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A mycoprotein-based high-protein vegan diet supports equivalent daily myofibrillar protein synthesis rates compared with an isonitrogenous omnivorous diet in older adults: a randomised controlled trial

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Abstract

Animal-derived dietary protein ingestion and physical activity stimulate myofibrillar protein synthesis rates in older adults. We determined whether a non-animal-derived diet can support daily myofibrillar protein synthesis rates to the same extent as an omnivorous diet. Nineteen healthy older adults (aged 66 (SEM 1) years; BMI 24 (SEM 1) kg/m^2; twelve males, seven females) participated in a randomised, parallel-group, controlled trial during which they consumed a 3-d isoenergetic high-protein (1·8 g/kg body mass per d) diet, where the protein was provided from predominantly (71 %) animal (OMNI; *n* 9; six males, three females) or exclusively vegan (VEG; *n* 10; six males, four females; mycoprotein providing 57 % of daily protein intake) sources. During the dietary control period, participants conducted a daily bout of unilateral resistance-type leg extension exercise. Before the dietary control period, participants ingested 400 ml of deuterated water, with 50-ml doses consumed daily thereafter. Saliva samples were collected throughout to determine body water ^{2}H enrichments, and muscle samples were collected from rested and exercised muscle to determine daily myofibrillar protein synthesis rates. Deuterated water dosing resulted in body water ^{2}H enrichments of approximately 0·78 (SEM 0·03) %. Daily myofibrillar protein synthesis rates were 13 (SEM 8) ($P = 0·169$) and 12 (SEM 4) % ($P = 0·016$) greater in the exercised compared with rested leg (1·59 (SEM 0·12) *v.* 1·77 (SEM 0·12) and 1·76 (SEM 0·14) *v.* 1·93 (SEM 0·12) %/d) in OMNI and VEG groups, respectively. Daily myofibrillar protein synthesis rates did not differ between OMNI and VEG in either rested or exercised muscle ($P > 0·05$). Over the course of a 3-d intervention, omnivorous- or vegan-derived dietary protein sources can support equivalent rested and exercised daily myofibrillar protein synthesis rates in healthy older adults consuming a high-protein diet.

Key words: **Sarcopenia: Skeletal muscle: Muscle protein synthesis: Dietary protein: Mycoprotein**

Ageing is associated with a progressive loss of skeletal muscle mass, termed sarcopenia[1]. The association between muscle loss and increased incidence of falls, fractures and metabolic disease indicates that the burden of our ageing society on healthcare systems will increase dramatically in the coming decades[1–4]. Importantly, it also underlines the critical role that muscle mass and quality play in healthy ageing.

Muscle mass is regulated by the dynamic balance between daily muscle protein synthesis (MPS) and muscle protein breakdown rates. Ageing muscle displays a blunted responsiveness to the major acute anabolic stimuli, dietary protein ingestion[5–8] and physical activity (particularly resistance exercise)[9,10]. This 'anabolic resistance' is now generally accepted as a key physiological mechanism responsible for age-related sarcopenia[11]. Consuming dietary protein and performing physical activity in close temporal proximity can synergistically augment MPS rates in older adults[12,13]. Moreover, by increasing the per meal dose of protein[14–16], consuming high quality or fortified protein sources[17,18] or strategic timing of protein ingestion[19], the muscle protein synthesis response to each meal can be augmented, though how this translates to daily MPS rates has received less attention.

Our understanding of how dietary protein and physical activity regulate muscle protein turnover in older adults is largely derived from studies using animal-derived protein sources.

Abbreviations: FSR, fractional synthesis rate; MPS, muscle protein synthesis.

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Indeed, studies evaluating the anabolic potential of animal *v.* non-animal-derived proteins typically compare meat, milk, casein and/or whey with soya[15,20] or wheat[21] proteins only. Despite limited non-animal protein sources having been investigated, there is the widespread assumption that animal-derived proteins are more anabolic compared with plant-based proteins[22]. Mycoprotein is a sustainably produced protein-rich whole food source, cultivated by the continuous flow fermentation of the filamentous fungus *Fusarium venenatum*, that is relatively high in protein (45 % protein, 20·9 % essential amino acids, 24·6 % non-essential amino acids, 9 % branched chain amino acids, 3·9 % leucine; online Supplementary Table S1), high in fibre (25 %; two-thirds β-glucan and one-third chitin), and with a relatively low energy density[23]. A full description of the nutritional properties of mycoprotein can be found in the review by Coelho *et al.*[24]. We recently reported that an ingested bolus of mycoprotein is effectively digested and its amino acids absorbed[25], which results in a robust stimulation of MPS rates in rested and exercised muscle of young men[26]. This suggests that mycoprotein may be a suitable alternative to animal- or plant-derived proteins to incorporate within the diet of older adults to support daily MPS rates.

In the present work, we applied an oral deuterated water approach[27] to determine whether a mycoprotein-based high-protein vegan diet could support rested and exercised daily MPS in older adults to the same extent as an isonitrogenous omnivorous protein diet. We hypothesised that in older adults consuming a high-protein diet, exercise would increase daily MPS rates compared with rested muscle, and by a similar extent irrespective of whether dietary protein was primarily obtained from animal or non-animal sources.

Methods

Participants

Nineteen healthy older adults (aged 66 (SEM 1) years, BMI: 24 (SEM 1) kg/m^2, seven females and twelve males) were included in the study, and their characteristics are presented in Table 1. Participants (within the age range of 55–75 years) attended the laboratory for a medical screening, where height, body mass (bm) and blood pressure were measured, a fasting venous blood sample collected, and a general medical questionnaire was completed, all to assess their eligibility for participation and to ensure no adverse health conditions were present. Exclusion criteria included a (family) history of deep vein thrombosis/CVD, metabolic disorders (e.g. type 2 diabetes), musculoskeletal/orthopaedic disorders, a BMI of above 30 kg/m^2 or below 18 kg/m^2, hypertension (defined as >150/90 mmHg), participation in a structured resistance training programme within 6 months prior to the study, any musculoskeletal injury of the legs within 12 months prior to the study, habitual use of anticoagulants and consumption of any nutritional supplement shortly prior to the study. Blood markers were screened to exclude any participants who displayed evidence of impaired renal function, blood/clotting, autoimmune disorders or high glycated Hb. Overall, thirty-four participants were screened of which one was excluded based on the above criteria and fourteen either did not take part or did not complete the study. Participants completed the International Physical Activity Questionnaire[28] and were provided with a diet diary to record habitual nutritional intake for 3 d (two weekdays and one weekend day). Detailed instructions from a member of the research team were provided to assist participants in collecting these data. Dietary analyses for the calculation of energy and macronutrient intakes were completed using specialised nutrition software (Nutritics Professional Nutritional Analysis Software; Swords Co.). All subjects were informed of the nature and possible risks of the experimental procedures before providing written informed consent. The present study was registered as a clinical trial with clinicaltrials.gov (NCT04325178). The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Sport and Health Sciences Ethics committee of the University of Exeter (180314/B/01).

