Long-chain omega-3 fatty acids as an essential link between musculoskeletal and cardio-metabolic health in older adults.

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Abstract

This narrative review aims to critically evaluate scientific evidence exploring the therapeutic role(s) of long-chain omega-3 polyunsaturated fatty acids (Ω-3PUFA) in the context of ageing, and specifically, sarcopenia. We highlight that beyond impairments in physical function and a lack of independence, the age-related decline in muscle mass has ramifications for cardio-metabolic health. Specifically, skeletal muscle is crucial in regulating blood glucose homeostasis (and by extension reducing type 2 diabetes mellitus (T2DM) risk) and providing gluconeogenic precursors that are critical for survival during muscle wasting conditions (i.e. AIDS). Recent interest in the potential anabolic action of Ω-3PUFA is based on findings from experimental studies that measured acute changes in the stimulation of muscle protein synthesis (MPS) and/or chronic changes in muscle mass and strength in response to fish oil-derived Ω-3PUFA supplementation. Key findings include a potentiated response of MPS to amino acid provision or resistance-based exercise with Ω-3PUFA in healthy older adults that extrapolated to longer-term changes in muscle mass and strength. The key mechanism(s) underpinning this enhanced response of MPS remains to be fully elucidated, but is likely driven by the incorporation of exogenous Ω-3PUFA into the muscle phospholipid membrane and subsequent upregulation of cell signaling protein known to control MPS. In conclusion, multiple lines of evidence suggest that dietary Ω-3PUFA provide an essential link between musculoskeletal and cardio-metabolic health in older adults. Given that western diets are typically meagre in Ω-3PUFA content, nutritional recommendations for maintaining muscle health with advancing age should place greater emphasis on dietary Ω-3PUFA intake.

Introduction

The amount and type of dietary fat consumed is widely recognised to play an important role in determining metabolic health in humans [1]. Fatty acids are hydrocarbon chains of varying lengths with a carboxyl group and methyl group at opposing ends. The presence of one or several double bonds in (unsaturated) fatty acids impact on their conformation, as well as their function. Very long-chain or long-chain omega-3 polyunsaturated fatty acids, abbreviated Ω-3PUFA throughout this review, are a class of fatty acids distinguished by two or more double bonds at the methyl end of the carbon chain. The most abundant species of Ω-3PUFA are eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA). EPA consists of a 20-carbon chain with 5 double bonds, DHA a 22-carbon chain with 6 double bonds, and ALA an 18-carbon chain with 3 double bonds. As humans are unable to endogenously synthesise ALA, it is defined as an essential fatty acid that must be acquired from the diet. The most commonly cited health benefit associated with increasing dietary Ω-3PUFA intake relates to a reduction in cardiovascular disease (CVD) risk [2], as mediated by improvements in the regulation of blood pressure, vascular function and cardiac rhythm, although recent evidence has cast doubt on some of these claims. Recent evidence also proposes a physiological role for Ω-3PUFA in regulating skeletal muscle protein metabolism [3] and, by extension, muscle mass [4], muscle strength [5] and muscle function. Other papers in this volume focus on the impact of dietary fatty acids on liver fat content and metabolism [6] and regional/ectopic fat depots in human adipose tissue [please insert Petrus 19 PNS here]. This review focuses on human skeletal muscle tissue and, specifically, the role of Ω-3PUFA in the context of sarcopenia and sarcopenic obesity. Our narrative is divided into three distinct themes. First, we identify food sources of Ω-3PUFA and their consumption at the population level. Next, we provide a holistic overview of the importance of skeletal muscle tissue for cardio-metabolic health, physical function and disease prevention in humans. Finally, we critique available evidence that evaluates the role of Ω-3PUFA as a component of non-pharmacological strategies designed to tackle sarcopenia and sarcopenic obesity.

Dietary Sources of Long Chain Omega-3 Polyunsaturated Fatty Acids

Commonly consumed food sources rich in Ω-3PUFA include oily fish such as mackerel, sardines, trout and salmon **(Figure 1)**. In comparison, canned tuna contains a lower Ω-3PUFA content and is no longer categorised as an oil-rich fish. While other non-fish food sources such as walnuts also contain Ω-3PUFA, the Ω-3PUFA are shorter chain (often ALA) which, in humans, are poorly converted to EPA and then DHA through processes of elongation and desaturation. Interestingly, this conversion is poorer in men than women [7].

