53% (0.00%)	21.84% (0.00%)	0.31% (-0.02 to 0.64%)	21.71% (0.00%)	21.46% (0.00%)	-0.25% (-0.60 to 0.11%)	0.34
Palmitelaidic Acid (%)	0.12% (0.00%)	0.11% (0.00%)	0.01% (-0.02 to 0.02%)	0.11% (0.00%)	0.11% (0.00%)	0.00% (-0.02 to 0.02%)	0.12% (0.00%)	0.12% (0.00%)	0.00% (-0.02 to 0.01%)	0.996
Palmitoleic Acid (%)	0.26% (0.00%)	0.28% (0.00%)	0.02% (-0.01to 0.06%)	0.27% (0.00%)	0.28% (0.00%)	0.01% (-0.03 to 0.04%)	0.29% (0.00%)	0.29% (0.00%)	0.00% (-0.03to 0.03%)	0.645

Stearic Acid (%)	17.06% (0.00%)	16.93% (0.00%)	-0.13% (-0.15 to 0.24%)	16.72% (0.00%)	16.57% (0.00%)	-0.15% (-0.43 to 0.12%)	16.89% (0.00%)	16.67% (0.00%)	-0.22% (19.66 to 20.34%)	0.540
Elaidic Acid (%)	0.47% (0.00%)	0.52% (0.00%)	-0.04% (-0.03 to 0.12%)	0.49% (0.00%)	0.45% (0.00%)	-0.04% (-0.16 to 0.7%)	0.55% (0.00%)	0.60% (0.00%)	0.04% (-0.07 0.16%)	0.39
Oleic Acid (%)	15.65% (0.00%)	15.89% (0.01%)	0.25% (-0.19 to 0.69%)	15.90% (0.01%)	15.57% (0.01%)	-0.32% (-0.66 to 0.01%)	15.50% (0.00%)	15.45% (0.00%)	-0.06% (-0.37 to 0.26%)	0.402
Linoelaidic Acid (%)	0.25% (0.00%)	0.21% (0.00%)	-0.04% (-0.14 to 0.07%)	0.20% (0.00%)	0.22% (0.00%)	0.01% (-0.04 to 0.07%)	0.21% (0.00%)	0.25% (0.00%)	0.05% (-0.04 to 0.13%)	0.008
Linoleic Acid (%)	11.34% (0.01%)	10.80% (0.01%)	0.54% (-0.98 to -0.10%)	10.74% (0.01%)	10.35% (0.01%)	-0.40% (-0.86 to 0.07%)	11.19% (0.01%)	11.24% (0.01%)	0.06% (-0.47 to 0.58%)	0.194
Arachidic Acid (%)	0.16% (0.00%)	0.14% (0.00%)	-0.01% (-0.04 to 0.01%)	0.16% (0.00%)	0.16% (0.00%)	0.00% (-0.02 to 0.02%)	0.16% (0.10%)	0.16% (0.00%)	0.00% (-0.03 to 0.03%)	0.367
gamma-Linolenic Acid (%)	0.10% (0.00%)	0.11% (0.00%)	0.00% (-0.03 to 0.03%)	0.11% (0.00%)	0.12% (0.00%)	0.00% (-0.01 to 0.02%)	0.11% (0.00%)	0.11% (0.00%)	-0.01% (-0.03 to 0.02%)	0.430
Eicosenoic Acid (%)	0.28% (0.00%)	0.25% (0.00%)	-0.02% (-0.06 to 0.02%)	0.26% (0.00%)	0.24% (0.00%)	-0.02% (-0.05 to 0.01%)	0.26% (0.00%)	0.24% (0.00%)	-0.02% (-0.04 to 0.01%)	0.962
alpha-Linolenic Acid (%)	0.18% (0.00%)	0.18% (0.00%)	0.00% (-0.03 to 0.03%)	0.17% (0.00%)	0.15% (0.00%)	-0.02% (-0.04 to 0.01%)	0.15% (0.00%)	0.16% (0.00%)	0.01% (-0.02 to 0.4%)	<0.001
Eicosadienoic Acid (%)	0.25% (0.00%)	0.21% (0.00%)	-0.04% (-0.08 to 0.00%)	0.26% (0.00%)	0.22% (0.00%)	-0.04% (-0.07 to -0.01%)	0.27% (0.00%)	0.28% (0.00%)	0.01% (-0.04 to 0.05)	0.005
Behenic Acid (%)	0.29% (0.00%)	0.27% (0.00%)	-0.01% (-0.05 to 0.02%)	0.31% (0.00%)	0.32% (0.00%)	0.02% (-0.05 to 0.08%)	0.30% (0.00%)	0.30% (0.00%)	0.00% (-0.04 to 0.05%)	0.407
Dihomo-g-linolenic Acid (%)	1.83% (0.00%)	1.52% (0.00%)	-0.30% (-0.40 to -0.21)	1.62% (0.00%)	1.38% (0.00%)	-0.24% (-0.34 to -0.13%)	1.76% (0.00%)	1.79% (0.00%)	0.03% (-0.09 to 0.15%)	<0.001

Arachidonic Acid (%)	15.58% (0.01%)	12.98% (0.01%)	-2.60% (-3.18 to -2.03%)	14.98% (0.02%)	13.54% (0.02%)	-1.44% (-1.93 to -0.96%)	15.52% (0.01%)	15.51% (0.01%)	-0.01% (-0.46 to 0.44%)	<0.001
Lignoceric Acid (%)	0.71% (0.00%)	0.68% (0.00%)	-0.04% (-0.13 to 0.06%)	0.78% (0.00%)	0.85% (0.00%)	0.07% (-0.03 to 0.17%)	0.72% (0.00%)	0.72% (0.00%)	0.00% (-0.07 to 0.08%)	0.053
Nervonic Acid (%)	0.78% (0.00%)	0.78% (0.005)	0.0% (-0.09 to 0.10%)	0.94% (0.00%)	0.94% (0.00%)	0.0% (-0.12 to 0.12%)	0.84% (0.00%)	0.85% (0.00%)	0.01% (-0.10 to 0.13%)	0.656
Docosatetraenoic Acid (%)	2.61% (0.00%)	1.77% (0.00%)	-0.84% (-1.00 to -0.67%)	2.51% (0.00%)	1.90% (0.00%)	-0.61% (-0.79 to -0.43%)	2.83% (0.00%)	2.83% (0.00%)	0.0% (-0.14 to 0.15%)	<0.001
Docosapentaenoic Acid - n6 (%)	0.51% (0.00%)	0.34% (0.00%)	-0.18% (-0.12 to -0.14%)	0.50% (0.00%)	0.41% (0.00%)	-0.09% (-0.15 to -0.03%)	0.58% (0.00%)	0.58% (0.00%)	-0.01% (-0.06 to 0.05%)	<0.001
Trans Fat Index	0.72% (0.00%)	0.73% (0.00%)	0.01% (-0.13 to 0.15%)	0.69% (0.00%)	0.67% (0.00%)	-0.02% (-0.16 to 0.11%)	0.76% (0.00%)	0.85% (0.00%)	0.09% (-0.09 to 0.26%)	0.083
Omega 6:3 Ratio	3.11% (0.70%)	2.07% (0.44%)	-1.04% (-144.79 to -63.48%)	2.83% (0.72%)	1.97% (0.45%)	-0.87% (-122.17 to -50.88%)	3.40% (0.78%)	3.26% (0.67%)	-0.14% (-34.99 to 6.56%)	<0.001
AA: EPA Ratio	16.33% (6.72%)	5.01% (2.38%)	-11.31% (-14.15 to -8.83%)	12.31% (5.75%)	5.38% (3.13%)	-6.93% (-9.34 to -4.51%)	16.31% (5.65%)	16.15% (5.43%)	-0.17% (-2.00 to1.67)	<0.001
Total Omega 3	10.36% (0.01%)	14.32% (0.02%)	3.96% (2.90 to5.03%)	11.30% (0.01%)	14.48% (0.02%)	3.18% (2.17 to4.19%)	9.86% (0.01%)	10.23% (0.01%)	0.37% (-0.19 to 0.93%)	<0.001
Total Omega 6	32.22% (0.01%)	27.73% (0.01%)	-4.49% (-5.30 to -3.68%)	30.73% (0.02%)	27.61% (0.02%)	-3.12 (-3.92 to -2.32%)	32.26% (0.02%)	32.33% (0.01%)	0.07% (-0.60 to 0.74%)	<0.001
Total Saturated	39.62% (0.00%)	39.89% (0.01%)	0.27% (-0.41 to 0.95%)	39.80% (0.00%)	40.00% (0.00%)	0.20% (-0.05 to 0.45%)	40.09% (0.01%)	39.63% (0.00%)	-0.46% (-1.13 to 0.20%)	0.311
Total Monounsaturated	16.96% (0.00%)	17.22% (0.01%)	0.25% (-0.22 to 0.73%)	17.37% (0.01%)	17.13% (0.01%)	-0.25% (-0.61 to 0.12%)	16.90% (0.01%)	16.84% (0.00%)	-0.06% (-0.44 to 0.32%)	0.259

Total Trans	0.84% (0.00%)	0.84% (0.00%)	0.01% (-0.13 to 0.14%)	0.80% (0.00%)	0.78% (0.00%)	-0.02% (-0.15 to 0.11%)	0.89% (0.00%)	0.97% (0.00%)	0.08% (-0.10 to 0.26%)	0.143

Differences in fatty acids composition and dietary intake data at baseline and 16 weeks. Data are presented as mean (SD). * Denotes a significant difference from control ($p<0.05$).

a. Adjusted mean difference for the post-outcome variable when controlling the pre-outcome variable.

b. Significant difference for the ANCOVA

4.4.4 Body weight and body composition

Body weight and body composition (BMI, weight, fat mass, fat percentage, fat free mass) data are presented in **Table 4.4.** No main effect was seen in the ANCOVA for BMI, body mass, fat mass, fat percentage, or muscle thickness (all $p > 0.05$).

4.4.5 Muscle function and strength

Muscle function and strength data are presented in **Table 4.5.** For knee extensor maximal torque a main effect was noted in the ANCOVA ($p = 0.041$), with post-hoc tests identifying that maximal torque was higher in the fish, but not krill, oil group, relative to the control group, at 16 weeks. Relative to control, at 16 weeks in the fish oil group there was an adjusted mean difference of 11.6 Nm (95% CI, 0.5 to 22.7, $p = 0.01$), and of 6.7 Nm (95% CI, -4.0 to 17.5, $p = 0.12$) for krill oil. No differences between krill oil and fish oil groups were noted (4.8 (95% CI, -5.7 to 15.4, $p = 0.26$)).

Handgrip strength data are also reported in **Table 4.5.** A main effect was observed in the ANCOVA ($p < 0.05$), with post-hoc analysis showing that handgrip strength was higher in the fish oil group, but not krill, oil group, relative to the control, group at 16 weeks. Relative to control, at 16 weeks in the fish oil group there was an adjusted mean difference of 2.2 kg (95% CI, 0.2 to 4.2, $p = 0.007$), and of 1.2 kg (95% CI, -0.6 to 3.1, $p = 0.10$) for krill oil. No differences between krill oil and fish oil groups were noted (0.9 kg (95% CI, - 0.9 to 2.8, $p = 0.21$)).

STS (30s sit-to-stand test) and gait data are presented in **Table 4.5.** No main effect in ANCOVA was observed, p = 0.27 was observed for STS test data. For cadence a main effect was noted in the ANCOVA (p = 0.006), with post-hoc tests identifying that cadence was higher in the fish and krill oil, relative to the control, group at 16 weeks. Relative to control, at 16 weeks in the fish oil group there was a mean difference of 5.3 steps/min (95% CI, 1.2 to 9.4, p = <0.001), and in the krill oil group of 4.0 steps/min (95% CI, 0.1 to 8.0, p = 0.01). No differences between krill oil and fish oil groups were noted (1.2 steps/min (95% CI, -2.6 to 5.1, p = 0.33)). For velocity no statistically significant main effect was noted in the ANCOVA (p = 0.056), but due to this indicating a possible effect we carried out post-hoc tests to help in the development of future studies in this area. These post-hoc tests indicated that velocity was higher in the fish and krill oil, relative to the control, group at 16 weeks. Relative to control, at 16 weeks in the fish oil group there was a mean difference of 0.09 m/s (95% CI, −0.003 to 0.184; *p* = 0.02), and in the krill oil group of 0.06 m/s (95% CI, −0.023 to 0.156; *p* = 0.07). No differences between krill oil and fish oil groups were noted (0.024 m/s (95% CI, −0.066 to 0.114; *p* = 0.33)). No main effects were seen in the ANCOVA for step length (p = 0.70), for stride length *(*p = 0.64), for swing time (p = 0.07), and for stance time (p = 0.13).

Table 4.4 Body Weight and Body Composition in control, krill oil and fish oil groups at baseline, and after 16 weeks.

Outcomes Variables	Krill Oil Group (n=20)			Fish Oil Group (n=17)			Control Oil Group (n=16)			a. Sig (ANCOVA)
	Pre	Post	Means and Confidence Interval for Difference (95%CI)	Pre	Post	Means and Confidence Interval for Difference (95%CI)	Pre	Post	Means and Confidence Interval for Difference (95%CI)	
b. BMI (kg/m2)	24.03 (2.29)	23.89 (2.44)	-0.14(-0.52 to 0.24)	24.77 (2.68)	24.63 (2.70)	-0.14 (-0.33 to 0.05)	23.23 (3.52)	23.48 (3.40)	0.24 (-0.14 to 0.63)	.246
Weight (kg)	68.84 (12.47)	68.69 (12.53)	-0.14(-1.22 to 0.93)	71.04 (14.18)	70.54 (14.13)	-0.49 (-1.16 to 0.17)	66.28 (12.44)	66.95 (12.18)	0.67 (-0.27 to 1.62)	.247
Fat Mass (kg)	18.63 (4.99)	18.78 (5.11)	0.15 (-0.55 to 0.86)	19.20 (5.63)	18.83 (6.23)	-0.37 (-1.26 to 0.52)	17.46 (6.95)	18.09 (6.91)	0.63 (-0.27 to 1.53)	.243
Fat Percentage (%)	27.50 (7.20)	27.77 (7.28)	0.27 (-0.44 to 0.98)	27.32 (7.14)	26.88 (7.81)	-0.44 (-1.73 to 0.85)	25.91 (8.11)	26.59 (8.18)	0.68 (-0.30 to 1.66)	.272
Fat Free Mass (kg)	50.21 (12.23)	49.91 (12.26)	-0.3 (-0.87 to 0.27)	51.83 (12.83)	51.77 (12.76)	-0.06 (-0.77 to 0.64)	48.81 (9.33)	48.88 (9.36)	0.06 (-0.46 to 0.60)	.666
Muscle Thickness (mm)	18.40 (3.35)	18.22 (3.17)	-0.16 (-1.02 to 0.68)	18.13 (4.72)	18.94 (3.51)	0.80 (-0.45 to 2.05)	17.58 (3.34)	17.82 (2.61)	0.23 (-1.01to 1.49)	.302

Differences in Body weight and body composition between groups before and after 16 weeks. Data are presented as mean (SD).

a. Significant effect in the (ANCOVA) test.

b. BMI: body mass index.