Pre-testing

Following screening and admittance, all participants underwent a single pre-testing session, which took place ≥5 d before the start of the experimental period. Participants reported to the laboratory for familiarisation with the exercise equipment and unilateral resistance-type exercise protocol to be used and to determine body composition (using Air Displacement Plethysmography; BodPod, Life Measurement Inc.). The exercise protocol consisted of five sets

Table 1. Participants' characteristics and work done during three consecutive days of unilateral resistance-type exercise (fifteen sets of thirty maximal isokinetic extension contractions)
(Mean values with their standard errors)

	OMNI (*n* 9; 3 female, 6 male)		VEG (*n* 10; 4 female, 6 male)		
	Mean	SEM	Mean	SEM	*P*
Age (years)	64	2	68	2	0·21
Body mass (kg)	70	3	69	3	0·86
Height (cm)	172	3	166	3	0·17
BMI (kg/m^2)	23·6	0·6	25·1	0·7	0·16
Fat (% body mass)	20	3	25	3	0·27
Lean mass (kg)	56	4	52	4	0·45
Total work done (J)	32 457	3450	26 657	2809	0·30

OMNI, omnivorous diet; VEG, non-animal-derived diet.

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of thirty repetitions of maximal concentric isokinetic leg extension contractions, with 90-s rest between each set, on a Biodex System 3 isokinetic dynamometer (Biodex Medical Systems) at a speed of 60° per s over a central 80° range of motion using their dominant leg. The dominant leg was selected as we deemed it likely that they would be better able to execute contractions in a leg that they were more accustomed to loading and therefore create more mechanical tension[29]. The exercise protocol was selected as we have previously shown that it stimulates MPS rates[30], allowing us to model the effect on rested tissue alongside exercised tissue. As the exercise is maximally concentric in nature, we would expect it to largely obviate any muscle damage and therefore represent a protein accretional stimulus as opposed to one directed at correcting muscle damage[31]. In addition, the lack of muscle damage permitted maximal exercise on three consecutive mornings, was practical for the population in question and allowed us to stimulate cumulative myofibrillar protein synthesis rates over the 3 d. Verbal encouragement was provided throughout the familiarisation and experimental testing to encourage maximal effort. Work done (J) was recorded for each completed set, and fatigue was calculated as the percentage decrement in work done between the first and last set.

Experimental design

Participants were randomly assigned to two parallel groups, A or B, by the lead investigator and completed a single condition. The study was an open-label design, with it not practically possible to blind the participants or the investigators. We took a theoretically optimal approach to stimulating daily MPS rates, providing a high-protein diet distributed throughout the day, and with protein consumed post-resistance exercise[32–34]. This necessitated that we clamped both protein and energy intake across groups. Participants received one of two dietary interventions which differed with respect to the primary sources of dietary protein consumed: predominantly animal-derived protein sources including milk protein supplementation (OMNI; *n* 9), or exclusively vegan-derived protein sources including mycoprotein supplementation (VEG; *n* 10). Three participants were vegetarian and were therefore allocated to the VEG group. A graphical representation of the experimental study design can be seen in Fig. 1. All participants were asked to refrain from alcohol and caffeine consumption, and from strenuous exercise (except for the exercise prescribed within the protocol) for 2 d prior and throughout the experimental protocol, though to keep all other daily habitual activities as normal.

Participants attended the laboratory each day for a 5-d (Monday–Friday) experimental period with days 2–4 (i.e. 3 d, Tuesday–Thursday, inclusive) involving the dietary control. Each day of the 3-d dietary control period participants attended the laboratory to conduct a single bout of unilateral leg extension exercise as described above. Immediately afterwards, volunteers received a protein-rich breakfast (approximately 20-g protein) and were then provided with their food for the remainder of the day. To measure daily myofibrillar protein synthesis rates, participants underwent a D_2O dosing protocol (described below), in line with our previous work[27], and muscle biopsies were collected before commencing the controlled diet (i.e. Tuesday approximately 08.00 hours; single muscle biopsy from the (to-be) rested leg) and following (i.e. Friday approximately 08.00 hours; bilateral biopsies from the rested and exercised legs). We chose a duration of 3 d to reduce the burden on participants, in terms of sampling and dietary imposition, whilst also remaining confident that this allowed sufficient time to robustly measure daily MPS rates[35] and detect differences between groups.

Muscle biopsies were obtained under local anaesthesia, using a percutaneous Bergstrom biopsy needle technique[6], from the *m. vastus lateralis* approximately 15 cm above the patella and approximately 3 cm below the fascia. Muscle tissue was quickly assessed and any blood or non-muscle tissue was dissected and discarded. The muscle samples were immediately frozen in liquid N_2 and stored at −80°C until further analysis.

