The Journal of Nutrition xxx (xxxx) xxx

THE JOURNAL OF NUTRITION

journal homepage: https://jn.nutrition.org/

Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

A Well-Balanced Vegan Diet Does not Compromise Daily Mixed Muscle Protein Synthesis Rates when Compared with an Omnivorous Diet in Active Older Adults: A Randomized Controlled Cross-Over Trial

Jacintha Domić [1,*], Philippe JM Pinckaers [2], Pol Grootswagers [1], Els Siebelink [1], Johanna C Gerdessen [3], Luc JC van Loon [2], Lisette CPGM de Groot [1]

[1] *Division of Human Nutrition and Health, Wageningen University, Wageningen, The Netherlands;* [2] *Department of Human Biology, School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University Medical Centre+, Maastricht, The Netherlands;* [3] *Operations Research and Logistics Group, Department of Business Science, Wageningen University, Wageningen, The Netherlands*

A B S T R A C T

Background: Plant-based foods have reduced protein digestibility and frequently display unbalanced amino acid profiles. Plant-based foods are therefore considered inferior to animal-based foods in their anabolic potential. No study has assessed the anabolic potential of a vegan diet that provides a large variety of plant-based protein sources in older adults.

Objectives: To investigate the effect of a 10-d vegan diet on daily mixed muscle protein synthesis (MPS) rates in comparison with an isocaloric, isonitrogenous, omnivorous diet in community-dwelling older adults.

Methods: This cross-over trial assessed 34 community-dwelling older adults (72 ± 4 y, 18 males, 16 females), who were randomly assigned to consume a 10-d controlled vegan diet, followed by a controlled omnivorous diet (60% animal protein), or vice versa. One day before the study diets, participants consumed 400 mL deuterated water, followed by daily doses of 50 mL. Subsequent plasma and muscle samples were collected during the intervention period. Physical activity levels were assessed using accelerometry. Secondary outcomes were cardiometabolic risk factors and appetite. Statistical analyses were performed using linear mixed models, and results are presented as means ± standard errors.

Results: Integrated MPS rates did not differ between the vegan (1.23 ± 0.04%/d) and omnivorous (1.29 ± 0.04%/d) diets ($P = 0.2542$). Plasma low-density lipoprotein (Δ0.23 ± 0.03, $P < 0.0001$), high-density lipoprotein (Δ0.03 ± 0.14, $P = 0.0387$), and total cholesterol (Δ0.25 ± 0.04, $P < 0.0001$) levels were significantly lower succeeding the vegan diet than the omnivorous diet. There were no significant differences between the omnivorous and the vegan diet in fasting plasma triglyceride, glucose and insulin levels, homeostasis model assessment of insulin resistance, and systolic and diastolic blood pressure ($P > 0.05$). Physical activity levels were high (12,460 ± 4512 steps/d).

Conclusions: A well-balanced vegan diet providing a variety of plant-based protein sources does not compromise daily MPS rates when compared with an isocaloric, isonitrogenous omnivorous diet in physically active, older adults.

This trial was registered at clinicaltrials.gov as NCT05624333 (https://clinicaltrials.gov/study/NCT05624333).

Keywords: plant-based, protein, aging, sarcopenia, sustainable diet, animal-based

Introduction

The progressive loss of skeletal muscle mass, strength, and function among older individuals, referred to as sarcopenia, is an emerging public health issue. Sarcopenia prevalence estimates vary ≤~40% in community-dwelling older adults [1]. Sarcopenic individuals are at risk of falls, prolonged hospital stays, and other complications [2–4]. A widely used strategy in preventing and counteracting sarcopenia is enhancing dietary protein consumption later in life, thereby focusing on the timing, amino acid (AA) composition, and origin of the protein source [5–8]. Dietary protein ingestion promotes skeletal muscle accretion by upregulating muscle protein synthesis (MPS) and inhibiting muscle protein breakdown [5]. The increased demand for dietary

Abbreviations: AA, amino acid; D_2O, deuterium oxide; En%, percentage of energy; FSR, fractional synthesis rate; MPS, muscle protein synthesis; VAS, visual analogue scale.

* Corresponding author. *E-mail address:* jacintha.domic@wur.nl (J. Domić).

https://doi.org/10.1016/j.tjnut.2024.12.019
Received 23 October 2024; Received in revised form 26 November 2024; Accepted 19 December 2024; Available online xxxx

protein later in life predominantly results from anabolic resistance, an impaired response to anabolic stimuli, including protein ingestion [9].

