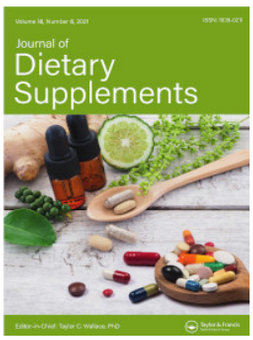

Journal of Dietary Supplements

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ijds20

Effects of a Long Chain n-3 Polyunsaturated Fatty Acid-rich Multi-ingredient Nutrition Supplement on Body Composition and Physical Function in Older Adults with Low Skeletal Muscle Mass

Alessandro Scotto di Palumbo, Fionn T. McSwiney, Michelle Hone, Aoibheann M. McMorrow, Gina Lynch, Giuseppe De Vito & Brendan Egan

To cite this article: Alessandro Scotto di Palumbo, Fionn T. McSwiney, Michelle Hone, Aoibheann M. McMorrow, Gina Lynch, Giuseppe De Vito & Brendan Egan (2021): Effects of a Long Chain n-3 Polyunsaturated Fatty Acid-rich Multi-ingredient Nutrition Supplement on Body Composition and Physical Function in Older Adults with Low Skeletal Muscle Mass, Journal of Dietary Supplements, DOI: 10.1080/19390211.2021.1897057

To link to this article: https://doi.org/10.1080/19390211.2021.1897057

Published online: 24 Mar 2021.

JOURNAL OF DIETARY SUPPLEMENTS
https://doi.org/10.1080/19390211.2021.1897057

Taylor & Francis
Taylor & Francis Group

ARTICLE

OPEN ACCESS Check for updates

Effects of a Long Chain n-3 Polyunsaturated Fatty Acid-rich Multi-ingredient Nutrition Supplement on Body Composition and Physical Function in Older Adults with Low Skeletal Muscle Mass

Alessandro Scotto di Palumbo[a], Fionn T. McSwiney[b], Michelle Hone[b], Aoibheann M. McMorrow[a], Gina Lynch[a], Giuseppe De Vito[a,c], and Brendan Egan[b,d,e]

[a]Institute for Sport and Health, School of Public Health, Physiotherapy and Sports Science, University College Dublin, Dublin 4, Ireland; [b]School of Health and Human Performance, Dublin City University, Dublin 9, Ireland; [c]Department of Biomedical Sciences, University of Padova, Padova, Italy; [d]National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland; [e]Florida Institute for Human and Machine Cognition, Pensacola, FL, USA

ABSTRACT

Six months of supplementation with a multi-ingredient nutrition supplement was investigated in older adults with low skeletal muscle mass given the recently purported benefits of such approaches. Community-dwelling older adults (age, 74.9 ± 3.6 y; M/F, 18/19) participated in a double-blind, placebo-controlled, randomized trial involving daily consumption of either fruit juice placebo (PLA) or supplement (SUPP) in the form of a 200-mL carton of a juice-based emulsion of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) (3000 mg as 1500 mg docosahexaenoic acid and 1500 mg eicosapentaenoic acid), whey protein isolate (8 g), vitamin D3 (400 IU), and resveratrol (150 mg). Body composition, physical function, and circulating markers of metabolic health were assessed at baseline (PRE), and after 3 (MID) and 6 (POST) months of supplementation. Lean body mass (LBM) was unchanged in either group, but fat mass increased in SUPP by 1.41 (0.75, 2.07) kg at POST (+6.4%; $p < .001$; $d = 0.20$). Hand-grip strength was maintained in SUPP, but declined in PLA by 2.50 (0.81, 4.19) kg at POST (−6.8%; $p = .002$; $d = 0.38$). Short physical performance battery score was unchanged in PLA, but increased in SUPP by 1.13 (0.41, 1.84) above PRE at POST ($p = .001$; $d = 0.47$). Circulating markers of metabolic health were unchanged in response to the intervention in either PLA or SUPP. Long-term supplementation with an LC n-3 PUFA-rich multi-ingredient nutrition supplement demonstrates potential efficacy for improving physical function in older adults in the absence of exercise training and independent of a change in LBM.

KEYWORDS

Docosahexaenoic acid
eicosapentaenoic acid
omega-3
lean body mass
strength

CONTACT Brendan Egan brendan.egan@dcu.ie School of Health and Human Performance, Dublin City University, Dublin 9, Ireland

Introduction

Age-related declines in lean body mass (LBM) and muscle strength and function, termed sarcopenia, are associated with the onset or worsening of symptoms related to metabolic syndrome (abdominal obesity, high-blood pressure, type 2 diabetes, and dyslipidemia) (Zhang et al. 2018) and reduced quality of life (Cruz-Jentoft et al. 2019). The etiology of sarcopenia is multifactorial, including declining physical function, the loss of muscle mass and type 2 muscle fibers, increased inflammation, altered hormonal profile, inadequate nutrition intake, and anabolic resistance (Cruz-Jentoft et al. 2019). Anabolic resistance refers to the anabolic processes of muscle protein synthesis (MPS) being attenuated due to a blunted anabolic response to dietary protein and insulin in older adults (Breen and Phillips 2011). A combination of reduced protein intake and anabolic resistance constitutes a catabolic environment that accelerates muscle loss and increases the likelihood of sarcopenia (Breen and Phillips 2011).

To date, pharmacological interventions have limited efficacy in counteracting the effects of sarcopenia (Kilsby et al. 2017), whereas most nutrition guidelines focus on the provision of additional protein (Deutz et al. 2014). This standard therapeutic approach through supplemental protein to stimulate MPS and facilitate muscle growth or maintenance in older adults has efficacy, but anabolic resistance may contribute to suboptimal therapeutic effects of protein supplementation interventions in older adults (Breen and Phillips 2011; Boirie et al. 2014). Indeed, there are a number of other nutrients that may be efficacious in this context (Cruz-Jentoft et al. 2020) and, therefore, a multi-ingredient nutrition strategy may offer additional therapeutic potential by targeting multiple factors associated with the development and consequences of age-related declines in muscle health and cognitive parameters (Veronese et al. 2019; Cruz-Jentoft et al. 2020).

Recent research has explored the potential role of interventions such as long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) (Logan and Spriet 2015; Smith et al. 2015), whey protein (Zhu et al. 2015), vitamin D (Dhesi et al. 2004), and resveratrol (Alway et al. 2017) in addition to multi-ingredient supplements alone (Negro et al. 2019) or combined with exercise training interventions (Verreijen et al. 2015; Rondanelli et al. 2016; Bell et al. 2017). These studies generally aim to either augment the training-induced improvements, or curb the decline in the absence of exercise, in LBM and muscle function in older adults, each with varying degrees of success. Supplementation with LC n-3 PUFA has been proposed as a targeted approach to reduce anabolic resistance and enhance the MPS response to feeding (McGlory et al. 2019; Rossato et al. 2020). In the absence of an exercise training intervention, supplementation with ~3 g/dayof LC n-3 PUFA for 3 to 6 months increased LBM and muscle function in older adults (>60 yrs) (Logan and Spriet 2015; Smith et al. 2015). Moreover, supplementation with other nutrients such as vitamin D3 combined with whey protein (Bauer et al. 2015), and resveratrol (Pollack et al. 2017), have promising effects on skeletal muscle and metabolic health in older adults, even in the absence of exercise.