Table 4.5 Muscle Function, Strength and Gait Data in control, krill oil and fish oil groups at baseline, and after 16 weeks.

Outcomes Variables	Krill Oil Group (n=20)			Fish Oil Group (n=17)			Control Oil Group (n=16)			a. Sig (ANCOVA)
	Pre	Post	Means and Confidence Interval for Difference (95%CI)	Pre	Post	Means and Confidence Interval for Difference (95%CI)	Pre	Post	Means and Confidence Interval for Difference (95%CI)	
Torque (N/m)	105.77 (29.57)	109.74 (27.95)	3.76 (-0.98 to 8.51)	109.43 (38.32)	117.98* (41.66)	8.55 (1.97 to 15.12)	100.9 1(38.10)	98.41 (35.39)	-2.49 (-10.75 to 5.75)	.041
Grip Strength (kg)	26.89 (7.88)	28.49 (6.64)	1.6 (0.37 to 2.82)	31 (10.58)	33.19* (10.29)	2.19 (0.97 to 3.41)	29.26 (8.69)	29.37 (8.71)	0.11 (-1.04 to 1.27)	.027
b. STS	14.45 (1.84)	16.55 (3.15)	2.1 (1.10 to 3.09)	15.41 (2.37)	16.17 (2.87)	0.76 (-0.66 to 2.19)	14.93 (3.04)	15.62 (3.46)	0.68 (-0.93 to 2.31)	.277
Cadence (steps/min)	111.62 (11.35)	113.97* (8.31)	2.35 (-0.92 to 5.62)	113.01 (10.28)	116.21* (9.71)	3.19 (0.99 to 5.39)	111.61 (13.74)	109.86 (10.87)	-1.74(-4.77 to1.28)	.006
Step Length (cm)	69.84 (7.15)	70.91 (7.76)	1.06 (-1.06 to 3.19)	74.35 (6.57)	74.41 (5.45)	0.06 (-1.80 to 1.93)	71.37 (8.21)	71.09 (8.85)	-0.27 (-2.48 to 1.92)	.707
Stride Length (cm)	140.06 (14.42)	142.42 (15.53)	0.02 (-1.66 to 6.38)	149.15 (13.05)	149.53 (11.04)	-0.00 (-3.31to 4.06)	143.68 (16.79)	143.12 (17.74)	-0.55 (-4.86 to 3.74)	.649
Swing Time (sec)	0.39 (0.03)	0.39 (0.02)	-0.00 (-0.01 to 0.00)	0.39 (0.03)	0.39 (0.02)	-0.00 (-0.01to 0.00)	0.40 (0.03)	0.40 (0.03)	0.00 (-0.00 to 0.01)	.072

Stance Time (sec)	0.68 (0.08)	0.66 (0.06)	-0.01 (-0.04 to 0.00)	0.65 (0.08)	0.64 (0.06)	-0.00 (-0.03 to 0.02)	0.68 (0.08)	0.69 (0.06)	0.00 (-0.01to 0.03)	.134
Velocity (m/s)	1.30 (0.21)	1.34 (0.18)	0.04 (−0.02 to 0.10)	1.39 (0.16)	1.43 (0.15)	0.04 (−0.00 to 0.09)	1.33 (0.25)	1.30 (0.21)	−0.03 (−0.09 to 0.03)	.056

Differences in Muscle function and Strength between groups before and after 16 weeks. Data are presented as mean (SD). * Denotes a significant difference from control group post-intervention ($p<0.05$).

a. Significant effect in the ANCOVA test.

b. STS: 30-second sit -to -stand test.

4.5 Discussion

This double-blind, randomised controlled trial indicates that 16 weeks of daily supplementation with 4 g/day of either krill oil or fish oil resulted in beneficial effects on muscle function. Specifically, fish oil supplementation resulted in improvements in knee extensor maximal voluntary contraction and handgrip strength, while both krill oil and fish oil supplementation resulted in comparable improvements in gait velocity and cadence in healthy older men and women. No differences between krill and fish oil were noted. These findings provide evidence that LCn-3 PUFA supplementation, with no difference between fish and krill oil, may serve as a viable nutritional strategy to support functional capacity and potentially mitigate age-related declines in muscle performance among older adults.

Based on our systematic review of the literature (chapter2), this is the first study to directly compare the effects of krill oil and fish oil supplementation on muscle mass and strength in older adults. The study findings indicate that participants in the fish oil group exhibited improvements in knee extensor maximal torque and handgrip strength relative to the placebo control group, whereas the krill oil group did not show significant improvements compared to control. However, there was no statistically significant difference between the fish oil and krill oil groups in direct comparison. This suggests that while fish oil supplementation may enhance muscle strength in older adults, the lack of effect with krill oil should be interpreted cautiously and may reflect limitations in statistical power due to the relatively small sample size and short duration of this pilot work.

The observed improvement in knee extensor maximal torque and grip strength following LCn-3 PUFA supplementation aligns with previous clinical trials. For example, Smith *et al.* demonstrated increases in knee extensor maximal torque and grip strength, after six months of supplementation with 4 g/day of fish oil, in a cohort of healthy older adults (Smith *et al.*, 2015). Similarly, an RCT, in healthy older adults, reported an increased knee extensor

maximal torque and grip strength after six months with 4 g/day of krill oil supplementation (Alkhedhairi *et al.*, 2022), further supporting that the lack of effect noted in the current study is due to low statistical power coupled with a shorter duration. The findings of a general benefit of LCn-3 PUFA are corroborated by a multiple meta-analysis, which indicated a statistically significant improvement in handgrip strength among older adults receiving LCn-3 fatty acid supplementation (Ma *et al.*, 2021). This is further supported by our meta-analysis in chapter 2, which confirmed broader muscle strength benefits in older adults receiving LCn-3 PUFA supplementation.

While the current data suggests significant improvements in muscle strength, no effects on body composition parameters or ultrasound-derived measures of muscle thickness were observed. The absence of detectable changes in muscle mass may reflect the limitations of BIA, which has lower sensitivity and precision compared to other techniques. In contrast, ultrasound is a well-validated method for assessing muscle size, and in previous studies such as Alkhedhairi *et al.*, increases in muscle size were detected with ultrasound but not with BIA (Alkhedhairi *et al.*, 2022). This was not, however, the case in the current study. The lack of observable changes in the current study might, therefore, be due to its relatively short duration (16 weeks), especially when compared to longer trials like those of Smith et al and Alkhedhairi et al, which spanned six months (Alkhedhairi *et al.*, 2022, Smith *et al.*, 2015). Similarly, Logan *et al.* reported no significant changes in body composition following fish oil supplementation, over an intervention period of 12 weeks (Logan and Spriet, 2015).

No significant effect of krill oil or fish oil supplementation was observed on performance of the 30-second sit-to-stand test. This test is known to have limited responsiveness to change in relatively healthy older adults. Previous work has indicated that while the 30STS is a valid measure of lower limb strength, it may not be sensitive enough to detect subtle improvements in muscle function within this population (Park and Shin, 2024). Furthermore, our study may

not have been adequately powered to detect subtle effects. This aligns with the findings of Krzyminska, who also reported no significant difference of LCn-3 PUFA supplementation with 1.3 g/day for 12 weeks in the repeated chair rise test in older adults (Krzyminska-Siemaszko *et al.*, 2015). Similarly, Logan et al, observed no effect of fish oil supplementation with 3g/day for 12 weeks, on the 30-second sit-to-stand test in their randomized controlled trial involving older women (Logan and Spriet, 2015).

In contrast, the current study found significant improvements in cadence and gait speed in both the krill oil and fish oil groups compared to the placebo control group, indicating that both LCn-3 PUFA supplements were equally effective in enhancing this functional parameter. This finding is consistent with a meta-analysis conducted by Hang *et al.*, which reported increased gait speed in older adults following LCn-3 PUFA supplementation at doses above 2 g/day for durations exceeding six months (Huang *et al.*, 2020). Additionally, Hutchins *et al.* demonstrated that n-3 fatty acid supplementation (2 capsules Fish oil, 1.2 g/day EPA and DHA) for 6 months, had a beneficial effect on walking speed in older women compared to placebo (2 capsules olive oil, 1.8 g/day) (Hutchins-Wiese *et al.*, 2013). Importantly, the present study utilised the GAITRite system an objective and highly sensitive electronic walkway to assess gait speed, offering greater measurement precision than traditional methods like stopwatch timing. This methodological strength may explain the differences of the findings from Alkhedhairi *et al.*, study, where no significant effect was detected on gait speed using manual timing (Alkhedhairi *et al.*, 2022). The improvements noted in the current study are likely clinically meaningful. Perera *et al.* estimated the minimal clinically important difference (MCID) in gait speed to be 0.05 - 0.10 meters per second (m/s), representing small to substantial meaningful change respectively (Perera *et al.*, 2006), with increases in the current study, relative to control of 0.07 m/s suggesting a clinically meaningful improvement. Given that gait speed is a well-established predictor of

frailty, functional decline, and fall risk in older adults (Studenski *et al.*, 2011) this is promising but should be confirmed in larger trials.

Additional research is also needed to understand the underlying mechanisms by which LCn-3 PUFA supplementation influences muscle strength and function. There are a multitude of possible mechanisms of action, including increases in muscle blood flow (Walser *et al.*, 2006), increases in muscle protein synthesis (Smith et al., 2011a), increases in mitochondrial content and function (Herbst *et al.*, 2014), and improvements in the composition of the extracellular matrix, with effects likely to be multifactorial in nature (Yoshino *et al.*, 2016, Gray and Mittendorfer, 2018). Recent data has emerged to suggest that there may be neuromuscular effects of LCn-3 PUFA. Fish oil supplementation shortened the electromechanical delay and increased the rate of force development during maximal voluntary isometric contractions in healthy people participating in an exercise training program (Rodacki *et al.*, 2012). Furthermore, the work of Alkhedhairi et al indicate that the mechanisms may be neuromuscular in origin as indicated by an increase in the M-wave, a measure of muscle membrane excitability. These data, however, have limitations and further work is needed using more advanced neuromuscular techniques (Piasecki *et al.*, 2021).

Several limitations should be acknowledged. First, the current study was a pilot study and was not powered to detect differences in all outcomes. Second, the intervention period may have been too short to observe structural muscle adaptations. Third, the use of bioelectrical impedance analysis (BIA), while convenient, may lack the sensitivity of more precise methods like DXA or MRI, potentially underestimating changes in muscle composition. Lastly, the relatively high functional capacity of participants may have masked benefits that would be more evident in people with frailty or sarcopenia.

Future studies should consider longer intervention periods, larger and more diverse cohorts, and inclusion of resistance training. Investigating the time course of strength versus hypertrophy adaptations, with gold standard techniques, and identifying the molecular pathways involved particularly those related to neuromuscular transmission will help optimize the therapeutic potential of LCn-3 PUFA supplementation.

4.6 Conclusion

This double-blind, randomized controlled trial contributes valuable evidence to the growing body of literature supporting the use of LCn-3 PUFA supplementation as a safe and effective intervention for maintaining muscle function and mobility in older adults, while demonstrating that fish oil supplementation showed superior effects on muscle strength compared to krill oil, although the magnitude of difference was small, despite both interventions producing similar improvements in gait parameters.

Chapter 5: General discussion

5.1 Summary and general discussion

Sarcopenia, the age-related loss of muscle strength and mass, is a significant global health concern that represents a considerable healthcare cost burden (Rosenberg, 1997, Fielding *et al.*, 2011, Petermann-Rocha *et al.*, 2022a, Janssen *et al.*, 2004). The condition arises from multiple factors including hormonal changes, chronic low-grade inflammation, physical inactivity, inadequate protein intake, and disrupted muscle protein metabolism (Walston, 2012, Morley *et al.*, 2014a). While current treatments like resistance exercise and protein supplementation offer modest benefits (Dennison *et al.*, 2018) (Dennison *et al.*, 2017), they are not suitable for all older adults. This has led to growing interest in LCn-3 PUFAs, particularly EPA and DHA, due to their anti-inflammatory properties and potential role in increasing MPS responses to feeding (Calder, 2010, Serhan *et al.*, 2008). Observational studies also suggest a link between higher fatty fish, rich in LCn-3 PUFA, intake and better muscle function in older adults (Robinson et al., 2008b).

This thesis aimed to comprehensively examine the effects of LCn-3 PUFA on muscle health in older adults, including comparison of different marine sources (fish oil versus krill oil), through three studies: a systematic review and meta-analysis of existing evidence to explore the impact of LCn-3 PUFA relative to control oil supplementation on muscle strength, with secondary outcomes of muscle mass and physical function in older individuals under conditions of habitual physical activity/exercise. In addition, the second study investigated, through regression analyses of data from UK biobank, the association of n-3 fatty acid intake and the n-6/n-3 fatty acid intake ratio with muscle strength and mass in older adults. Further, the final study compared the effects of krill oil and fish oil supplementation on muscle function in older adults, in a double blind RCT recruiting healthy older adults of both sexes.

Through these three complementary study designs, this thesis makes the following novel contributions to the scientific understanding of n-3 fatty acids and muscle health in older adults:

From Chapter 2 (Systematic Review and Meta-analysis):

a. First systematic review and meta-analysis isolating the independent effects of LCn-3 PUFA supplementation on muscle outcomes in older adults, excluding confounding exercise interventions and multi-nutrient supplements.

From Chapter 3 (UK Biobank Cross-sectional Analysis):

b. Largest cross-sectional analysis to date (n = 61,381 participants) investigating associations between dietary n-3 fatty acid intake, n-6/n-3 ratio, and muscle mass and strength in older adults.

c. First identification of age-specific effects: positive associations between n-3 intake and grip strength were present in adults aged ≥ 60 years but absent in those aged ≥ 65 years.

d. Discovery that associations between n-3 intake and muscle outcomes differ by sarcopenia status: in men with sarcopenia, positive associations were observed only with muscle mass index (0.197 kg/m²; 95% CI: 0.05 to 0.33), not with grip strength.

From Chapter 4 (Randomised Controlled Trial):

e. First direct head-to-head comparison of krill oil versus fish oil supplementation on muscle function outcomes in older adults in a randomised controlled trial.

f. Discovery of differential effects between marine oil sources: fish oil, but not krill oil, significantly improved knee extensor strength (11.6 Nm; 95% CI, 0.5 to 22.7, p = 0.01) and handgrip strength (2.2 kg; 95% CI, 0.2 to 4.2, p = 0.007), while both sources similarly improved gait parameters.

Overall contribution:

Comprehensive evidence from three complementary study designs demonstrating that LCn-3 PUFA supplementation may be more effective for prevention rather than treatment of muscle decline, with strongest benefits in healthy older adults.