Dietary intervention

BMR was estimated using the Henry equations based on age, sex and weight[36]. The International Physical Activity Questionnaire was used to calculate a physical activity level (PAL) factor[37]. Individual energy requirements were then calculated by multiplying the participant's BMR by their physical activity level factor. Thereafter, an individual 3-d meal plan was designed for each participant with all food prepared, weighed and packaged

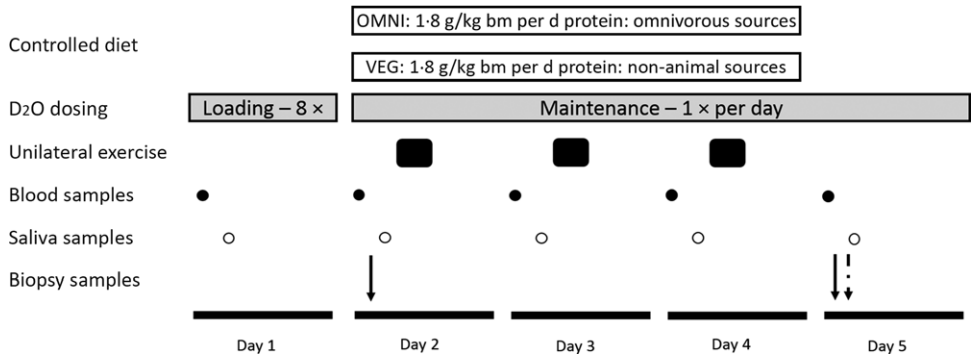

Fig. 1. Schematic representation of the experimental protocol. Nineteen healthy older adults (aged 66 (SEM 1) years) consumed a 3-d fully controlled, isoenergetic and high-protein (1·8 g/kg body mass per d) diet, where the protein was provided predominantly from animal (OMNI; *n* 9) or exclusively non-animal (VEG; *n* 10) sources. During the dietary control period (days 2–4), participants conducted a single bout of unilateral isokinetic knee extension exercise (5 × 30 contractions) each morning. On day 1, participants loaded 400 ml of deuterated water, with 50 ml of maintenance doses consumed daily thereafter. Saliva (○) and blood samples (●) were collected daily, and muscle biopsies were collected from both the rested (↓) and exercised (⇣) legs to determine daily myofibrillar protein synthesis rates.

Table 2. Nutritional content of the participants' habitual diets and of the intervention diets*
(Mean values with their standard errors)

	OMNI (*n* 9; 3 female, 6 male)		VEG (*n* 10; 4 female, 6 male)		
	Mean	SEM	Mean	SEM	*P*
Habitual diet					
Energy (MJ/d (kcal/d))	9·7 (2314)	0·7 (164)	8·2 (1949)	0·6 (145)	0·12
Protein (g/d)	81	6	82	6	0·90
Protein (g/kg bm per d)	1·17	0·08	1·27	0·10	0·42
Carbohydrate (g/d)	264	32	222	20	0·31
Fat (g/d)	94	6	72	8	0·04
Fibre (g/d)	27	2	31	2	0·22
Intervention diet					
Energy (MJ/d (kcal/d))	10 (2382)	0·6 (139)	9·6 (2296)	0·6 (136)	0·67
Protein (g/d)	127	5	125	6	0·79
Protein (g/kg bm per d)	1·8	0·00	1·8	0·00	N/A
Carbohydrate (g/d)	305	20	274	20	0·28
Fat (g/d)	65	4	67	4	0·60
Fibre (g/d)	32	2	68	3	<0·0001

OMNI, omnivorous diet; VEG, non-animal-derived diet; bm, body mass; N/A, not applicable.
*In OMNI, participants consumed an omnivorous diet with the majority of their protein coming from animal-derived sources. In VEG, participants consumed a diet derived from non-animal sources with the majority of their protein coming from Quorn products and mycoprotein. Both groups received 1·8 g/kg bm per d protein.

in-house in the Nutritional Physiology Unit's research kitchen facility. Nutritional information for the two diets is provided in Tables 2 and 3. Subjects consumed a diet containing 1·8 g of protein per kg of bm per d (with 23–26 % of energy being provided by fat and 49–54 % from carbohydrates in OMNI, and with 23–28 % and 42–52 % of energy being provided by fat and carbohydrates, respectively, in VEG (variation due to different energy requirements and the matching of protein intake). The meals were identical between the two groups, aside from meat or dairy products providing the primary protein source in lunches and dinners for the OMNI group and this being replaced by Quorn Foods™ mycoprotein containing products or supplementary mycoprotein in the VEG group (provided by Marlow Foods Ltd). The OMNI group received 39 g of supplemental milk protein daily (31 g of protein, 2 g of carbohydrate, <1 g of fat, 548 kJ (131 kcal)) to drink prior to sleep, which was replaced with 70 g of supplementary mycoprotein (31 g of protein, 7 g of carbohydrate, 9 g of fat, 971 kJ (232 kcal)) to drink in the VEG group. A small amount of mycoprote was also added to the breakfast in the VEG group to more closely equate the protein in the breakfast meal across groups. Breakfast was consumed within 1 h of completing the unilateral resistance-type exercise and provided 19 (SEM 1) and 21 (SEM 1) g protein per d in OMNI and VEG, respectively. The OMNI group consumed meals based on chicken, pork and dairy products. In the VEG group, this was substituted for Quorn deli ham, Quorn pieces, Quorn burgers, Quorn BBQ strips, Quorn sausages and Quorn nuggets from their vegan range (which does not contain any egg products). A document and diary detailing the plan were provided to the subjects to log mealtimes and provide recipe information/instructions. Compliance to the intervention was ascertained verbally on each morning of the intervention during a detailed discussion with the researcher. There were no major deviations from the diet and no major incidents of GI distress reported by participants who completed the study. Three out of the four participants who withdrew from the intervention (all in VEG) did so because they disliked the diet with one citing bloating as the primary reason. Participants' bm was measured wearing light clothing at the start and end of the 3-d control diet period (seca 703 column scale, seca GmbH & Co. KG). Each morning, the researchers discussed with the participants any questions or issues that may have arisen, before the next day of food was provided.