<<< Insert Figure 1 here >>>

Dietary guidelines in the UK recommend two, 140g, portions of fish per week, one of which should be of oily source [8]. However, the latest National Diet and Nutrition Survey [9] indicates that, on average, adults aged 19-64 years consume only 56g of oily fish on a weekly basis (excluding canned tuna), while older adults aged 65+ consume 84g of oily fish per week. While the average oily fish intake falls alarmingly short of this 140g recommendation, also noteworthy is the median intake for both age groups is 0g per week, with the majority of UK adults avoiding dietary intake of oily fish altogether. Evidence from the EPIC-Norfolk study highlights that cod liver oil (a source of Ω-3PUFA) was the most popular supplement (consumed by 32% of men and 45% of women) [10]. However, it is worth noting that over-the-counter fish oil preparations do not always contain the dose advertised on the label, and that the fatty acids can often be extensively oxidised, compromising their proposed biological function [11, 12].

The Scientific Advisory Committee on Nutrition (SACN) recommends a long chain Ω-3PUFA intake of 450 mg/day. In comparison, UK intakes of EPA and DHA are estimated at 244 mg/day (131 mg/day from oil-rich fish) [13], with potentially lower intakes in ethnic minority groups. Hence, there is ample scope to explore strategies to increase Ω-3PUFA intakes in the UK diet, potentially through enrichment strategies targeting foods such as dairy and meat (especially poultry) [13], with a view to improving cardio-metabolic health. While Ω-3PUFA intake is low in the Western population, Ω-6PUFA consumption remains comparatively high, through regular intake of seed oils and food products. It is understood that the ratio of Ω-6:Ω-3PUFA has recently shifted from a balanced 1:1 to ~20:1, with implications for metabolism, specifically the production of pro-inflammatory molecules, such as prostaglandins and leukotrienes [14].

Importance of Skeletal Muscle Tissue for Cardiometabolic Health and Physical Function

The term cardio-metabolic risk describes a family of risk factors of metabolic origin that increase the risk of developing CVD such as coronary heart disease, stroke, type 2 diabetes mellitus (T2DM) and chronic kidney disease. Skeletal muscle tissue plays a crucial, albeit often underappreciated, role in maintaining cardio-metabolic health and offsetting morbidities commonly associated with advancing age [15]. Accounting for ~40% of total body mass [16], skeletal muscle is described as a plastic tissue that is capable of (mal)adaptation to physical (in)activity and diet. As the primary site of blood glucose disposal, skeletal muscle accounts for ~30% of postprandial glucose uptake [17]. Low muscle mass is associated with a reduced resting metabolic rate that can lead to the accumulation of fat mass [15]. Therefore, the maintenance of skeletal muscle mass over the lifecourse is critical in regulating

blood glucose homeostasis and reducing the risk of T2DM, as well as other associated cardio-metabolic diseases. In addition, skeletal muscle serves as the body's primary storage site for amino acids and, during starvation or in the context of conditions such as acquired immune deficiency disorder (AIDS) by providing gluconeogenic precursors that are crucial for survival [18]. Beyond metabolic health, it is widely recognised that skeletal muscle is crucial in preserving physical function, mobility and ultimately independence during older age.

An inevitable, albeit partially modifiable, feature of the ageing process concerns the progressive decline in skeletal muscle mass, strength and function. Muscle atrophy begins as early as the fourth decade of life [19], continues at a rate of ~1% of total muscle mass per year until 70 years [20], and increases to ~1.5% of total muscle mass per year above 80 years old [21]. Alarmingly, the decline in muscle strength with advancing age typically exceeds the decline in muscle mass, with annual declines of 3-4% in strength commonly reported [22]. Once the decline in muscle strength and muscle mass fall below critical thresholds, older adults are classified as sarcopenic [23]. This condition is associated with a 2-3 fold increase in risk of falling, bone fractures, loss of independence and increased mortality [24, 25]. According to a recent report, additional health and social care costs associated with sarcopenia in the UK are currently estimated to be £2.5 billion per year [26].

In 2016, sarcopenia was recognised as an independent geriatric condition, with its own International Classification of Disease code. Compounding this progressive loss of functional ability, the age-related decline in muscle mass and strength is associated with an increased cardio-metabolic health risk. In this regard, a recent study demonstrated that low muscle strength was associated with increased risk of all-cause mortality from cardiovascular disease (CVD), cancer and respiratory disease [27]. Similarly, low muscle strength has been associated with higher incidence of T2DM [27], with findings more equivocal for low muscle mass [28, 29]. Conversely, the increased risk of CVD mortality observed in patients with T2DM is attenuated in those individuals with greater grip strength [30]. Taken together, these observational data provide compelling evidence that the maintenance of muscle mass and strength with advancing age is critical for the management of cardio-metabolic health risk.

