Proceedings of the Nutrition Society, Page 1 of 11 doi:10.1017/S0029665123004834

The Nutrition Society Summer Conference 2023 hosted at The Hilton Liverpool on 3–6 July 2023

Conference on 'Nutrition at key stages of the lifecycle' Symposium six: Nutrition in later life

Long-chain *n*-3 PUFA ingestion for the stimulation of muscle protein synthesis in healthy older adults

Oliver C. Witard[1]*, Milena Banic[2], Nidia Rodriguez-Sanchez[2], Miriam van Dijk[3] and Stuart D. R. Galloway[2]

[1]*Centre for Human and Applied Physiological Sciences, King's College London, London, UK*
[2]*Physiology, Exercise and Nutrition Research Group, University of Stirling, Stirling, Scotland, UK*
[3]*Nutricia, Utrecht, The Netherlands*

This review aims to critically evaluate the efficacy of long-chain *n*-3 PUFA ingestion in modulating muscle protein synthesis (MPS), with application to maintaining skeletal muscle mass, strength and function into later life. Ageing is associated with a gradual decline in muscle mass, specifically atrophy of type II fibres, that is exacerbated by periods of (in)voluntary muscle disuse. At the metabolic level, in otherwise healthy older adults, muscle atrophy is underpinned by anabolic resistance which describes the impaired MPS response to non-pharmacological anabolic stimuli, namely, physical activity/exercise and amino acid provision. Accumulating evidence implicates a mechanistic role for *n*-3 PUFA in upregulating MPS under stimulated conditions (post-prandial state or following exercise) via incorporation of EPA and DHA into the skeletal muscle phospholipid membrane. In some instances, these changes in MPS with chronic *n*-3 PUFA ingestion have translated into clinically relevant improvements in muscle mass, strength and function; an observation evidently more prevalent in healthy older women than men. This apparent sexual dimorphism in the adaptive response of skeletal muscle metabolism to EPA and DHA ingestion may be related to a greater propensity for females to incorporate *n*-3 PUFA into human tissue and/or the larger dose of ingested *n*-3 PUFA when expressed relative to body mass or lean body mass. Future experimental studies are warranted to characterise the optimal dosing and duration of *n*-3 PUFA ingestion to prescribe tailored recommendations regarding *n*-3 PUFA nutrition for healthy musculoskeletal ageing into later life.

EPA: DHA: Muscle protein turnover: Mammalian target of rapamycin complex 1

Omega-3 polyunsaturated fatty acids *n*-3 PUFA are a family of biologically active long-chain fatty acids. The most abundant and bioactive of the *n*-3 PUFA species are EPA (20 : 5 *n*-3), DHA (22 : 6 *n*-3) and α-linolenic acid (ALA; 18 : 3 *n*-3)[1]. Recently, *n*-3 PUFA have received considerable scientific attention in the context of promoting the retention of muscle mass into later life, with clinical application to the management of sarcopoenia[2–4]. The scientific rationale that underpins the link between *n*-3 PUFA and muscle metabolism stems from two distinct, but likely inter-related, biological actions of *n*-3 PUFA[5]. First, *n*-3 PUFA are readily

Abbreviations: ALA, α-linolenic acid; CSA, cross-sectional area; MPB, muscle protein breakdown; MPS, muscle protein synthesis; mTORC1, mammalian target of rapamycin complex 1.

***Corresponding author:** Oliver C. Witard, email oliver.witard@kcl.ac.uk

incorporated into the phospholipid membrane of all cell types, including skeletal muscle cells and serve to upregulate the activity of cell signalling pathways known to control the remodelling of muscle tissue[6,7]. Secondly, the anti-inflammatory properties of *n*-3 PUFA are particularly relevant in more compromised older adults that exhibit low-grade inflammation[8]. Given that these physiological processes underpin the muscle anabolic response to key stimuli, namely amino acid provision and physical activity, there is current interest in the application of *n*-3 PUFA ingestion for healthy musculoskeletal ageing.

Common food sources that are rich in *n*-3 PUFA include linseed/flaxseed oil, chia seeds, walnuts, rapeseed/canola oil and soyabean oil which are all good plant-based sources of ALA. Moreover, cold water fatty fish such as mackerel, sardines, salmon, trout and herring are good marine sources of EPA and DHA. Since ALA conversion to EPA and DHA in the liver is not particularly efficient in human subjects[9], the ingestion of foods containing EPA and DHA is recommended. The European Food Safety Authority set an adequate intake for EPA and DHA for adults at 250 mg/d, typically achieved through ingestion of one portion of fatty fish per week. The EPA and DHA content of fatty fish flesh is dependent upon their dietary intake. Farmed fatty fish such as salmon has a slightly higher EPA and DHA content than wild-caught salmon due to the *n*-3 PUFA added to marine diets of farmed salmon. A typical 4·5 oz (130 g) serving of farmed salmon will supply about 1·86 g EPA and about 0·89 g of DHA, whereas other seafood sources such as shrimps, scallops and mussels contain far less EPA and DHA (about 150–300 mg total EPA and DHA per serving). In the absence of fatty fish consumption, other dietary sources such as the plant-based sources mentioned earlier, microalgae, krill oil, fish oil supplements or seed oils rich in stearidonic acid (18 : 4 *n*-3) such as echium oil[10–12] could go some way to help achieve sufficient EPA and DHA. It has been reported that stearidonic acid is more readily converted to EPA in comparison with conversion from ALA to EPA. However, conversion of both ALA and stearidonic acid to DHA appears limited[13]. Hence, there are several potential plant and marine sources of *n*-3 PUFA to increase dietary EPA and DHA intake in human subjects, with marine sources likely best for elevating both EPA and DHA content of human tissues.

The aims of this review are 2-fold. First, to explain the metabolic fate of ingested *n*-3 PUFA in skeletal muscle with emphasis on the uptake and incorporation kinetics of EPA and DHA into the muscle phospholipid membrane. Once taken up by the muscle cell, a notable biological action of EPA includes the stimulation of muscle protein synthesis (MPS) as mediated by an upregulation of intracellular cell signalling proteins (i.e. mammalian target of rapamycin complex 1 (mTORC1)) known to trigger the translation initiation step of MPS. Hence, the secondary aim of this review is to critically evaluate existing evidence that investigates the efficacy of *n*-3 PUFA ingestion in stimulating MPS in healthy older adults, with application to mitigating the age-related decline in skeletal mass in men and women. We propose mechanistic and practical explanations behind the thesis that older females may benefit from *n*-3 PUFA ingestion to a greater extent than older men in terms of stimulating a muscle protein synthetic response to key anabolic stimuli.

Age and sex-specific changes in muscle protein synthesis

The natural biological ageing process is associated with a gradual decline in skeletal muscle mass, strength and functional capacity in later life. Accordingly, although muscle mass usually peaks in early adulthood (between 20 and 30 years of age), the onset of muscle mass loss can begin as early as the fourth decade of life[14]. Muscle atrophy typically proceeds at a rate of about 1 % of total muscle mass per year until the age of 70 years[15], increasing to about 1·5 % of total muscle mass per year beyond 80 years of age[16]. At the ultrastructural level, muscle atrophy is primarily underpinned by a decline in cross-sectional area (CSA) of the larger force-generating type II muscle fibres rather than oxidative type I fibres[17,18]. In comparison, the age-associated decline in muscle strength proceeds at an even faster rate (2–3 % per year)[19] due primarily to additional age-associated changes in muscle architecture, innervation and deposition of non-contractile tissue (i.e. fat and connective tissue). Perhaps under-appreciated is the notion that periods of muscle disuse, ranging in severity from injury-induced immobilisation to illness-inflicted bedrest (i.e. cancer cachexia), exacerbate the trajectory of muscle atrophy with advancing age[20]. Hence, once muscle mass and strength fall below a critical level – often referred to as the disability threshold – the functional capacity of older adults is likely impaired, manifesting in a reduction in mobility, loss of independence and increased risk of falls and bone fractures in later life.