Deuterated water dosing protocol

The deuterated water dosing protocol was based on our previous work[27]. Day 1 (Monday) of the experimental protocol acted as a D_2O loading day where participants consumed 400 ml of 70 % D_2O separated over the day as 8 × 50 ml boluses (CK Isotopes Ltd). Upon arrival at the laboratory (07.30 hours) background saliva samples were collected before the first bolus of D_2O was ingested. The first dose of D_2O was consumed at approximately 08.00 hours with the remaining loading day doses being consumed every 1 h (doses 2 and 3) and then every 1·5 h thereafter. Participants stayed at the laboratory until four out of the eight loading day D_2O doses had been consumed, with the remaining D_2O doses being consumed at home under instruction of timings (i.e. leaving 1·5 h between each). Every day following the loading day, participants consumed a maintenance dose of D_2O (50 ml) upon waking (approximately 08.00 hours). A small proportion of participants reported mild feelings of dizziness during the latter part of the loading day, which subsided by the following morning (4/19). At least 90 min (approximately 09.30 hours) after the daily D_2O maintenance dose, a daily saliva sample was collected using a cotton mouth swab (Celluron) which the participant lightly chewed for approximately 1 min until saturated with saliva. The saturated sponge was placed into an empty syringe where the swab was squeezed to release the saliva into a collection tube and stored at −80°C until further analysis. The saliva samples were used to assess the body water ^2H enrichment. To ensure uniformity and

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Table 3. Dietary intake, meal by meal, during the intervention*
(Mean values with their standard errors)

	Energy				Protein				Cholesterol				Fat				Fibre	
	kJ (kcal)		kJ/kg (kcal/kg)		g		g/kg		g		g/kg		g		g/kg		g	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
OMNI																		
Breakfast	2092 (500)	113 (27)	30 (7·1)	1·3 (0·3)	19	1	0·3	0·0	75	4	1·1	0·0	11	1	0·2	0·0	11	1
Lunch	2063 (493)	146 (35)	29 (6·9)	1·7 (0·4)	33	2	0·5	0·0	67	6	0·9	0·1	9	1	0·1	0·0	4	0
Dinner	3837 (917)	230 (55)	54 (13)	2·1 (0·5)	40	3	0·6	0·0	91	6	1·3	0·1	41	3	0·6	0·0	11	1
Snacks	1979 (473)	234 (56)	28 (6·6)	2·1 (0·5)	35	1	0·5	0·0	73	10	1·0	0·1	3	1	0·0	0·0	6	1
Total	9966 (2382)	582 (139)	141 (33·6)	2·9 (0·7)	127	5	1·8	0·0	305	20	4·3	0·1	65	4	0·9	0·0	32	2
VEG																		
Breakfast	1874 (448)	109 (26)	27 (6·5)	1·3 (0·3)	21	1	0·3	0·0	61	4	0·9	0·1	11	1	0·2	0·0	15	1
Lunch	2469 (590)	146 (35)	36 (8·5)	1·3 (0·3)	33	2	0·5	0·0	74	5	1·1	0·0	15	1	0·2	0·0	13	1
Dinner	3523 (842)	243 (58)	51 (12·1)	2·1 (0·5)	39	3	0·6	0·0	95	7	1·4	0·1	30	2	0·4	0·0	19	1
Snacks	1741 (416)	192 (46)	25 (5·9)	2·1 (0·5)	33	1	0·5	0·0	45	8	0·6	0·1	11	1	0·2	0·0	21	1
Total	9606 (2296)	569 (136)	138 (33)	3·8 (0·9)	125	5	1·8	0·0	274	20	3·9	0·2	67	4	1·0	0·0	68	3

OMNI, omnivorous diet; VEG, non-animal-derived diet.
*In OMNI, participants consumed an omnivorous diet with the majority of their protein coming from animal-derived sources. In VEG, participants consumed a diet derived from non-animal sources with the majority of their protein coming from Quorn products and mycoprotein. Both groups received 1·8 g/kg body mass per d protein.

compliance with the D_2O protocol, participants were provided with bottles of D_2O labelled with the specific time and date to be taken and were required to return bottles each day.

Body water ^2H enrichment

Body water ^2H enrichment was measured using the saliva samples collected daily throughout the study, at the University of Texas Medical Branch. A ThermoFisher Delta V Advantage isotope ratio mass spectrometer , equipped with a Finnigan GasBench II (Thermo Fisher Scientific), was used for stable hydrogen isotope ratio measurements. After uncapping a 12-ml Exetainer (Labco Limited), 5 mg of activated charcoal (Thermo Fisher Scientific) and 200 mg of Cu powder (Thermo Fisher Scientific) were introduced into the Exetainer followed with a platinum catalytic rod (Thermo Fisher Scientific). The activated charcoal and Cu powder were added to remove any potential contaminants in the samples that might poison the platinum catalyst. After putting 200 ul of sample into the Exetainer, the Exetainer was recapped and placed into the GasBench II and flushed with 2 % H_2 in helium for 7 min. The sample was allowed to equilibrate with the flushed gas at room temperature (approximately 4–6 h). At the end of the equilibration, an aliquot of the headspace in the Exetainer was injected into the ThermoFisher Delta V Advantage mass spectrometer system for stable hydrogen isotope ratio measurement against the reference gas H_2. A standard calibration curve was prepared using 99·9 % ^2H-enriched water (Sigma-Aldrich), and the ^2H enrichment in duplicate saliva samples was determined[38].

Myofibrillar-bound ^2H alanine enrichments

Myofibrillar protein-enriched fraction was extracted from approximately 50 mg of wet weight muscle tissue by hand-homogenisation on ice using a pestle in a standard homogenisation buffer (TRIS-HCL 50 mM, EDTA 1 mM, EGTA 1 mM, β-glycerophosphate 10 mM, NaF 50 mM, activated sodium orthovanadate 0·5 mM, cOmplete protease inhibitor cocktail tablet (Roche Holding AG)) (7·5 µl/mg). The samples were centrifuged at 2200 ***g*** for 10 min at 4°C, the pellet was then washed with 500 µl of homogenisation buffer and centrifuged at 700 ***g*** for 10 min at 4°C. The myofibrillar protein was solubilised by adding 750 µl of 0·3 M NaOH and heating for 30 min at 50°C with samples being vortexed every 10 min. Samples were then centrifuged for 10 min at 10 000 ***g*** and 4°C, the supernatant containing the myofibrillar protein was kept and the collagen protein pellet was discarded. The myofibrillar proteins were precipitated by the addition of 500 µl of 1 M PCA and centrifuged at 700 ***g*** and 4°C for 10 min. Myofibrillar proteins were then washed with 70 % ethanol twice and hydrolysed overnight in 2 ml of 6 M HCl at 110°C. The free amino acids from the hydrolysed myofibrillar protein pellet were dried under vacuum with a Speed-Vac rotary dryer (Savant Instruments) for 3 h at 80°C radiant cover. The free amino acids were subsequently dissolved in 1·5 ml of 25 % acetic acid solution and passed over cation exchange AG 50 W-X8 resin columns (mesh size: 100–200, ionic form: hydrogen; Bio-Rad Laboratories) and eluted with 6 M NH_4OH. Following this, the purified amino acids were dried and derivatised to tert-butyldimethylsilyl derivatives via the addition of 50 µl of MTBSTFA + 1 % tert-butyl-dimethylchlorosilane and 50 µl of acetonitrile, which was then vortex-mixed and heated at 95°C for 40 min. The samples were transferred to a GC vial. The level of