Across the thesis, LCn-3 PUFA supplementation demonstrated variable effects on muscle strength depending on the specific measure and population studied. The systematic review found no significant impact on grip strength (SMD 0.57, 95% CI [−0.10, 1.25], p = 0.10), though there was a tentative trend suggesting potential beneficial effects, with some studies indicating benefits on lower body muscle strength. This finding aligns with previous meta-analyses that included broader criteria and reported no effect on grip strength in older adults (Huang *et al.*, 2020, Cornish *et al.*, 2022). For example, in studies with and without exercise and/or multi-nutrient supplements were included, with no effect of LCn-3 PUFA on grip strength (on the basis of 3 studies with 97 participants) (Huang *et al.*, 2020). The cross-sectional analysis in chapter 3 revealed positive associations between higher n-3 fatty acid intake and grip strength in both men (0.114 kg; 95% CI: 0.02 to 0.21) and women (0.115 kg; 95% CI: 0.05 to 0.18), consistent with previous study from the Hertfordshire cohort by Robinson *et al.* who analysed 2,983 older adults from Hertfordshire, UK and found that each additional portion of fatty fish consumed per week was associated with a higher grip strength of 0.43 kg in men (95% CI: 0.13−0.74) and 0.48 kg in women (95% CI: 0.24−0.72), after adjusting for height, age, and birth weight (Robinson et al., 2008b). However, these associations were notably absent

among individuals with established sarcopenia or those over 65 years of age. The RCT, in chapter 4, provided the strongest evidence for strength benefits, with fish oil supplementation significantly improving knee extensor strength by 11.6 Nm (95% CI, 0.5 to 22.7, p = 0.01) and handgrip strength by 2.2 kg (95% CI, 0.2 to 4.2, p = 0.007) compared to control. These findings are consistent with previous work by Smith *et al.* who demonstrated increases in knee extensor torque and grip strength after six months of 4 g/day fish oil supplementation in healthy older adults (Smith *et al.*, 2015).

In terms of muscle mass, the overall evidence, from this thesis and the wider literature, on the effects of LCn-3 PUFAs is mixed, with some studies reporting benefits across different study designs. The systematic review found that studies measuring knee extensor muscle mass n= 3 showed increases with LCn-3 PUFA supplementation, with MRI-measured thigh muscle volume (Smith *et al.*, 2015), ultrasound-measured vastus lateralis muscle thickness (Alkhedhairi *et al.*, 2022). However, studies measuring whole-body lean/muscle mass n= 2 reported mixed results, with outcomes including bioelectrical impedance-evaluated lean mass, skeletal muscle mass, and fat-free mass (Logan and Spriet, 2015, Xu *et al.*, 2022). These findings align with the study by Smith et al, which showed beneficial effects of n-3 fatty acids on muscle mass, particularly a 3.6% increase in thigh muscle volume measured using MRI after 6 months of fish oil supplementation in older adults (Smith *et al.*, 2015). In contrast, the cross-sectional analysis found no association between n-3 fatty acid intake and muscle mass index in the general population. However, sensitivity analyses revealed a positive association in men over 60 with sarcopenia (0.197 kg/m²; 95% CI: 0.05 to 0.33), suggesting possible therapeutic effects in this subgroup. In addition, the analyses from chapter 3, found no consistent associations between the n-6/n-3 fatty acid intake ratio and muscle mass in either men or women, although relatively weak negative associations were seen with muscle mass index in men and

with grip strength in women. These findings are broadly consistent with prior studies that also reported no significant associations between the n-6/n-3 fatty acid intake ratio and muscle health or sarcopenia status (Jang *et al.*, 2020, Zhang *et al.*, 2021, Dupont *et al.*, 2023, Das *et al.*, 2021). The RCT in chapter 4 showed no significant effects on body composition parameters or ultrasound-derived measures of muscle thickness. In contrast, previous studies by Saleh *et al.* demonstrated that increases in muscle size were detected with ultrasound but not with BIA (Alkhedhairi *et al.*, 2022), though this finding was not replicated in the current study. The lack of observable changes in the current study might be attributed to its relatively short duration (16 weeks), particularly when compared to longer trials such as that conducted by Alkhedhairi *et al.*, which spanned six months (Alkhedhairi *et al.*, 2022).

Regarding muscle function, the effects of LCn-3 PUFA showed mixed results across different functional measures. The systematic review found that studies examining functional abilities n= 4 reported mixed results, with some finding benefits on walking speed (Hutchins-Wiese *et al.*, 2013) and timed up-and-go (TUG) tests (Logan and Spriet, 2015, Xu *et al.*, 2022), while others found no effect on chair rise tests (Hutchins-Wiese *et al.*, 2013, Logan and Spriet, 2015) or the short physical performance battery test (Alkhedhairi *et al.*, 2022). This variability may reflect the health status of participants as all had no functional limitations at baseline. This also aligns with the Do-HEALTH study, involving 2157 older adults who received 3 years of LCn-3 PUFA supplementation, which found no significant impact on the short-performance physical battery test score. The participants, however, had baseline scores of 11–12 out of 12 and were highly physically active (Bischoff-Ferrari *et al.*, 2020). The RC, in chapter 4, demonstrated significant improvements in gait parameters, with both krill oil and fish oil enhancing cadence in the fish oil (5.3 steps/min, 95% CI 1.2 to 9.4, p = 0.00) and in the krill oil (4.0 steps/min, 95% CI 0.1 to 8.0, p =

0.01) and for velocity in the fish oil (9.0 cm/s, 95% CI -0.3 to 18.4, p = 0.02) and in the krill oil (6.6 cm/s, 95% CI -2.3 to 15.6, p = 0.07) groups. These findings are consistent with a meta-analysis conducted by Hang *et al.*, which reported increased gait speed in older adults following LCn-3 PUFA supplementation at doses above 2 g/day for durations exceeding six months (Huang *et al.*, 2020). However, no significant effects were observed on the 30-second sit-to-stand test. This finding aligns with the findings of Logan et al, who observed no effect of fish oil supplementation with 3g/day for 12 weeks, on the 30-second sit-to-stand test in their randomized controlled trial involving older female (Logan and Spriet, 2015). This is likely reflective of the limited responsiveness of this measure in healthy older adult populations with good baseline functional capacity.

5.2 Implications of the study

The findings from this thesis raise important practical considerations for translating research evidence into real-world recommendations for older adults seeking to maintain muscle health through n-3 fatty acid intake.

The beneficial effects demonstrated in Chapter 4 required 4 g/day of fish oil (providing ~1.9 g/day EPA+DHA), achievable through 2–3 portions (140 g each) of oily fish per week (Scientific Advisory Committee on Nutrition, 2004). However, UK older adults consume substantially less than recommended levels, with many consuming no oily fish (Public Health England, 2018). Dietary sources offer additional nutritional benefits including high-quality protein, vitamin D, and selenium that may synergistically support muscle health (Liao et al., 2017), but face barriers including cost (£4–24/week for fish vs £5–15/month for

standard fish oil supplements), availability, preparation skills, and concerns about contaminants (Colombo and Mazal, 2020). Supplementation provides standardised dosing and convenience but lacks the broader nutritional profile of whole foods. A pragmatic approach would prioritise dietary sources while recognising supplements as viable alternatives for those unable to consume adequate oily fish (EFSA and Allergies, 2012).

From a healthcare system perspective, cost-effectiveness analyses would be essential before recommending widespread supplementation. Given that preventive benefits appear strongest in healthy older adults, targeted supplementation to at-risk individuals may optimise cost-effectiveness by potentially reducing falls, fractures, and hospitalisations associated with muscle decline (Beaudart et al., 2017).

Environmental sustainability represents a critical consideration. Wild fish stocks face substantial pressure, with many species overfished or at maximum sustainable levels (FAO, 2022). Fish oil supplements typically derive from small pelagic fish (anchovies, sardines, mackerel), and while these are more abundant, large-scale supplementation increases demand with potential ecosystem impacts (Tacon and Metian, 2013). Krill oil raises particular concerns as Antarctic krill are keystone species supporting Southern Ocean ecosystems including whales, seals, and penguins; expansion of harvesting to meet growing supplement demand poses ecological risks amid climate change uncertainties (Nicol et al., 2012, Kawaguchi and Nicol, 2020). Farmed fish require feed containing fish meal and oil, often at ratios exceeding 3:1, potentially exacerbating pressure on marine resources despite industry efforts to incorporate alternative feeds (Cashion et al., 2017).

Algal oils produced through microalgae fermentation offer sustainable alternatives by avoiding marine resource depletion and contaminant concerns (Lenihan-Geels et al., 2013). However, they remain more expensive than conventional supplements, and research confirming equivalent efficacy for muscle health outcomes in older adults is needed. Public health recommendations should prioritise consuming oily fish from well-managed, certified sustainable sources (Marine Stewardship Council), with supplements reserved for those unable to meet dietary needs, while supporting research into sustainable n-3 PUFA production methods. These recommendations must balance individual health benefits against collective environmental responsibilities (Farmery et al., 2022).

5.3 Limitations of the current thesis

Several important limitations must be acknowledged across the studies conducted in this thesis.

The systematic review (chapter 2) was constrained by the limited number of available studies and substantial heterogeneity in methodologies, with study durations of 12 weeks to 6 months and doses of ~1–3 g/day LCn-3 PUFA providing insufficient data to investigate dose-response relationships. The cross-sectional analysis (chapter 3), despite its large sample size, was limited by self-reported dietary data and the healthier, less diverse characteristics of UK Biobank participants, which may limit generalizability. Small subgroup sample sizes in individuals with sarcopenia and those aged 65+ further limited reliability of specific findings. The RCT (chapter 4), being a pilot study with a relatively homogeneous sample of healthy older adults with good baseline functional capacity, may have masked subtle improvements that could be detected in a larger,

adequately powered trial involving people with functional limitations. Technical limitations included the use of bioelectrical impedance analysis instead of gold-standard methods like DXA or MRI, which may have underestimated body composition changes and contributed to null findings in muscle mass outcomes. Additionally, the 30-second sit-to-stand test shows limited responsiveness to change in this healthy population.

A fundamental limitation of LCn-3 PUFA supplementation research, including the present thesis, is the challenge of attributing observed effects specifically to EPA and DHA versus other bioactive components or their interactions. This is particularly relevant for krill oil, which contains astaxanthin (with independent anti-inflammatory and antioxidant effects), phospholipids (which may enhance bioavailability), and choline (essential for neurotransmitter synthesis) (Sarıyer et al., 2025). The differential effects observed between krill oil and fish oil in Chapter 4 could theoretically be explained by: (1) superior bioavailability of phospholipid-bound EPA/DHA in krill oil, (2) independent or synergistic effects of astaxanthin, (3) benefits from choline content, or (4) combinations of these factors.

5.4 Future work

Based on the findings and limitations identified in this thesis, several important research priorities emerge for advancing the field: Large-scale, long-term RCTs (>6 months) are needed in diverse populations of older adults, including those with muscle weakness, frailty, or sarcopenia. These studies should include clinically relevant outcomes such as falls, fractures, and healthcare utilization to establish cost-effectiveness. Mechanistic investigations should utilize advanced techniques to assess neuromuscular function, muscle inflammation markers, and fibre type-specific adaptations. While direct comparisons between krill oil and fish oil have been established in healthy older adults, future studies should compare these sources in

populations with sarcopenia, frailty, or functional limitations to determine if source-specific benefits vary by health status. Implementation research is needed to translate findings into clinical practice through practical screening tools and cost-effectiveness analyses. Moreover, combination intervention studies should explore synergistic effects of LCn-3 PUFA supplementation with resistance exercise training or other interventions to maximize therapeutic potential. Finally, research employing factorial designs comparing pure EPA and DHA supplementation, astaxanthin alone, phospholipid carriers alone, and combinations thereof would be necessary to definitively isolate the contribution of individual components. Additionally, studies using equal EPA and DHA doses (rather than equal oil doses) across different marine oil sources, combined with comprehensive biomarker analysis correlating omega-3 index with muscle outcomes, would help clarify whether LCn-3 PUFA are sufficient or whether other components enhance their efficacy (Ulven et al., 2015, Sarıyer et al., 2025).

5.5 Conclusion

This thesis provides substantial evidence that LCn-3 PUFA supplementation from marine sources represents an effective and safe intervention for supporting muscle health in older adults. The most notable benefits were seen in muscle strength and physical function, especially among healthy individuals. The results indicate that LCn-3 PUFAs may be more effective in preventing muscle loss than treating existing decline. Although uncertainties remain around the ideal dosage, treatment duration, and specific target groups, the findings support the inclusion of LCn-3 PUFA supplementation in broader healthy ageing approaches. Future studies should prioritize long-term trials in more diverse and clinically vulnerable populations, such as those already experiencing muscle weakness or sarcopenia, to inform clinical guidelines.

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Appendices

Appendix 2- A: Prisma checklist (chapter 2)

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual	

Section and Topic	Item #	Checklist item	Location where item is reported
		studies and syntheses.	
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	
	23b	Discuss any limitations of the evidence included in the review.	
	23c	Discuss any limitations of the review processes used.	
	23d	Discuss implications of the results for practice, policy, and future research.	
OTHER INFORMATION			
Registration and	24a	Provide registration information for the review, including register name and	

Section and Topic	Item #	Checklist item	Location where item is reported
protocol		registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

Appendix 2-B: Prospero registration for systematic and meta-analysis review (chapter 2)

NIHR | National Institute for Health and Care Research

PROSPERO
International prospective register of systematic reviews

The effect of omega-3 fatty acid supplementation on muscle mass and strength in older adults: a systematic review

Maha Timraz, Ahmad Binmahfoz, Stuart Gray

Citation
Maha Timraz, Ahmad Binmahfoz, Stuart Gray. The effect of omega-3 fatty acid supplementation on muscle mass and strength in older adults: a systematic review. PROSPERO 2024 Available from https://www.crd.york.ac.uk/PROSPERO/view/CRD42021267011

REVIEW TITLE AND BASIC DETAILS

Review title
The effect of omega-3 fatty acid supplementation on muscle mass and strength in older adults: a systematic review

Review objectives
Does omega-3 fatty acid, relative to a control oil, supplementation affect muscle mass and strength in older people?

SEARCHING AND SCREENING

Searches
Systematic searches will be conducted in the following databases: PubMed, EMBASE, CINAHL, Scopus, Web of Science and the Cochrane Central Register of Controlled Trials (CENTRAL).
First, keyword and categorical searches will be performed for (i) muscle mass or strength, (ii) omega-3 fatty acid supplementation, (iii) older adult.
Then, categories (i) to (iii) will be combined using 'and'.
We will restrict our search to papers published in the English language, with no restriction on the

Appendix 2-C: Search terms (chapter 2)

Search Number	Search Term
1	"Older adult"[Mesh] OR elderly OR older OR older adults OR elder OR aged
2	"Fish oil"[Mesh] OR “Krill oil” OR “Omega 3 fatty acid” OR “n3 PUFA” OR “DHA*” OR “EPA”
3	"Muscle AND mass” OR “lean"[Mesh] OR volume” OR “grip*” OR “strength” OR “fat free*” OR “DXA” OR “DEXA*”
4	1 AND 2 AND 3

Appendix 2-D: Extraction form (chapter 2)

General Information

Date form completed *(dd/mm/yyyy)*	2015
Name/ID of person extracting data	Maha Timraz
Reference citation	Logan SL, Spriet LL (2015) Omega-3 FattyAcid Supplementation for 12 Weeks Increases Resting and Exercise Metabolic Rate in Healthy Community-Dwelling Older Females. PLoS ONE 10(12): e0144828. doi: 10.1371/journal.pone.0144828
Study author contact details	Department of Human Health and Nutritional Sciences, 50 Stone Road East, University of Guelph, Guelph, Ontario, N1G 2W1, Canada
Publication type *(e.g. full report, abstract, letter)*	*Full Report*
Notes:	

Appendix 2-E: The evidence certainty for the effects of LCn-3 PUFA supplements on hand grip strength was rated very low according to GRADE (chapter2).