While resistance exercise training is a potent anabolic stimulus across the lifespan (Henwood and Taaffe 2005; Kosek et al. 2006; Stewart et al. 2014), the aim of the present study was to examine the efficacy of a targeted multi-ingredient nutrition

supplement on body composition, physical function, and markers of metabolic health in older adults in the absence of a formal exercise training intervention. Specifically, this study investigated 6 months of daily consumption of a formulation of LC n-3 PUFA, vitamin D, resveratrol, and whey protein isolate. Importantly, because there are sensory and digestive issues that contribute to inadequate energy and protein intake in older adults (Furman 2006), the provision of the supplement was in the form of a convenient, juice-based beverage that aimed to enhance compliance to supplementation.

Materials and methods

Experimental design

A double-blind, placebo-controlled, randomized trial (ClinicalTrials.gov Identifier: NCT02001831) investigated the impact of a 6-month intervention with a bespoke multi-ingredient nutrition supplement (Smartfish® AS, Norway) on body composition and physical and cognitive function in pre-sarcopenic older ($\geq$70 yrs) Irish adults. The outcomes related to cognitive function have been previously reported (Moran et al. 2018). Participants were recruited via a combination of methods including an advertisement placed in a national newspaper, *The Irish Times*; invitations issued on the University College Dublin alumni website; and recruitment flyers distributed to local organizations for older adults and retirement groups. Ethical approval was granted by the University College Dublin Human Research Ethics Committee (permit number: LS-13-28-Egan), and the study was conducted in accordance with the Declaration of Helsinki. All participants provided their written informed consent prior to commencing participation in the study.

The supplement (SUPP) consisted of a 200-mL carton of juice-based drink consumed each day that contained LC n-3 PUFA, whey protein isolate, vitamin D3, and resveratrol. The placebo (PLA) consisted of the fruit juice alone. Thirty-seven participants (age, 74.9 ± 3.6 yrs) completed the 6-month intervention comprising 16 in PLA (M/F, 8/8) and 21 in SUPP (M/F, 10/11) (Table 1). Body composition and physical function were assessed at baseline (PRE) and after 3 months (MID) and 6 months (POST) of daily consumption of PLA or SUPP.

Participants

Participants were required to not currently be, or recently have been (previous 8 weeks), consuming fish oil, vitamin D3, or whey protein supplements. Eligibility for participation was determined as being aged 70 yrs or older; having a body mass index between 20 and 30 kg/m^2; being sedentary as defined by $\leq$125 min per weekof activity on the CHAMPS-18 questionnaire; being defined as medically stable (Greig et al. 1994); being community-dwelling, independent, mobile, and capable of completing the trial; scoring >23 on the Mini Mental State Examination (MMSE) (Folstein et al. 1975); and having low skeletal muscle mass (Janssen et al. 2004). The latter prescreening was performed using bioelectrical impedance analysis measurement of skeletal muscle mass index

Table 1. Participant characteristics at baseline.

	PLA $n = 16$	SUPP $n = 21$	All $n = 37$	PLA vs SUPP p value
Anthropometry				
M/F	8/8	11/10	19/18	
Age (y)	74.8 ± 3.9	75.0 ± 3.4	74.9 ± 3.6	.838
Height (m)	1.67 ± 0.10	1.67 ± 0.11	1.67 ± 0.10	.908
Body mass (kg)	71.0 ± 12.9	71.4 ± 16.3	71.2 ± 14.7	.942
BMI (kg/m^2)	25.2 ± 3.3	25.3 ± 3.5	25.3 ± 3.4	.958
Body fat (%)	33.0 ± 8.4	33.6 ± 6.7	33.4 ± 7.4	.808
Fat mass (kg)	23.1 ± 7.8	23.4 ± 6.9	23.3 ± 7.2	.902
LBM (kg)	45.41 ± 9.51	45.26 ± 11.49	45.32 ± 10.54	.965
ALM (kg)	19.57 ± 4.55	19.38 ± 5.62	19.47 ± 5.12	.912
SMMI (kg/m^2)	6.83 ± 1.02	6.79 ± 1.39	6.81 ± 1.24	.938
Physical function				
Hand-grip strength (kg)	32.0 ± 7.3	30.5 ± 9.7	31.1 ± 8.7	.619
Gait speed (m/s)	1.05 ± 0.14	1.09 ± 0.15	1.07 ± 0.15	.377
Sit-to-stand (s)	10.5 ± 1.3	12.5 ± 3.6	11.6 ± 3.0	.035
Balance (s)	24.8 ± 8.2	20.3 ± 12.5	22.2 ± 11.0	.240
SPPB score	10.7 ± 1.6	9.4 ± 2.4	10.0 ± 2.2	.076
Blood markers				
Total cholesterol (mM)	4.29 ± 1.11	4.85 ± 1.04	4.58 ± 1.09	.135
HDL-C (mM)	1.51 ± 0.37	1.66 ± 0.55	1.59 ± 0.47	.357
LDL-C (mM)	2.94 ± 1.04	3.04 ± 0.95	2.99 ± 0.98	.754
TG (mM)	1.00 ± 0.45	0.94 ± 0.31	0.97 ± 0.38	.677
hsCRP (mg/L)	1.35 ± 0.82	1.03 ± 1.43	1.19 ± 1.16	.439
Glucose (mM)	5.22 ± 0.74	5.15 ± 0.73	5.18 ± 0.72	.782
Insulin (mU/L)	7.13 ± 5.47	6.24 ± 3.73	6.64 ± 4.55	.587
HOMA-IR	1.67 ± 1.37	1.48 ± 1.15	1.58 ± 1.12	.632
Dietary intake				
Energy (kcal)	1720 ± 397	1680 ± 489	1699 ± 439	.825
Carbohydrate (g)	191 ± 48	193 ± 86	192 ± 69	.943
Protein (g)	72 ± 14	80 ± 22	76 ± 19	.276
Protein (g/kg)	1.01 ± 0.26	1.13 ± 0.34	1.07 ± 0.30	.356
Fat (g)	70 ± 24	62 ± 14	66 ± 19	.313

Data are reported as mean ± standard deviation. ALM, appendicular lean mass; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment–insulin resistance; hsCRP, high-sensitivity C-reactive protein; LBM, lean body mass; LDL-C, low-density lipoprotein cholesterol; M/F, male/female; SMMI, skeletal muscle mass index; SPPB; Short Physical Performance Battery; TG, triglycerides.

(Janssen et al. 2000), and low skeletal muscle mass was defined by cutoffs of $\leq$6.75 kg/m^2 in females and $\leq$10.75 kg/m^2 in males (Janssen et al. 2004).

Of the 106 individuals who were prescreened for the study, 55 did not meet the inclusion criteria and 51 were therefore randomly assigned to PLA ($n = 25$) or SUPP ($n = 26$) (Figure 1), 37 of whom completed the 6-month intervention (PLA, $n = 16$; SUPP, $n = 21$) (Table 1). The principal investigator, blind to the assessments, conducted the random allocation procedure, which included stratified randomization by sex. Sealed envelopes were drawn from an opaque container, which contained an equal distribution of PLA and SUPP. Once an envelope had been drawn it was not returned prior to the subsequent randomization.