The primary metabolic mechanism that underpins the decline in skeletal muscle mass with advancing age in healthy older adults is an impaired response of MPS to key anabolic stimuli, namely nutrition and/or physical activity/exercise[21]. This phenomenon is termed anabolic resistance. In theory, two metabolic drivers of muscle atrophy exist. First, an increase in the rate of muscle protein degradation into amino acid precursors (termed muscle protein breakdown, MPB) and secondly, a decrease in the rate by which amino acids are synthesised into muscle protein (termed MPS). While some[22], but not all, trials[23] have reported the suppressive action of insulin on MPB to be diminished in older adults, studies have shown no clear difference in basal post-absorptive rates of MPS between healthy young and older adults[24,25]. In contrast, the response of MPS to ingesting meal-like quantities of protein[21,26] and/or other anabolic stimuli such as muscle loading[27–29] is impaired in older adults compared to their younger counterparts[30,31]. In theory, over time, anabolic resistance would contribute to the gradual loss of skeletal muscle mass with advancing age[32]. Hence, nutritional

https://doi.org/10.1017/S0029665123004834 Published online by Cambridge University Press

interventions focused on combatting the age-related decline in skeletal muscle mass in otherwise healthy older adults are primarily targeted at restoring a youthful response of MPS to anabolic stimuli. Regarding compromised populations, such as cancer cachexic patients, the role of MPB in mediating muscle atrophy appears to be more pronounced. However, the metabolic action of *n*-3 PUFA in this specific clinical sub-population is beyond the scope of this review and interested readers are directed to the following excellent reviews[3,4].

Sex differences in skeletal muscle mass and MPS rates appear to be evident in older adults[33]. At the ultrastructural level, when stratified by sex, the magnitude of type II muscle fibre atrophy was shown to be more pronounced in older women than men relative to their younger counterparts[34]. The age-related decline in muscle mass in women coincides with the onset of the menopause is accelerated during the transition into menopause[35], and then proceeds at a slower rate than in men[36]. Although the magnitude of muscle hypertrophy in response to resistance exercise training is reduced in older women compared with older men[37], the degree of muscle atrophy in response to muscle disuse is reduced in older women than men[38,39]. Moreover, based on a limited number of relevant studies, sex differences in the response of MPS to anabolic stimuli are evident in healthy older adults. Although basal rates of MPS appear to be greater in older women than older men[40], the MPS response to the intravenous infusion of amino acids and insulin[40], the ingestion of a mixed macronutrient meal[40] and an acute bout of resistance exercise[41] has been shown to be impaired to a greater extent in older women than age-matched men. As such, older women appear more susceptible to anabolic resistance than older men[40] which appears to be mediated by lower oestrogen levels in post-menopausal women[42]. Sex comparisons in the response of MPB are yet to be elucidated in older adults. Nonetheless, taken together these combined data underscore the potential need for sex-specific nutritional recommendations to mitigate the age-related decline in muscle mass. Specifically, given recent evidence regarding the anabolic properties of *n*-3 PUFA in stimulating MPS, the role of dietary *n*-3 PUFA ingestion in mitigating muscle loss in later life has received considerable attention, especially in older women.

Incorporation of *n*-3 PUFA into skeletal muscle membranes

The most plausible mechanism linking *n*-3 PUFA ingestion with the modulation of muscle protein metabolism relates to the incorporation of EPA and DHA into the skeletal muscle phospholipid membrane, and subsequent modulation of intracellular anabolic cell signalling pathways. This notion is supported by two key lines of evidence. First, using an animal model of tumour-bearing mice, we recently demonstrated that the incorporation and subsequent uptake of EPA and DHA into both plasma and skeletal muscle membranes was directly proportional to the relative EPA and DHA content of a controlled diet following a 3-week experimental period[43]. Similar findings were reported in human studies whereby the provision of a fish oil containing medical food formulation over 7 d resulted in an increased percentage incorporation of EPA into the phospholipid membrane of leucocytes in healthy Caucasian adults[44]. Hence, a direct relationship appears to exist between dietary *n*-3 PUFA intake and the propensity for tissue EPA/DHA incorporation.

Secondly, the uptake and incorporation of *n*-3 PUFA into human skeletal muscle cells is evidently congruent with the kinase activity of cell signalling proteins known to trigger MPS. The time course of changes in *n*-3 PUFA incorporation appears to differ between blood and muscle tissue. Consistent with the slower turnover rates of skeletal muscle tissue compared with blood, McGlory *et al.* demonstrated that ≥2-weeks of *n*-3 PUFA intake is required to increase the EPA/DHA content of skeletal muscle, whereas the incorporation of EPA and DHA into whole blood was increased after only 1 week of fish oil supplementation in healthy young adults[7]. In this study[7], about 2-fold change in the proportion of *n*-3 PUFA incorporated into the skeletal muscle cell was observed after 4 weeks of fish oil supplementation, although no plateau in EPA/DHA incorporation was reached. Importantly, this incorporation of EPA/DHA into the skeletal muscle membrane was accompanied by an increased kinase activity of candidate cell signalling proteins known to regulate MPS, namely focal adhesion kinase and mTORC1. Interestingly, a recent study in healthy females that administered 5 g/d of fish oil (3·50 g/d EPA, 0·90 g/d DHA) over an extended 8-week time course reported a plateau in muscle phospholipid *n*-3 PUFA incorporation after 6 weeks of fish oil supplementation[45]. Moreover, other studies that provided lower doses of *n*-3 PUFA over an extended timeframe reported similar proportional increases in *n*-3 PUFA at the end of an 8-week[46] and 12-week[6] supplementation period. In contrast, only 6 d of high-dose *n*-3 PUFA ingestion resulted in increased muscle tissue *n*-3 PUFA content[47]. Taken together, these studies suggest that the duration and dose of *n*-3 PUFA supplementation are cogent factors that modulate the magnitude of *n*-3 PUFA incorporation into skeletal muscle, and thus provide the primary biological rationale that underpins the regulation of MPS with *n*-3 PUFA ingestion (Table 1).