Certainty assessment							№ of patients		Effect	Certainty
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	krill oil	placebo	Absolute-Relative (95% CI)	
Hand Grip Strength (follow-up: range 12 weeks to 24 weeks)										
4	randomised trials	not serious	serious	not serious	serious	publication bias strongly suspected	175	113	SMD **0.68 SD higher** (0.31 lower to 1.67 higher)	⊕◯◯◯ Very low

CI: confidence interval; **SMD:** standardised mean difference

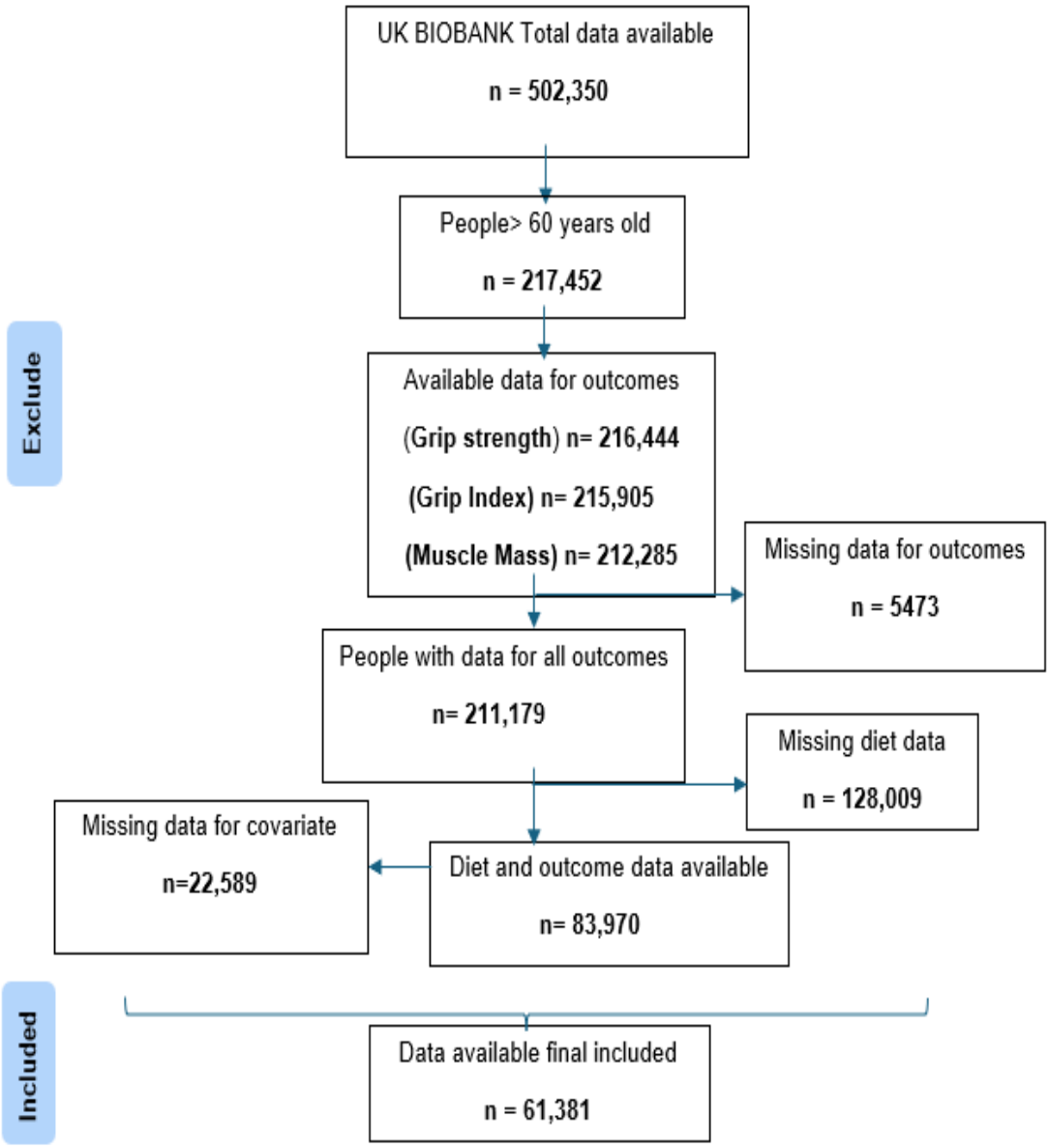
Appendix 3-A: Overview of Self-Reported Long-Term Health Conditions Included in the Multimorbidity Count (chapter 3)

Long term condition grouping	Conditions included as reported by participants
1. Painful conditions	Back pain Joint pain Back pain Joint pain Headaches (not migraine) Sciatica Plantar fasciitis Carpal tunnel syndrome Fibromyalgia Arthritis Shingles Disc problem Prolapsed disc/slipped disc Spine arthritis/spondylitis Ankylosing spondylitis Back problem Osteoarthritis Gout Cervical spondylosis Trigeminal neuralgia Disc degeneration Trapped nerve/compressed nerve
2. Hypertension	Hypertension Essential Hypertension
3. Depression	Depression Postnatal Depression
4. Asthma	Asthma
5. Atrial Fibrillation	Atrial Fibrillation
6. Coronary Heart Disease	Heart attack/Myocardial Infarction Angina
7. Dyspepsia	Gastro-oesophageal reflux (GORD)/gastric reflux Oesophagitis /Barrett's oesophagus Gastric stomach ulcers Gastric erosions/gastritis Duodenal ulcer Dyspepsia/indigestion Hiatus hernia Helicobacter pylori
8. Diabetes	Diabetic nephropathy Diabetic neuropathy/ulcers Diabetes Type 1 diabetes Type 2 diabetes Diabetic eye disease
9. Thyroid disorders	Thyroid problem (not cancer) Hyperthyroidism/thyrotoxicosis Hypothyroidism/myxoedema Grave's disease Thyroid goitre Thyroiditis
10. Connective tissue disorders	Myositis/myopathy Systemic Lupus Erythematosus Connective tissue disorder Sjogrens syndrome/sicca syndrome Dermatopolymyositis Scleroderma/systemic sclerosis Rheumatoid arthritis Psoriatic arthropathy Dermatomyositis Polymyositis

	Polymyalgia Rheumatica Malabsorption/coeliac disease
11. Chronic Obstructive Pulmonary Disease (COPD)	COPD/chronic obstructive airways disease Emphysema/ chronic bronchitis Emphysema
12. Anxiety	Anxiety/panic attacks Nervous breakdown Post-traumatic stress disorder Obsessive compulsive disorder Stress Insomnia Psychological/psychiatric problem
13. Irritable bowel syndrome	Irritable bowel syndrome
14. Alcohol problems	Alcohol dependency Alcoholic liver disease/alcoholic cirrhosis
15. Other psychoactive substance abuse	Opioid dependency Other substance abuse/dependency
16. Treated constipation	Constipation
17. Stroke/Transient Ischaemic Attack (TIA)	Stroke TIA Subarachnoid haemorrhage Brain haemorrhage Ischaemic stroke
18. Chronic kidney disease	Polycystic kidney Diabetic nephropathy Renal/kidney failure Renal failure requiring dialysis. Renal failure not requiring dialysis. Kidney nephropathy Immunoglobulin A (IgA) nephropathy
19. Diverticular disease	Diverticular disease Diverticulitis
20. Peripheral vascular disease	Peripheral vascular disease Leg claudication/intermittent claudication
21. Heart failure	Cardiomyopathy Hypertrophic cardiomyopathy Heart failure/pulmonary oedema
22. Prostate disorders	Prostate problem (not cancer) Enlarged prostate. Benign prostatic hypertrophy
23. Glaucoma	Glaucoma
24. Epilepsy	Epilepsy
25. Dementia	Dementia Alzheimer's disease Cognitive impairment
26. Schizophrenia/bipolar disorder	Schizophrenia Mania/ Bipolar disorder Manic depression
27. Psoriasis/eczema	Eczema Dermatitis Psoriasis
28. Inflammatory Bowel Disease	Inflammatory Bowel Disease Crohn's disease Ulcerative colitis
29. Migraine	Migraine
30. Chronic sinusitis	Chronic sinusitis
31. Anorexia or bulimia	Anorexia Bulimia Other eating disorders
32. Bronchiectasis	Bronchiectasis
33. Parkinson's disease	Parkinson's disease
34. Multiple Sclerosis	Multiple Sclerosis
35. Viral Hepatitis	Infective/viral hepatitis

	Hepatitis B Hepatitis C Hepatitis D Hepatitis E
36. Chronic Liver disease	Oesophageal varices Non infective hepatitis Liver failure/cirrhosis Primary biliary cirrhosis
37. Osteoporosis	Osteoporosis
38. Chronic fatigue syndrome	Chronic fatigue syndrome
39. Endometriosis	Endometriosis
40. Meniere's disease	Meniere's disease
41. Pernicious Anaemia	Pernicious Anaemia
42. Polycystic ovary	Polycystic ovary
43. Cancer	Lifetime diagnosis

Appendix 3-B: flowchart for participants included in the study (chapter 3)



Appendix 3-C: Cohort characteristics by quintiles of n-3 fatty acid intake (chapter 3)

	n-3 fatty acids intake categories in g per day					
	<1.15	1.15-1.90	1.91-2.65	2.66-3.4	>3.40	Total
N (%)	6,330 (10.3%)	23,056 (37.6%)	17,939 (29.2%)	8,128 (13.2%)	5,928 (9.7%)	61,381 (100.0%)
Age (years)	63.9 (2.7)	63.8 (2.7)	63.8 (2.7)	63.9 (2.8)	63.9 (2.8)	63.8 (2.7)
Sex, n (%)						
Women	4,282 (67.6%)	13,004 (56.4%)	9,122 (50.9%)	3,942 (48.5%)	2,844 (48.0%)	33,194 (54.1%)
Men	2,048 (32.4%)	10,052 (43.6%)	8,817 (49.1%)	4,186 (51.5%)	3,084 (52.0%)	28,187 (45.9%)
Deprivation index, n (%)						
Least deprived	2,409 (38.1%)	9,161 (39.7%)	6,867 (38.3%)	3,136 (38.6%)	2,335 (39.4%)	23,908 (39.0%)
Middle deprived	2,238 (35.4%)	8,216 (35.6%)	6,465 (36.0%)	2,881 (35.4%)	2,091 (35.3%)	21,891 (35.7%)
Most deprived	1,683 (26.6%)	5,679 (24.6%)	4,607 (25.7%)	2,111 (26.0%)	1,502 (25.3%)	15,582 (25.4%)
Ethnicity, n (%)						
White	6,181 (97.6%)	22,638 (98.2%)	17,651 (98.4%)	7,994 (98.4%)	5,795 (97.8%)	60,259 (98.2%)
Mixed	46 (0.7%)	146 (0.6%)	102 (0.6%)	47 (0.6%)	47 (0.8%)	388 (0.6%)
South Asian	64 (1.0%)	174 (0.8%)	112 (0.6%)	47 (0.6%)	46 (0.8%)	443 (0.7%)
Black	26 (0.4%)	66 (0.3%)	52 (0.3%)	26 (0.3%)	33 (0.6%)	203 (0.3%)
Chinese	13 (0.2%)	32 (0.1%)	22 (0.1%)	14 (0.2%)	7 (0.1%)	88 (0.1%)
lpaq_totalpaactive						
Inactive	774 (15.2%)	2,818 (14.7%)	2,277 (15.1%)	1,004 (14.6%)	678 (13.4%)	7,551 (14.7%)
active	4,327 (84.8%)	16,373 (85.3%)	12,784 (84.9%)	5,891 (85.4%)	4,371(86.6%)	43,746 (85.3%)
Smoking status, n (%)						
Never	3,222 (50.9%)	11,988 (52.0%)	9,321 (52.0%)	4,263 (52.4%)	3,113 (52.5%)	31,907 (52.0%)
Previous	2,690 (42.5%)	9,778 (42.4%)	7,643 (42.6%)	3,393 (41.7%)	2,526 (42.6%)	26,030 (42.4%)
Current	418 (6.6%)	1,290 (5.6%)	975 (5.4%)	472 (5.8%)	289 (4.9%)	3,444 (5.6%)
Alcohol consumption (Units/week)	16.61 (19.03)	15.96 (17.05)	15.60 (16.41)	16.09 (16.69)	15.97 (17.02)	15.94 (17.03)
Multimorbidity, n (%)						
0 LTC	1,831 (28.9%)	6,434 (27.9%)	4,987 (27.8%)	2,261 (27.8%)	1,659(28.0%)	17,172 (28.0%)
1LTC	2,145 (33.9%)	7,909 (34.3%)	6,145 (34.3%)	2,808 (34.5%)	2,032(34.3%)	21,039 (34.3%)
2+ LTCs	2,354 (37.2%)	8,713 (37.8%)	6,807 (37.9%)	3,059 (37.6%)	2,237(37.7%)	23,170 (37.7%)
Total Energy intake (Kcal/day)	1,829 (317)	2,057 (360)	2,276(422)	2,416 (484)	2,489 (527)	2,187 (454)

Data presented as mean and (SD) for continuous variables and as frequency observations and % for categorical variables. LTC: Long Term Condition

Appendix 3-D: Cohort characteristics by quintiles of n-3 fatty acid intake in people with sarcopenia (chapter 3).

	n-3 fatty acids intake categories in g per day					
	<1.15	1.15-1.90	1.91-2.65	2.66-3.4	>3.40	Total
N (%)	183 (13.4%)	537 (39.3%)	382 (28.0%)	175 (12.8%)	89 (6.5%)	1,366 (100.0%)
Age (years)	64.6 (2.8)	64.5 (2.8)	64.4 (2.8)	64.3 (2.8)	64.6 (3.1)	64.5 (2.8)
Sex, n (%)						
Women	158 (86.3%)	420 (78.2%)	276 (72.3%)	130 (74.3%)	69 (77.5%)	1,053 (77.1%)
Men	25 (13.7%)	117 (21.8%)	106 (27.7%)	45 (25.7%)	20 (22.5%)	313 (22.9%)
Deprivation index, n (%)						
Least deprived	44 (24.0%)	168 (31.3%)	114 (29.8%)	47 (26.9%)	35 (39.3%)	408 (29.9%)
Middle deprived	67 (36.6%)	188 (35.0%)	125 (32.7%)	63 (36.0%)	27 (30.3%)	470 (34.4%)
Most deprived	72 (39.3%)	181 (33.7%)	143 (37.4%)	65 (37.1%)	27 (30.3%)	488 (35.7%)
Ethnicity						
White	176 (96.2%)	513 (95.5%)	357 (93.5%)	162 (92.6%)	86 (96.6%)	1,294 (94.7%)
Mixed	3 (1.6%)	5 (0.9%)	9 (2.4%)	5 (2.9%)	0 (0.0%)	22 (1.6%)
South Asian	3 (1.6%)	15 (2.8%)	13 (3.4%)	4 (2.3%)	2 (2.2%)	37 (2.7%)
Black	0 (0.0%)	3 (0.6%)	2 (0.5%)	2 (1.1%)	1 (1.1%)	8 (0.6%)
Chinese	1 (0.5%)	1 (0.2%)	1 (0.3%)	2 (1.1%)	0 (0.0%)	5 (0.4%)
lpaq_totalpaactive						
Inactive	43 (30.9%)	128 (32.0%)	95 (33.2%)	41 (29.3%)	23 (34.3%)	330 (32.0%)
active	96 (69.1%)	272 (68.0%)	191 (66.8%)	99 (70.7%)	44 (65.7%)	702 (68.0%)
Smoking status, n (%)						
Never	92 (50.3%)	296 (55.1%)	200 (52.4%)	79 (45.1%)	44 (49.4%)	711 (52.0%)
Previous	75 (41.0%)	204 (38.0%)	163 (42.7%)	80 (45.7%)	38 (42.7%)	560 (41.0%)
Current	16 (8.7%)	37 (6.9%)	19 (5.0%)	16 (9.1%)	7 (7.9%)	95 (7.0%)
Alcohol consumption (Units/week)	11.3 (1.8)	9.9 (1.0)	10.3 (1.8)	9.7 (1.5)	10.1 (1.4)	10.2 (1.2)
Multimorbidity, n (%)						
0 LTC	23 (12.6%)	52 (9.7%)	42 (11.0%)	24 (13.7%)	9 (10.1%)	150 (11.0%)
1LTC	48 (26.2%)	108 (20.1%)	86 (22.5%)	45 (25.7%)	28 (31.5%)	315 (23.1%)
2+ LTCs	112 (61.2%)	377 (70.2%)	254 (66.5%)	106 (60.6%)	52 (58.4%)	901 (66.0%)
Total Energy intake (Kcal/day)	1,745.27 (316.8)	1,971.46 (361.8)	2,187.88 (440.5)	2,361.51 (481.8)	2,411.27 (541.2)	2,080.31 (455.7)

Data presented as mean and (SD) for continuous variables and as frequency observations and % for categorical variables.