enrichment of d4-alanine was analysed using a ThermoFisher Delta V Advantage isotope ratio mass spectrometer fitted with a Trace 1310 GC with an online high-temperature thermal conversion oven (HTC) at 1420°C. The sample (1 µl) was injected in splitless mode at an injection port temperature of 250°C. The peaks were resolved on a 30 m × 0·25 mm internal diameter × 0·25 µm film Agilent Technologies DB-5 capillary column (temperature programme: 110°C for 1 min; 10°C/min ramp to 180°C; 5°C/min ramp to 220°C; 20°C/min ramp to 300°C; hold for 2 min) prior to pyrolysis. Helium was used as the carrier gas with a constant flow of 1 ml/min. Any amino acid eluting from the gas chromatograph was converted to H_2 before entry into the isotope ratio mass spectrometer. The enrichment of the alanine tracer was measured by monitoring the ion masses 2 and 3 to determine the ^2H:^1H ratios in the samples and referenced to the calibration curve. The calibration curve consisted of a series of known concentrations of d4-alanine and was applied to assess both the linearity of the mass spectrometer and to control for the loss of tracer. The isotopic abundances were expressed as the δ notation, δ^2H per mil (‰) deviation from VSMOW (Vienna Standard Mean Ocean Water) standard[38]. Values of δ per mil given by the isotope ratio mass spectrometer were transformed into MPE.

Calculations

Myofibrillar fractional synthesis rates (FSR) were calculated based on the incorporation of the mean body water ^2H enrichment over the 3-d intervention as a precursor pool into myofibrillar-bound proteins. Ourselves and others have previously shown the body water ^2H pool is a valid precursor pool for the calculation of myofibrillar protein synthesis rates (corrected by a factor of 3·7 based on ^2H labelling of alanine during *de novo* synthesis) which shows excellent agreement with either plasma or muscle-free ($^2H_4$) alanine as alternative precursor pool selections[27,32,39]. FSR was calculated using the standard precursor-product method and expressed as daily rates as follows:

$$FSR\ (\%\ /\mathrm{d}) = \left(\frac{E_{m2} - E_{m1}}{E_{precursor} \times t}\right) \times 100,$$

where E_{m1} and E_{m2} are the myofibrillar muscle protein-bound enrichments pre (one leg only) and post (either the rested or exercised leg) the dietary intervention. $E_{precursor}$ represents mean body water ^2H enrichment corrected by a factor of 3·7. t represents the time between biopsies (i.e. 3 d).

Statistics

A two-sided power analysis based on previous research[32] showed that eight per group was sufficient to detect expected differences in myofibrillar protein synthesis rates between rested and exercised legs when using a two-factor ANOVA ($P < 0·05$, 95 % power, $f = 1·68$; G*power version 3.1.9.2). Our primary measure was myofibrillar protein synthesis rates, with all other measures representing secondary measures. All data are presented as mean values with their standard errors and all statistical analyses were conducted in GraphPad Prism version 7.0 (GraphPad Software). An independent-samples t test was used to compare each of the participants' characteristics across groups. A two-way (OMNI *v.* VEG and time (pre-post)) ANOVA was used to compare bm during the nutritional intervention. A two-way (OMNI *v.* VEG and time (days 1–4)) ANOVA was used to compare changes in body water ^2H enrichments. A three-way (OMNI *v.* VEG, pre *v.* post and rested *v.* exercised leg) mixed-effects model was used to compare myofibrillar protein-bound (^2H) alanine enrichments. A two-way (OMNI *v.* VEG and rested *v.* exercised leg) ANOVA, independent-samples t tests and paired t tests were used to compare myofibrillar protein synthesis rates. When a significant interaction was found, Sidak's or Tukey's *post hoc* tests were applied to locate individual differences. Statistical significance was set at $P < 0·05$.

Results

Participants' characteristics and daily exercise protocol

No differences in age, weight, height, BMI or body composition were detected between groups (all $P > 0·05$) and groups were also well balanced for sex (Table 1). Data relating to work done during the exercise protocol are displayed in Table 1. No differences in total work performed during the experimental resistance exercise bouts (32 547 (SEM 3450) J in OMNI *v.* 27 851 (SEM 2809) J in VEG; $P = 0·302$), or in fatigue during each trial (all $P > 0·05$) or over the week ($P = 0·392$) were detected between groups. Total work done was lower on day 2 than day 3 ($P = 0·026$), with no differences detected between days 1 and 2 ($P = 0·503$), or 1 and 3 ($P = 0·934$). Body mass did not change in response to the nutritional intervention in either group ($P = 0·703$ and $P = 0·175$ in OMNI and VEG, respectively).

Nutritional intervention

Habitual diet did not differ between groups for energy intake, protein intake or carbohydrate intake (all $P > 0·05$), although fat intake was higher in the OMNI group than VEG ($P = 0·039$; Table 2). Energy intake did not change between participants' habitual diets and the diet they received during the intervention ($P = 0·142$). Daily protein intake was higher (by design) during the intervention diet than in participants' habitual diets (1·8 (SEM 0·0) *v.* 1·2 (SEM 0·1) g/d, respectively; $P < 0·0001$). Daily carbohydrate intake was also higher during the intervention diet than in participants' habitual diets ($P = 0·037$). Time and interaction effects were detected (both $P < 0·05$) such that fat intake decreased from habitual levels during the intervention diet in the OMNI group only ($P < 0·0001$).