Intervention

SUPP provided liquid nutrition support as a 200-mL emulsion (≈200 kcal) comprising 3000 mg of LC n-3 PUFA (as 1500 mg docosahexaenoic acid [DHA] and 1500 mg

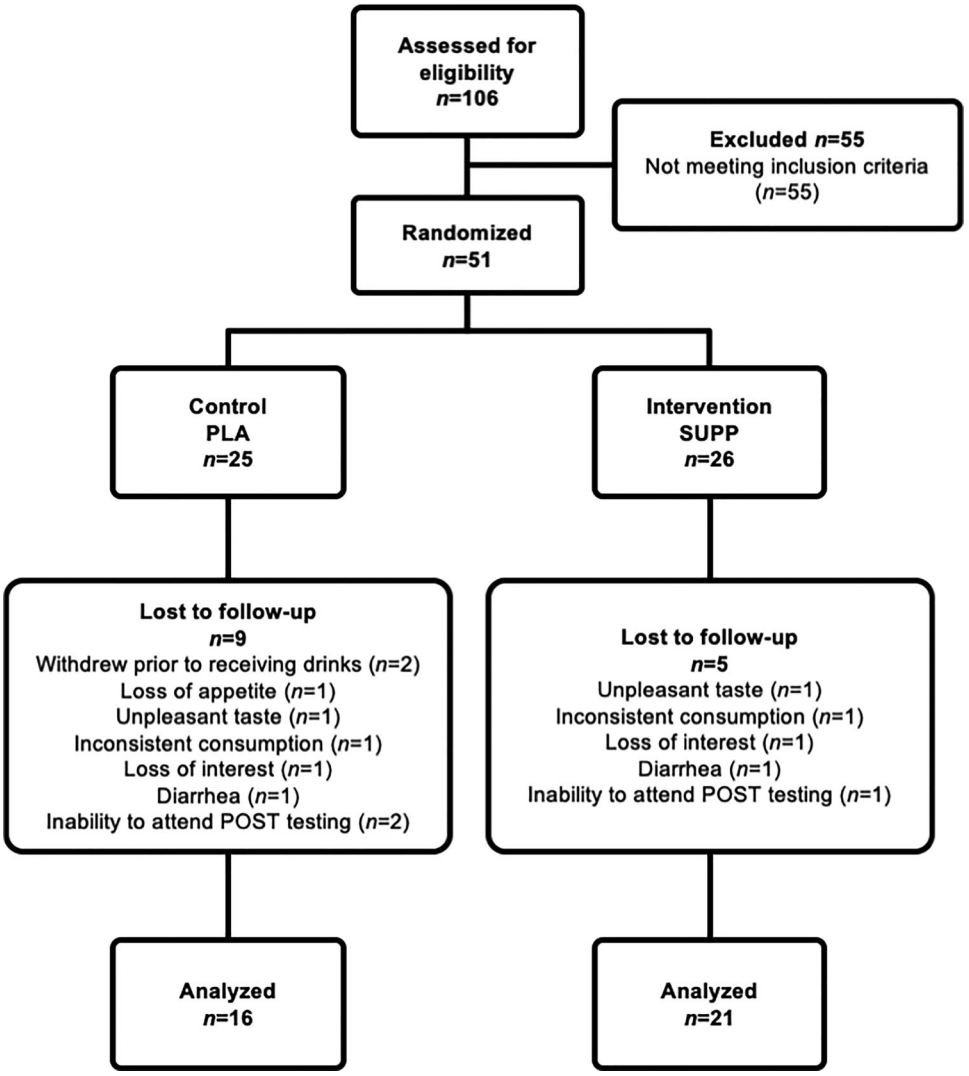

Figure 1. Flow chart for study participation.

eicosapentaenoic acid [EPA], both Pure Arctic 360, Denomega, Oslo, Norway; protected against oxidation through nano-sized, stabilized emulsion droplets), 8 g of whey protein isolate (Lacprodan DI-7017; Arla Foods, Aarhus, Denmark), 400 IU of vitamin D3 stabilized with tocopherol (DSM Nutritional Products, Basel, Switzerland), and 150 mg of resveratrol (resVida; DSM Nutritional Products, Basel, Switzerland) and 28 g carbohydrate from pomegranate and apple juice.

PLA provided 200 mL of juice only (≈110 kcal). Smartfish AS, a Norwegian biotech company, provided both the PLA and SUPP as ready-to-drink, palatable, pomegranate and apple juice–based formulations, presented in identically sealed TetraPak cartons. The formulations were indistinguishable in appearance and taste, and participants were required to consume their allotted formulation once daily for a period of 6 months.

Research staff and participants were blind to group allocation until completion of the data collection. Participants received 3 months worth of drinks immediately following their baseline (PRE) assessment, and these were replenished following their 3-month (MID) assessment.

Assessment procedures

Prior to PRE assessment, each participant completed a 4-day semi-weighed food diary (Hone et al. 2020), which were analyzed using an online nutrition analysis software package (Nutritics Dietary Analysis Software; Nutritics, Dublin, Ireland) (Table 1). Participants in both PLA and SUPP groups were advised to maintain their current dietary habits for the duration of the study, but no further assessment of dietary intake was performed.

Assessments on all three testing occasions (PRE, MID, POST) lasted approximately 45 min and were conducted using standardized protocols by the same researchers at each visit. All assessments took in the same laboratory between 0630 h and 0930 h in a fasted state. Consumption of coffee and tea was not permitted before or during testing.

Participants were advised to consume 500 mL of water 2 h prior to arriving at the lab and engage with minimal ambulation. Upon arrival, body mass was measured to the nearest 0.1 kg using a calibrated digital scales (SECA, Hamburg, Germany), and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain, Pembrokeshire, UK). Body composition was then assessed by dual-energy X-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare, Chicago, IL, USA)

Next, with participants remaining in a supine position, a blood sample was taken from a superficial forearm vein by venipuncture. Approximately 4 mL of blood collected in a pre-chilled silicone-coated and lithium heparin–coated tubes (Plus Blood Collection Tubes; Becton Dickinson, Franklin Lakes, NJ, USA) for the separation of serum and plasma, respectively, by centrifugation at 3000 g for 15 min at 4 °C. Aliquots were frozen and stored at −80 °C until batch analysis. Serum samples were analyzed to determine glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, and high sensitivity C-reactive protein (hsCRP) using the RX Daytona^{TM} chemical autoanalyzer and appropriate reagents (Randox Laboratories, Crumlin, UK). Serum insulin was determined by an enzyme-linked immunosorbent assay (10-1113-01; Mercodia, Uppsala, Sweden). Homeostatic model assessment–insulin resistance (HOMA-IR) was calculated based on the fasting glucose and insulin concentrations (Matthews et al. 1985). To assess compliance to the intervention, total lipid was extracted from plasma and transmethylated with borontrifluoride in methanol; fatty acid composition was determined by gas liquid chromatography with a Shimadzu GC2010 (Shimadzu, Japan) as previously described (Phillips et al. 2009).

Lower body physical function was assessed by the Short Physical Performance Battery (SPPB) (Guralnik et al. 1994), which evaluated lower body physical function. The SPPB consists of three components: habitual gait speed (2.4 m), standing balance (non-tandem, semi-tandem, and tandem), and five-repetition sit-to-stand chair rise test. Hand-grip strength was measured in the dominant hand to the nearest 0.5 kg using a hydraulic hand dynamometer (JAMAR, Duluth, MN, USA), with participants

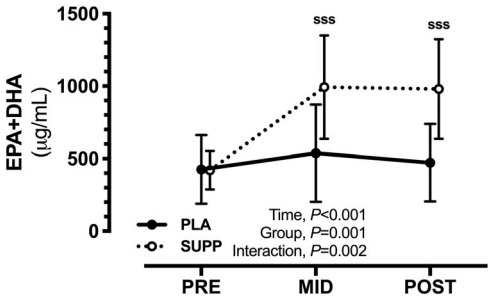

Figure 2. Changes in plasma concentrations of total (EPA + DHA) LC n-3 PUFA in response to the intervention. $^{sss}p < .001$ for SUPP vs PRE.
EPA indicates eicosapentaenoic acid; DHA, docosahexaenoic acid; LC n-3 PUFA, long chain n-3 polyunsaturated fatty acids; SUPP, supplement; PRE, baseline.

seated in an upright position and the elbow flexed to a 90-degree angle. Three attempts were performed, with the best score of the three being used for analysis.