The decline in muscle mass with advancing age often occurs in concert with an increase in fat mass. This age-related phenomenon is referred to as sarcopenic obesity. It is well established that obesity independently increases the risk of many cardio-metabolic health outcomes such as myocardial infarction, stroke, some cancers and all-cause mortality [31-33]. Evidence also suggests that when sarcopenia and obesity are combined, the debilitating effects are additive. For example, whilst sarcopenia and obesity are independently associated with increased risk of all-cause mortality (sarcopenia hazard ratio (HR) 1.41 (95% CI 1.22-1.63) and obesity HR 1.21 (95% CI 1.03-1.42)

compared to lean non-sarcopenic individuals, all-cause mortality risk is even greater (HR 1.72 (95% CI 1.35-2.18)) in sarcopenic obese men [34]. Therefore, it seems prudent to target the maintenance/increase of muscle mass, strength and function alongside the loss of fat mass to optimal levels in older adult populations. Before establishing targeted interventions to offset the age-related decline in muscle mass and increase in fat mass, it is important to understand the causal mechanism(s) that underpin the decline in muscle mass with advanced age.

Causal Mechanisms that Underpin the Decline in Muscle Mass, Strength and Function with Age

Although sarcopenia affects ~10-30% of community-dwelling men and women aged 60+ worldwide, the underlying pathology of this clinical condition is not fully understood. Clearly, the underlying cause of sarcopenia is multifactorial, with interconnected and complex contributing factors. In terms of muscle atrophy, contributing factors include, but are not limited to, chronic low-grade inflammation, elevated levels of oxidative stress, DNA damage, mitochondrial dysfunction and hormonal changes [35]. Ultimately however, from a metabolic standpoint, the decline in muscle mass with advanced age is underpinned by a state of negative muscle protein balance.

Two possible metabolic drivers of negative muscle protein balance exist. First, an impaired stimulation of muscle protein synthesis (MPS), defined as the rate by which freely available amino acids in the blood or muscle amino acid pools are incorporated into functional muscle protein. Second, an upregulation of muscle protein breakdown (MPB), defined as the rate by which muscle protein is degraded into amino acid precursors. There is general consensus that basal, post-absorptive rates of MPS are comparable between young and older adults [36-38]. In contrast, several studies have reported suppressed postprandial rates of MPS in response to amino acid feeding in older adults compared with their younger counterparts [39]. The concept of this so-called ‘anabolic resistance’ has been conceived from this observation and describes the age-related impairment in response of MPS to ingesting a meal-like (~20 g) quantity of protein and/or other typically robust anabolic stimuli such as mechanical loading, i.e., structured exercise training. At the molecular level, this age-related impairment in MPS appears to be mediated by a dysregulation in the Akt-mTOR (mechanistic target of rapamycin) cell signalling cascade that controls the rate limiting translation initiation step of MPS [40]. As such, anabolic resistance is widely regarded as one of the key drivers of sarcopenia. Moreover, as further evidence of the interplay between mechanisms underlying sarcopenia, animal studies have demonstrated that low grade inflammation, which is particularly prevalent in sarcopenic obese individuals, impairs the stimulation of MPS in response to food intake [41]. Hence, there is a clear

biological rationale to establish non-pharmacological lifestyle-friendly interventions that target overcoming both anabolic resistance and low grade inflammation in older adults.

In practical terms, the progressive decline in muscle mass and strength is exacerbated by periods of muscle disuse [42, 43]. Examples of skeletal muscle disuse range in duration and severity from short-term periods of limb immobilisation caused by injury (i.e. accidental falls) to longer-term periods of bed-rest inflicted by illness and/or cardio-metabolic disease. A reduction in physical activity, as typically quantified by step count, provides another important, albeit less extreme, example of muscle disuse. Accordingly, age-related anabolic resistance is exacerbated by reducing physical activity levels [44], limb immobilisation [42, 45] and bedrest [46]. Moreover, recent evidence suggests that age-related anabolic resistance is further exacerbated in overweight and/or obese older adults [47] **(Figure 2)** and in response to a period of high-fat feeding [48]. Thus, it follows that optimising diet and lifestyle strategies for maintaining muscle health is of critical importance in sarcopenic older adults. In this regard, given the potent anti-inflammatory properties of Ω-3PUFA [49] and recent evidence that Ω-3PUFA exhibit anabolic properties [50, 51], the role of dietary Ω-3PUFA intake in combating sarcopenia has received considerable recent attention.