During the intervention, diet participants consumed 10 (SEM 0·6) and 9·6 (SEM 0·6) MJ (0·14 (SEM 0·00) and 0·14 (SEM 0·00) MJ/kg; 2382 (SEM 139) and 2296 (SEM 137) kcal) per d in OMNI and VEG, respectively, with no differences between groups ($P = 0·667$). By design, daily protein intake (i.e. 127 (SEM 5) and 125 (SEM 5) g/d, respectively) was identical between groups during the intervention. Participants consumed 305 (SEM 20) and 274 (SEM 20) g carbohydrate, and 65 (SEM 4) and 67 (SEM 4) g fat per d in OMNI and VEG, respectively, with no differences between groups (both $P > 0·05$). Fibre intake was higher in the VEG intervention diet than

the OMNI intervention diet (68 (SEM 3) g *v.* 32 (SEM 2) g; $P < 0{\cdot}0001$) as a result of the high natural fibre content of mycoprotein (Table 3).

In VEG, of the 125 (SEM 6) g of protein consumed per d, 71 (SEM 2) g was derived from mycoprotein (30 (SEM 2) g from mycoprotein within 329 (SEM 23) g of Quorn products, and 41 (SEM 1) g protein from supplementary isolated mycoprotein) corresponding to 57 (SEM 1) % total protein intake. Remaining protein was provided by wheat and potato protein in Quorn products and from the protein present in the other elements of the diet. Overall, Quorn products provided 50 (SEM 3) g of daily protein and accounted for 39 (SEM 2) % of total daily protein intake.

In OMNI, of the 127 (SEM 5) g of protein consumed per d, 90 (SEM 3) g was provided by animal-derived sources and 38 (SEM 3) g from non-animal sources, corresponding to 71 (SEM 1) and 29 (SEM 1) % from animal and non-animal-derived sources, respectively. Meat products provided 40 (SEM 0) g and dairy products (including the milk protein supplement) provided 50 (SEM 3) g of protein per d, corresponding to 32 (SEM 1) and 39 (SEM 1) % of total protein, respectively. The milk protein supplement alone provided 31 (SEM 0) g of protein per d, 25 (SEM 1) % of total protein.

Body water ^{2}H enrichments

Saliva ^{2}H enrichments throughout the experimental protocol are depicted in Fig. 2. From baseline (i.e. background) enrichments of 0·00044 (SEM 0·00017) and 0·00030 (SEM 0·00010) % in OMNI and VEG, respectively, body water ^{2}H enrichments increased ($P < 0{\cdot}0001$; effect of time) and reached 0·77 (SEM 0·03), 0·79 (SEM 0·03), 0·79 (SEM 0·04) and 0·85 (SEM 0·03) % on days 2–5 in OMNI, and 0·70 (SEM 0·06), 0·75 (SEM 0·05), 0·74 (SEM 0·05) and 0·78 (SEM 0·05) % on days 2–5 in VEG, with no differences between groups (treatment and treatment × time interaction both $P > 0{\cdot}05$).

Daily myofibrillar protein synthesis rates

Myofibrillar protein-bound (^{2}H) alanine enrichments increased over time ($P < 0{\cdot}0001$) and to a greater extent in the exercised compared with control leg (time × leg interaction; $P = 0{\cdot}015$). Myofibrillar protein-bound (^{2}H) alanine enrichments increased in the OMNI group by 358 (SEM 76) % (from 0·058 (SEM 0·014) to 0·206 (SEM 0·027) MPE) in rested and 394 (SEM 74) % (from 0·058 (SEM 0·014) to 0·216 (SEM 0·023) MPE) in exercised muscle, and in the VEG group by 263 (SEM 42) % (from 0·070 (SEM 0·014) to 0·229 (SEM 0·0·031) MPE) in rested and 299 (SEM 44) % (from 0·070 (SEM 0·014) to 0·235 (SEM 0·028) MPE) in exercised muscle, with no differences between groups (all OMNI *v.* VEG interactions; $P > 0{\cdot}05$).

Saliva ^{2}H enrichments were used as a precursor pool and myofibrillar protein-bound (^{2}H) alanine enrichments as the product to calculate daily myofibrillar FSR (Fig. 3). Daily myofibrillar FSR were 13 (SEM 8) ($P = 0{\cdot}169$) and 12 (SEM 4) % ($P = 0{\cdot}016$) greater in the exercised compared with rested leg (1·59 (SEM 0·12) *v.* 1·77 (SEM 0·12) %/d and 1·75 (SEM 0·14) *v.* 1·93 (SEM 0·12) %/d) in OMNI and VEG groups, respectively. Daily myofibrillar protein synthesis rates did not differ between groups in either rested ($P = 0{\cdot}38$) or exercised ($P = 0{\cdot}33$) muscle.

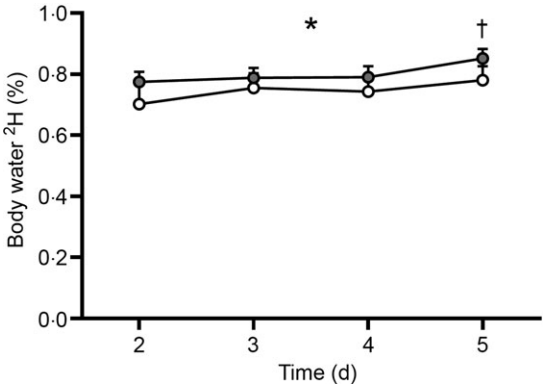

Fig. 2. Saliva ^{2}H enrichments over time during oral deuterated water dosing in nineteen healthy older adults (aged 66 (SEM 1) years) consuming a 3-d fully controlled isoenergetic high-protein (1·8 g/kg body mass per d) diet, where the protein was provided predominantly from animal (OMNI; *n* 9) or exclusively non-animal (VEG; *n* 10) sources. During the dietary control period (days 2–4), participants conducted a single bout of maximal unilateral concentric isokinetic knee extension exercise (5 × 30 contractions) each morning. On day 1, participants were loaded with a total of 400 ml of deuterated water, with 50 ml of maintenance doses consumed daily thereafter. Data were analysed with mixed-effects ANOVA. Values are means, with their standard errors represented by vertical bars. * Main effect of time ($P < 0{\cdot}05$). † Significant difference to preceding time points ($P < 0{\cdot}05$). Treatment × time interaction effect, $P = 0{\cdot}5565$; treatment, $P = 0{\cdot}3847$; time, $P = 0{\cdot}0026$. ●, OMNI; ○, VEG.