Appendix 3-E: The association of n-3 fatty acid intake with hand grip strength and muscle mass in older men (chapter3).

	Model 0	Model 1	Model 2	Model 3
Grip strength (kg)	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
n-3 <1.15 g/day	36.8 (36.5; 37.1)	36.7 (36.4; 37.1)	36.7 (36.4; 37.0)	37.2 (36.8; 37.5)
n-3 1.15-1.90 g/day	37.2 (37.0; 37.3)	37.2 (37.0; 37.3)	37.2 (37.0; 37.3)	37.4 (37.2; 37.5)
n-3 1.91-2.65 g/day	37.7 (37.5; 37.8)	37.7 (37.5; 37.9)	37.7 (37.5; 37.9)	37.7 (37.6; 37.9)
n-3 2.66-3.4 g/day	38.0 (37.8; 38.2)	38.0 (37.8; 38.2)	38.0 (37.8; 38.2)	37.9 (37.6; 38.1)
n-3 >3.40 g/day	37.7 (37.4; 38.0)	37.7 (37.4; 38.0)	37.7 (37.4; 38.0)	37.5 (37.2; 37.8)
Grip Strength Index (kg/m^2)				
n-3 <1.15 g/day	21.2 (21.0; 21.3)	21.1 (20.9; 21.3)	21.1 (20.9; 21.3)	21.2 (21.0; 21.4)
n-3 1.15-1.90 g/day	21.2 (21.2; 21.3)	21.2 (21.2; 21.3)	21.2 (21.2; 21.3)	21.3 (21.2; 21.4)
n-3 1.91-2.65 g/day	21.4 (21.3: 21.5)	21.4 (21.3; 21.5)	21.4 (21.3; 21.5)	21.5 (21.4; 21.6)
n-3 2.66-3.4 g/day	21.5 (21.4; 21.7)	21.5 (21.4; 21.7)	21.5 (21.4; 21.7)	21.5 (21.4; 21.7)
n-3 >3.40 g/day	21.4 (21.2: 21.5)	21.4 (21.2; 21.5)	21.4 (21.2.;21.5)	21.4 (21.2; 21.5)
Muscle Mass Index (kg/m^2)				
n-3 <1.15 g/day	8.83 (8.79; 8.86)	8.82 (8.78; 8.85)	8.82 (8.79; 8.86)	8.90 (8.87; 8.94)
n-3 1.15-1.90 g/day	8.91 (8.89; 8.92)	8.91 (8.89; 8.92)	8.91 (8.89; 8.92)	8.94 (8.92; 8.96)
n-3 1.91-2.65 g/day	8.96 (8.94; 8.98)	8.96 (8.95; 8.98)	8.96 (8.95; 8.98)	8.94 (8.93; 8.96)
n-3 2.66-3.4 g/day	9.00 (8.98: 9.02)	9.00 (8.98; 9.03)	9.00 (8.98; 9.03)	8.95 (8.92; 8.97)
n-3 >3.40 g/day	9.01 (8.99; 9.04)	9.02 (8.99; 9.04)	9.02 (8.99; 9.04)	8.96 (8.93; 8.99)

Data presented as adjusted means. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity.

Appendix 3-F: The association of n-3 fatty acid intake with hand grip strength and muscle mass in older women (chapter3).

	Model 0	Model 1	Model 2	Model 3
Grip strength (kg)	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
n-3 <1.15 g/day	21.2 (21.1; 21.4)	21.2 (21.0; 21.4)	21.2 (21.0; 21.4)	21.5 (21.3; 21.7)
n-3 1.15-1.90 g/day	21.6 (21.5; 21.7)	21.6 (21.5; 21.7)	21.6 (21.5; 21.7)	21.7 (21.6; 21.8)
n-3 1.91-2.65 g/day	21.8 (21.7; 21.9)	21.8 (21.7; 22.0)	21.8 (21.7; 22.0)	21.9 (21.8; 22.0)
n-3 2.66-3.4 g/day	22.1 (21.9; 22.3)	22.1 (21.9; 22.3)	22.1 (21.9; 22.3)	22.1 (21.9; 22.3)
n-3 >3.40 g/day	22.0 (21.8; 22.2)	22.0 (21.8; 22.2)	22.0 (21.8; 22.2)	21.9 (21.7; 22.1)
Grip Strength Index (kg/m^2)				
n-3 <1.15 g/day	13.1 (13.0; 13.2)	13.1 (13.0; 13.2)	13.1 (13.0; 13.2)	13.3 (13.2; 13.4)
n-3 1.15-1.90 g/day	13.3 (13.2; 13.3)	13.3 (13.2; 13.3)	13.3 (13.2; 13.3)	13.4 (13.3; 31.4)
n-3 1.91-2.65 g/day	13.4 (13.3;13.5)	13.4 (13.3; 13.5)	13.4 (13.3; 13.5)	13.5 (13.4; 13.5)
n-3 2.66-3.4 g/day	13.5 (13.4; 13.6)	13.5 (13.4; 13.6)	13.5 (13.4; 13.6)	13.6 (13.4; 13.7)
n-3 >3.40 g/day	13.5 (13.4; 13.6)	13.5 (13.4; 13.6)	13.5 (13.4; 13.6)	13.5 (13.3; 13.6)
Muscle Mass Index (kg/m^2)				
n-3 <1.15 g/day	6.31 (6.29; 6.33)	6.31 (6.29; 6.33)	6.31 (6.29; 6.33)	6.37 (6.35; 6.40)
n-3 1.15-1.90 g/day	6.38 (6.37; 6.39)	6.38 (6.37; 6.39)	6.38 (6.37; 6.39)	6.41 (6.40; 6.42)
n-3 1.91-2.65 g/day	6.42 (6.41; 6.44)	6.42 (6.41; 6.44)	6.42 (6.41; 6.44)	6.40 (6.38; 6.41)
n-3 2.66-3.4 g/day	6.44 (6.42: 6.46)	6.44 (6.42; 6.46)	6.44 (6.42; 6.46)	6.38 (6.36; 6.40)
n-3 >3.40 g/day	6.44 (6.41; 6.46)	6.44 (6.41; 6.46)	6.44 (6.41; 6.46)	6.37 (6.34; 6.39)

Data presented as adjusted means. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity.

Appendix 3-G: The association of n-3 fatty acid intake with hand grip strength and muscle mass in older men and women with sarcopenia (chapter 3).

	Men - Model 3 (n=313)		Women - Model 3 (n=1,053)	
Grip strength (kg)	**Beta (95% CI)**	**p**	**Beta (95% CI)**	**p**
n-3 <1.15 g/day	Ref.		Ref.	
n-3 1.15-1.90 g/day	2.793 (0.398; 5.189)	0.022	0.545 (-0.292; 1.383)	0.202
n-3 1.91-2.65 g/day	2.784 (0.339; 5.229)	0.026	0.506 (-0.431; 1.444)	0.290
n-3 2.66-3.4 g/day	2.008 (-1.782; 4.799)	0.158	0.985 (-0.134; 2.104)	0.084
n-3 >3.40 g/day	2.833 (-0.373; 6.040)	0.083	0.490 (-0.895; 1.877)	0.488
Trend for quintiles	0.181 (-0. 440; 0. 803)	0.566	0.163 (-0.119; 0.446)	0.256
Grip strength Index (kg/m^2)				
n-3 <1.15 g/day	Ref.		Ref.	
n-3 1.15-1.90 g/day	1.427 (0.018; 2.836)	0.047	0.363 (-0.156; 0.883)	0.170
n-3 1.91-2.65 g/day	1.559 (0.120; 2.997)	0.034	0.353 (-0.228; 0.936)	0.234
n-3 2.66-3.4 g/day	1.069 (-0.571; 2.711)	0.201	0.594 (-0.099; 1.289)	0.093
n-3 >3.40 g/day	1.640 (-0.245; 3.527)	0.088	0.301 (-0.559; 1.162)	0.492
Trend for quintiles	0.141 (-0.223; 0.506)	0.446	0.097 (-0.077; 0.272)	0.276
Muscle Mass Index (kg/m^2)				
n-3 <1.15 g/day	Ref.		Ref.	
n-3 1.15-1.90 g/day	0.773 (0.228; 1.319)	0.006	0.037 (-0.160; 0.235)	0.708
n-3 1.91-2.65 g/day	0.892 (0.336; 1.449)	0.002	-0.038 (-0.260; 0.183)	0.733
n-3 2.66-3.4 g/day	0.956 (0.320; 1.591)	0.003	-0.180 (-0.445; 0.083)	0.180
n-3 >3.40 g/day	1.228 (0.498; 1.958)	0.001	-0.177 (-0.505; 0.150)	0.288
Trend for quintiles	0.197 (0.055; 0.338)	0.006	-0.062 (-0.129; 0.004)	0.068

Data presented as adjusted beta-coefficients. Analyses were adjusted for model 3 including age, sex, deprivation index, ethnicity, assessment month, lifestyle factors (smoking and alcohol intake), multimorbidity, total energy intake and physical activity.

Appendix 3-H: The association of n-3 fatty acid intake with hand grip strength and muscle mass in older men and women with sarcopenia (chapter 3).

	Men - Model 3	Women - Model 3
Grip strength (kg)	**Mean (95% CI)**	**Mean (95% CI)**
n-3 <1.15 g/day	18.8 (16.5; 21.1)	11.9 (11.2; 12.6)
n-3 1.15-1.90 g/day	21.6 (20.7; 22.5)	12.5 (12.0; 12.9)
n-3 1.91-2.65 g/day	21.6 (20.7; 22.5)	12.4 (11.9; 13.0)
n-3 2.66-3.4 g/day	20.8 (19.3; 22.3)	12.9 (12.1; 13.7)
n-3 >3.40 g/day	21.6 (19.5; 23.8)	12.4 (11.3; 13.5)
Grip strength Index (kg/m2)		
n-3 <1.15 g/day	11.0 (9.75; 12.4)	7.44 (6.99; 7.90)
n-3 1.15-1.90 g/day	12.5 (11.9; 13.0)	7.81 (7.53; 8.08)
n-3 1.91-2.65 g/day	12.6 (12.1; 13.1)	7.80 (7.45; 8.14)
n-3 2.66-3.4 g/day	12.1 (11.2; 13.0)	8.04 (7.55; 8.53)
n-3 >3.40 g/day	12.7 (11.4; 13.9)	7.74 (7.04; 8.44)
Muscle Mass Index (kg/m2)		
n-3 <1.15 g/day	8.05 (7.53; 8.56)	5.97 (5.80; 6.15)
n-3 1.15-1.90 g/day	8.82 (8.61; 9.03)	6.01 (5.91; 6.12)
n-3 1.91-2.65 g/day	8.94 (8.74; 9.14)	5.94 (5.80; 6.07)
n-3 2.66-3.4 g/day	9.00 (8.67; 9.34)	5.79 (5.61; 5.98)
n-3 >3.40 g/day	9.28 (8.79; 9.76)	5.80 (5.53; 6.06)

Data presented as adjusted means. Analyses were adjusted for model 3 including age, sex, deprivation index, ethnicity, assessment month, lifestyle factors (smoking and alcohol intake), multimorbidity, total energy intake and physical activity.

Appendix 3-I: The association of the n-6/n-3 fatty acid intake ratio with hand grip strength and muscle mass in older men (chapter 3).