Proteins from plant origin are generally considered to be inferior in their quality to those of animal origin [10,11]. The quantity and the quality of a protein source define the ability of a protein to carry out its functions when digested and absorbed, such as upregulating MPS. The capacity of a protein source to stimulate MPS is largely determined by the protein digestion and AA absorption kinetics, and the AA composition of the protein [12]. Although animal-based foods typically exhibit complete AA profiles, plant-based foods show high heterogeneity in their AA profiles, often limiting in 1 or more essential AA. Furthermore, plant-based foods often exhibit inconvenient food matrices that may interfere with protein digestion, because of the abundance of, for example, antinutritional factors [13,14].

Despite the suggested lesser anabolic potential, augmenting the consumption of plant-based foods increases dietary intake of numerous important other nutrients and bioactive compounds and, therefore, holds several health benefits. For example, observational studies showed plant-based diets to be associated with a reduced risk of cardiovascular disease [15,16]. Concurrently, decreasing animal-based food consumption lowers the environmental impact of the diet. Plant-based diets are, therefore, endorsed by the EAT-Lancet commission and the European Union [17,18]. Nevertheless, considering the suggested lower anabolic properties of plant-based foods, we have previously discussed that a diet in which all animal-based foods are eliminated, that is a vegan diet, may aggravate sarcopenia in older individuals [19].

As yet, intervention studies regarding the anabolic potential of plant-based foods have mainly focused on isolated protein supplements [20–27]. Nevertheless, proteins are generally consumed as part of a whole foods diet rather than being consumed in its isolated form. Furthermore, combining multiple plant-based sources to achieve a more optimal AA profile within a meal has been suggested to improve its anabolic properties [10,11,28]. As such, several studies have recently shifted their focus toward the anabolic effect of whole plant-based foods in the form of mixed meals or diets in young [29] and older [30–32] individuals. Recent work from our groups showed 47% higher postprandial mixed MPS rates after ingestion of an omnivorous meal when compared with the ingestion of an isocaloric and isonitrogenic vegan meal in older adults [30]. However, it remains unknown how a vegan diet, providing a large variety of plant-based protein sources, impacts daily MPS rates in older individuals when applied for a more prolonged period. In the present randomized controlled cross-over trial, we compared daily integrated mixed MPS rates although consuming a well-balanced vegan diet compared with an isocaloric, isonitrogenous, healthful omnivorous diet for 10 d in community-dwelling older adults.

Methods

Participants

Participants were recruited via an existing volunteer database of Wageningen University and Research and advertisements in local newspapers and online media. Recruitment was done between October 2021 and December 2022. All participants were informed regarding the purpose of the study, the study procedures, and the potential risks before providing informed consent. After providing informed consent, eligibility was assessed by a member of the research team using a screening questionnaire that included questions regarding the in- and exclusion criteria of the study and their medical history, and by measuring body weight and height to the nearest 0.1 kg and 0.1 m, respectively, using a calibrated digital scale (Seca 704, Seca GmbH & Co) and a wall-mounted stadiometer. Volunteers were considered eligible if they were aged between 65 and 79 y old, community-dwelling, and had a BMI between 20 and 35 kg/m^2 at the moment of screening. Volunteers were excluded from participation in the study if they: *1*) followed a vegetarian or vegan diet during the 6 mo before the study; *2*) followed a high- or low-protein diet during the 6 mo before the study; *3*) participated in a structured exercise program during the 3 mo before the study; *4*) had ≥5% of body weight loss during the 3 mo before the study; *5*) were diagnosed with diabetes, renal disease, neurological or neuromuscular disorders, serious cardiovascular disease, cancer, and/or chronic obstructive lung disease; *6*) chronically used medication that affects muscle function; *7*) used heavy anticoagulants; *8*) were allergic or intolerant to any product included in the diets; or *9*) did not have a general physician. In total, 38 participants were included in the study (see Supplemental Figure 1 for the Consolidated Standards of Reporting Trials flow diagram). The study was approved by the Medical Ethical Committee Brabant (reference number NL82653.028.22), and the procedures were in accordance with the Declaration of Helsinki of 1975, as revised in October 2013. The trial is registered at clinicaltrials.gov (https://clinicaltrials.gov/study/NCT05624333).