Statistical analysis

The primary outcome measure was change in LBM from PRE to POST. Secondary outcomes included changes in body composition, physical function, and markers of metabolic health in fasted blood samples. To estimate sample size, using a Type I error rate (α) of 0.05 and a power (1-β) of 0.8, our two allocation groups and three testing occasions required a sample size of $n = 28$ per group to detect a medium effect ($f = 0.25$) (G*Power v3.1).

All statistical analyses and graphical representation of data were performed using Prism v8.4 (GraphPad Software Inc, San Diego, CA, USA). The threshold for statistical significance was set at $p \leq .05$ for all tests. Normality of data was assessed with the Shapiro-Wilk normality test, for which all data passed. Data are presented as mean ± SD, or mean difference (lower, higher 95% confidence limits of mean) where indicated. Independent samples *t* tests were used to compare males and females for differences at PRE. A two-way (group*time) mixed analysis of variance (ANOVA) was used to assess differences between PLA and SUPP for variables with serial measurements. When a main effect of time or a group*time interaction effect was indicated, post hoc testing was performed with Tukey's correction, and multiplicity-adjusted *p* values are reported for the respective comparisons between (PLA, SUPP) and within (PRE, MID, POST) groups. Sphericity was not assumed, and the Greenhouse-Geiser correction was applied to all ANOVA analyses. When a significant difference was detected, standardized differences in the mean were used to assess magnitudes of effects at respective time points. These effect sizes were calculated using Cohen's *d*, and interpreted as *trivial* for <0.2, *small* for ≥0.2 to <0.5, *moderate* for ≥0.5 to <0.8, and *large* for ≥0.8.

Results

Compliance with supplementation reported by completion of a daily tick-box diary was 98% ± 1% and 89% ± 4% from PRE to MID and MID to POST, respectively, for PLA and 96% ± 2% and 91% ± 7% from PRE to MID and MID to POST, respectively, for

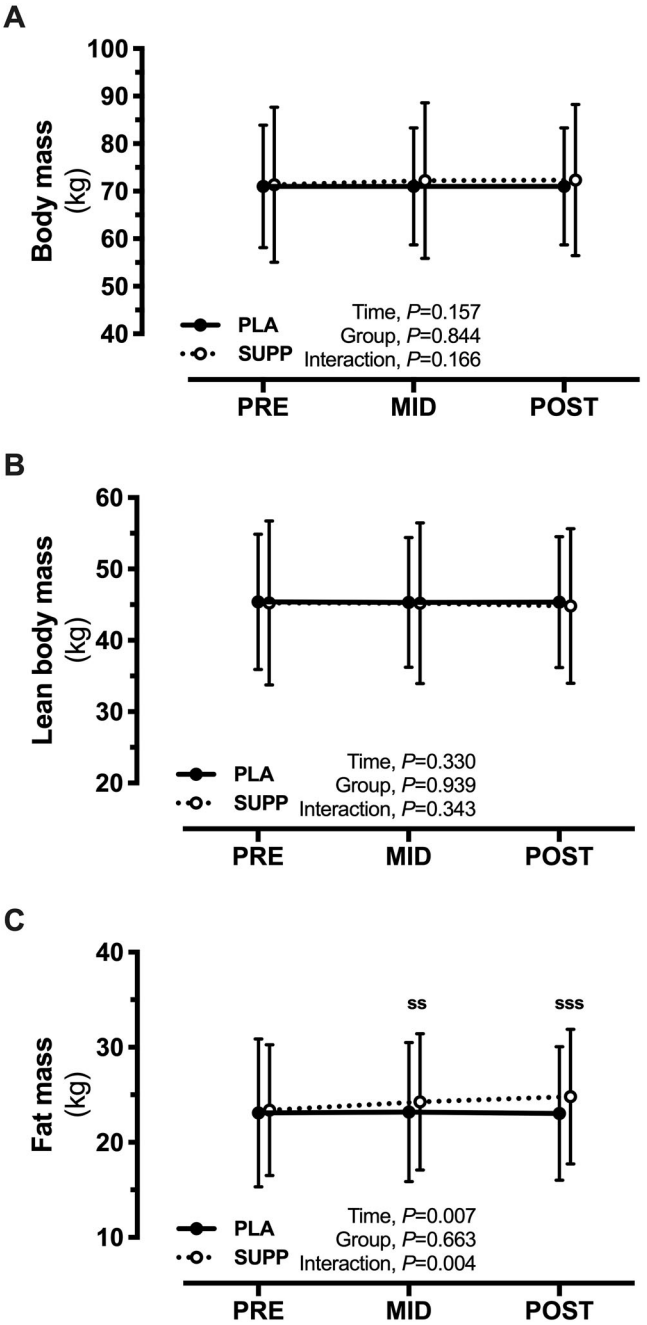

Figure 3. Changes in body composition assessed by (A) body mass, (B) lean body mass, and (C) fat mass in response to the intervention. $^{ss}p < .01$ and $^{sss}p < .001$ for SUPP vs PRE.
SUPP indicates supplement; PRE, baseline.

SUPP. Plasma EPA + DHA concentrations were unchanged in PLA but increased in SUPP (interaction effect, $p = 0.001$) by 574 (329, 819) ug/mL($p < .001$) at MID and remained 560 (315, 805) ug/mLabove PRE ($p < .001$) at POST (Figure 2).

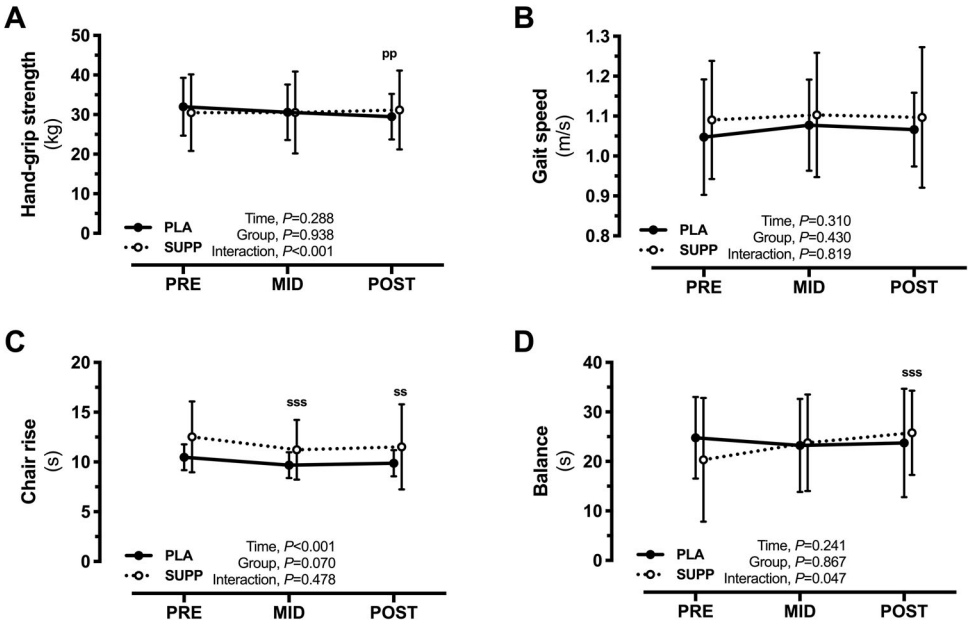

Figure 4. Changes in physical function assessed by (A) hand-grip strength, (B) gait speed, (C) chair rise test, and (D) balance. $^{ss}p < .01$ and $^{sss}p < .001$ for SUPP vs PRE. $^{pp}p < .01$ PLA vs PRE.
SUPP indicates supplement; PRE, baseline; PLA, placebo.