Outcomes	Model 0 (n= 27,813)		Model 1 (n= 27,813)		Model 2 (n= 27,813)		Model 3 (n= 27,813)	
Grip strength (kg)	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**
<=2.4	Ref.		Ref.		Ref.		Ref.	
2.41-4.8	0.323 (-0.270; 0.918)	0.286	0.350 (-0.243; 0.944)	0.248	0.340 (-0.252; 0.933)	0.260	0.137 (-0.484; 0.759)	0.665
4.81-7.2	0.130 (-0.447; 0.708)	0.658	0.163 (-0.415; 0.741)	0.581	0.169 (-0.406; 0.746)	0.563	-0.010 (-0.616; 0.595)	0.973
7.21-9.6	0.295 (-0.309; 0.899)	0.339	0.322 (-0.281; 0.927)	0.295	0.337 (-0.265; 0.940)	0.273	0.116 (-0.516; 0.750)	0.718
>9.6	0.108 (-0.653; 0.871)	0.780	0.084 (-0.677; 0.847)	0.827	0.072 (-0.687; 0.833)	0.851	-0.175 (0.066; 0.678)	0.667
Trend for quintiles	-0.012 (-0.125; 0.100)	0.825	-0.015 (-0.128; 0.097)	0.792	-0.008 (-0.121; 0.104)	0.885	-0.032 (-0.152; 0.086)	0.590
Grip Strength Index (kg/m2)								
<=2.4	Ref.		Ref.		Ref.		Ref.	
2.41-4.8	0.089 (-0.239; 0.417)	0.595	0.102 (-0.225; 0.431)	0.540	0.097 (-0.229; 0.425)	0.559	0.001 (-0.342; 0.346)	0.992
4.81-7.2	-0.008 (-0.327; 0.311)	0.960	0.007 (-0.311; 0.327)	0.961	0.011 (-0.307; 0.330)	0.944	-0.055 (-0.390; 0.280)	0.747
7.21-9.6	0.096 (-0.237; 0.430)	0.572	0.109 (-0.224; 0.444)	0.519	0.117 (-0.216; 0.450)	0.491	0.029 (-0.321; 0.379)	0.870
>9.6	0.001 (-0.419; 0.422)	0.994	-0.012 (-0.433; 0.409)	0.955	-0.018 (-0.438; 0.402)	0.933	-0.144 (-0.586; 0.297)	0.523
Trend for quintiles	-0.003 (-0.066; 0.058)	0.903	0.005 (-0.067; 0.057)	0.864	-0.002 (-0.064; 0.060)	0.949	-0.009 (-0.075; 0.056)	0.789
Muscle Mass Index (kg/m2)								
<=2.4	Ref.		Ref.		Ref.		Ref.	
2.41-4.8	-0.038 (-0.099; 0.022)	0.220	-0.035 (-0.096; 0.025)	0.256	-0.033 (-0.094; 0.026)	0.272	-0.081 (-0.144; -0.017)	0.012
4.81-7.2	-0.022 (-0.082; 0.036)	0.450	-0.019 (-0.079; 0.039)	0.512	-0.020 (-0.079; 0.038)	0.490	-0.070 (-0.132; 0.009)	0.024
7.21-9.6	-0.054 (-0.116; 0.007)	0.084	-0.052 (0.114; 0.009)	0.099	-0.054 (-0.115; 0.007)	0.085	-0.104 (-0.168; -0.040)	0.001
>9.6	-0.053 (-0.132; 0.024)	0.177	-0.058 (-0.136; 0,019)	0.144	-0.056 (-0.134; 0.021)	0.153	-0.120 (-0.201; -0.38)	0.004
Trend for quintiles	-0.008 (-0.020; 0.002)	0.144	-0.009 (-0.020; 0.002)	0.117	-0.010 (-0.021; 0.001)	0.083	-0.016 (-0.028; -0.004)	0.007

Data presented as adjusted beta-coefficients. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity.

Appendix 3-J: The association of the n-6/n-3 fatty acid intake ratio with hand grip strength and muscle mass in older women (chapter 3).

Outcomes	Model 0 (n=32,041)		Model 1 (n=32,041)		Model 2 (n=32,041)		Model 3 (n=32,041)	
Grip strength (kg)	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**
<=2.4	Ref.		Ref.		Ref.		Ref.	
2.41-4.8	0.025 (-0.307; 0.357)	0.882	0.029 (-0.302; 0.361)	0.862	0.480 (-0.281; 0.377)	0.775	0.129 (-0.236; 0.495)	0.489
4.81-7.2	-0.221 (-0.545; 0.101)	0.179	-0.184 (-0.506; 0.138)	0.264	-0.142 (-0.463; 0.178)	0.383	-0.088 (-0.445; 0.268)	0.626
7.21-9.6	-0.196 (-0.543; 0.149)	0.265	-0.151 (-0.497; 0.194)	0.392	-0.128 (-0.473; 0.215)	0.463	-0.040 (-0.423; 0.342)	0.837
>9.6	-0.247(-0.739; 0.244)	0.324	-0.243(-0.734; 0.247)	0.331	-0.212 (-0.700; 0.275)	0.393	-0.170 (-0.716; 0.375)	0.540
Trend for quintiles	-0.107 (-0.180; -0.033)	0.004	0.091 (-0.165; -0.018)	0.014	-0.085 (-0.158; -0.012)	0.022	-0.081 (-0.162; -0.000)	0.049
Grip Strength Index (kg/m^2)								
<=2.4	Ref.		Ref.		Ref.		Ref.	
2.41-4.8	-0.012 (-0.211; 0.187)	0.094	-0.010 (-0.209; 0.189)	0.919	0.000 (-0.197; 0.198)	0.996	0.061 (-0.158; 0.282)	0.581
4.81-7.2	-0.143(-0.337; 0.050)	0.148	-0.123 (-0.317; 0.070)	0.211	-0.100 (-0.293; 0.093)	0.310	-0.042 (-0.257; 0.172)	0.700
7.21-9.6	-0.128 (-0.336; 0.080)	0.228	-0.103 (-0.311; 0.103)	0.327	-0.091 (-0.298; 0.115)	0.388	-0.015 (-0.245; 0.215)	0.898
>9.6	-0.133 (-0.429; 0.161)	0.375	-0.131 (-0.426; 0.164)	0.384	-0.113 (-0.407; 0.180)	0.448	-0.062 (-0.390; 0.265)	0.709
Trend for quintiles	-0.057 (-0.101; 0.013)	0.011	-0.048 (-0.092; 0.004)	0.030	-0.045 (-0.089; -0.001)	0.043	-0.036 (-0.085; 0.012)	0.145
Muscle Mass Index (kg/m^2)								
<=2.4	Ref.		Ref.		Ref.		Ref.	
2.41-4.8	0.019 (-0.021; 0.060)	0.352	0.020 (-0.020; 0.061)	0.333	0.018 (-0.022; 0.059)	0.376	-0.021 (0.066; 0.023)	0.360
4.81-7.2	0.033 (-0.006; 0.073)	0.103	0.032 (-0.007; 0.072)	0.112	0.028 (-0.011; 0.068)	0.160	-0.015 (-0.059; 0.028)	0.480
7.21-9.6	0.026 (-0.016; 0.069)	0.230	0.024 (-0.018; 0.067)	0.259	0.022 (-0.020; 0.065)	0.300	-0.019 (-0.061; 0.028)	0.379
>9.6	-0.007 (-0.068; 0.053)	0.809	-0.008 (-0.069; 0.052)	0.789	-0.011 (-0.071; 0.049)	0.718	-0.026 (-0.073; 0.020)	0. 271
Trend for quintiles	0.003 (-0.005; 0.012)	0.488	0.002 (-0.006; 0.011)	0.614	0.001 (-0.007; 0.010)	0.706	-0.005 (-0.015; 0.004)	0.274

Data presented as adjusted beta-coefficients. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity.

Appendix3-K: The association of the n-6/n-3 fatty acid intake ratio with hand grip strength and muscle mass in older men (chapter 3).

	Model 0 (n= 27,813)	Model 1 (n= 27,813)	Model 2 (n= 27,813)	Model 3 (n= 27,813)
Grip strength (kg)	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
<=2.4	37.3 (36.7; 37.8)	36.3 (36.7; 37.8)	37.3 (36.7; 37.8)	37.5 (36.9; 38.1)
2.41-4.8	37.6 (37.4; 37.8)	37.6 (37.4; 37.8)	37.6 (37.4; 37.8)	37.7 (37.5; 37.8)
4.81-7.2	37.4 (37.3; 37.5)	37.4 (37.3; 37.5)	37.4 (37.3; 37.5)	37.5 (37.4; 37.6)
7.21-9.6	38.6 (37.4; 38.8)	37.6 (37.4; 37.8)	38.6 (37.4; 38.8)	37.6 (37.4; 37.9)
>9.6	37.4 (36.9; 37.9)	37.3 (36.8; 37.9)	37.3 (36.8; 38.8)	37.3 (36.8; 37.9)
Grip Strength Index (kg/m^2)				
<=2.4	21.3 (21.0; 21.6)	21.3 (21.0; 21.6)	21.3 (21.0; 21.6)	21.4 (21.1; 21.7)
2.41-4.8	21.4 (21.3; 21.5)	21.4 (21.3; 21.5)	21.4 (21.3; 21.5)	21.4 (21.3; 21.5)
4.81-7.2	21.3 (21.2; 21.4)	21.3 (21.2; 21.4)	21.3 (21.2; 21.4)	21.4 (21.3; 21.4)
7.21-9.6	21.4 (21.3; 21.5)	21.4 (21.3; 21.5)	21.4 (21.3; 21.5)	21.4 (21.3; 21.6)
>9.6	21.3 (21.0; 21.6)	21.3 (21.0; 21.6)	21.3 (21.0; 21.6)	21.3 (21.0; 21.6)
Muscle Mass Index (kg/m^2)				
<=2.4	8.98 (8.92; 9.92)	8.97 (8.92; 9.03)	8.97 (8.92; 9.03)	9.02 (8.92; 8.96)
2.41-4.8	8.94 (8.92; 8.96)	8.94 (8.92; 8.96)	8.94 (8.92; 8.96)	8.94 (8.94; 8.96)
4.81-7.2	8.95 (8.94; 8.97)	8.95 (8.94; 8.97)	8.95 (8.94; 8.97)	8.95 (8.89; 8.94)
7.21-9.6	9.92 (8.90: 8.94)	8.92 (8.90; 8.94)	8.92 (8.90; 8.94)	8.92 (8.90; 8.94)
>9.6	9.92 (8.87; 8.97)	8.91 (8.86; 8.97)	8.92 (8.86; 8.97)	8.90 (8.85; 8.95)

Data presented as adjusted means. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity.

Appendix 3-L: The association of the n-6/n-3 fatty acid intake ratio with hand grip strength and muscle mass in older women (chapter 3).

	Model 0 (n=32,041)	Model 1 (n=32,041)	Model 2 (n=32,041)	Model 3 (n=32,041)
Grip strength (kg)	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
<=2.4	21.8 (21.5; 22.2)	21.8 (21.5; 22.1)	21.8 (21.5; 22.1)	21.8 (21.5; 22.2)
2.41-4.8	21.9 (21.8; 22.0)	21.8 (21.7; 21.0)	21.8 (21.7; 21.9)	22.0 (21.8; 22.1)
4.81-7.2	21.6 (21.5; 21.7)	21.6 (21.6; 21.7)	21.6 (21.6; 21.7)	21.7 (21.7; 21.8)
7.21-9.6	21.6 (21.5; 21.8)	21.7 (21.5; 21.8)	21.7 (21.5; 21.8)	21.8 (21.6; 22.0)
>9.6	21.6 (21.2; 22.0)	21.6 (21.2; 22.0)	21.6 (21.2; 22.0)	21.7 (21.2; 22.1)
Grip Strength Index (kg/m^2)				
<=2.4	13.4 (13.3; 13.6)	13.4 (13.2; 13.6)	13.4 (13.2; 13.6)	13.4 (13.2; 13.6)
2.41-4.8	13.4 (13.4; 13.5)	13.4 (13.4; 13.5)	13.4 (13.3; 13.5)	13.5 (13.4; 13.6)
4.81-7.2	13.3 (13.3; 13.4)	13.3 (13.3; 13.4)	13.3 (13.3; 13.4)	13.4 (13.3; 13.4)
7.21-9.6	13.3 (13.2; 13.4)	13.3 (13.2; 13.4)	13.3 (13.2; 13.4)	13.4 (13.3; 13.5)
>9.6	13.3 (13.1; 13.5)	13.3 (13.1; 13.5)	13.3 (13.1; 13.5)	13.4 (13.1; 13.6)
Muscle Mass Index (kg/m^2)				
<=2.4	6.37 (6.33; 6.41)	6.37 (6.33; 6.41)	6.37 (6.33; 6.41)	6.41 (6.37; 6.46)
2.41-4.8	6.39 (6.37; 6.40)	6.39 (6.38; 6.40)	6.39 (6.38; 6.40)	6.39 (6.38; 6.41)
4.81-7.2	6.40 (6.39; 6.41)	6.40 (6.39; 6.41)	6.40 (6.39; 6.41)	6.40 (6.39; 6.41)
7.21-9.6	6.40 (6.38; 6.41)	6.39 (6.38; 6.41)	6.40 (6.38; 6.41)	6.39 (6.37; 6.41)
>9.6	6.36 (6.31; 6.41)	6.36 (6.31; 6.41)	6.36 (6.31; 6.41)	6.36 (6.31; 6.41)

Data presented as adjusted means. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity.

Appendix 3-M: The association of the n-6/n-3 fatty acid intake ratio with hand grip strength and muscle mass in older men and women with sarcopenia (chapter 3).

	Men - Model 3 (n=313)		Women - Model 3 (n=1,053)	
Grip strength (kg)	**B (95% CI)**	**p**	**B (95% CI)**	**p**
<=2.4	Ref.		Ref.	
2.41-4.8	1.448 (-4.026; 6.924)	0.603	-0.336 (-1.954; 1.281)	0.683
4.81-7.2	1.807 (-3.538; 7.153)	0.506	-0.108 (-1.692; 1.476)	0.893
7.21-9.6	2.403 (-3.025; 7.833)	0.384	-0.104 (-1.829; 1.620)	0.906
>9.6	2.238 (-3. 970; 8.448)	0.478	-2.618 (-4.791; -0.445)	0.018
Trend for quintiles	0.455 (-0. 281; 1. 192)	0.225	-0.194 (-0.539; 0.150)	0.269
Grip strength Index (kg/m^2)				
<=2.4	Ref.		Ref.	
2.41-4.8	0.691 (-2.525; 3.909)	0.672	-0.237 (-1.240; 0.765)	0.642
4.81-7.2	0.786 (-2.354; 3.928)	0.622	-0.057 (-1.040; 0.924)	0.908
7.21-9.6	1.150 (-2.040; 4.340)	0.478	-0.094 (-1.164; 0.975)	0.863
>9.6	1.310 (-2.338; 4.959)	0.480	-1.719 (-3.067; -0.372)	0.012
Trend for quintiles	0.242 (-0.190; 0.675)	0.271	-0.125 (-0.339; 0.088)	0.249
Muscle Mass Index (kg/m^2)				
<=2.4	Ref.		Ref.	
2.41-4.8	-0.022 (-1.281; 1.235)	0.972	0.037 (-0.045; 0.721)	0.084
4.81-7.2	0.073 (-1.155; 1.301)	0.907	0.378 (0.003; 0.753)	0.048
7.21-9.6	-0.200 (-1.448; 1.047)	0.752	0.266 (-0.142; 0.675)	0.201
>9.6	0.296 (-1.130; 1.723)	0.683	-0.005 (-0.519; 0.509)	0.984
Trend for quintiles	-0.025 (-0.196; 0.144)	0.765	-0.017 (-0.099; 0.064)	0.674

Data presented as adjusted beta coefficients. Analyses were adjusted for model 3 including age, sex, deprivation index, ethnicity, assessment month, lifestyle factors (smoking and alcohol intake), multimorbidity, total energy intake and physical activity.

Appendix 3-N: The association of the n-6/n-3 fatty acid intake ratio with hand grip strength and muscle mass in older men and women with sarcopenia (chapter 3).