Study design

This dietary controlled intervention study had an open-label randomized cross-over design. The study was conducted between November 2021 and February 2023. A general overview of the design is presented in Figure 1. The total duration of the study was 28 d. During the week before the diets were initiated, baseline measurements were performed, as described in more detail in the following paragraphs. The deuterium oxide (D_2O) dosing protocol was initiated 1 d before the participants would start with their diet. Subsequently, all participants consumed a strictly controlled, a priori calculated, vegan diet, as well as a strictly controlled omnivorous diet for 10 d. Test days were performed before and after each diet. No washout period was included because we considered the 10-d treatment period as sufficient to allow for the washout of the first treatment before the final measurements of the second treatment. Additionally, if a washout period had been included, an extra muscle biopsy would have had to be performed, increasing the burden on the participants. Participants were randomly assigned to consume the vegan diet first, followed by the omnivorous diet, or vice versa. Randomization was done on a 1:1 ratio, stratified by sex, and based on a computer-derived random allocation sequence (SAS, version 9.4) generated by an independent researcher using a block size of 6.

Study diets

For both diets, a 5-d menu-cycle was designed for 12 levels of energy intake ranging from 6.5 to 12.0 MJ/d, with increments of

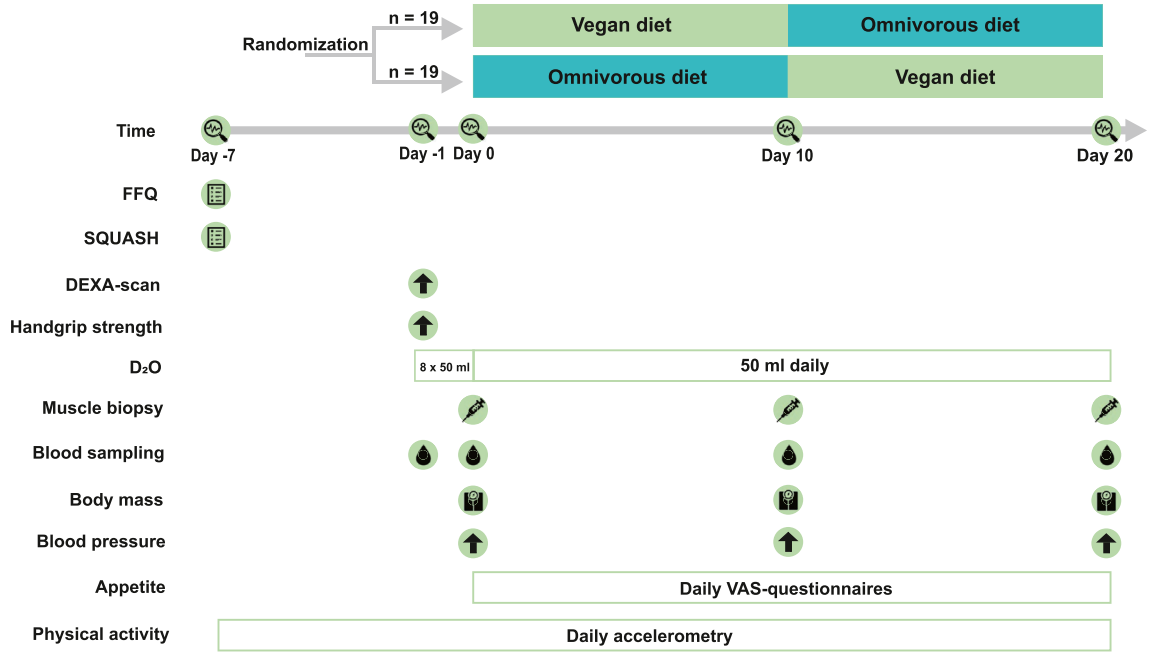

FIGURE 1. Study design. D_2O, deuterium oxide; DEXA, Dual Energy X-ray Absorptiometry; FFQ, food frequency questionnaire; SQUASH, Short Questionnaire to Assess Health-enhancing physical activity; VAS, visual analogue scale.