Alternative factors modulate the rate of *n*-3 PUFA incorporation into the skeletal muscle membrane including, (1) the cellular fraction (i.e. mitochondrial, sarcolemmal) measured, (2) the specific *n*-3 PUFA species (EPA *v.* DHA) of interest and (3) the sex of the individual. Regarding cellular fraction, Herbst *et al.* detected an increased incorporation of EPA and DHA into mitochondrial membranes of skeletal muscle after 12 weeks of fish oil supplementation[48]. Consistent with these findings, Gerling *et al.* demonstrated an increased enrichment of EPA and DHA into the skeletal muscle mitochondrial membrane, whereas only DHA incorporation was detected in the sarcolemmal fraction[6]. Evidence

https://doi.org/10.1017/S0029665123004834 Published online by Cambridge University Press

Proceedings of the Nutrition Society

Table 1. Summary of studies characterising the incorporation on *n*-3 PUFA into blood cell fractions

	Fraction measured	Study population	Daily dose	Length	EPA % of total fatty acids			DHA % of total fatty acids			*n*-3 PUFA % of total fatty acids		
					Pre	Post	Fold change	Pre	Post	Fold change	Pre	Post	Fold change
Da Boit *et al.* (2017)[60]	Erythrocytes	Older men	2·1 g EPA + 0·6 g DHA	18 weeks	1·5	4·3	**2·9**	5·9	6·4	**1·1**			
Da Boit *et al.* (2017)[60]	Erythrocytes	Older women	2·1 g EPA + 0·6 g DHA	18 weeks	1·3	4·7	**3·5**	5·5	6·5	**1·2**			
Jannas-Vela *et al.* (2017)[78]	Erythrocytes	Young men	2 g EPA + 1 g DHA	12 weeks	0·6	3·1	**5·2**	2·9	4·6	**1·6**			
Logan *et al.* (2015)[79]	Serum	Older women	2 g EPA + 1 g DHA	12 weeks	0·9	6·0	**6·9**	1·8	3·8	**2·1**			
McGlory *et al.* (2014)[7]	Whole blood	Young men	3·5 g EPA + 0·9 g DHA	4 weeks	0·9	4·8	**5·4**	2·7	3·6	**1·3**	5·9	11·2	**1·9**
McGlory *et al.* (2016)[61]	Whole blood	Young men	3·5 g EPA + 0·9 g DHA	8 weeks	1·1	5·1	**4·7**	3·5	4·5	**1·3**	6·7	12·6	**1·9**
McGlory *et al.* (2019)[45]	Erythrocyte phospholipids	Young women	2·97 g EPA + 2·03 g DHA (liquid)	4 weeks							118*	328*	**2·8**
McGlory *et al.* (2019)[45]	Erythrocyte phospholipids	Young women	2·97 g EPA + 2·03 g DHA (liquid)	6 weeks							118*	343*	**2·9**
McGlory *et al.* (2019)[45]	Erythrocyte phospholipids	Young women	2·97 g EPA + 2·03 g DHA (liquid)	8 weeks							118*	310*	**2·6**
Metherel *et al.* (2009)[50]	Plasma	Young men	3·2 g EPA + 1·6 g DHA	4 weeks	0·4	4·1	**10·5**	1·1	2·8	**2·6**	2·5	9·0	**3·7**
Metherel *et al.* (2009)[50]	Plasma	Young women	3·2 g EPA + 1·6 g DHA	4 weeks	0·3	4·7	**15·7**	1·3	3·4	**2·5**	2·5	9·6	**3·8**
Metherel *et al.* (2009)[50]	Whole blood	Young men	3·2 g EPA + 1·6 g DHA	4 weeks	0·4	3·3	**8·4**	1·7	3·3	**1·9**	3·6	8·7	**2·4**
Metherel *et al.* (2009)[50]	Whole blood	Young women	3·2 g EPA + 1·6 g DHA	4 weeks	0·3	4·0	**13·3**	2·1	3·6	**1·8**	3·5	9·6	**2·7**
Metherel *et al.* (2009)[50]	Erythrocytes	Young men	3·2 g EPA + 1·6 g DHA	4 weeks	0·4	2·1	**5·4**	2·5	3·5	**1·4**	5·1	8·1	**1·6**
Metherel *et al.* (2009)[50]	Erythrocytes	Young women	3·2 g EPA + 1·6 g DHA	4 weeks	0·3	2·4	**7·8**	3·4	4·3	**1·3**	5·4	9·2	**1·7**

n-3 PUFA, long-chain n-3 PUFA.
Data are represented as % of total fatty acids, except *, where data are represented as nM/ml blood phospholipid content.

from human studies also suggests that EPA exhibits a faster rate of tissue incorporation compared to DHA. Accordingly, several studies[49–51] have reported a more rapid uptake of EPA than DHA in multiple plasma and serum fractions and erythrocytes. This differential uptake and incorporation profile of EPA and DHA in blood appears to follow a similar pattern in skeletal muscle[7,48,52] which could be attributed to a preferential incorporation of distinct *n*-3 PUFA species into different phospholipid fractions. For instance, EPA has been shown to be preferentially incorporated into phosphatidylcholine species of erythrocytes that are primarily located on the outer membrane, whereas DHA is preferentially incorporated into phosphatidylethanolamine species that are located on the inner membrane and requires DHA to be transported through the cellular membrane for incorporation[53,54]. Although no data exist to confirm this thesis, this additional step in the biological fate of DHA has been proposed to underpin the consistent observation of slower incorporation rates of DHA *v.* EPA into the phospholipid membrane[54].

The notion that the anabolic action of *n*-3 PUFA is primarily ascribed to EPA is supported by two lines of evidence from *in vitro* studies conducted in C_2C_{12} myotubes but is not universally accepted. First, Kamolrat and Gray reported a 25 % increase in MPS rates in the EPA condition *v.* control cells, with no effect of DHA on MPS[55]. Furthermore, MPB rates were 22 % lower after incubation with EPA compared to control, with no changes in MPB in the DHA condition. Secondly, Jeromson *et al.* demonstrated that EPA treatment enhanced protein accretion via a suppression of MPB, while DHA elicited no impact on rates of MPS or MPB[56]. However, Wang *et al.* reported a greater attenuation of protein degradation with DHA compared to EPA, as mediated via the upregulation of the PPARγ/NF-κB pathway. The authors attributed these distinct effects to the longer carbon chain of DHA compared to EPA[57]. Future human *in vivo* studies are warranted to fully elucidate the distinct physiological effects of EPA and DHA in terms of anabolic cell signalling, with application to mitigating age-related anabolic resistance.

The suggestion that sex differences exist with regards to tissue *n*-3 PUFA incorporation is supported by a series of preliminary observations. For example, Metherel *et al.* reported higher baseline DHA levels in erythrocytes in females than males[50]. Moreover, a more pronounced increase in DHA incorporation has been observed in females following *n*-3 PUFA supplementation[50,58] that may be linked to a higher conversion of ALA to EPA and DHA in the liver of females. A recent study also demonstrated a greater abundance of precursors necessary for DHA synthesis following EPA supplementation in females than males[59] and thus may serve as an explanation for the apparent sex-based differences in baseline DHA levels. Conversely, Da Boit *et al.* reported no sex differences in the incorporation of EPA and DHA into blood and skeletal muscle following 12 weeks of *n*-3 PUFA supplementation, despite observing improvements in maximal isometric torque and muscle quality

https://doi.org/10.1017/S0029665123004834 Published online by Cambridge University Press

in older women only[60]. Acknowledging the relatively small sample size in human studies, a trend appears to exist regarding a greater propensity for EPA and DHA incorporation in females than males (Table 1). However, given that previous studies did not adjust the dose of ingested *n*-3 PUFA for individual body mass, the greater incorporation of *n*-3 PUFA in females may merely be a function of the smaller stature and subsequent higher relative dose of *n*-3 PUFA in women compared to men. Taken together, these data highlight the practical importance of establishing the optimum supplementation strategy to achieve a maximal, rapid and sustained incorporation of EPA/DHA into the muscle membrane, while accounting for the different biological roles of EPA and DHA and potential sex-specific differences in the biological fate of ingested *n*-3 PUFA.