<<< *Insert figure 2 here* [47, 52-55] >>>

Diverse Biological Roles of Long Chain Omega-3 Fatty Acids

A key determinant of physiological function at the cellular level includes the fatty acid composition of the phospholipid cell membrane. Membrane fatty acid composition is modulated by metabolic, genetic and hormonal factors, and of particular relevance to this review, dietary intake of fatty acids. As detailed in *Dietary Sources of Very Long Chain Omega-3 Fatty Acids*, the western diet is generally rich in Ω-6PUFA (e.g. linoleic acid) relative to Ω-3PUFA. This pattern is reflected in the constituent fatty acid composition of cell membranes which typically range from 10-20% for Ω-6PUFA and 2-5% Ω-3PUFA [56]. The membrane composition of Ω-3PUFA can be elevated in a dose-dependent manner by dietary intake of Ω-3PUFA [57]. Functionally, the most important Ω-3PUFA are EPA and DHA and many research studies have investigated the physiological properties of EPA/DHA, primarily due to their potential to reduce inflammation [56].

Whilst inflammation is an important defence mechanism of the immune system to protect humans from infection, unresolved pathological inflammation can result in damage and disease. For example, and as detailed previously, low grade chronic inflammation has been implicated in the aetiology of sarcopenia but also many cardiometabolic conditions. There is a host of research demonstrating that increasing Ω-3PUFA intake serves to reduce inflammation, as reviewed previously [56]. As

inflammation has been associated with many cardio-metabolic conditions, it has been suggested that Ω-3PUFA supplementation may be of therapeutic use. For example, early observational studies in Inuits demonstrated that even though this population consumed very high fat diets, the prevalence of heart disease was low, with this inverse relationship attributed to the high dietary Ω-3PUFA intake [58, 59]. In contrast, a recent meta-analysis demonstrated that increasing EPA and DHA consumption has minimal, or no effect, on mortality or cardiovascular health [60], with the authors calling for a halt in further studies until ongoing large trials are fully reported.

In addition to their anti-inflammatory properties and role in regulating immune function, Ω-3PUFA exhibit other physiological roles due to their incorporation into all cell types. Therefore, it is not surprising that the physiological roles of EPA and DHA are not limited to the immune system. For example, DHA is vital for fetal brain and retinal development given the high propensity for DHA incorporation in brain and retinal membrane phospholipids that are crucial for the functional development of these tissues [61]. Since the recent observation that EPA and DHA supplementation results in an increased incorporation of EPA and DHA in muscle cells [51], there has been a growing interest in the physiological effects of such a change for muscle health with advancing age.

Role of Long Chain Omega 3 Fatty Acids in Prevention and Treatment of Sarcopenia

Dietary Ω-3PUFA have received considerable recent attention in the context of optimising diet for the management of sarcopenia. Extending early epidemiological data, which found that fatty fish consumption was positively associated, in a dose-response manner, with grip strength [62], two seminal experimental studies in healthy young, middle-aged and older adults sparked interest in the potential muscle anabolic action of Ω-3PUFA [63, 64]. These proof-of-principle, acute metabolic, studies were conducted under controlled laboratory conditions and measured rates of MPS under basal (fasted and rested) and simulated fed conditions before and after 8 weeks of fish oil (4 g/day) derived Ω-3 PUFA supplementation (1.86 g EPA, 1.50 g DHA per day). Amino acids and insulin were infused intravenously to partially mimic the ingestion of a protein-rich mixed macronutrient meal. Whereas the basal response of MPS was not modulated by Ω-3PUFA, the feeding-induced increase in MPS was potentiated by 30-60% after 8 weeks of fish oil supplementation compared with before supplementation [63, 64].

Perhaps surprisingly, at least from a mechanistic standpoint, in these studies [63, 64] no changes in tumero necrosis factor alpha (TNF-α) or C-reactive protein (CRP) concentrations as systemic markers of inflammation were observed over the 8 week period of fish oil supplementation. However, the phosphorylation status of intramuscular cell signalling proteins known to upregulate MPS (e.g.

mTORC1-p70S6k1) was potentiated in response to simulated feeding following dietary fish oil supplementation. Consistent with this observation, our laboratory reported an increase in the proportion of Ω-3PUFA, specifically EPA — to increase in the muscle cell following 4 weeks of fish oil (5 g/day) derived Ω-3PUFA in healthy young men [51]. Such structural modifications to the muscle cell membrane also were associated with an increased phosphorylation of mTORC1 — a nutrient-sensitive intramuscular cell signalling protein, and focal adhesion kinase — a mechanically sensitive kinase known to regulate MPS. Therefore, the primary causal mechanism that appears to underpin the anabolic action of Ω-3PUFA relates to modifying the lipid profile of the muscle phospholipid membrane and subsequently upregulating the activity of intracellular signaling proteins, rather than an anti-inflammatory response.