	Men - Model 3 (n=313)	Women - Model 3 (n=1,053)
Grip strength (kg)	**Mean (95% CI)**	**Mean (95% CI)**
<=2.4	19.5 (14.2; 24.8)	12.7 (11.1; 14.2)
2.41-4.8	20.9 (19.7; 22.1)	12.3 (11.8; 12.8)
4.81-7.2	21.3 (20.6; 22.0)	12.6 (12.2; 12.9)
7.21-9.6	21.9 (20.6; 23.2)	12.9 (11.8; 13.3)
>9.6	21.7 (18.4; 25.0)	10.0 (8.56; 11.6)
Grip strength Index (kg/m^2)		
<=2.4	11.6 (8.48; 14.7)	7.94 (6.99; 8.89)
2.41-4.8	12.2 (11.5; 12.9)	7.70 (7.38; 8.02)
4.81-7.2	12.3 (11.9; 12.8)	7.88 (7.64; 8.12)
7.21-9.6	12.7 (11.9; 13.5)	7.84 (7.36; 8.32)
>9.6	12.9 (10.9; 14.8)	6.22 (5.27; 7.17)
Muscle Mass Index (kg/m^2)		
<=2.4	8.86 (7.65; 10.0)	5.62 (5.25; 5.98)
2.41-4.8	8.84 (8.57; 9.12)	5.95 (5.83; 6.08)
4.81-7.2	8.94 (8.77; 9.10)	6.00 (5.90; 6.09)
7.21-9.6	8.66 (8.36; 8.96)	5.88 (5.70; 6.07)
>9.6	9.16 (8.40; 9.92)	5.61 (5.25; 5.98)

Data presented as adjusted means. Analyses were adjusted for model 3 including age, sex, deprivation index, ethnicity, assessment month, lifestyle factors (smoking and alcohol intake), multimorbidity, total energy intake and physical activity.

Appendix 3-O: The association of n-3 fatty acid intake with hand grip strength and muscle mass in older men and women aged ≥65 (chapter 3).

	Men (n=12,254) n-3 fatty acid intake Model 3		**Men (n=171) n-3 fatty acid intake with sarcopenia Model 3**		**Women (n=12,457) n-3 fatty acid intake Model 3**		**Women (n=519) n-3 fatty acid intake with sarcopenia Model 3**	
Grip strength (kg)	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**
n-3 <1.15 g/day	Ref.		Ref.		Ref.		Ref.	
n-3 1.15-1.90 g/day	0.292 (-0.278; 0.863)	0.315	3.643 (-0.052; 7.339)	0.053	0.131 (-0.215; 0.477)	0.459	0.583 (-0.484; 1.651)	0.283
n-3 1.91-2.65 g/day	0.596 (0.001; 1.193)	0.051	4.342 (0.537; 8.147)	0.026	0.259 (-0.118;0.637)	0.178	0.128 (-1.068;1.325)	0.833
n-3 2.66-3.4 g/day	0.641 (-0.023;1.307)	0.059	2.165 (-2.014; 6.345)	0.307	0.497 (0.048; 0.946)	0.030	0.855 (-0.608; 2.318)	0.251
n-3 >3.40 g/day	0.236 (-0.472; 0.495)	0.513	2.330 (-2.190; 6.851)	0.310	0.278 (-0.215; 0.773)	0.269	0.492 (-0.608; 2.318)	0.611
Trend for quintiles	0.072 (-0.067; 0.213)	0.309	-0.081(-0.940; 0.777)	0.851	0.102 (-0.001; 0.206)	0.053	0.106 (-0.268; 0.480)	0.577
Grip strength Index (kg/m^2)								
n-3 <1.15 g/day	Ref.		Ref.		Ref.		Ref.	
n-3 1.15-1.90 g/day	0.093 (-0.224;0.410)	0.565	1.896 (-0.256;4.049)	0.084	0.064 (-0.145;0.274)	0.547	0.393 (-0.266;1.054)	0.242
n-3 1.91-2.65 g/day	0.241 (-0.090;0.574)	0.154	2.455 (0.238;4.671)	0.030	0.124 (-0.103;0.353)	0.284	0.169 (-0.570;0.909)	0.653
n-3 2.66-3.4 g/day	0.244 (-0.125;0.614)	0.195	1.201 (-1.233;3.636)	0.331	0.262 (-0.009;0.533)	0.058	0.539 (-0.365;1.444)	0.242
n-3 >3.40 g/day	0.078 (-0.315;0.472)	0.698	1.309 (-1.324;3.942)	0.327	0.150 (-0.148;0.449)	0.323	0.335 (-0.843;1.513)	0.577
Trend for quintiles	0.030 (-0.047;0.108)	0.448	-0.004 (-0.503;0.494)	0.986	0.032 (-0.008;0.117)	0.087	0.075 (-0.156;0.306)	0.523
Muscle Mass Index (kg/m^2)								
n-3 <1.15 g/day	Ref.		Ref.		Ref.		Ref.	
n-3 1.15-1.90 g/day	0.369 (-0.233; 0.097)	0.230	0.338 (-0.455; 1.131)	0.401	0.049 (0.005;0.092)	0.027	0.067 (-0.198;0.333)	0.617
n-3 1.91-2.65 g/day	0.030 (-0.032; 0.093)	0.342	0.808 (-0.008; 1.625)	0.052	0.039 (-0.008;0.086)	0.104	0.088 (-0.209;0.386)	0.561
n-3 2.66-3.4 g/day	0.048 (-0.021; 0.119)	0.173	0.398 (-0.499; 1.295)	0.382	0.033 (-0.022;0.090)	0.238	-0.150 (-0.515;0.213)	0.416
n-3 >3.40 g/day	0.044 (-0.030; 0.119)	0.248	0.911 (-0.059; 1.881)	0.066	0.021 (-0.040;0.083)	0.504	-0.096 (-0.571;0.377)	0.689
Trend for quintiles	0.007 (-0.007; 0.021)	0.364	0.169 (-0.013; 0.351)	0.070	0.000 (-0.012;0.013)	0.933	-0.037 (-0.130;0.055)	0.430

Data presented as adjusted beta coefficients. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity.

Appendix 3-P: The association of n-3 fatty acid intake with hand grip strength and muscle mass in older men and women aged ≥65 with and without sarcopenia (chapter 3).

	Men (n=12,254) n-3 fatty acid Model 3	Men (n=171) n-3 fatty acid with sarcopenia - Model 3	Women (n=12,457) n-3 fatty acid Model 3	Women (n=519) n-3 fatty acid with sarcopenia - Model 3
Grip strength (kg)	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
n-3 <1.15 g/day	36.0 (35.4; 36.5)	17.6 (14.1; 21.1)	20.8 (20.5; 21.1)	12.1 (11.1; 13.0)
n-3 1.15-1.90 g/day	36.2 (36.0; 36.5)	21.2 (19.9; 22.5)	20.9 (20.8; 21.1)	12.6 (12.1; 13.2)
n-3 1.91-2.65 g/day	36.5 (36.3; 36.8)	21.9 (20.5; 23.3)	21.1 (20.8; 21.3)	12.2 (11.5; 12.9)
n-3 2.66-3.4 g/day	36.6 (36.2; 37.0)	19.7 (17.5; 22.0)	21.3 (21.0; 21.6)	12.9 (11.9; 14.0)
n-3 >3.40 g/day	36.2 (35.8; 36.6)	19.9 (17.1; 22.7)	21.1 (20.7; 21.4)	12.6 (10.9; 14.2)
Grip strength Index (kg/m^2)				
n-3 <1.15 g/day	20.6 (20.3; 20.9)	10.4 (8.38; 12.4)	12.8 (12.7; 13.0)	7.53 (6.95; 8.10)
n-3 1.15-1.90 g/day	20.7 (20.6; 20.8)	12.3 (11.5; 13.0)	12.9 (12.8; 13.0)	7.92 (7.56; 8.27)
n-3 1.91-2.65 g/day	20.9 (20.7; 21.0)	12.8 (12.0; 13.6)	13.0 (12.8; 13.1)	7.69 (7.25; 8.14)
n-3 2.66-3.4 g/day	20.9 (20.7; 21.1)	11.6 (10.3; 12.9)	13.1 (12.9; 13.3)	8.06 (7.41; 8.72)
n-3 >3.40 g/day	20.7 (20.5; 20.9)	11.7 (10.1; 13.3)	13.0 (12.8; 13.2)	7.86 (6.86; 8.86)
Muscle Mass Index (kg/m^2)				
n-3 <1.15 g/day	8.86 (8.80; 8.91)	8.25 (7.51; 9.00)	6.30 (6.26; 6.34)	5.90 (5.67; 6.13)
n-3 1.15-1.90 g/day	8.89 (8.87; 8.92)	8.59 (8.31; 8.88)	6.35 (6.32; 6.37)	5.96 (5.82; 6.11)
n-3 1.91-2.65 g/day	8.89 (8.86; 8.91)	9.06 (8.77; 9.36)	6.34 (6.31; 6.36)	5.98 (5.80; 6.17)
n-3 2.66-3.4 g/day	8.91 (8.87; 8.94)	8.65 (8.17; 9.13)	6.33 (6.29; 6.37)	5.75 (5.48; 6.01)
n-3 >3.40 g/day	8.90 (8.86; 8.95)	9.16 (8.57; 9.76)	6.32 (6.27; 6.36)	5.80 (5.40; 6.20)

Data presented as adjusted means. Analyses were adjusted for model 3 including age, sex, deprivation index, ethnicity, assessment month, lifestyle factors (smoking and alcohol intake), multimorbidity, total energy intake and physical activity.

Appendix 3-Q: The association of the n-6/n-3 fatty acid intake ratio with hand grip strength and muscle mass in older men and women aged ≥65 with and without sarcopenia (chapter 3).

	Men (n= 12,057) n-6/n-3 ratio Model 3		Men (n=171) n-6/n-3 ratio with sarcopenia Model 3		Women (n= 11,888) n-6/n-3 ratio Model 3		Women (n=519) n-6/n-3 ratio with sarcopenia Model 3	
Grip strength (kg)	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**	**B (95% CI)**	**p**
<=2.4	Ref.		Ref.		Ref.		Ref.	
2.41-4.8	-0.197 (-1.069; 0.674)	0.657	3.368 (-3.176; 9.912)	0.310	0.116 (-0.453;0.685)	0.689	0.705 (-1.308; 2.719)	0.491
4.81-7.2	-0.221 (-1.069; 0.626)	0.608	3.338 (-2.903; 9.580)	0.292	-0.128 (-0.684;0.427)	0.650	0.471 (-1.503; 2.447)	0.639
7.21-9.6	-0.091 (-0.986; 0.804)	0.842	2.430 (-3.979; 8.841)	0.454	0.147 (-0.455;0.750)	0.631	0.288 (-1.904; 2.481)	0.796
>9.6	-0.959 (-2.142; 0.223)	0.112	4.316 (-3.518; 12.151)	0.278	-0.164 (-1.033;0.704)	0.711	-1.467 (-4.404;1.469)	0.327
Trend for quintiles	-0.057 (-0. 234;0.118)	0.521	0.059 (-0.963; 1.083)	0.908	-0.033 (-0.164;0.097)	0.621	-0.264 (-0.724;0.194)	0.258
Grip strength Index (kg/m^2)								
<=2.4	Ref.		Ref.		Ref.		Ref.	
2.41-4.8	-0.180 (-0.665; 0.303)	0.464	1.669 (-2.136; 5.475)	0.387	0.037 (-0.306;0.382)	0.829	0.448 (-0.795;1.693)	0.479
4.81-7.2	-0.179 (-0.651; 0.291)	0.455	1.608 (-2.021; 5.238)	0.382	-0.090 (-0.427;0.245)	0.597	0.318 (-1.192;1.518)	0.608
7.21-9.6	-0.107 (-0.604; 0.390)	0.673	1.139 (-2.588; 4.867)	0.546	0.081 (-0.282;0.446)	0.659	0.162 (-1.192;1.518)	0.813
>9.6	-0.585 (-1.242; 0.728)	0.081	2.537 (-2.019; 7.093)	0.273	-0.079 (-0.604;0.446)	0.768	-0.945 (-2.760;0.870)	0.307
Trend for quintiles	-0.031 (-0.129; 0.669)	0.530	0.043 (-0.551; 0.638)	0.885	-0.010 (-0.089;0.068)	0.800	-0.170 (-0.454;0.113)	0.237
Muscle Mass Index (kg/m^2)								
<=2.4	Ref.		Ref.		Ref.		Ref.	
2.41-4.8	-0.111 (-0.203; -0.019)	0.017	-0.186 (-1.557;1.184)	0.788	0.014 (-0.056;0.086)	0.683	0.401 (-0.100;0.903)	0.117
4.81-7.2	-0.074 (-0.164; 0.014)	0.101	-0.275 (-1.583;1.032)	0.677	0.019 (-0.050;0.088)	0.588	0.342 (-0.150;0.834)	0.173
7.21-9.6	-0.139 (-0.234; -0.045)	0.004	-0.814 (-2.157;0.529)	0.233	0.004 (-0.071;0.079)	0.910	0.215 (-0.331;0.761)	0.439
>9.6	-0.094 (-0.219; 0.030)	0.137	0.573 (-1.068;2.215)	0.491	0.001 (-0.110;0.107)	0.981	0.322 (-0.409;1.055)	0.386
Trend for quintiles	-0.013 (-0.032; 0.005)	0.158	-0.128 (-0.347;0.091)	0.249	-0.001(-0.018;0.014)	0.825	-0.017 (-0.131;0.097)	0.770

Data presented as adjusted beta coefficients. Model 0 was unadjusted. Model 1 was adjusted for age, deprivation index, ethnicity, assessment month, and lifestyle factors (smoking and alcohol intake). Model 2 included additional adjustment for multimorbidity, and Model 3 further adjusted for total energy intake and physical activity.

Appendix3-R: The association of the n-6/n-3 fatty acid intake ratio with hand grip strength and muscle mass in older men and women aged ≥65 with and without sarcopenia (chapter 3).

	Men (n= 12,057) n-6/n-3 ratio Model 3	Men (n=171) n-6/n-3 with sarcopenia - Model 3	Women (n= 11,888) n-6/n-3 ratio Model 3	Women (n=519) n-6/n-3 ratio with sarcopenia Model 3
Grip strength (kg)	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
<=2.4	36.6 (35.7; 37.4)	17.8 (11.6; 23.9)	21.0 (20.5; 21.5)	12.0 (10.1; 13.9)
2.41-4.8	36.4 (36.1; 36.7)	21.1 (19.5; 22.7)	21.1 (20.9; 21.3)	12.7 (12.1; 13.4)
4.81-7.2	36.3 (36.2; 36.5)	21.1 (20.0; 22.3)	20.9 (20.7; 21.0)	12.5 (12.0; 13.0)
7.21-9.6	36.5 (36.1; 36.8)	20.2 (18.2; 22.2)	21.2 (20.9; 21.4)	12.3 (11.2; 13.4)
>9.6	35.6 (34.8; 36.5)	22.1 (17.1; 27.1)	20.9 (20.2; 21.5)	10.5 (8.34; 12.8)
Grip strength Index (kg/m^2)				
<=2.4	20.9 (20.5; 21.4)	10.7 (7.12; 14.2)	13.0 (12.7; 13.3)	7.52 (6.34; 8.69)
2.41-4.8	20.8 (20.6; 20.9)	12.3 (11.4; 13.3)	13.0 (12.9; 13.1)	7.97 (7.56; 8.37)
4.81-7.2	20.8 (20.7; 20.9)	12.3 (11.6; 12.9)	12.9 (12.8; 13.0)	7.83 (7.52; 8.14)
7.21-9.6	20.8 (20.6; 21.0)	11.8 (10.6; 13.0)	13.1 (12.9; 13.2)	8.68 (7.02; 8.34)
>9.6	20.4 (19.9; 20.8)	13.2 (10.3; 16.1)	12.9 (12.5; 13.3)	7.57 (5.18; 7.96)
Muscle Mass Index (kg/m^2)				
<=2.4	8.98 (8.90; 9.07)	9.10 (7.81; 10.3)	6.32 (6.25; 6.38)	5.59 (5.11; 6.06)
2.41-4.8	8.87 (8.84; 8.90)	8.92 (8.58; 9.25)	6.33 (6.31; 6.36)	5.99 (5.83; 6.15)
4.81-7.2	8.91 (8.89; 8.93)	8.83 (8.59; 9.07)	6.34 (6.32; 6.36)	5.93 (5.81; 6.06)
7.21-9.6	8.85 (8.81; 8.88)	8.29 (7.86; 8.71)	6.32 (6.29; 6.36)	5.80 (5.54; 6.07)
>9.6	9.89 (8.80; 9.98)	9.68 (8.62; 10.7)	6.32 (6.23; 6.40)	5.91 (5.35; 6.47)

Data presented as adjusted means. Analyses were adjusted for model 3 including age, sex, deprivation index, ethnicity, assessment month, lifestyle factors (smoking and alcohol intake), multimorbidity, total energy intake and physical activity.