Influence of *n*-3 PUFA ingestion on muscle protein synthesis

The preponderance of evidence garnered from high-quality experiential studies conducted in human volunteers suggests a modulatory role of *n*-3 PUFA ingestion in stimulating MPS rates, particularly in healthy older adults (Table 2). Initial interest in the anabolic action of *n*-3 PUFA in older adults essentially stemmed from a seminal study conducted by Smith *et al.* in healthy older men and women[46]. This tightly controlled repeated-measures clinical trial measured mixed (i.e. myofibrillar, mitochondrial and sarcoplasmic protein fractions combined) MPS rates under basal (fasted) and simulated fed conditions immediately before and after 8 weeks of fish oil-derived *n*-3 PUFA supplementation *v.* a placebo-controlled corn oil supplementation regimen. The simulated fed condition was represented by the intravenous infusion of amino acids and insulin (i.e. hyperaminoacidemic–hyperinsulinemic clamp) to mimic the nutrient profile and delivery kinetics of a protein-rich mixed macronutrient meal. Basal rates of MPS did not change following 8 weeks of *n*-3 PUFA supplementation. However, the main finding of this study was a potentiation in the simulated feeding-induced increase in MPS rates after 8 weeks of *n*-3 PUFA supplementation compared with before supplementation with no pre–post supplementation changes in the MPS response observed in the corn oil (placebo control) trial. Moreover, the increased stimulation of MPS with *n*-3 PUFA ingestion was accompanied by an upregulation in the phosphorylation status of intracellular cell signalling proteins (mTORC1–p70S6K1) known to trigger the rate-limiting translation initiation step of MPS. Interestingly, no changes in TNF-α or C-reactive protein concentration were observed with *n*-3 PUFA ingestion. Hence, from a mechanistic standpoint, the anti-inflammatory effects of *n*-3 PUFA did not appear to mediate the increased stimulation of MPS following 8 weeks of fish oil supplementation, at least in this cohort of healthy older adults. As such, this proof-of-concept study provided preliminary evidence regarding the metabolic action of chronic (8 weeks) *n*-3 PUFA ingestion in stimulating MPS, at least when the protein synthetic machinery of skeletal muscle is exposed to nutritional stimuli such as amino acids and insulin. In practical terms, these data provided the scientific platform to systematically investigate the efficacy of *n*-3 PUFA ingestion as an adjunct nutritional strategy to mitigate anabolic resistance in older adults.

To further interrogate the efficacy of chronic *n*-3 PUFA ingestion in modulating the stimulation of MPS, we and others conducted a series of arguably more physiologically relevant metabolic studies in young[61] and older[60,62–66] adults, utilising protein feeding (rather than intravenous hyperaminoacidemic–hyperinsulinemic clamp)[61,62] and/or supervised bouts of exercise[61,63–65] as primary anabolic stimuli. Two of these studies were conducted under tightly controlled laboratory conditions and utilised the intravenous tracer infusion of $^{13}C_6$ labelled phenylalanine to measure the acute 3–4-h response of MPS to a single bout of resistance exercise combined with protein feeding both in the presence or absence of prior (8–16 weeks) *n*-3 PUFA supplementation[61,64]. In terms of experimental setup, study participants were confined to the laboratory setting for the duration of the trial while connected to an infusion pump, hence it could be argued that these studies[61,64] were low in ecological validity and thus primarily served as proof-of-concept from an efficacy perspective. Nevertheless, in healthy older (76 (SD 5) years) men (*n* 5) and women (*n* 7), *n*-3 PUFA supplementation (3·9 g/d) was shown to markedly (30 %) augment the acute (3-h) response of MPS (myofibrillar and mitochondrial protein fractions) to resistance exercise combined with the ingestion of two standardised mixed macronutrient (41.84 kJ/kg of 50 % carbohydrate, 30 % fat and 20 % protein) meals[64]. Conversely, in resistance exercise trained young men, 8 weeks of *n*-3 PUFA (5 g/d) supplementation failed to elicit any measurable changes in the acute response of myofibrillar-MPS to a large bolus of whey protein ingested immediately post-exercise in comparison to a coconut oil control condition[61]. These seemingly discrepant findings may be attributed to several methodological considerations. First, due to financial constraints, measurements of MPS were only conducted post *n*-3 PUFA or coconut oil supplementation in the study by McGlory *et al.*[61]. Hence, we sacrificed a degree of statistical power afforded by utilising a more robust repeated-measures design that may have detected statistical differences in MPS between *n*-3 PUFA and coconut oil supplementation groups. Secondly, we cannot discount the possibility that ingestion of 30 g whey protein saturated MPS rates in our cohort of resistance-trained and 'anabolic sensitive' young men[61]. Thus, future studies are warranted to investigate the potential anabolic action of *n*-3 PUFA under conditions of suboptimal protein intake (<20 g) in both young and older adults. Finally, in the study by Lalia *et al.*, data values for MPS were remarkably high (i.e. about 2-fold greater than values presented by multiple comparable studies across various laboratories using an identical tracer protocol in older adults[52,67–69]) and an open-label design was implemented without placebo control. Therefore, the validity of this dataset should be

https://doi.org/10.1017/S0029665123004834 Published online by Cambridge University Press

Table 2. Summary of experimental studies investigating the impact of *n*-3 PUFA ingestion on muscle protein synthesis

References	Participants	Study design/intervention	Muscle fraction/time period for MPS measurement	Primary result	Primary conclusion
Proof-of-concept acute metabolic studies using tracer infusions					
Smith *et al.* (2011)[52]	Young and middle-aged (25–45 y) adults	Single arm pre–post study 8 wk FO (4 g/d, 1·86 g/d EPA, 1·50 g/d DHA) intervention in capsule form	Mixed 3 h basal Mixed 3 h post-intravenous infusion (human insulin: 20 mU m^2 BSA/min; amino acids: 105 mg·kg/FFM/h)	**Basal:** Pre: 0·022%/h; Post: 0·025%/h **Stimulated:** Pre: 0·062%/h; Post: 0·083%/h	Eight wk of FO-derived *n*-3 PUFA ingestion potentiates response of MPS under simulated fed conditions in young and middle-aged adults
Smith *et al.* (2011)[46]	Older (71 (SD 2) y) adults	Single blinded, parallel RCT 8 wk FO (4 g/d, 1·86 g/d EPA, 1·50 g/d DHA) or CO (4 g/d) supplementation in capsule form	Mixed 3 h basal Mixed 3 h post-intravenous infusion (human insulin: 20 mU m^2 BSA/min; amino acids: 105 mg·kg/FFM/h)	**Basal (FO):** Pre: 0·051%/h; Post: 0·053%/h **Stimulated (FO):** Pre: 0·060%/h; Post: 0·084%/h **Basal (CO):** Pre: 0·051%/h; Post: 0·053%/h **Stimulated (CO):** Pre: 0·063%/h; Post: 0·060%/h	Eight wk of FO-derived *n*-3 PUFA ingestion potentiates response of MPS under simulated fed conditions in young and middle-aged adults
Physiologically relevant acute metabolic studies using tracer infusions					
McGlory *et al.* (2016)[61]	Resistance-trained young (23 (SD 2) y) males (*n* 20)	Randomised between-groups repeated measures design. 8 wk FO (5 g/d, 3·50 g/d EPA, 0·90 g/d DHA) or CNO (5 g/d) supplementation in capsule form	Myofibrillar 3 h basal Myofibrillar 3 h post 30 g of ingested whey protein (FED) Myofibrillar 3 h post a single bout of REx followed by 30 g of ingested protein (FEDEX)	**Basal (FO):** 0·025%/h; **(CNO):** 0·024%/h **FED (FO):** 0·069%/h; **(CNO):** 0·056%/h **FEDEX (FO):** 0·091%/h; **(CNO):** 0·077%/h	No statistical effect of FO-derived *n*-3 PUFA ingestion on MPS rates under basal conditions or when muscle is stimulated by a 30 g whey protein feed with or without resistance exercise
Lalia *et al.* (2017)[64]	Older (65–85 y, *n* 12) adults	Single arm pre–post open label study 16 wk *n*-3 PUFA (3·9 g/d) supplementation in capsule form	Mixed, myofibrillar and mitochondrial 3 h basal mixed, myofibrillar and mitochondrial 15–18 h post-acute bout resistance exercise	**BASAL:** Post>Pre* **POST EXERCISE:** Post>Pre*	Prolonged *n*-3 PUFA supplementation augments basal and post-exercise MPS rates in older adults
Free-living intermediate metabolic studies using deuterium oxide tracer methodology					
McGlory *et al.* (2019)[45]	Young physically active women	Randomised controlled, double-blinded, parallel trial 8 wk *n*-3 PUFA (5·0 g/d, 3·0 g/d EPA, 2·0 g/d DHA) or SO (5·0 g/d) supplementation in beverage form combined with immobilisation and RET	Myofibrillar 2-wk pre, during and post (+RET) leg immobilisation	**PRE:** *n*-3 PUFA = SO* **DURING:** *n*-3 PUFA>SO* **POST:** *n*-3 PUFA>SO*	Prolonged *n*-3 PUFA supplementation increases integrated MPS rates during 2 wk of immobilisation and 2 wk of RET-induced remobilisation