LBM was unchanged in either group by the intervention (Figure 3B). Fat mass was unchanged in PLA but increased in SUPP (interaction effect, $p = .004$) by 0.86 (0.20, 1.52) kg ($p = .008$; $d = 0.12$) at MID and by 1.41 (0.75, 2.07) kg above PRE ($p < .001$; $d = 0.20$) at POST (Figure 3C), which represented 3.7% and 6.4% increases in fat mass, respectively.

An interaction effect was observed for hand-grip strength ($p < .001$; Figure 4A) wherein hand-grip strength was maintained throughout the 6-month period in SUPP but declined in PLA by 2.50 (0.81, 4.19) kg, or 6.8%, at POST compared to PRE ($p = .002$; $d = 0.38$).

SPPB score was unchanged in PLA but increased in SUPP (interaction effect, $p = .032$) by 0.97 (0.26, 1.69) (+14.7%; $p = .005$; $d = 0.39$) by MID and by 1.13 (0.41, 1.84) above PRE (+16.5%; $p = .001$; $d = 0.47$) at POST. Of the SPPB components, gait speed was unchanged by the intervention (Figure 4B), but improvements in chair rise time (9.8%; $p = .003$, $d = 0.25$) and balance (21.0%; $p = .007$, $d = 0.51$) were observed in SUPP (Figure 4C and D).

There was no change in response to the intervention in either PLA or SUPP for glucose, insulin, HOMA-IR, total cholesterol, HDL-C, LDL-C, triglycerides, or hsCRP (Table 2).

Discussion

The main findings of the present study were that 6 months of daily consumption of a multi-ingredient nutrition supplement by older adults with low muscle mass had no effect on LBM but resulted in an increase in fat mass. Moreover, performance in the

Table 2. Changes in serum concentrations of markers of metabolic health after 3 (MID) and 6 (POST) months of intervention.

	PLA			SUPP					
	PRE	MID	POST	PRE	MID	POST	Time *p* value	Group *p* value	T*G *p* value
Total cholesterol (mM)	4.29 ± 1.11	4.21 ± 1.10	4.12 ± 1.13	4.85 ± 1.04	4.64 ± 1.37	4.74 ± 1.14	.675	.108	.877
HDL-C (mM)	1.51 ± 0.37	1.42 ± 0.35	1.39 ± 0.41	1.66 ± 0.55	1.56 ± 0.56	1.52 ± 0.51	.037	.346	.986
LDL-C (mM)	2.94 ± 1.04	2.92 ± 0.87	2.95 ± 1.17	3.04 ± 0.95	2.88 ± 0.95	2.66 ± 0.87	.618	.808	.366
TG (mM)	1.00 ± 0.45	0.99 ± 0.35	0.93 ± 0.54	0.94 ± 0.31	0.91 ± 0.46	0.86 ± 0.39	.447	.576	.973
hsCRP (mg/L)	1.35 ± 0.82	1.40 ± 1.09	1.27 ± 1.05	1.03 ± 1.43	1.65 ± 2.56	1.13 ± 1.16	.400	.855	.584
Glucose (mM)	5.22 ± 0.74	5.23 ± 0.51	5.39 ± 1.17	5.15 ± 0.73	5.16 ± 0.74	4.95 ± 0.90	.990	.312	.479
Insulin (mU/L)	7.13 ± 5.47	7.26 ± 6.02	7.30 ± 6.20	6.24 ± 3.73	5.79 ± 2.92	6.46 ± 3.21	.831	.480	.830
HOMA-IR	1.69 ± 1.37	1.66 ± 1.36	1.61 ± 1.17	1.48 ± 1.15	1.33 ± 0.73	1.40 ± 0.78	.810	.472	.916

Data are reported as mean ± standard deviation. HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment–insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; PLA, placebo; PRE, baseline; SUPP, supplement.

SPPB improved and a decline in hand-grip strength was prevented when compared to the placebo condition, evidently independent of a change in LBM.

Nutrition supplements designed to prevent the age-associated decline in LBM in older adults have had varying degrees of success. Supplementation with LC n-3 PUFA (≈3 g/day) (Logan and Spriet 2015; Smith et al. 2015) or essential amino acids (15–16 g/day) (Solerte et al. 2008; Dillon et al. 2009) has increased LBM following 3–16 months of supplementation, whereas 30 g/dayof whey protein for 24 months had no effect (Zhu et al. 2015). Here, we demonstrate that multi-ingredient nutrition supplementation commenced in older adults with low muscle mass had no effect on LBM, but it resulted in an increase in fat mass following 6 months of daily supplementation. Daily protein intake at PRE was consistent with our previous work in older Irish adults (Hone et al. 2020), which observed that habitual protein intake in this population fails to meet the daily protein intake guideline of ≈1.2 g/kg/dayfor this age range (Deutz et al. 2014). Changes in LBM occur in response to temporal fluctuations in MPS and muscle protein breakdown in response to nutrient intake, exercise, and inactivity, which determine whether a net gain or loss in muscle mass is observed (Rennie et al. 2004; Breen and Phillips 2011). Therefore, unless a nutrition supplement contributes to positive nitrogen balance, either by providing an anabolic stimulus, improving anabolic sensitivity, and/or enabling an older adult to meet the daily protein intake guidelines, an increase in LBM is unlikely to be observed (Breen and Phillips 2011). That said, the present study assessed body composition via DXA, which is less sensitive for detecting small changes in LBM over time compared to magnetic resonance imaging (MRI) (Delmonico et al. 2008; Tavoian et al. 2019). This point is notable given that a previous study demonstrating ≈3.4 g/dayof LC n-3 PUFA to result in a 3.6% increase in muscle mass utilized MRI for the determination of thigh muscle volume (Smith et al. 2015). However, DXA has also been used previously when observing a 4% increase in LBM with 3 g/dayof LC n-3 PUFA (Logan and Spriet 2015). Both of those studies of LC n-3 PUFA supplementation also observed improvements in muscle function coincident with the increase in LBM. In females aged 60 to 76 yrs, 3 months of supplementation with 3 g/dayof LC n-3 PUFA (2 g EPA, 1 g DHA) resulted in a 7% improvement in timed-up-and-go performance. Similarly, in 60- to 85-year-old men and women, compared to a control group 6 months