0.5 MJ/d, using a mixed integer linear programming model, as described in detail elsewhere [33]. The Dutch food composition database [34] was used to derive the energy and nutrient composition of the food products, and nutrient values for composite foods were calculated using the nutrient calculation program Compl-eat [35]. Participants were allocated to one of the energy groups based on their basal metabolic rate as estimated by the Schofield formula [36], and their habitual food intake and physical activity levels. Habitual food intake was estimated by a food frequency questionnaire [37], and habitual physical activity levels were estimated using the short-questionnaire to assess health-enhancing physical activity [38]. Participants were instructed to maintain their habitual physical activity levels throughout the study. Nevertheless, if energy expenditure was increased substantially on an incidental day during the study due to increased physical activity levels, participants were provided with bread buns that were specifically produced to have a similar macronutrient composition as the diets. The diets that were fully provided to the participants covered 95% of the energy requirements of the participants. Participants were instructed to cover the remaining 5% with foods from a predefined list. The food items on this list had a protein content below 0.5 g per portion. The participants were instructed to record these food items, the bread buns, and any other deviations from the protocol in a logbook.

Participants consumed their hot meal during lunch time at the Wageningen University on weekdays under supervision of members of the research team. Take-home packages were provided containing breakfast, bread meals, snacks, and beverages that had to be fully consumed before their subsequent visit. On Fridays, the take-home packages contained all meals for Saturday, Sunday, and Monday morning as well. The participants received instructions regarding the preparation of the meals and the time range during which the meals and snacks had to be consumed to ensure that protein intake was spread out evenly throughout the day. Body weight was additionally measured twice a week to the nearest 0.1 kg using a calibrated digital scale to allow for adjustment of energy group allocation if body weight of a participant would change substantially although consuming the study diets.

Diet composition and duplicate portions

The macronutrient compositions of both diets aimed to have a protein content of 15% of daily energy intake (en%). The diets were composed based on the Dutch national dietary guidelines [39]. The protein content of the omnivorous diet consisted for ~60% of animal protein, in line with the average habitual dietary protein intake of Dutch older adults [40,41]. The a priori calculated approximate daily protein distribution was as follows: 20%, 30%, 20%, and 30% of the daily protein intake consumed during breakfast, the hot meal, the bread meal, and snacks (3 snacks providing each 10% of total protein), respectively. The diets solely consisted of food products, and thus did not include any nutritional supplements. The main sources of protein in the vegan diet were soy-based dairy alternatives, legumes, nuts, cereals, and plant-based meat analogs based on (isolated) pea protein and/or rice protein. The main sources of protein in the omnivorous diet were dairy products, cheese, chicken, beef, and pork sausage. The menu cycles including quantities of both diets are presented in Supplemental Tables 1–3.

During the intervention, duplicate portions of both diets were collected for a fictional participant with an energy level of 9 MJ/d. The duplicate portions were analyzed externally for nitrogen, AA, fat, carbohydrate, and dietary fiber contents by an independent professional food and feed analytical company (Nutrilab B.V.) using validated methods. Nitrogen content of the diets were analyzed using the Dumas method (NEN-EN-ISO 16634). The protein contents were subsequently calculated using the

nitrogen-to-protein conversion factor of 6.25 [42]. The total and free AAs, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, ornithine, phenylalanine, proline, serine, threonine, tyrosine, and valine were analyzed using IC-UV according to the ISO standard 13903:2005. Tryptophan was analyzed separately in each sample via alkaline hydrolysis using HPLC.

Physical activity

Participants were instructed to maintain their habitual physical activity levels during the study. Physical activity levels were assessed from 1 wk before the diets were consumed until the end of the study using accelerometry (ActiGraph). Additionally, participants recorded their physical activity in a diary during this period.

Body composition

Body mass was measured in a fasted state using a calibrated digital scale at baseline, and on each subsequent test morning. Furthermore, a dual energy X-ray absorptiometry scan (LUNAR Prodigy, GE Health Care) was performed at baseline in a fasted state to assess bone mineral density and body composition.

Handgrip strength

Handgrip strength (kg) was measured at baseline with the use of a hand dynamometer (Jamar). Both hands were measured 3 consecutive times to the nearest 0.5 kg. The average of these measurements was reported. During the measurement, participants were seated in an upright position with the arm in a 90-degree angle.