Brook *et al.* (2021)[63]	Older (67 (SD 1) y, *n* 16) women	Randomised controlled, double-blinded, parallel trial 6 wk *n*-3 PUFA (3·7 g/d) or CO (3·7 g/d) supplementation in capsule form combined with single leg resistance training	Myofibrillar and ASR 2 wk (4–6 wk) in T and UT legs	**T (*n*-3 PUFA):** 7·1%/d **T (CO):** 6·5%/d **UT (*n*-3 PUFA):** 5·6%/d **Trained (CO):** 5·5%/d	Prolonged *n*-3 PUFA supplementation enhances free-living MPS rates during RET
Murphy *et al.* (2021)[62]	Older (>65 y, *n* 28) adults	Randomised controlled, double-blinded, parallel trial 24 wk *n*-3 PUFA (4·0 g/d) *v.* PRO *v.* CHO in beverage form	Myofibrillar 1 wk (wk 23–24)	***n*-3 PUFA:** 1·74%/d **PRO:** 1·79%/d **CHO:** 1·61%/d	No beneficial impact of 24 wk *n*-3 PUFA supplementation on integrated MPS rates in older adults at risk of sarcopoenia

ASR, absolute synthesis rates; BSA, body surface area; CHO, carbohydrate; CNO, coconut oil; CO, corn oil; d, day; FFM, fat-free mass; FO, fish oil; mo, months; PRO, protein; RET, resistance exercise training; SO, sunflower oil; T, trained; UT, untrained; wk, weeks; y, years.
*Data values not presented in paper.

considered with caution. Nevertheless, taken together, these acute metabolic data indicate that *n*-3 PUFA ingestion may serve as a more effective intervention to increase the response of MPS to nutritional and exercise stimuli in 'anabolically resistant' older adults than 'anabolically sensitive' young adults.

The reintroduction of deuterium oxide tracer methodology into the field of nutrition, exercise and muscle protein metabolism[70] has provided a unique platform for laboratories worldwide to conduct measurements of free-living MPS rates that integrate post-absorptive and post-prandial physiological states over several days or weeks. Accordingly, a series of recent randomised, placebo-controlled, repeated measures studies have investigated the influence of chronic (6–24 weeks) *n*-3 PUFA ingestion on free-living integrative rates of MPS in healthy young[45] and older adults[60,62,63]. Given the relatively longitudinal nature of these study designs, whole-body measurements of muscle mass, strength and function have been conducted simultaneously with tissue-specific measurements of MPS, alongside other metabolic and molecular parameters understood to regulate muscle mass and quality such as surrogate markers of MPB[63], myonuclei number[63], satellite cell activation[63] and transcriptional changes in genes related to extracellular matrix organisation[64,71]. In theory, the measurement of MPS over this intermediate time period (rather than an acute 3–4-h period) should better translate to clinically relevant chronic changes in muscle mass (and by extension strength and function) with direct application to the maintenance of muscle mass in later life.

Perhaps the most comprehensive of these deuterium oxide tracer studies, albeit relatively small-scale in cohort size (*n* 16 participants, eight per group), was conducted by Brook *et al.* in healthy older women that embarked on a 6-week programme of structured and progressive resistance exercise training[63]. The treatment regimen consisted of 3·7 g/d of *n*-3 PUFA supplementation and the control group was assigned to a dose-matched *n*-6-rich corn oil supplementation regimen. Integrated rates of MPS were determined during the early (0–2 week) and latter (4–6 week) phases of resistance training. No effect of *n*-3 PUFA ingestion on MPS rates was observed during the early phase of training. However, a trend for an increased stimulation of MPS was observed during the latter phase of training in the *n*-3 PUFA group that was accompanied by an upregulation of eukaryotic initiation factor 4E binding protein 1 as a downstream (of mTORC1) anabolic signalling protein. Moreover, thigh fat-free mass and type II muscle fibre CSA increased following short-term resistance training in the *n*-3 PUFA group only, whereas no differences in myonuclear number, satellite cell number and static markers of MPB (calpain, MAFbx and ubiquitin) measured 60–90 min after the final exercise training bout were observed between *n*-3 PUFA and corn oil regimens. Consistent with this observation[63], McGlory *et al.* reported higher free-living integrated rates of MPS during 2 weeks of leg immobilisation (used as a model of muscle disuse) and 2 weeks of remobilisation (resistance

https://doi.org/10.1017/S0029665123004834 Published online by Cambridge University Press

exercise training) following *n*-3 PUFA supplementation in a cohort of physically active young women[45]. This increased stimulation of MPS with *n*-3 PUFA ingestion translated to an attenuated decline in muscle (quadriceps) volume and CSA during the muscle disuse period and an accelerated restoration of muscle mass during rehabilitation[45]. Hence, taken together the augmentation of resistance exercise training-induced muscle mass in older adults, specifically of type II fibres, with *n*-3 PUFA ingestion observed by Brook *et al.* and others[72] appears to be mediated by an increased stimulation of MPS rather than suppression of MPB and/or via satellite cell recruitment[63]. Larger-scale, follow-up studies are warranted to investigate the impact of *n*-3 PUFA ingestion on free-living integrated MPS rates and muscle mass in response to discrete (injury-induced leg immobilisation) or chronic (illness-induced hospitalisation) periods of muscle disuse in older adults.