Discussion

The present study demonstrates that performing a single bout of unilateral knee extensor resistance exercise daily for a 3-d period modestly stimulates daily myofibrillar protein synthesis rates compared with the rested control leg in older adults consuming a high-protein (1·8 g/kg bm per d) diet. Importantly, we report the novel finding that daily myofibrillar protein synthesis rates in both rested and exercised muscle of older adults during the dietary intervention period were equivalent regardless of whether dietary protein was obtained primarily from animal or exclusively from non-animal sources.

The continuous infusion of stable isotopically labelled amino acids into human volunteers to measure hour-to-hour MPS rates has revealed important insights concerning the physiological aetiology of age-related sarcopenia. Specifically, a failure of senescent muscle tissue to respond appropriately to the anabolic properties of bolus dietary protein ingestion ('anabolic resistance') is now widely accepted as a key physiological mechanism responsible for sarcopenia[5,11,40]. Such experimental approaches have also demonstrated that anabolic resistance can effectively be overcome (or compensated for) on a meal-by-meal basis by consuming higher amounts of protein[14,41] and/or consuming protein in close temporal proximity to resistance-type exercise[12,41]. However, such studies have relied on single time point measurements made over only a few hours and confined to laboratory conditions. Fewer data are available exploring whether increased dietary protein intake with concurrent physical activity in older adults translates to improvements in MPS rates throughout multiple days (including basal, postprandial and overnight periods), and thus encompassing all free-living influences (e.g. multiple meals, habitual physical activity, diurnal metabolic fluctuations, sleep). In the present

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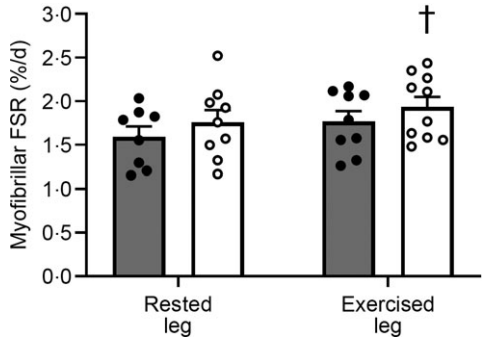

Fig. 3. Daily free-living myofibrillar protein fractional synthesis rates (FSR) calculated from the body water ^{2}H precursor pool in nineteen healthy older adults (aged 66 (SEM 1) years) consuming a 3-d fully controlled isoenergetic high-protein (1·8 g/kg body mass per d) diet, where the protein was provided predominantly from animal (OMNI; *n* 9) or exclusively non-animal (VEG; *n* 10) sources, in rested and exercised (single bout of 5 × 30 maximal unilateral isokinetic knee extension contractions on three consecutive days) muscle. Data were analysed with two-way ANOVA, with paired *t* tests used to detect differences between rested and exercised legs in each respective groups. Values are means, with their standard errors represented by vertical bars. † Effect of exercise in the VEG groups ($P < 0·05$). Treatment × exercise interaction effect, $P = 0·9917$; treatment, $P = 0·1874$; exercise, $P = 0·1632$. OMNI paired *t* test, rested leg *v.* exercised leg, $P = 0·1694$. VEG paired *t* test, rested leg *v.* exercised leg, $P = 0·0162$ (†). ▬, OMNI; ▭, VEG.

work, we applied a deuterated water stable isotope approach in older adults consuming a fully controlled high-protein (1·8 g/kg bm per d) diet and daily unilateral resistance exercise (with the non-exercised leg serving as a rested, within-subject control) to determine daily and free-living myofibrillar protein synthesis rates over a 3-d experimental period. In agreement with our previous work in young adults[27], we observed the deuterated water dosing regimen rapidly increased body water ^{2}H enrichments to near steady-state levels of approximately 0·78 %, with a slight increase over time. The steady-state enrichment of body water served as a precursor for the calculation of daily myofibrillar protein synthesis rates from muscle biopsy samples (Fig. 2). We report a single bout of unilateral resistance exercise performed daily each morning for 3 d increased daily myofibrillar protein synthesis rates by approximately 13 % in healthy older men and women (Fig. 3), though this effect only reached statistical significance in the vegan group (though numerically similar across groups).

The absence of myofibrillar protein synthesis data collected under habitual (i.e. lower; 1·2 g/kg bm per d for the present volunteers) protein intake conditions precludes us from confirming that the high-protein dietary intervention *per se* (i.e. irrespective of protein source) had a stimulatory effect. However, recent work has reported that higher protein diets in the absence of exercise (1·8 *v.* 1·0 g/kg bm per d; achieved via protein supplementation, and in line with the intervention *v.* habitual intakes of the present volunteers) elevates daily, free-living myofibrillar protein synthesis rates in older adults[42]. Further, such studies have also demonstrated that resistance exercise can elevate daily MPS rates across a range of low-moderate or protein-supplemented protein intakes (1·0–1·8 g/kg bm per d) in older adults of varying health statuses[42–45]. Our data, therefore, support and extend on these observations by demonstrating that daily resistance exercise retains the capacity to augment daily myofibrillar protein synthesis rates (though perhaps more modestly) in healthy older adults consuming a strategically timed (i.e. protein consumed directly after exercise, distributed throughout the day and before sleep[19,46,47]) high-protein diet designed to nutritionally maximise daily myofibrillar protein synthesis rates. Collectively, therefore, the data indicate that chronic maintenance of higher daily MPS rates mechanistically underpin the cross-sectional and longitudinal observational studies that reliably show more active[48–50] and/or higher protein consuming (at least above the currently accepted RDA/RDI)[51,52] older adults experience slower rates of annual muscle loss.