of supplementation with ≈3.4 g/dayof LC n-3 PUFA (1.86 g EPA, 1.5 g DHA) resulted in a 2.3 kg increase in hand-grip strength and a 4% increase in a composite score of one repetition maximum strength (Smith et al. 2015). A limitation of the present study is that the sample size of participants completing the intervention (PLA, $n = 16$; SUPP, $n = 21$) is underpowered relative to the a priori sample size required ($n = 28$ per group). Low statistical power due to the smaller than required sample size likely resulted in this analysis being underpowered for null hypothesis statistical testing and thereby increased the likelihood of a Type II error (i.e., false negative) for the primary outcome of change in LBM. However, the n size in the present study compares favorably with the previous studies demonstrating an increased in LBM with LC n-3 PUFA supplementation: i.e., placebo, $n = 12$ vs. fish oil, $n = 12$ (Logan and Spriet 2015), and control, $n = 15$ vs. n-3 PUFA, $n = 29$ (Smith et al. 2015). Additionally, independent of the ANOVA outcome, examination of the PRE–POST change in LBM reveals −0.05 (−0.61, 0.51) kg in PLA and −0.46 (−1.09, 0.18) kg in SUPP, but this directional decrease in LBM in SUPP compared to PLA was not significant ($p = .343$). Therefore, we contend that despite being somewhat underpowered, the probability is low that increasing the n size to the required sample size would have revealed an increase in LBM with SUPP, and this study can be summarized as a null finding for effects on LBM.

Despite no measurable change in LBM in the present study, SUPP improved balance and chair rise time, and therefore overall score in the SPPB, while also maintaining hand-grip strength whereas PLA observed declines in hand-grip strength. Declines in hand-grip strength are predictive of declines in mobility, functional status, and mortality within community-dwelling populations (Rijk et al. 2016), with scores <27 kg for males and <16 kg for females being the sarcopenia cutoffs (Cruz-Jentoft et al. 2019). The 6.8% reduction in hand-grip strength within PLA in 6 months is particularly noteworthy, as it is greater than the reported ≈1.5% to 5.0% annual reduction in muscle strength observed within persons >50 yrs (Keller and Engelhardt 2019). On the SPPB, it is worth noting that performance in the chair rise test was lower at PRE in SUPP, and similarly the SPPB score was ≈1.3 points lower in SUPP compared to PLA. Because a 1-point difference in SPPB is clinically meaningful and the SPPB is subject to ceiling effects in high-functioning older adults (Beaudart et al., 2019), care must be taken with the interpretation of change within SUPP relative to PLA.

Whereas the association between LBM and physical function is inconsistent and discordance has been observed (Janssen et al. 2004; Woo et al. 2007; Bouchard et al. 2011), a negative association between body fatness and physical function within older adults has been consistently observed (Baumgartner et al. 1998; Visser et al. 1998; Lebon et al. 2016). In the absence of a change in physical activity or exercise habits, the addition of the multi-ingredient nutrition supplement may have resulted in increases in fat mass due to the provision of ≈200 additional calories per day, which was ≈90 kcal more per day than PLA. A limitation of the present study, however, is that we did not measure physical activity or dietary intake throughout the intervention period, which would be required in order to better inform the mechanism of this gain in fat mass. Given the improvements in physical function observed, and that the increases in fat mass in SUPP were *trivial to small* effects, the effects on fat mass would not provide

cause for concern in the timeframe under observation. Moreover, blood markers of metabolic health were not negatively impacted. However, given that this intervention was only 6 months in duration, potentially negative consequences of longer-term supplementation, if resulting in continuous weight gain, cannot be ruled out.

LC n-3 PUFA supplementation is effective at lowering concentrations of C-reactive protein (CRP) within persons with dyslipidemia and high baseline concentrations of CRP (Guo et al. 2019), and improving glycated hemoglobin (HbA1c) and blood lipid profile within persons with type 2 diabetes (Chen et al. 2015). Despite increased concentrations of EPA + DHA at 3 and 6 months in SUPP, the present study observed no changes in hsCRP, insulin resistance, or blood lipid concentrations. The fact this study enrolled non-obese, medically stable older adults for whom elevated CRP, type 2 diabetes, and/or dyslipidemia were not preexisting concerns likely contributed to the lack of effect observed.

While a multi-ingredient approach has the potential to influence more than one important pathway or mechanism through which older adults experience functional decline (Bell et al. 2017; Veronese et al. 2019), beyond LC n-3 PUFA (Logan and Spriet 2015; Smith et al. 2015), the individual doses of vitamin D, resveratrol, and whey protein were likely inadequate in the present formulation. For example, a meta-analysis demonstrated that interventional studies containing vitamin D doses twice that (800–1000 IU) of the present supplement were most effective at benefiting balance and lower body strength (Muir and Montero-Odasso 2011). Whey protein supplemented at more than twice to three times that (>20–30 g) of the present supplement would provide sufficient essential amino acids and, specifically, leucine to elicit the desired MPS response (Breen and Phillips 2011) and would be particularly efficacious within older adults who struggle to meet protein guidelines (≈1.2 g/kg/day) (Deutz et al. 2014). Last, resveratrol, while promising (Pollack et al. 2017), has generally produced equivocal effects in older adults due to variations in dosing, duration, and clinical outcomes (Novelle et al. 2015). Due to the intervention being multi-ingredient by design, a de facto limitation of this study is that it is unknown whether the findings resulted from the synergistic nature of the supplement or through LC n-3 PUFA, resveratrol, vitamin D, or whey protein in isolation.

In conclusion, the present study investigated the effect of 6-months of daily supplementation with a multi-ingredient nutrition supplement containing LC n-3 PUFA, vitamin D, resveratrol, and whey protein in older adults. Our findings demonstrate that this intervention had no measurable effect on LBM but increased fat mass. Moreover, even in the absence of an exercise intervention, supplementation demonstrated efficacy for physical function by improving performance in the SPPB and preventing the decline in hand-grip strength observed in the 6-month period in the placebo condition.

Disclosure statement

The authors declare no conflict of interest.

Author contributions

Conceptualization, GDV and BE; methodology, GDV and BE; formal analysis, ASP, MH, AMM, and BE; investigation, ASP, FTM, MH, AMM, and GL; writing—original draft preparation, ASP

and BE; writing—review and editing, FTM and BE; supervision, GDV and BE; project administration, GDV and BE; funding acquisition, GDV and BE. All authors have read and agreed to the published version of the manuscript.

Funding

The research was funded by Norwegian biotech company Smartfish® AS, who also provided the ready-to-drink juice formulations of the nutrition supplement and placebo. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

About the authors

Alessandro Scotto di Palumbo, completed his PhD in the study of acute exercise, exercise training and metabolic health. He is currently employed at Sapienza University of Rome, Italy.

Fionn T. McSwiney, completed his PhD in the study of a low carbohydrate ketogenic diet and its impact of endurance exercise performance, body composition and circulating markers of health in athletes. He is currently employed by Eli Lilly & Company in Cork, Ireland.

Michelle Hone, completed her PhD in the study of leucine supplementation and dietary protein distribution in recovery and adaptation to exercise in trained men and older adults. She is founder and currently CEO of the The Fit Clinic in Dublin, Ireland.

Aoibheann M. McMorrow, completed her PhD in the study of an anti-inflammatory dietary intervention on the metabolic phenotype of overweight and obese adolescents. She is currently employed as a registered dietitian at St. James' Hospital in Dublin, Ireland.

Gina Lynch, completed her PhD in the study of inflammation and skeletal muscle dysfunction with potential mitigation by n-3 polyunsaturated fatty acids. She is currently employed by AbbVie in Sligo, Ireland.