Two larger-scale (≥50 participants) randomised placebo-controlled trials also investigated the impact of chronic (18–24 week) *n*-3 PUFA supplementation on free-living integrated rates of MPS in combination with validated readouts of muscle mass (i.e. muscle anatomical CSA, appendicular lean mass) in healthy older adults[60,62]. Based on previous evidence highlighting the interaction of bioavailable *n*-3 PUFA and amino acids in modulating MPS[52,55,73], Murphy *et al.* administered a treatment condition consisting of a leucine-rich whey protein-based beverage that was fortified with *n*-3 PUFA (3·8 g/d + vitamin D) *v.* protein/leucine and isoenergetic controls twice daily over a 24-week period[62]. No exercise training stimulus was included in this study. In comparison, Da Boit *et al.* combined *n*-3 PUFA supplementation with an 18-week progressive resistance exercise training programme[60]. As a note of caution, neither study stipulated MPS as the primary endpoint measurement and only a relatively small subset of participants (i.e. five participants in the *n*-3 PUFA group[62]) volunteered for muscle biopsies, thus allowing for measurement of MPS. Hence, by the authors' own admission, the MPS datasets from these studies should be interpreted with caution. Nonetheless, studies were consistent in reporting no beneficial impact of chronic (18–24-week) *n*-3 PUFA supplementation on integrated MPS rates, appendicular lean mass[62] and muscle anatomical CSA[60] in older adult men and women. These findings may be considered somewhat surprising given that a comparable large-scale (*n* 60) study by Smith *et al.*[74] previously demonstrated that supplementation with a similar dose (3·4 g) of *n*-3 PUFA over the same 24-week period increased thigh muscle volume in a cohort of healthy older adults. Moreover, this favourable change in muscle mass with *n*-3 PUFA supplementation was accompanied by an increase in skeletal muscle gene expression of pathways involved in mitochondrial function (i.e. uncoupling protein 3, ubiquinol-cytochrome c reductase core protein 1) and extracellular matrix organisation, and a decreased gene expression of pathways related to calpain and ubiquitin-mediated proteolysis and the inhibition of mTORC1[71]. Hence, in these studies[71,74] the favourable effects of *n*-3 PUFA on muscle mass and function appeared to be transcriptionally regulated. The inconsistent findings regarding the efficacy of *n*-3 PUFA ingestion in modulating muscle mass are difficult to reconcile given the subtle, yet potentially important methodological differences in study design (i.e. participant characteristics including sex, *n*-3 PUFA dosing regimen, inclusion or absence of resistance training), but may be attributed to inter-individual variability in the skeletal muscle adaptive response to *n*-3 PUFA ingestion[75]. Follow-up studies designed to investigate the anabolic effects of *n*-3 PUFA should include omics (i.e. metabolomics, transcriptomics and proteomics) analysis to further interrogate the magnitude of inter-individual variability in response of MPS, muscle mass, strength and function to *n*-3 PUFA ingestion in healthy and more compromised older adult populations.

An interesting and remarkably consistent observation across published studies investigating the efficacy of *n*-3 PUFA ingestion to stimulate MPS and/or elicit favourable changes in muscle mass, strength and function relates to evidence of a sex-specific response. This notion is supported by several lines of scientific evidence. First, whereas fish oil-derived (3 g/d) *n*-3 PUFA supplementation was shown to enhance improvements in maximal isometric strength and muscle quality (calculated as strength per unit of muscle area) following 18 weeks of resistance exercise training in older women, a comparable response was not observed in older men[60]. Secondly, and consistent with this observation, 12 weeks of fish oil-derived (2 g/d) *n*-3 supplementation was shown to enhance muscle strength (increases in maximal torque and rate of torque development) and functional (sit-to-stand performance) outcomes following 90 d of resistance training in a large cohort (*n* 45) of older women[76]. Thirdly, a relatively large-scale (*n* 44) study by Smith *et al.* reported that daily ingestion of *n*-3 PUFA (1·86 g EPA and 1·50 g DHA) over 6 months elicited clinically relevant improvements in muscle volume (about 3·5 %) and function (about 6 %) in an older adult cohort that consisted primarily (67 %) of females[74]. These remarkable findings, particularly in light of the absence of resistance training, imply that 6 months of *n*-3 PUFA ingestion has the potential to offset 2–3 years of the 'expected' age-associated decline in skeletal muscle mass (about 0·5–1·0 %/year) and function (about 2–3 %/year)[19].

As alluded to earlier (see 'Incorporation of n-3 PUFA into skeletal muscle membranes' section), the most intuitive mechanism to explain these sex-specific observations relates to sex differences in the incorporation of *n*-3 PUFA into the phospholipid membrane. However, to this end, although a greater uptake of EPA in plasma, whole blood and erythrocytes[50,77] following *n*-3 PUFA supplementation has been reported in women than men, the propensity for EPA and DHA incorporation into the muscle phospholipid membrane has been shown to be comparable between sexes[60], at least in older adults. Alternatively, the apparent sex-specific response of skeletal muscle adaptation to *n*-3 PUFA ingestion may merely be a function of a higher dose relative to body mass administered to female participants. In

https://doi.org/10.1017/S0029665123004834 Published online by Cambridge University Press

all previous studies[45,52,60,63,64,71,74], the dose of ingested *n*-3 PUFA was administered on an absolute basis (typically between 3 and 5 g/d), irrespective of body mass. Assuming a lower body mass in older adult women than men, the higher relative dose of *n*-3 PUFA administered to females provides a plausible explanation for the observation of a more favourable muscle adaptive response to *n*-3 PUFA in older women. Clearly, further studies are warranted to confirm this apparent sex difference in muscle adaptive response to *n*-3 PUFA ingestion and fully elucidate the mechanism(s) that underpin any sexual dimorphism in response to *n*-3 PUFA ingestion.

Conclusion

Sarcopoenia is underpinned by the age-related decline in type II muscle fibres, to which females are particularly susceptible. The biological rationale linking *n*-3 PUFA with muscle protein turnover relates to the incorporation of EPA and DHA into the muscle phospholipid membrane rather than any anti-inflammatory properties of *n*-3 PUFA. However, to this end, no study has explored fibre type differences in the incorporation of *n*-3 PUFA. The efficacy of *n*-3 PUFA ingestion to elicit favourable changes in muscle protein turnover in healthy older adults appears to be more pronounced in females than in males. On a clinical basis, the efficacy of *n*-3 PUFA ingestion to elicit favourable changes in muscle protein turnover warrants further investigation in cancer cachexic patients given evidence of a metabolic action of EPA in blunting MPB. Specifically, dose–response studies are required to determine the optimal dosing of *n*-3 PUFA ingestion to promote a range of musculoskeletal health outcomes (muscle mass, strength, physical function and MPS rates) in healthy and compromised older adult sub-populations.

Acknowledgements

We sincerely acknowledge the Nutrition Society for the invitation to contribute to the Proceedings of the Nutrition Society following the 2023 Nutrition Society Summer meeting in Liverpool. The authors would like to thank Vanessa Rapson and Iris Zieler for proofreading the manuscript.

Financial Support

None.

Conflict of Interest

None.

Authorship

All authors wrote and approved the manuscript.