Appendix 4-A: Flyer for the comparison of the effects of krill oil and fish oil supplementation on muscle function in older adults: a randomised controlled pilot study (chapter4)

University of Glasgow | College of Medical, Veterinary & Life Sciences

Volunteers Wanted!

Do you want to help research in muscle ageing?

We are investigating the effects of krill oil and fish oil supplementation on muscle function in older adults.

We are looking for volunteers who are:

- **aged 60 years or older.**
- **BMI less than 30kg/m^2.**
- **Healthy with no history of cancer, kidney disease, liver disease, diabetes, cardiovascular disease or allergy to seafood.**

Daily supplements will be taken over 16 weeks period.

We will measure your muscle strength and size before and after the supplements

We will provide you £50 upon completion to compensate you for your time

For further information please contact:

Maha Timraz

cams-fishkrill@glasgow.ac.uk

Appendix 4-B: Health screen questionnaire (chapter 4)

HEALTH SCREEN FOR STUDY VOLUNTEERS Number .....

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present**, do you have any health problem for which you are:
   - (a) on medication, prescribed or otherwise ..... Yes ☐ No ☐
   - (b) attending your general practitioner ..... Yes ☐ No ☐
   - (c) on a hospital waiting list ..... Yes ☐ No ☐

2. **In the past two years**, have you had any illness which required you to:
   - (a) consult your GP ..... Yes ☐ No ☐
   - (b) attend a hospital outpatient department ..... Yes ☐ No ☐
   - (c) be admitted to hospital ..... Yes ☐ No ☐

3. **Have you ever** had or been diagnosed with any of the following:
   - (a) Convulsions/epilepsy ..... Yes ☐ No ☐
   - (b) Asthma/Respiratory disorders ..... Yes ☐ No ☐
   - (c) Eczema ..... Yes ☐ No ☐
   - (d) Diabetes ..... Yes ☐ No ☐
   - (e) A blood disorder ..... Yes ☐ No ☐
   - (f) Head injury ..... Yes ☐ No ☐
   - (g) Digestive problems ..... Yes ☐ No ☐
   - (h) Heart problems ..... Yes ☐ No ☐
   - (i) Problems with bones or joints ..... Yes ☐ No ☐
   - (j) Disturbance of balance/coordination ..... Yes ☐ No ☐
   - (k) Numbness in hands or feet ..... Yes ☐ No ☐
   - (l) Disturbance of vision ..... Yes ☐ No ☐
   - (m) Ear / hearing problems ..... Yes ☐ No ☐
   - (n) Thyroid and adrenal problems ..... Yes ☐ No ☐
   - (o) Kidney or liver problems ..... Yes ☐ No ☐
   - (p) Allergies ..... Yes ☐ No ☐
   - (q) High cholesterol ..... Yes ☐ No ☐
   - (r) High triacylglycerol or any other form of dyslipidaemia ... Yes ☐ No ☐

4. **Has any**, otherwise healthy, member of your family died suddenly during or soon after exercise? ..... Yes ☐ No ☐

5. **Are you**:
   - (a) taking omega-3 fatty acid supplements in the last six months? Yes ☐ No ☐
   - (b) consuming oily fish more than once a week? ..... Yes ☐ No ☐

☐ ☐ ☐ ☐

V1 02_03_2022

6. **How much** physical activity/exercise do you do on a weekly basis?…… (hours)

If you answered YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.)

……
……
……

Thank you for your cooperation!

University of Glasgow

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Appendix 4-C: Participant consent form (chapter 4)

University of Glasgow | College of Medical, Veterinary & Life Sciences

Project Number:

Participant Identification Number for this trial:

Title of Project: **A comparison of the effects of krill oil and fish oil supplementation on muscle function in older adults: a randomised controlled pilot study**

Name of Researcher(s):

Miss. Maha Timraz
Prof. Stuart Gray
Pro. Emilie Combet
Dr. Terry Quinn
Dr. Julien LeKernec

CONSENT FORM

	Please initial box
I confirm that I have read and understood the Participant Information Sheet version xxxx dated xxxxx	☐
I confirm that I have read and understood the Privacy Notice version xxx dated xxxx I have had the opportunity to think about the information and ask questions and understand the answers I have been given.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.	☐
I confirm that I agree to the way my data will be collected and processed and that data will be stored for up to 10 years in University archiving facilities in accordance with relevant Data Protection policies and regulations.	☐
I understand that all data and information I provide will be kept confidential and will be seen only by study researchers and regulators whose job it is to check the work of researchers.	☐
I agree that my name, contact details and data described in the information sheet will be kept for the purposes of this research project.	☐
I understand that if I withdraw from the study, my data collected up to that point will be retained and used for the remainder of the study.	☐

I agree to take part in the study. ☐

I understand and agree with how my collected samples will be processed and handled for purposes of this study. ☐

I agree to a sample of my blood being stored for a period of 10 years at University of Glasgow facility/facilities. ☐

Name of participant	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

(1 copy for participant; 1 copy for researcher)

Appendix 4-D: The International physical activity Questionnaire (chapter 4)

University of Glasgow

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ
International collaboration on IPAQ is on-going and an International Physical Activity Prevalence Study is in progress. For further information see the IPAQ website.

More Information
More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). Assessment of Physical Activity: An International Perspective. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

days per week

☐ No vigorous physical activities → **Skip to question 3**

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

hours per day
_____ **minutes per day**

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

☐ **days per week** No moderate physical activities → **Skip to question 5**

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**
_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → **Skip to question 7**

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**
_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Appendix 4-E: Participant information sheet (chapter 4)

University of Glasgow | College of Medical, Veterinary & Life Sciences

PARTICIPANT INFORMATION SHEET

1. **Study title**

A comparison of the effects of krill oil and fish oil supplementation on muscle function in older adults: a randomised controlled pilot study.

2. **Invitation paragraph**

We invite you to participate in an investigation which we believe to be of potential importance. In order to help you to understand what the investigation is about, please read the following information carefully. Be sure you understand it before you formally agree to participate. If there are any points that need further explanation, please ask a member of the research team. It is important that you understand what you are volunteering to do and are completely happy with all the information before you sign this form.

3. **What is the purpose of the study?**

The aim of the current study is to compare the effects of krill oil and fish oil supplementation on muscle function in older adults. A second aim is to investigate the mechanisms underlying these beneficial effects.

4. **Why have I been invited to participate?**

You have been selected as a possible participant in this investigation because you are healthy and aged 60 and older, with a BMI less than 30, not taking any fish oil and krill oil supplementation and not participating in any resistance exercise (weightlifting) training.

5. **Do I have to take part?**

It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and you will be asked to sign a consent form. If you do decide to take part, you are still free to withdraw at any time and without giving a reason.

6. **What will happen to me if I take part?**

You will be asked to visit the laboratory, at the University of Glasgow, at the beginning and the end of a 16-week period for various tests. We will randomly assign you to a fish oil group or krill oil or control group. If you are assigned to either the fish oil or krill oil group you will receive 4g/day of krill oil or fish oil supplements capsules, if you are assigned to the control group will receive 4g/day of vegetable oil supplement capsules. You will not know which supplement you receive. We ask all participants to maintain their habitual diet and physical activity patterns.

Initial visit (~1hour)
On your first visit around 1 week before you start the supplementation programme, you will be introduced to the laboratory personnel and familiarised with the study. You will be asked to complete questionnaires about your health and physical activity habits to make sure you are suitable to join the study. We will also measure your BMI and blood pressure for the same purpose.

Baseline visit (~3hours):
We will collect a blood sample, measure your body composition and strength, functional abilities, and measure the electrical activity in your thigh muscle. We will also ask you questions about your normal diet.

Intervention (16 weeks):
We will then ask you to consume 4g/day of the provided supplements for 16 weeks. During this period we will also ask you to record any fish you eat.

Post-intervention visit (~3hours):
The same measurements performed during the baseline visit will be repeated after the completion of the supplementation.

Measurements:

Blood test
A small needle will be inserted into an antecubital vein and a baseline blood sample collected. A total of 15ml (3 teaspoons) of blood will be taken during each visit. We will measure blood lipids, sugars and measures of inflammation.

Muscle size
You will lie on an examination couch. We will then use a small handheld ultrasound device to measure the thickness of the thigh muscle

Muscle strength:
You will sit in a chair with your legs at a 90-degree angle. A strap will be placed around the right ankle which will be connected to a force sensor. We will ask you to contract maximally with the leg fixed in position and record force for 10 seconds. You will perform 3 contractions, with 1min rest between contractions. We will also ask you to perform 3 maximal contractions, on each hand, with the hand grip dynamometer.

Functional abilities:
We will ask you to perform three tests. First – Balance test: you will stand in 3 different positions for 10 seconds. Second - the timed up and go: you will stand up from a chair and walk for 3m then return back to the chair and sit down. Third - 4m walk test: you will be asked to walk for 4m at your normal pace

Muscle electrical activity: You will sit in a chair with your legs at a 90-degree angle. A strap will be placed around the right ankle which will be connected to a force sensor. We will ask you to contract at a variety of easy intensity levels with the leg fixed in position with a needle electrode (similar to an acupuncture needle) inserted into the thigh muscle to measure the electrical activity of the muscle fibres.

7. What are the possible disadvantages and risks of taking part?

Fish oil supplements can cause mild side effects, including: a fishy aftertaste, bad breath, heartburn, nausea or diarrhea although this is unlikely with the doses used in the current study. If you experience any unusual sensations and feel unwell or do not want to continue for any other reason during the supplementation you should stop taking the supplement immediately and let us know.

Blood sampling may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood.

8. What are the possible benefits of taking part?

Krill oil and fish oil may increase your muscle strength. We will be able to provide feedback about your muscle strength.

9. Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the University will have your name and address removed so that you cannot be recognised from it. In addition, your records, samples and results will be identified by a number and not your name.

10. What will happen to my data?

All study data will be held in accordance with The General Data Protection Regulation (2018). The data will be stored in archiving facilities in line with the University of Glasgow retention policy of up to 10 years. After this period, further retention may be agreed or your data will be securely destroyed in accordance with the relevant standard procedures.

11. What will happen to the results of the research study?

The results from this study will be presented at scientific meetings and published in scientific journals. The results will also be used in the research project reports of the PhD student undertaking this research. A copy of the published results will be sent to you upon request. You will not be identifiable in any of the data presented or published from this study.

12. Who is organising and funding the research?

University of Glasgow.

13. Who has reviewed the study?

This study has been reviewed and approved by the Ethics committee of the College of Medical, Veterinary and Life Sciences at the University of Glasgow.

14. Contact for Further Information

Miss. Maha Timraz.
Institute of Cardiovascular and Medical Sciences
College of Medical, Veterinary and Life Sciences
University of Glasgow
Glasgow G12 8TA
Phone
E-mail: cams-fishkrill@glasgow.ac.uk

Appendix 4-F: Privacy Notice (chapter4).

Privacy Notice for A comparison of the effects of krill oil and fish oil supplementation on muscle function in older adults: a randomised controlled pilot study

Your Personal Data
The University of Glasgow *will be what's known as the 'Data Controller' of your personal data processed in relation to the study of a* comparison of the effects of krill oil and fish oil supplementation on muscle function in older adults. *This privacy notice will explain how The University of Glasgow will process your personal data.*

Why we need it
We are collecting your basic personal data such as name, contact details and health data in order to investigate the effects of krill and fish oil supplements on muscle function. We will only collect data that we need in order to provide and oversee this service to you.

Legal basis for processing your data
We must have a legal basis for processing all personal data. In this instance, the legal basis is consent. We must have a consent to use your body measurement as part of this research study. As your health data is sensitive personal information, we are unable to use this information we are unable to use this information without prior consent from you.

What we do with it and who we share it with
All the personal data you submit is processed by staff at the University of Glasgow in the United Kingdom. In addition, this data will be stored securely on an encrypted USB stick. As, stated above your name will be replaced with a unique ID number. Only the main researcher will have knowledge of which unique ID number belongs to you. Therefore, your personal data will be anonymous to all other researchers.

How long do we keep it for
Your data will be retained by the University for 6 months after the conference has ended or the survey has concluded

What are your rights?*
You can request access to the information we process about you at any time. If at any point you believe that the information we process relating to you is incorrect, you can request to see this information and may in some instances request to have it restricted, corrected or, erased. You may also have the right to object to the processing of data and the right to data portability.
If you wish to exercise any of these rights, please contact dp@gla.ac.uk.
*Please note that the ability to take the supplementation of these rights will vary and depend on the legal basis on which the processing is being carried out.

Complaints

If you wish to raise a complaint on how we have handled your personal data, you can contact the University Data Protection Officer who will investigate the matter.
Our Data Protection Officer can be contacted at dataprotectionofficer@glasgow.ac.uk
If you are not satisfied with our response or believe we are not processing your personal data in accordance with the law, you can complain to the Information Commissioner's Office (ICO) https://ico.org.uk/

☐ I consent to the University processing my personal data for the purposes detailed above.

☐ I have read and understand how my personal data will be used.

Signed:
……

Date:……

Appendix 4-G: Food Frequency Questionnaire (FFQ) (chapter 4)

CONFIDENTIAL STUDY NUMBER: ☐☐☐☐☐☐☐☐

FOOD FREQUENCY QUESTIONNAIRE

This questionnaire asks for some background information about you, especially about what you eat.

Your answers will be treated as strictly confidential and will be used only for medical research.

PLEASE COMPLETE USING BLACK INK/PEN

Date of birth: ☐☐ ☐☐ ☐☐☐☐

Please enter: **M if you are Male**
F if you are Female ☐

Please answer every question. If you are uncertain about how to answer a question then do the best you can, but please do not leave a question blank.

CAMB/PQ/6/1205

Appendix 4-H: Fish Log (chapter4).

Fish consumption Log

Please note every time you consume any fish (oily or non-oily) by writing in these boxes. For example, one salmon fillet or portion haddock.

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1							
Week 2							
Week 3							
Week 4							
Week 5							
Week 6							
Week 7							

Week 8							
Week 9							
Week 10							
Week 11							
Week 12							
Week 13							
Week 14							
Week 15							
Week 16							