In recent years, we [65, 66] and others [67] have extended these acute metabolic studies to investigate the anabolic and/or anti-catabolic potential of Ω-3PUFA in young and older adults using more physiologically relevant experimental study designs **(Figure 3)**. Rather than the intravenous infusion of amino acids and insulin to simulate feeding, anabolic stimuli included either an orally ingested dose of intact protein, a standardised mixed macronutrient meal and/or a resistance exercise session(s) administered over a period of 1-4 days. Informed by our *in vitro* experiment with fully differentiated C2C12 cells whereby EPA, rather than DHA, was shown to both upregulate the MPS response to a leucine stimulus and downregulate MPB [68], these studies have primarily administered high dose (3-5g/day) fish oil supplements that are rich in EPA content. Accordingly, Lalia et al. [67] reported that fish oil supplementation (3.9 g/day) augmented the acute response of MPS to conducting a single bout of resistance exercise alongside feeding a protein-containing meal by ~30% in older adults. As a note of caution, data values for MPS (expressed as fractional synthesis rate) were remarkably high in this study, calling into question the validity of these findings.

However, study findings regarding the influence of Ω-3PUFA supplementation on postprandial rates of MPS have been equivocal, which may be attributed to differences in study design (i.e., the duration and dose of Ω-3PUFA supplementation, choice of control supplement and technique used to measure MPS) and/or participant characteristics. For instance, we observed no differences in p70S6K1 kinase activity or free-living integrated rates of MPS measured over 4 days (assessed by recently re-introduced and less invasive orally administered deuterium oxide tracer methodology) between two groups of older adults that combined resistance training with either fish oil (3 g/day) or safflower oil (3 g/day) supplementation [65]. In addition, we demonstrated that 8 weeks of fish oil (5 g/day) derived Ω-3PUFA (3.5 g/day EPA) supplementation failed to modulate the 4 hour (as measured by the precursor-product method with intravenous infusion of labelled phenylalanine) MPS response to ingestion of a 30g whey protein bolus under both rested and post-exercise conditions in trained young

men [66]. Follow-up studies designed with a mechanistic focus are warranted to further explore these findings. We cannot discount the possibility that ingesting 30g of whey protein saturated the muscle protein synthetic machinery in our cohort of "nutrient-sensitive" trained young men [52], and although more relevant to simulating daily lifestyle patterns, free-living measurements of MPS integrating postabsorptive and postprandial physiological states might have "diluted" the chance of detecting any subtle, but physiological relevant, anabolic action of Ω-3PUFA [65]. Taken together, based on currently available evidence, these data indicate the anabolic action of Ω-3PUFA may confer greater application to older adults who exhibit a state of anabolic resistance **(Figure 3)**.

<<< Insert figure 3 here [47, 63, 64, 66, 67] >>>

The anabolic action of Ω-3PUFA in ageing muscle has been partially supported by a series of longitudinal studies that obtained clinically-relevant endpoint measurements of muscle mass, strength and function, particularly when older women were studied. Expanding upon their initial work, Smith and colleagues [4] have demonstrated that daily ingestion of Ω-3PUFA (1.86g EPA and 1.50g DHA) over 6 months increased thigh volume by ~3.5% and handgrip strength by ~6% in older adults, despite the absence of structured exercise training. The clinical implications of these remarkable data are particularly significant given that, as mentioned previously, handgrip strength [69] and general strength [70] are known predictors of all-cause mortality. Moreover, we demonstrated that improvements in muscle strength and quality (calculated as peak torque relative to muscle anatomical cross sectional area), but not muscle mass, following 18 weeks of structured bi-weekly resistance exercise training were augmented with dietary fish oil-derived Ω-3PUFA supplementation in older women [65]. However, no such benefit of Ω-3PUFA ingestion was observed when older men were studied. Consistent with this observation, an earlier study supplemented older women with 2g/day of fish oil during 90 days of resistance training and reported greater strength gains compared with training alone [5]. However, we contend that these data from Rodacki and coworkers [5] should be treated with caution since no placebo group was included in the study design, the changes in blood Ω-3PUFA composition were minimal, and no direct measures of muscle mass or MPS were collected. It follows that further studies are warranted to, first confirm this apparent sex-difference in the muscle adaptive response to resistance training with Ω-3PUFA ingestion and, second, determine the mechanism(s) that underpins this apparent sexual dimorphism in response to ingested Ω-3PUFA.