Aside from total daily (or per meal) dietary protein intake, an important contemporary research focus is from *where* dietary protein should (or could) be obtained. Government and societal priorities are increasing the demand to reduce animal-derived protein consumption in favour of sustainable alternatives[53]. This is particularly pertinent for older adults where the scientific consensus is advocating for a near 50 % increase in the UK/USA RDI for dietary protein[11,54,55]. Limited comparisons to date have suggested that, on a gram-for-gram basis, plant-based protein sources are inferior to animal-derived protein sources with respect to their capacity to stimulate MPS rates upon ingestion, attributed to their typically lower leucine contents[15,20,21]. This implies that more total protein would be required within a vegan diet to support equivalent daily MPS rates, a circumstance itself that has implications for environmental sustainability and dietary feasibility. Indeed, by supplementing with more acutely anabolic proteins (whey *v.* collagen), it has been shown that daily MPS rates can be augmented to a greater degree, even under isonitrogenous conditions[42]. We have recently shown that the fungal-derived protein source, mycoprotein, robustly stimulates MPS rates in young men. In the present work, we therefore hypothesised that the incorporation of mycoprotein within a vegan, high-protein diet, would support daily myofibrillar protein synthesis rates to the same extent as a protein-matched diet based (more typically) on animal-derived protein consumption in older men and women. In support of this hypothesis, comparable daily free-living rates of myofibrillar protein synthesis between omnivorous and vegan diets were observed in both rested (1·59 *v.* 1·76 %, respectively) and exercised (1·77 *v.* 1·93 %, respectively) muscle (Fig. 3). Our data therefore provide the proof of concept that higher protein vegan diets can be adopted in healthy older adults without compromising rested myofibrillar protein synthesis rates, implying vegan diets can be equivalently capable of supporting the maintenance of muscle mass during ageing. Further, our data also indicate that for older adults living more active lifestyles and/or participating in structured (resistance) exercise training, implementing a higher protein vegan diet would not compromise prolonged muscle tissue adaptive responses. We therefore show that non-animal-derived dietary proteins (the vast majority of which have not been investigated in relation to their impact on MPS) are not necessarily inferior in their capacity to stimulate daily myofibrillar protein synthesis rates when incorporated into the daily diet, even in older adults. Our work also captures the total muscle anabolism achieved with two divergent diets in a free-living scenario over multiple days, thereby incorporating the diurnal variation and multiple feeding-fasting cycles that are potentially missed by intravenous isotope infusion studies. The necessarily short-term nature of such metabolic studies as presently reported

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(i.e. 3 d) means we cannot rule out the possibility that a longer duration of study (or increased statistical power) may have yielded significant differences across groups. However, it is also true that numerically our data indicated greater rates of myofibrillar protein synthesis in the vegan group. Irrespective, it is of clear importance that future work is performed to confirm our mechanistic findings as to the longer-term impact of such diets on muscle mass and strength in older adults.

Some important design aspects and limitations of the present study require further consideration and context. We chose to provide a dietary protein intake (amount, type, timing and distribution) that we considered (close to) 'optimal' for maximising daily myofibrillar protein synthesis rates. While this amount (i.e. 1·8 g/kg bm per d) is well above the RDA, it is in line with the currently asserted optimal dietary protein intakes to support active and healthy muscle ageing[56,57] and is only about 50 % above habitual intakes typically reported by healthy and active UK adults[58], including those within the present study (see Table 2). Though the feasibility of applying such a diet in community-dwelling older adults can be debated, the approach allowed us to perform a proof-of-concept experiment investigating whether manipulating the *type* of protein only would impair daily myofibrillar rates under these ('optimal') conditions. As a result, we can conclude that protein type may become less relevant when consuming a high-protein diet, where the amount and timing of protein are at an 'optimal' level. Further research is necessary to establish whether such findings hold true under lower and/or less optimal protein intake conditions. Indeed, given the typically lower leucine (and total essential amino acid/branched chain amino acid) contents of non-animal protein sources[59], it may be expected that differences across sources would become most apparent when protein intake is suboptimal. However, due to the relatively high leucine content of mycoprotein and the proportion of protein across groups that was obtained from identical sources (i.e. the control group was omnivorous), we estimated that daily leucine intakes were sufficient (and perhaps surplus) and comparable across groups (approximately 10 g of leucine per d, an average of approximately 2·5 g split between four meals, in both groups). The latter may, at least in part, explain the lack of differences between diets in daily myofibrillar protein synthesis rates.

The inclusion of mycoprotein as the basis for the vegan dietary intervention is also of relevance. To date, mycoprotein is the only vegan protein source whose bolus ingestion has been shown to acutely stimulate postprandial MPS rates to a comparable extent as an animal-derived control[26]. Accordingly, it cannot be assumed that our current data are generalisable to vegan diets predicated on other protein sources, particularly those lower in leucine and/or other essential amino acids. Future work is required to investigate the impact of a range of vegan protein sources both on acute postprandial and daily MPS rates. Finally, we employed unilateral isodynamic maximal concentric contractions as a model of exercise to ensure a daily stimulation of MPS rates in line with our previous work[30]. We reasoned that this would increase the cumulative stimulatory effect across the 3-d intervention without inducing excessive muscle damage. While effective for the experimental purposes herein, there will be value in translating our data to multiple modalities of exercise to further the ability to make applied recommendations to support active and healthy ageing.

To conclude, the present work reports that a single bout of resistance exercise performed daily each morning increases daily myofibrillar protein synthesis rates in older men and women consuming a high-protein diet. Obtaining the majority of dietary protein from animal-derived sources compared with exclusively vegan-based sources (primarily mycoprotein) did not modulate daily myofibrillar protein synthesis rates in rested or exercised muscle. Our data indicate that obtaining dietary protein from animal-derived sources is not an essential prerequisite to support daily myofibrillar protein synthesis rates in older adults.

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Aside from those mentioned above, the authors declare that there are no conflicts of interest.

Supplementary material

For supplementary materials referred to in this article, please visit https://doi.org/10.1017/S0007114520004481

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