Mixed muscle fractional protein synthesis rates

The primary outcome measure, daily mixed muscle fractional synthesis rate (FSR; %/d), was determined using a D_2O dosing protocol, as previously described [43]. In short, the participants ingested eight 50 mL doses of 70% deuterated water 1 d before the start of the diets with 1.5 h between each dose. On the remaining days, participants ingested 50 mL of 70% deuterated water in the afternoon to maintain body water deuterium enrichment. Blood samples and muscle biopsies were collected in a fasted state to assess plasma ^{2}H-alanine enrichments, and the incorporation of ^{2}H-alanine into mixed muscle proteins, respectively. Muscle biopsies were collected on the morning before the first diet started (day 0) and immediately after the first (day 10) and second (day 20) dietary intervention period. Muscle biopsies were collected from the middle region of the m. vastus lateralis muscle, 15 cm above the patella and ~2 cm below the entry through the fascia, using the percutaneous needle biopsy technique with a modified Bergström needle [44]. The first and the last muscle biopsies were taken from the same leg, the second biopsy was taken from the contralateral leg. Subsequently, the samples were frozen in liquid nitrogen and stored at −80°C until further analysis. Blood samples were collected in EDTA-containing tubes before the first dose of 70% deuterated water was consumed, and on day 0, 10, and 20. The blood samples were centrifuged at 1000 g for 10 min at 4°C to obtain plasma aliquots and stored at −80°C until further analysis. Further analyses were performed at the Stable Isotope Research Centre (Maastricht University Medical Centre +) and have been described in detail elsewhere [43]. In short, mixed muscle protein-bound ^{2}H-alanine enrichments were assessed using a gas chromatography-ratio mass spectrometer (MAT 253; Thermo Fisher Scientific) equipped with a pyrolysis oven using a 60-m DB-17MS column and 5-m precolumn (No. 122–4762; Agilent) and GC-Isolink. Plasma ^{2}H-alanine enrichments were assessed using GC-MS (Agilent 5975C MSD & 7890A GC). Mixed muscle protein FSR were calculated using the precursor-product method, as previously described, using the plasma ^{2}H-alanine enrichments as precursor [43].

Plasma insulin, glucose, and lipid concentrations

For the assessment of the secondary outcome measures, fasting insulin, glucose, and lipid profile (total cholesterol; triglycerides; LDL cholesterol; HDL cholesterol), blood samples were collected in Li-heparin- and NaFl-containing tubes on day 0, 10, and 20. The blood samples were centrifuged at 3000 g for 8 min at 22°C to obtain plasma aliquots for subsequent analysis. Plasma insulin was analyzed using a solid-phase enzyme-labeled chemiluminescent immunometric assay (IMMULITE® 2000, Siemens; average coefficient of variation (CV): 5.1%). Samples with an insulin concentration below the detection limit of 2 mU/L were set to 1 mU/L ($n = 4$). The Atellica® CH Glucose Hexokinase 3 assay (CV 1.1%) and The Atellica CH HDL/LDL Cholesterol Calibrator (CV: total cholesterol 0.9%; HDL cholesterol 2.0%; LDL cholesterol 2.3%; triglycerides 1.0%) were used for the assessment of plasma glucose and lipid profile, respectively. The plasma samples were analyzed singlicate at the hospital Gelderse Vallei. Subsequently, the insulin resistance index (HOMA-IR) was calculated according to the following formula: fasting insulin (mU/L) * fasting glucose (mmol/L) / 22.5 [45].

Blood pressure

The secondary outcomes systolic and diastolic blood pressure were measured after an overnight fast on day 0, 10, and 20 using an ambulatory blood pressure monitor (Omron Healthcare Europe B.V.). Participants were instructed not to drink coffee, smoke, or perform vigorous exercise in the 60 min before the measurement and were not allowed to speak during the measurement. Blood pressure was measured 3 consecutive times with 1 min rest intervals, after resting in a chair for 5 min. The mean of the second and third measurement was used for analysis. A fourth measurement was performed if the participant would speak or move during a measurement, or if a difference of ≥10 mmHg was observed between the second and third measurement. In that case, the mean of the third and fourth measurement was used for analysis.

Appetite

Visual analogue scales (VAS) with a length of 100 mm were used to assess the secondary outcome appetite during the consumption of both the vegan and the omnivorous diets. Five VAS regarding hunger, fullness, satiety, desire to eat, and prospective food consumption were used [46]. The VAS were anchored from lowest intensity ratings (that is, not hungry at all) to highest intensity ratings (that is, extremely hungry). The participants were instructed to fill out the VAS daily before and after the consumption of the hot meal.