Giuseppe De Vito, is a Full Professor of Human Physiology at University of Padova, Italy. His research investigates muscle function and neuromuscular control in both older and young subjects, as well as the study of sarcopenia and how it relates to both aging and chronic diseases such as in type 1 and 2 diabetes.

Brendan Egan, is an Associate Professor in Sport and Exercise Physiology at Dublin City University, Ireland, and Visiting Research Scientist at the Institute for Human and Machine Cognition, Pensacola FL USA. His research investigates skeletal muscle function and adaptation, with special interest in the synergy between nutrition and exercise interventions to optimise performance across the life course.

ORCID

Fionn T. McSwiney http://orcid.org/0000-0002-5007-162X
Brendan Egan http://orcid.org/0000-0001-8327-9016

References

Alway SE, McCrory JL, Kearcher K, Vickers A, Frear B, Gilleland DL, Bonner DE, Thomas JM, Donley DA, Lively MW, et al. 2017. Resveratrol enhances exercise-induced cellular and functional adaptations of skeletal muscle in older men and women. J Gerontol A Biol Sci Med Sci. 72(12):1595–1606. doi:10.1093/gerona/glx089.

Bauer JM, Verlaan S, Bautmans I, Brandt K, Donini LM, Maggio M, McMurdo MET, Mets T, Seal C, Wijers SL, et al. 2015. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: a randomized, double-blind, placebo-controlled trial. J Am Med Dir Assoc. 16(9):740–747. doi:10.1016/j.jamda.2015.05.021.

Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD. 1998. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 147(8):755–763. doi:10.1093/oxfordjournals.aje.a009520.

Beaudart C, Rolland Y, Cruz-Jentoft AJ, Bauer JM, Sieber C, Cooper C, Al-Daghri N, Araujo de Carvalho I, Bautmans I, Bernabei R, et al. 2019. Assessment of Muscle Function and Physical Performance in Daily Clinical Practice : a position paper endorsed by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO). Calcif Tissue Int. 105(1):1–14. doi:10.1007/s00223-019-00545-w.

Bell KE, Snijders T, Zulyniak M, Kumbhare D, Parise G, Chabowski A, Phillips SM. 2017. A whey protein-based multi-ingredient nutritional supplement stimulates gains in lean body mass and strength in healthy older men: a randomized controlled trial. PLoS One. 12(7): e0181387. doi:10.1371/journal.pone.0181387.

Boirie Y, Morio B, Caumon E, Cano NJ. 2014. Nutrition and protein energy homeostasis in elderly. Mech Ageing Dev. 136-137:76–84. doi:10.1016/j.mad.2014.01.008.

Bouchard DR, Choquette S, Dionne IJ, Brochu M. 2011. Is fat mass distribution related to impaired mobility in older men and women? Nutrition as a determinant of successful aging: The Quebec longitudinal study. Exp Aging Res. 37(3):346–357. doi:10.1093/gerona/62.12.1382.

Breen L, Phillips SM. 2011. Skeletal muscle protein metabolism in the elderly: Interventions to counteract the "'anabolic resistance' of ageing" . Nutr Metab (Lond)). 8:68. doi:10.1186/1743-7075-8-68.

Chen C, Yu X, Shao S. 2015. Effects of omega-3 fatty acid supplementation on glucose control and lipid levels in type 2 diabetes: a meta-analysis. PLoS One. 10(10):e0139565. doi:10.1371/journal.pone.0139565.

Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, Cooper C, Landi F, Rolland Y, Sayer AA, et al. 2019. Sarcopenia: Revised European consensus on definition and diagnosis. Age Ageing. 48(1):16–31. doi:10.1093/ageing/afy169.

Cruz-Jentoft AJ, Dawson Hughes B, Scott D, Sanders KM, Rizzoli R. 2020. Nutritional strategies for maintaining muscle mass and strength from middle age to later life: a narrative review. Maturitas. 132:57–64. doi:10.1016/j.maturitas.2019.11.007.

Delmonico MJ, Kostek MC, Johns J, Hurley BF, Conway JM. 2008. Can dual energy X-ray absorptiometry provide a valid assessment of changes in thigh muscle mass with strength training in older adults? Eur J Clin Nutr. 62(12):1372–1378. doi:10.1038/sj.ejcn.1602880.

Deutz NEP, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, Cederholm T, Cruz-Jentoft A, Krznariç Z, Nair KS, et al. 2014. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. Clin Nutr. 33(6): 929–936. doi:10.1016/j.clnu.2014.04.007.

Dhesi JK, Jackson SHD, Bearne LM, et al. 2004. Vitamin D supplementation improves neuromuscular function in older people who fall. Age Ageing. 33(6):589–595. doi:10.1093/ageing/afh209.

Dillon EL, Sheffield-Moore M, Paddon-Jones D, Gilkison C, Sanford AP, Casperson SL, Jiang J, Chinkes DL, Urban RJ. 2009. Amino acid supplementation increases lean body mass, basal muscle protein synthesis, and insulin-like growth factor-I expression in older women. J Clin Endocrinol Metab. 94(5):1630–1637. doi:10.1210/jc.2008-1564.

Folstein M, Folstein S, Hugh P. 1975. "Mini-mental State". A practical method for grading the cognitive state of patients for the clinician. Proc Int Offshore Polar Eng Conf. 12:189–198. doi: 10.1016/0022-3956. (75)90026-6

Furman E. 2006. Undernutrition in older adults across the continuum of care: nutritional assessment, barriers, and interventions. J Gerontol Nurs. 32(1):22–27. doi:10.3928/0098-9134-20060101-11.

Greig CA, Young A, Skelton DA, Pippet E, Butler FM, Mahmud SM. 1994. Exercise studies with elderly volunteers. Age Ageing. 23(3):185–189. doi:10.1093/ageing/23.3.185.

Guo X, Fei Li K, Lei Li J, Mei Li D. 2019. Effects of EPA and DHA on blood pressure and inflammatory factors: a meta-analysis of randomized controlled trials. Crit Rev Food Sci Nutr. 59(20):3380–3393. doi:10.1080/10408398.2018.1492901.

Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, Scherr PA, Wallace RB. 1994. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol. 49(2):M85–94. doi:10.1093/geronj/49.2.m85.

Henwood TR, Taaffe DR. 2005. Improved physical performance in older adults undertaking a short-term programme of high-velocity resistance training. Gerontology. 51(2):108–115. doi:10.1159/000082195.

Hone M, Nugent AP, Walton J, McNulty BA, Egan B. 2020. Habitual protein intake, protein distribution patterns and dietary sources in Irish adults with stratification by sex and age. J Hum Nutr Diet. 33(4):465–476. doi:10.1111/jhn.12736.

Janssen I, Baumgartner RN, Ross R, Rosenberg IH, Roubenoff R. 2004. Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. Am J Epidemiol. 159(4):413–421. doi:10.1093/aje/kwh058.

Janssen I, Heymsfield SB, Baumgartner RN, Ross R. 2000. Estimation of skeletal muscle mass by bioelectrical impedance analysis. J Appl Physiol. 89(2):465–471. doi:10.1152/jappl.2000.89.2.465.

Keller K, Engelhardt M. 2019. Strength and muscle mass loss with aging process. Age and strength loss. Muscle Ligaments and Tendons J. 03(04):346–350. doi:10.11138/mltj/2013.3.4.346.