References

1. Calder PC (2015) Functional roles of fatty acids and their effects on human health. *JPEN J Parenter Enteral Nutr* **39**, 18S–32S.
2. Witard OC, Combet E & Gray SR (2020) Long-chain n-3 fatty acids as an essential link between musculoskeletal and cardio-metabolic health in older adults. *Proc Nutr Soc* **79**, 47–55.
3. Ferguson EJ, Seigel JW & McGlory C (2021) Omega-3 fatty acids and human skeletal muscle. *Curr Opin Clin Nutr Metab Care* **24**, 114–119.
4. McGlory C *et al.* (2019) The influence of omega-3 fatty acids on skeletal muscle protein turnover in health, disuse, and disease. *Front Nutr* **6**, 144.
5. Calder PC (2012) Mechanisms of action of (n-3) fatty acids. *J Nutr* **142**, 592S–599S.
6. Gerling CJ, Mukai K, Chabowski A *et al.* (2019) Incorporation of omega-3 fatty acids into human skeletal muscle sarcolemmal and mitochondrial membranes following 12 weeks of fish oil supplementation. *Front Physiol* **10**, 348.
7. McGlory C, Galloway SD, Hamilton DL *et al.* (2014) Temporal changes in human skeletal muscle and blood lipid composition with fish oil supplementation. *Prostaglandins Leukot Essent Fatty Acids* **90**, 199–206.
8. Calder PC (2010) Omega-3 fatty acids and inflammatory processes. *Nutrients* **2**, 355–374.
9. Burdge GC & Calder PC (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* **45**, 581–597.
10. Kuhnt K, Weiß S, Kiehntopf M *et al.* (2016) Consumption of echium oil increases EPA and DPA in blood fractions more efficiently compared to linseed oil in humans. *Lipids Health Dis* **15**, 32–32.
11. Greupner T, Koch E, Kutzner L *et al.* (2019) Single-dose SDA-rich echium oil increases plasma EPA, DPAn3, and DHA concentrations. *Nutrients* **11**, 2346. doi: 10.3390/nu11102346
12. Rizzo G, Baroni L & Lombardo M (2023) Promising sources of plant-derived polyunsaturated fatty acids: a narrative review. *Int J Environ Res Public Health* **20**, 1683. doi: 10.3390/ijerph20031683
13. Baker EJ, Miles EA, Burdge GC *et al.* (2016) Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Prog Lipid Res* **64**, 30–56.
14. Janssen I, Heymsfield SB, Wang ZM *et al.* (2000) Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J Appl Physiol* **89**, 81–88.
15. Mitchell WK, Williams J, Atherton P *et al.* (2012) Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength: a quantitative review. *Front Physiol* **3**, 260.
16. Delmonico MJ, Harris TB, Visser M *et al.* (2009) Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr* **90**, 1579–1585.
17. Verdijk LB, Koopman R, Schaart G *et al.* (2007) Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. *Am J Physiol Endocrinol Metab* **292**, E151–E157.
18. Lexell J, Taylor CC & Sjöström M (1988) What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15– to 83-year-old men. *J Neurol Sci* **84**, 275–294.
19. Goodpaster BH, Park SW, Harris TB *et al.* (2006) The loss of skeletal muscle strength, mass, and quality in older

https://doi.org/10.1017/S0029665123004834 Published online by Cambridge University Press

adults: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci* **61**, 1059–1064.
20. Wall BT, Dirks ML, Snijders T *et al.* (2015) Short-term muscle disuse atrophy is not associated with increased intramuscular lipid deposition or a decline in the maximal activity of key mitochondrial enzymes in young and older males. *Exp Gerontol* **61**, 76–83.
21. Guillet C, Prod'homme M, Balage M *et al.* (2004) Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. *FASEB J* **18**, 1586–1587.
22. Wilkes EA, Selby AL, Atherton PJ *et al.* (2009) Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. *Am J Clin Nutr* **90**, 1343–1350.
23. Groen BBL, Horstman AM, Hamer HM *et al.* (2016) Increasing insulin availability does not augment postprandial muscle protein synthesis rates in healthy young and older Men. *J Clin Endocrinol Metab* **101**, 3978–3988.
24. Cuthbertson D, Smith K, Babraj J *et al.* (2005) Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* **19**, 422–424.
25. Volpi E, Sheffield-Moore M, Rasmussen BB *et al.* (2001) Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *JAMA* **286**, 1206–1212.
26. Cuthbertson DJ, Babraj J, Smith K *et al.* (2006) Anabolic signaling and protein synthesis in human skeletal muscle after dynamic shortening or lengthening exercise. *Am J Physiol Endocrinol Metab* **290**, E731–E738.
27. Durham WJ, Casperson SL, Dillon EL *et al.* (2010) Age-related anabolic resistance after endurance-type exercise in healthy humans. *FASEB J* **24**, 4117–4127.
28. Fry CS, Drummond MJ, Glynn EL *et al.* (2011) Aging impairs contraction-induced human skeletal muscle mTORC1 signaling and protein synthesis. *Skelet Muscle* **1**, 11.
29. Kumar V, Selby A, Rankin D *et al.* (2009) Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol* **587**, 211–217.
30. Wall BT, Gorissen SH, Pennings B *et al.* (2015) Aging is accompanied by a blunted muscle protein synthetic response to protein ingestion. *PLoS ONE* **10**, e0140903.
31. Shad BJ, Thompson JL & Breen L (2016) Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review. *Am J Physiol Endocrinol Metab* **311**, E803–E817.
32. Phillips SM, Glover EI & Rennie MJ (2009) Alterations of protein turnover underlying disuse atrophy in human skeletal muscle. *J Appl Physiol* **107**, 645–654.
33. Hansen M (2018) Female hormones: do they influence muscle and tendon protein metabolism? *Proc Nutr Soc* **77**, 32–41.
34. Martel GF, Roth SM, Ivey FM *et al.* (2006) Age and sex affect human muscle fibre adaptations to heavy-resistance strength training. *Exp Physiol* **91**, 457–464.
35. Forbes GB & Reina JC (1970) Adult lean body mass declines with age: some longitudinal observations. *Metabolism* **19**, 653–663.
36. Kyle UG, Genton L, Hans D *et al.* (2001) Age-related differences in fat-free mass, skeletal muscle, body cell mass and fat mass between 18 and 94 years. *Eur J Clin Nutr* **55**, 663–672.
37. Bamman MM, Hill VJ, Adams GR *et al.* (2003) Gender differences in resistance-training-induced myofiber hypertrophy among older adults. *J Gerontol A Biol Sci Med Sci* **58**, 108–116.
38. Yasuda N, Glover EI, Phillips SM *et al.* (2005) Sex-based differences in skeletal muscle function and morphology with short-term limb immobilization. *J Appl Physiol* **99**, 1085–1092.
39. Callahan DM, Tourville TW, Miller MS *et al.* (2015) Chronic disuse and skeletal muscle structure in older adults: sex-specific differences and relationships to contractile function. *Am J Physiol Cell Physiol* **308**, 932.
40. Smith GI, Atherton P, Villareal DT *et al.* (2008) Differences in muscle protein synthesis and anabolic signaling in the postabsorptive state and in response to food in 65–80 year old men and women. *PLoS ONE* **3**, e1875.
41. Smith GI, Reeds DN, Hall AM *et al.* (2012) Sexually dimorphic effect of aging on skeletal muscle protein synthesis. *Biol Sex Differ* **3**, 11–11.
42. Hansen M, Skovgaard D, Reitelseder S *et al.* (2012) Effects of estrogen replacement and lower androgen status on skeletal muscle collagen and myofibrillar protein synthesis in postmenopausal women. *J Gerontol A Biol Sci Med Sci* **67**, 1005–1013.
43. Dijk FJ, van Dijk M, Dorresteijn B *et al.* (2019) DPA shows comparable chemotherapy sensitizing effects as EPA upon cellular incorporation in tumor cells. *Oncotarget* **10**, 5983–5992.
44. Faber J, Berkhout M, Vos AP *et al.* (2011) Supplementation with a fish oil-enriched, high-protein medical food leads to rapid incorporation of EPA into white blood cells and modulates immune responses within one week in healthy men and women. *J Nutr* **141**, 964–970.
45. McGlory C, Gorissen SHM, Kamal M *et al.* (2019) Omega-3 fatty acid supplementation attenuates skeletal muscle disuse atrophy during two weeks of unilateral leg immobilization in healthy young women. *FASEB J* **33**, 4586–4597.
46. Smith GI, Atherton P, Reeds DN *et al.* (2011) Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. *Am J Clin Nutr* **93**, 402–412.
47. Wardle SL, Macnaughton LS, McGlory C *et al.* (2020) Human skeletal muscle metabolic responses to 6 days of high-fat overfeeding are associated with dietary n-3 PUFA content and muscle oxidative capacity. *Physiol Rep* **8**, e14529.
48. Herbst EA, Paglialunga S, Gerling C *et al.* (2014) Omega-3 supplementation alters mitochondrial membrane composition and respiration kinetics in human skeletal muscle. *J Physiol* **592**, 1341–1352.
49. Browning LM, Walker CG, Mander AP *et al.* (2012) Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish. *Am J Clin Nutr* **96**, 748–758.
50. Metherel AH, Armstrong JM, Patterson AC *et al.* (2009) Assessment of blood measures of n-3 polyunsaturated fatty acids with acute fish oil supplementation and washout in men and women. *Prostaglandins Leukot Essent Fatty Acids* **81**, 23–29.
51. Katan MB, Deslypere JP, van Birgelen AP *et al.* (1997) Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res* **38**, 2012–2022.
52. Smith GI, Atherton P, Reeds DN *et al.* (2011) Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia–hyperaminoacidaemia in healthy young and middle-aged men and women. *Clin Sci* **121**, 267–278.