Adverse events

Adverse events, including all unfavorable and/or unexpected symptoms, were recorded starting from the time the dietary intervention period started and followed up by the research physician.

Sample size calculation

The sample size calculation was performed in GPower version 3.1.9.4. On the basis of previous research [32,47], the SD was initially estimated to be ~0.16%/d. Considering an effect size of 10%, α-level of 0.05, and 80% power, and a drop-out rate of 20%, 24 participants were initially included in the study. However, on the basis of a blinded interim analysis, a larger sample size was considered necessary. Using the SD observed in the blinded interim analysis (0.31%/d), and a matching effect size, α-level, power, and drop-out rate as the initial sample size calculation, 14 additional participants were included. As such, a total of 38 participants were included in the study.

Statistical analysis

Data were collected using Castor Electronic Data Capture [48]. All entered data were double checked by a second member of the research team. Statistical analysis was performed in a blinded manner in R Studio version 1.4 according to the intention-to-treat principle. Descriptive statistics were used to explore and present the participant characteristics at baseline, and are presented as counts and percentages or mean ± SD. Linear mixed models were used to assess any potential change in daily physical activity levels over the study period. Participants were added to the model as a random factor, and study period (baseline, period 1, or period 2) was added as a fixed factor. Linear mixed models were used for the analysis of the primary and secondary outcomes as well. Participants were added to the model as a random factor, and diet, period, and sequence as fixed factors [49]. Endpoints were used to compare the effects of diet on body mass, fasting lipid, glucose, and insulin levels, HOMA-IR, and blood pressure, with baseline levels added as a covariate [50]. Regarding the assessment of appetite after consumption of the hot meals, appetite measures before consumption of the hot meal were added as covariate. No other covariates were added to the models. Models were checked for homoscedasticity and normality of residuals via QQ-plots and boxplots. Results from the linear mixed-model analysis are presented as estimated marginal means and their SE. A P value <0.05 was considered statistically significant. Although there was a slight increase in the possibility of a type I error, we did not correct for testing multiple outcomes [51]. Because linear mixed models handle missing data well via maximum likelihood estimation, missing values in outcome variables were not imputed before statistical analysis [52].

Results

Participant characteristics

Participant characteristics are presented in Table 1. In total, 38 participants were included in the study. Three participants dropped out before initiation of the study diets due to the following reasons: *1*) a medical condition unrelated to the study procedures (n = 1), *2*) a muscle biopsy that could not be performed (n = 1), or *3*) not willing to continue due to symptoms of dizziness resulting from the D_2O doses consumed on the dosing day (n = 1). One participant dropped out on the first morning of the dietary intervention period due to gastrointestinal discomfort (Supplemental Figure 1). Therefore, data of 34 participants were eventually available to be included in the analysis. Approximately half (53%) of the participants were male. Mean age and BMI of the participants were 72 ± 4 y and 26.7 ± 3.1 kg/m^2. Participants showed a habitual daily step count of 12,460 ± 4512, relating to an average daily activity level of 1.16 ± 0.15 metabolic equivalents. Both remained unchanged throughout the study period (P > 0.05). Habitual daily sedentary time was 408 ± 126 min/d at baseline, which significantly decreased with 27 ± 7 and 20 ± 7 min/d from baseline during the first and second period of the study (P = 0.0009), respectively, with no significant differences between the 2 study periods (P > 0.05). Overall, the baseline characteristics were similar between the sequence groups. Dizziness on the D_2O dosing day was reported by 9 participants. Gastrointestinal discomfort during the dietary intervention was reported 5 times. The habitual dietary intake of the participants, and the daily nutrient composition of the study diets are presented in Table 2.

TABLE 1
Baseline participant characteristics of the total study population and per sequence group.