Accumulating evidence also substantiates a "protective" role for Ω-3PUFA ingestion during short-term periods of muscle disuse. In this regard, an elegant recent study by McGlory and colleagues [71] investigated the influence of Ω-3PUFA supplementation on changes in muscle mass and integrated rates of MPS following 2 weeks of limb immobilisation in young women. The decline in muscle volume elicited by short-term limb immobilisation was attenuated by ~6% with Ω-3PUFA

supplementation (a decrease of 8%) vs. the sunflower oil control (a decrease of 14%). Moreover, following 2 weeks of rehabilitation whereby study participants resumed their habitual physical activity levels, muscle volume returned to baseline levels with Ω-3PUFA supplementation, but remained below baseline in the control group. Accompanying the retention of muscle volume during simulated muscle disuse atrophy was a higher integrated response of MPS both at immediate cessation of limb immobilisation and following two weeks of remobilisation. Interestingly, Ω-3PUFA supplementation had no protective effect on the decline in muscle strength. Consistent with this observation, albeit using an animal model, rats fed a Ω-3PUFA-rich diet during hindlimb suspension (simulating leg immobilisation) demonstrated an attenuated loss of muscle mass *vs.* rats fed a corn oil-rich diet [72]. Taken together, based on multiple lines of evidence, the preponderance of available data suggests that the optimal diet for maintaining muscle mass with age should consider the dietary intake of Ω-3PUFA. Future studies are warranted to investigate the impact of Ω-3PUFA ingestion on age-related changes in body composition in sarcopenic, obese, population groups.

Conclusions

Skeletal muscle plays an underappreciated role in cardio-metabolic health and disease. The age-related decline in muscle mass and muscle strength is explained, in part, by anabolic resistance. Convincing evidence exists that dietary Ω-3PUFA ingestion acutely increases the anabolic sensitivity of skeletal muscle in older adults with long-term data indicating a beneficial effect of Ω-3PUFA ingestion on muscle mass and/or function, particularly in women. Promising, albeit preliminary, evidence suggests that dietary Ω-3PUFA ingestion may form part of an effective non-pharmacological strategy to attenuate the decline in skeletal muscle mass associated with periods of muscle disuse, e.g. limb immobilisation. Moving forward, larger-scale experimental studies [73] should be repeated in more compromised populations (i.e., frail older adults, sarcopenic obese adults, etc.) to evaluate the application of Ω-3PUFA ingestion during more extreme periods of muscle disuse, i.e. bedrest during surgery and hospitalisation.

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588

589 **Figure legends**

590

591 **Figure 1.** Commonly consumed Ω-3PUFA rich food sources in the UK diet. Data extracted from
592 Composition of foods integrated dataset (CoFID) [74].
593 Ω-3PUFA, very long-chain omega-3 polyunsaturated fatty acids; ALA, alpha-linoleic acid; EPA,
594 eicosapentaenoic acid; DHA, docosahexaenoic acid.

595

596 **Figure 2.** Theoretical model of muscle "anabolic resistance" associated with ageing and obesity.
597 Data are generated from citations denoted by number in parentheses: [52] Young (18-35 years) adults
598 ingested 10, 20 or 40g of whey protein; [53] Older (65-75 years) ingested 10g of whey protein; [55]
599 Older (65-75 years) ingested 20 or 40g of soy protein; [47] Older (66-73 years) obese (BMI >30) adults
600 ingested 15g of milk protein isolate; [54] Young (23-30 years) obese (BMI >33) adults ingested 170g
601 of pork containing 36 g of protein.
602 Small protein feed, 10g of protein; moderate protein feed, 20g of protein, large protein feed, 36-40g
603 of protein. MPS, muscle protein synthesis; Yg, young adults. Old, Older adults.

604

605 **Figure 3:** Overview of findings from experimental studies that investigated the influence of fish oil-
606 derived Ω-3PUFA supplementation on the response of muscle protein synthesis (MPS) to amino acid
607 provision in young and older adults.
608 Data generated from citations denoted by number in parentheses: [64] Young and middle-aged (~39
609 years) adults consumed fish oil (4 g/day; 1.86 g/day EPA and 1.50 g/day DHA) capsules over 8 weeks
610 and MPS was measured pre and post supplementation in response to the intravenous infusion of
611 amino acids and insulin. [63] Older (≥ 65 years) adults consumed fish oil (4 g/day; 1.86 g/day EPA
612 and 1.50 g/day DHA) or corn oil capsules over 8 weeks and MPS was measured in response to the
613 intravenous infusion of amino acids and insulin. [66] Young (~ 21 years) adults consumed fish oil (5
614 g/day; 3.5 g/day EPA and 0.9 g/day DHA) or coconut oil capsules over 8 weeks and MPS was
615 measured in response to ingesting 30g of whey protein at rest and following resistance exercise. [67]
616 Older (65-85 years) adults consumed fish oil (3.9 g/day) capsules over 16 weeks and MPS was
617 measured in response to an acute bout of resistance exercise. [47] MPS was measured in response to
618 ingesting 15g of milk protein isolate in older (66-73 years) obese (BMI >30) adults.
619 FSR, fractional synthesis rate; Yg, young adults, Old, older adults; AA, amino acid, WP, whey
620 protein; REx, resistance exercise.