https://doi.org/10.1017/S0029665123004834 Published online by Cambridge University Press

53. Cartwright IJ, Pockley AG, Galloway JH *et al.* (1985) The effects of dietary omega-3 polyunsaturated fatty acids on erythrocyte membrane phospholipids, erythrocyte deformability and blood viscosity in healthy volunteers. *Atherosclerosis* **55**, 267–281.
54. Pal A, Metherel AH, Fiabane L *et al.* (2020) Do eicosapentaenoic acid and docosahexaenoic acid have the potential to compete against each other? *Nutrients* **12**, 3718. doi: 10.3390/nu12123718
55. Kamolrat T & Gray SR (2013) The effect of eicosapentaenoic and docosahexaenoic acid on protein synthesis and breakdown in murine C2C12 myotubes. *Biochem Biophys Res Commun* **432**, 593–598.
56. Jeromson S, Mackenzie I, Doherty MK *et al.* (2018) Lipid remodeling and an altered membrane-associated proteome may drive the differential effects of EPA and DHA treatment on skeletal muscle glucose uptake and protein accretion. *Am J Physiol Endocrinol Metab* **314**, E605–E619.
57. Wang Y, Lin QW, Zheng PP *et al.* (2013) DHA inhibits protein degradation more efficiently than EPA by regulating the PPARgamma/NFkappaB pathway in C2C12 myotubes. *Biomed Res Int* **2013**, 318981.
58. Patterson AC, Chalil A, Aristizabal Henao JJ *et al.* (2015) Omega-3 polyunsaturated fatty acid blood biomarkers increase linearly in men and women after tightly controlled intakes of 0·25, 0·5, and 1 g/d of EPA + DHA. *Nutr Res* **35**, 1040–1051.
59. Rotarescu RD, Rezaei K, Mutch DM *et al.* (2022) Increases in plasma n-3 tetracosapentaenoic acid and tetracosahexaenoic acid following 12 weeks of EPA, but not DHA, supplementation in women and men. *Prostaglandins Leukot Essent Fatty Acids* **185**, 102494.
60. Da Boit M, Sibson R, Sivasubramaniam S *et al.* (2017) Sex differences in the effect of fish-oil supplementation on the adaptive response to resistance exercise training in older people: a randomized controlled trial. *Am J Clin Nutr* **105**, 151–158.
61. McGlory C, Wardle SL, Macnaughton LS *et al.* (2016) Fish oil supplementation suppresses resistance exercise and feeding-induced increases in anabolic signaling without affecting myofibrillar protein synthesis in young men. *Physiol Rep* **4**. doi: 10.14814/phy2.12715
62. Murphy CH, Flanagan EM, De Vito G *et al.* (2021) Does supplementation with leucine-enriched protein alone and in combination with fish-oil-derived n-3 PUFA affect muscle mass, strength, physical performance, and muscle protein synthesis in well-nourished older adults? A randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* **113**, 1411–1427.
63. Brook MS, Din U, Tarum J *et al.* (2021) Omega-3 supplementation during unilateral resistance exercise training in older women: a within subject and double-blind placebo-controlled trial. *Clin Nutr ESPEN* **46**, 394–404.
64. Lalia AZ, Dasari S, Robinson MM *et al.* (2017) Influence of omega-3 fatty acids on skeletal muscle protein metabolism and mitochondrial bioenergetics in older adults. *Aging (Albany NY)* **9**, 1096–1129.
65. Dalle S, Van Roie E, Hiroux C *et al.* (2021) Omega-3 supplementation improves isometric strength but not muscle anabolic and catabolic signaling in response to resistance exercise in healthy older adults. *J Gerontol A Biol Sci Med Sci* **76**, 406–414.
66. Kunz HE, Michie KL, Gries KJ *et al.* (2022) A randomized trial of the effects of dietary n3-PUFAs on skeletal muscle function and acute exercise response in healthy older adults. *Nutrients* **14**, 3537. doi: 10.3390/nu14173537
67. Bukhari SS, Phillips BE, Wilkinson DJ *et al.* (2015) Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women, at rest and after exercise. *Am J Physiol Endocrinol Metab*, ajpendo.
68. Dreyer HC, Drummond MJ, Pennings B *et al.* (2008) Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. *Am J Physiol Endocrinol Metab* **294**, E392–E400.
69. Tipton KD, Ferrando AA, Phillips SM *et al.* (1999) Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol* **276**, E628–E634.
70. Wilkinson DJ, Brook MS, Smith K *et al.* (2017) Stable isotope tracers and exercise physiology: past, present and future. *J Physiol* **595**, 2873–2882.
71. Yoshino J, Smith GI, Kelly SC *et al.* (2016) Effect of dietary n-3 PUFA supplementation on the muscle transcriptome in older adults. *Physiol Rep* **4**, e12785. doi: 10.14814/phy2.12785
72. Bischoff-Ferrari HA, Vellas B, Rizzoli R *et al.* (2020) Effect of vitamin D supplementation, omega-3 fatty acid supplementation, or a strength-training exercise program on clinical outcomes in older adults: the DO-HEALTH randomized clinical trial. *JAMA* **324**, 1855–1868.
73. Kamolrat T & Gray SR (2013) Fish oil positively regulates anabolic signalling alongside an increase in whole-body gluconeogenesis in ageing skeletal muscle. *Eur J Nutr* **52**, 647–657.
74. Smith GI, Julliand S, Reeds DN *et al.* (2015) Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults. *Am J Clin Nutr*. **102**, 115–122.
75. Murphy CH, Connolly C, Flanagan EM *et al.* (2022) Interindividual variability in response to protein and fish oil supplementation in older adults: a randomized controlled trial. *J Cachexia Sarcopenia Muscle* **13**, 872–883.
76. Rodacki CL, Rodacki AL, Pereira G *et al.* (2012) Fish-oil supplementation enhances the effects of strength training in elderly women. *Am J Clin Nutr* **95**, 428–436.
77. Caslake MJ, Miles EA, Kofler BM *et al.* (2008) Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN study. *Am J Clin Nutr* **88**, 618–629.
78. Jannas-Vela S, Roke K, Boville S *et al.* (2017) Lack of effects of fish oil supplementation for 12 weeks on resting metabolic rate and substrate oxidation in healthy young men: a randomized controlled trial. *PLoS ONE* **12**, e0172576.
79. Logan SL & Spriet LL (2015) Omega-3 fatty acid supplementation for 12 weeks increases resting and exercise metabolic rate in healthy community-dwelling older females. *PLoS ONE* **10**, e0144828.

https://doi.org/10.1017/S0029665123004834 Published online by Cambridge University Press