	Total (n = 34)	VEG/OMNI (n = 16)	OMNI/VEG (n = 18)
Male, n (%)	18 (53%)	8 (50%)	10 (56%)
Age (y)	72 ± 4	70 ± 4	74 ± 3
BMI (kg/m^2)	26.7 ± 3.1	25.8 ± 3.2	27.3 ± 2.9
Lean body mass (kg)	49.1 ± 7.8	48.5 ± 6.4	49.7 ± 9.0
Fat percentage (%)	33.5 ± 5.9	32.4 ± 5.9	34.6 ± 6.0
Handgrip strength			
Left (kg)	30.8 ± 7.5	31.2 ± 6.5	30.5 ± 8.4
Right (kg)	31.4 ± 8.9	31.6 ± 8.1	31.2 ± 9.8
Daily step count	12,460 ± 4512	13,694 ± 4412	12,152 ± 4491
METs, kcal/(kg/h)	1.16 ± 0.15	1.16 ± 0.16	1.14 ± 0.14
Sedentary time (min/d)	408 ± 126	445 ± 109	367 ± 130
Smoking			
Never smoked	21 (62%)	12 (75%)	9 (50%)
Former smoker	11 (32%)	4 (25%)	7 (39%)
Current smoker	2 (6%)	0 (0%)	2 (11%)
Vitamin D use, n (%)	8 (24%)	3 (19%)	5 (28%)
Statin use, n (%)	4 (11%)	1 (6%)	3 (17%)
Blood pressure medication use, n (%)	7 (21%)	3 (19%)	4 (22%)

Continuous values presented as mean ± SD.
Abbreviations: MET, metabolic equivalent, OMNI/VEG, the group that consumed the omnivorous diet followed by the vegan diet; VEG/OMNI, the group that consumed the vegan diet followed by the omnivorous diet.

Mixed muscle fractional protein synthesis rates

Plasma ^{2}H-alanine enrichments increased significantly over time from 2.41 ± 0.08 MPE on day 0 to 3.26 ± 0.08 and 3.62 ± 0.08 MPE on day 10 and 20 for all participants (P < 0.0001), and did not significantly differ between the vegan and omnivorous diet (P > 0.05). Similarly, mixed muscle protein-bound ^{2}H-alanine enrichments significantly increased over time (P < 0.0001), and did not differ significantly between diets (P > 0.05). On day 10, mixed muscle protein-bound ^{2}H-alanine

TABLE 2
Habitual dietary intake of the participants, and analyzed macronutrient and amino acid composition of the study diets per day.

	Habitual[1]	Vegan diet	Omnivorous diet
Macronutrients			
Energy (MJ)	9.3 ± 2.3	8.8	9.1
Total protein[2] (en%)	15.5 ± 2.2	19.9	18.1
Total protein[3] (g/kg)	1.1 ± 0.2	1.3	1.2
Total protein (g)	83 ± 18	103	97
Animal protein (% total protein)	55.5 ± 9.7	0	60.4
Plant protein (% total protein)	44.5 ± 9.7	100	39.6
Fat (en%)	37.1 ± 3.7	42.7	39.2
Saturated fat (en%)	13.6 ± 2.5	11.4	12.9
Carbohydrates (en%)	41.9 ± 3.8	37.2	39.4
Fiber (en%)	2.1 ± 0.5	3.7	3.9
Alcohol (en%)	2.9 ± 3.1	0	0
Sodium[4] (mg)	NA	2427	2467
Amino acids (mg/g protein)			
Leucine	NA	70.6	86.1
Lysine	NA	53.5	72.9
Methionine	NA	9.9	16.8
Isoleucine	NA	39.3	46.7
Threonine	NA	33.9	43.1
Valine	NA	46.6	57.7
Tryptophan	NA	11.1	12.6
Phenylalanine	NA	49.1	50.6
Histidine	NA	22.4	31.6
Alanine	NA	42.2	51.2
Arginine	NA	72.9	72.7
Aspartic acid	NA	106	98
Cystine + cysteine	NA	12.4	11.1
Glutamic acid	NA	188	212
Glycine	NA	41.6	46.5
Tyrosine	NA	32.3	39.5
Serine	NA	48.3	51.4
Proline	NA	47.7	68.1
Ornithine	NA	0	0
Total	NA	928	1069
Total EAA	NA	336	418
EAA/AA ratio	NA	0.34	0.36

Abbreviations: AA, amino acid; EAA, essential amino acid; NA, not assessed.

[1] Habitual dietary intake as measured with a food frequency questionnaire at baseline, values presented as mean ± SD.

[2] Protein content of the study diets estimated by determining nitrogen content using the DUMAS approach and using the nitrogen-to-protein conversion factor of 6.25.

[3] Values calculated based on: habitual diet, mean body weight of total study population (78.5 kg); study diets, mean body weight of participants allocated to energy group 9 (79.1 kg).