621

622

623

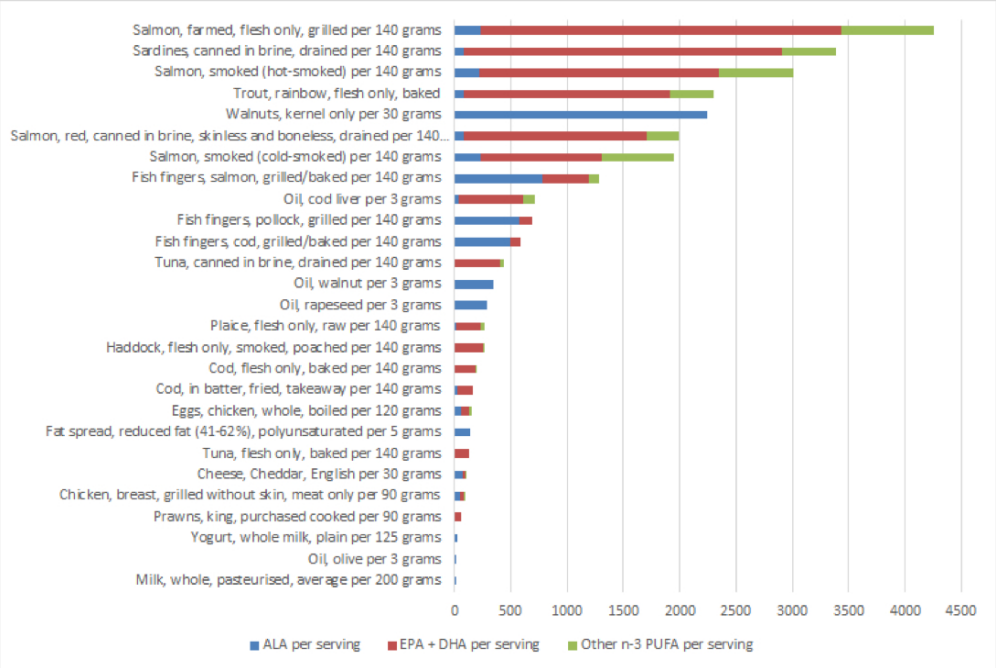
Salmon, farmed, flesh only, grilled per 140 grams
Sardines, canned in brine, drained per 140 grams
Salmon, smoked (hot-smoked) per 140 grams
Trout, rainbow, flesh only, baked
Walnuts, kernel only per 30 grams
Salmon, red, canned in brine, skinless and boneless, drained per 140...
Salmon, smoked (cold-smoked) per 140 grams
Fish fingers, salmon, grilled/baked per 140 grams
Oil, cod liver per 3 grams
Fish fingers, pollock, grilled per 140 grams
Fish fingers, cod, grilled/baked per 140 grams
Tuna, canned in brine, drained per 140 grams
Oil, walnut per 3 grams
Oil, rapeseed per 3 grams
Plaice, flesh only, raw per 140 grams
Haddock, flesh only, smoked, poached per 140 grams
Cod, flesh only, baked per 140 grams
Cod, in batter, fried, takeaway per 140 grams
Eggs, chicken, whole, boiled per 120 grams
Fat spread, reduced fat (41-62%), polyunsaturated per 5 grams
Tuna, flesh only, baked per 140 grams
Cheese, Cheddar, English per 30 grams
Chicken, breast, grilled without skin, meat only per 90 grams
Prawns, king, purchased cooked per 90 grams
Yogurt, whole milk, plain per 125 grams
Oil, olive per 3 grams
Milk, whole, pasteurised, average per 200 grams
0
500
1000
1500
2000
2500
3000
3500
4000
4500
ALA per serving
EPA + DHA per serving
Other n-3 PUFA per serving