Kilsby AJ, Sayer AA, Witham MD. 2017. Selecting potential pharmacological interventions in sarcopenia. Drugs Aging. 34(4):233–240. doi:10.1007/s40266-017-0444-z.

Kosek DJ, Kim JS, Petrella JK, Cross JM, Bamman MM. 2006. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. J Appl Physiol. 101(2):531–544. doi:10.1152/japplphysiol.01474.2005.

Lebon J, Barsalani R, Payette H, Brochu M, Dionne IJ. 2016. Inflammation and fat mass as determinants of changes in physical capacity and mobility in older adults displaying a large variability in body composition: the NuAge Study. Exp Aging Res. 42(5):403–417. doi:10.1080/0361073X.2016.1224649.

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(12):e0144828. doi:10.1371/journal.pone.0144828.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 28(7):412–419. doi:10.1007/BF00280883.

McGlory C, Calder PC, Nunes EA. 2019. The influence of omega-3 fatty acids on skeletal muscle protein turnover in health, disuse, and disease. Front Nutr. 6. doi:10.3389/fnut.2019.00144.

Moran C, Scotto di Palumbo A, Bramham J, Moran A, Rooney B, De Vito G, Egan B. 2018. Effects of a six-month multi-ingredient nutrition supplement intervention of omega-3 polyunsaturated fatty acids, vitamin D, resveratrol, and whey protein on cognitive function in older adults: a randomised, double-blind, controlled trial. J Prev Alzheimer's Dis. 5:175–183. doi:10.14283/jpad.2018.11.

Muir SW, Montero-Odasso M. 2011. Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. J Am Geriatr Soc. 59(12):2291–2300. doi:10.1111/j.1532-5415.2011.03733.x.

Negro M, Perna S, Spadaccini D, Castelli L, Calanni L, Barbero M, Cescon C, Rondanelli M, D'Antona G. 2019. Effects of 12 weeks of essential amino acids (EAA)-based multi-ingredient nutritional supplementation on muscle mass, muscle strength, muscle power and fatigue in healthy elderly subjects: a randomized controlled double-blind study. J Nutr Health Aging. 23(5):414–424. doi:10.1007/s12603-019-1163-4.

Novelle MG, Wahl D, Diéguez C, Bernier M, de Cabo R. 2015. Resveratrol supplementation: Where are we now and where should we go? Ageing Res Rev. 21:1–15. doi:10.1016/j.arr.2015.01.002.

Phillips CM, Goumidi L, Bertrais S, Ferguson JF, Field MR, Kelly ED, Peloso GM, Cupples LA, Shen J, Ordovas JM, et al. 2009. Complement component 3 polymorphisms interact with polyunsaturated fatty acids to modulate risk of metabolic syndrome. Am J Clin Nutr. 90(6): 1665–1673. doi:10.3945/ajcn.2009.28101.

Pollack RM, Barzilai N, Anghel V, Kulkarni AS, Golden A, O'Broin P, Sinclair DA, Bonkowski MS, Coleville AJ, Powell D, et al. 2017. Resveratrol improves vascular function and mitochondrial number but not glucose metabolism in older adults. J Gerontol A Biol Sci Med Sci. 72(12):1703–1709. doi:10.1093/gerona/glx041.

Rennie MJ, Wackerhage H, Spangenburg EE, Booth FW. 2004. Control of the size of the human muscle mass. Annu Rev Physiol. 66:799–828. doi:10.1146/annurev.physiol.66.052102.134444.

Rijk JM, Roos PR, Deckx L, van den Akker M, Buntinx F. 2016. Prognostic value of handgrip strength in people aged 60 years and older: a systematic review and meta-analysis. Geriatr Gerontol Int. 16(1):5–20. doi:10.1111/ggi.12508.

Rondanelli M, Klersy C, Terracol G, Talluri J, Maugeri R, Guido D, Faliva MA, Solerte BS, Fioravanti M, Lukaski H, et al. 2016. Whey protein, amino acids, and Vitamin D supplementation with physical activity increases fat-free mass and strength, functionality, and quality of life and decreases inflammation in sarcopenic elderly. Am J Clin Nutr. 103(3):830–840. doi:10.3945/ajcn.115.113357.

Rossato LT, Schoenfeld BJ, de Oliveira EP. 2020. Is there sufficient evidence to supplement omega-3 fatty acids to increase muscle mass and strength in young and older adults? Clin Nutr. 39(1):23–32. doi:10.1016/j.clnu.2019.01.001.

Smith GI, Julliand S, Reeds DN, Sinacore DR, Klein S, Mittendorfer B. 2015. Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults. Am J Clin Nutr. 102(1):115–122. doi:10.3945/ajcn.114.105833.

Solerte SB, Gazzaruso C, Bonacasa R, Rondanelli M, Zamboni M, Basso C, Locatelli E, Schifino N, Giustina A, Fioravanti M. 2008. Nutritional supplements with oral amino acid mixtures increases whole-body lean mass and insulin sensitivity in elderly subjects with sarcopenia. Am J Cardiol. 101:e69–77. doi:10.1016/j.amjcard.2008.03.004.

Stewart VH, Saunders DH, Greig CA. 2014. Responsiveness of muscle size and strength to physical training in very elderly people: a systematic review. Scand J Med Sci Sports. 24(1):e1-10–e10. doi:10.1111/sms.12123.

Tavoian D, Ampomah K, Amano S, Law TD, Clark BC. 2019. Changes in DXA-derived lean mass and MRI-derived cross-sectional area of the thigh are modestly associated. Sci Rep. 9(1): 10028. doi:10.1038/s41598-019-46428-w.

Veronese N, Stubbs B, Punzi L, Soysal P, Incalzi RA, Saller A, Maggi S. 2019. Effect of nutritional supplementations on physical performance and muscle strength parameters in older people: a systematic review and meta-analysis. Ageing Res Rev. 51:48–54. doi:10.1016/j.arr.2019.02.005.

Verreijen AM, Verlaan S, Engberink MF, Swinkels S, de Vogel-van den Bosch J, Weijs PJ. 2015. A high whey protein-, leucine-, and vitamin D-enriched supplement preserves muscle mass during intentional weight loss in obese older adults: a double-blind randomized controlled trial. Am J Clin Nutr. 101(2):279–286. doi:10.3945/ajcn.114.090290.

Visser M, Harris TB, Langlois J, Hannan MT, Roubenoff R, Felson DT, Wilson PW, Kiel DP. 1998. Body fat and skeletal muscle mass in relation to physical disability in very old men and women of the Framingham Heart Study. J Gerontol A Biol Sci Med Sci. 53(3):M214–221. doi:10.1093/gerona/53a.3.m214.

Woo J, Leung J, Kwok T. 2007. BMI, body composition, and physical functioning in older adults. Obesity (Silver Spring)). 15(7):1886–1894. doi:10.1038/oby.2007.223.

Zhang H, Lin S, Gao T, Zhong F, Cai J, Sun Y, Ma A. 2018. Association between sarcopenia and metabolic syndrome in middle-aged and older non-obese adults: a systematic review and meta-analysis. Nutrients. 10(3):364. doi:10.3390/nu10030364.

Zhu K, Kerr DA, Meng X, Devine A, Solah V, Binns CW, Prince RL. 2015. Two-year whey protein supplementation did not enhance muscle mass and physical function in well-nourished healthy older postmenopausal women. J Nutr. 145(11):2520–2526. doi:10.3945/jn.115.218297.