Figure 1

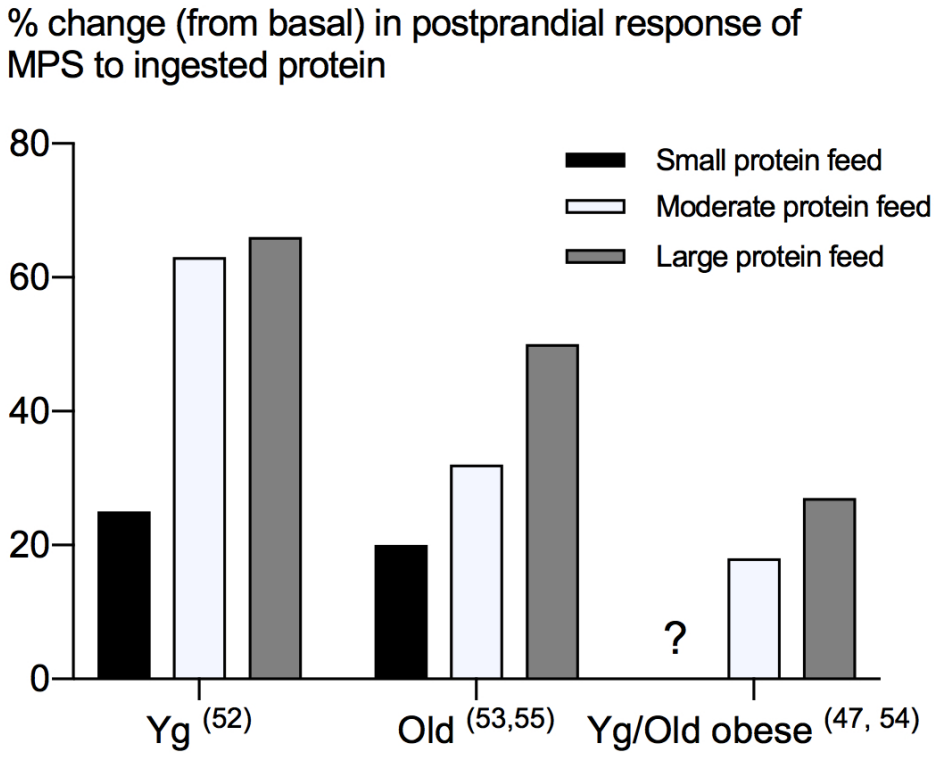

Figure 2

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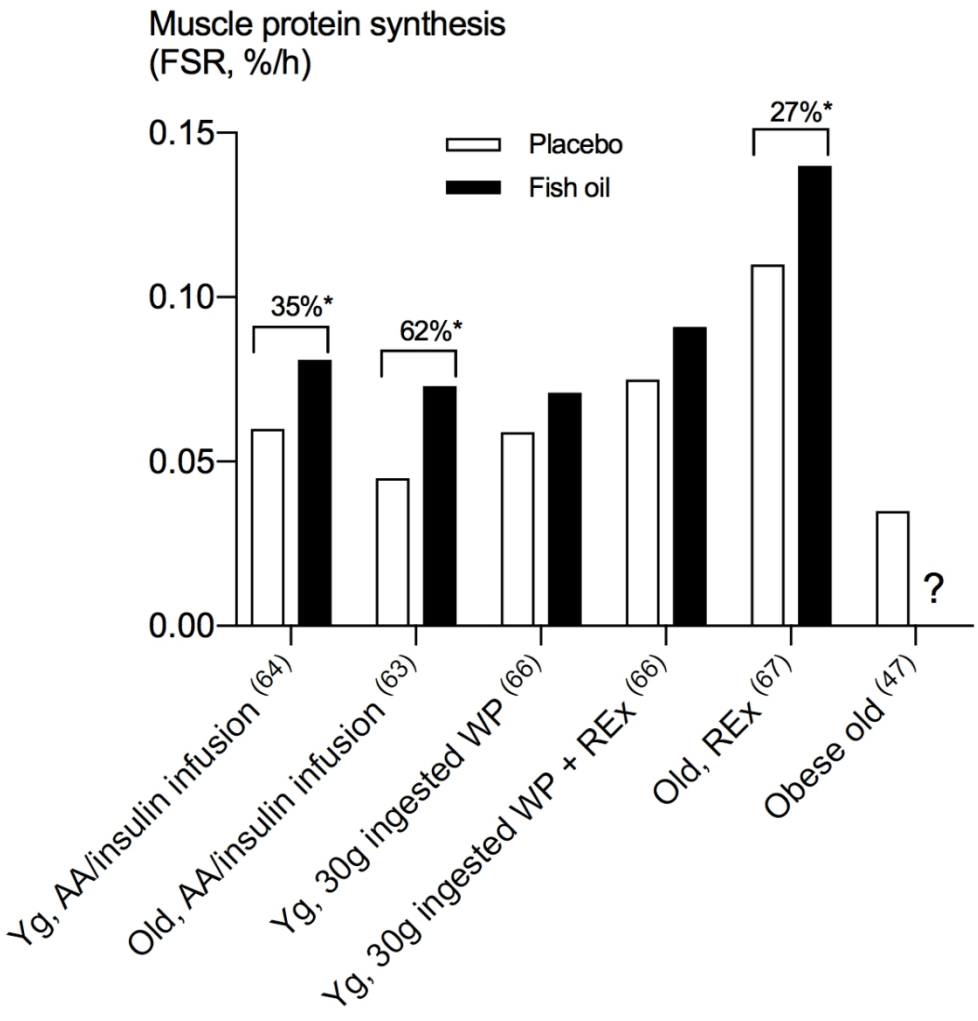

Figure 3

103x107mm (300 x 300 DPI)