[4] Sodium content was not analyzed, quantities are based on the a priori calculated nutrient content as described in the methods section.

enrichments were 0.36 ± 0.04 MPE and 0.38 ± 0.04 MPE after the vegan and omnivorous diet, respectively. These values were 0.63 ± 0.04 MPE and 0.65 ± 0.04 MPE after the vegan and omnivorous diet on day 20. There was no significant difference in mixed muscle FSR between the vegan and the omnivorous diet (vegan 1.23 ± 0.04 %/d; omnivorous, 1.29 ± 0.04 %/d; $P = 0.2542$). There was a significant period effect ($P = 0.048$), but the effect of diet did not differ per period (no significant carry-over effect: $P > 0.05$). The mixed muscle FSRs during the vegan and omnivorous diets are presented in Figure 2.

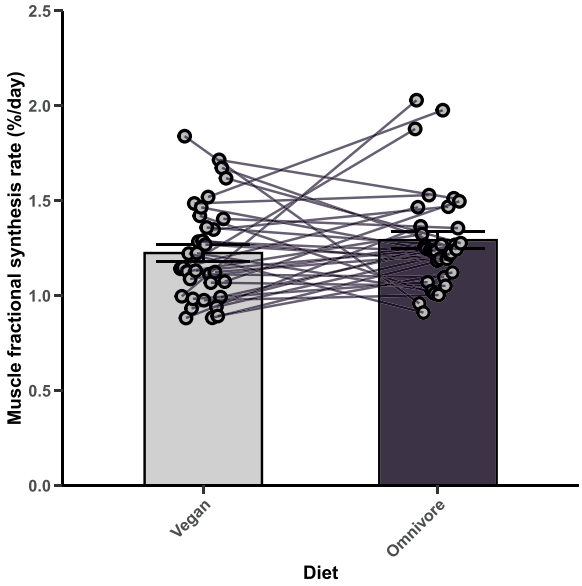

FIGURE 2. Daily mixed muscle protein fractional synthesis rates (%/d) on the vegan and omnivorous diet. Data in figure presented as mean ± SE and individual values. Statistical analysis with linear mixed models did not show any difference between the diets ($P = 0.2542$).

Cardiometabolic risk factors and body weight

Table 3 shows the difference in effects between the vegan and omnivorous diets on body mass and cardiometabolic outcome measures. Body mass was significantly lower on day 20 of the study period than on day 10 ($P = 0.0063$), but did not differ between diets ($P > 0.05$). Plasma LDL cholesterol (Δ0.23 ± 0.03, $P < 0.0001$), HDL cholesterol (Δ0.03 ± 0.14, $P = 0.0387$), and

TABLE 3
Cardiometabolic outcome measures.

	Baseline[1]	Vegan	Omnivorous	*P* value (vegan vs. omnivore)
Body mass (kg)	79.3 ± 2.0	77.2 ± 0.1	77.2 ± 0.1	0.7868
LDL-c (mmol/L)	3.11 ± 0.17	2.46 ± 0.05	2.68 ± 0.05	<0.0001
HDL-c (mmol/L)	1.60 ± 0.08	1.32 ± 0.02	1.35 ± 0.02	0.0387
Triglycerides (mmol/L)	1.23 ± 0.01	1.08 ± 0.04	1.05 ± 0.04	0.2803
Total cholesterol (mmol/L)	5.35 ± 0.19	4.32 ± 0.06	4.58 ± 0.06	<0.0001
Glucose (mmol/L)	5.57 ± 0.01	5.31 ± 0.06	5.40 ± 0.06	0.1584
Insulin (mU/L)	11.63 ± 2.28	9.30 ± 0.54	9.53 ± 0.54	0.6173
HOMA-IR	2.86 ± 0.55	2.20 ± 0.15	2.30 ± 0.15	0.4179
Systolic blood pressure (mmHg)	133 ± 3	130 ± 2	127 ± 2	0.1994
Diastolic blood pressure (mmHg)	72 ± 1	69 ± 1	68 ± 1	0.2930

Values for the study diets are presented as estimated marginal means and their SEs.

[1] Baseline values are crude values, presented as mean and their SEs. *P* values represent the significance of difference between the vegan and omnivorous diets and are obtained using linear mixed models (random factor: participants; fixed factors: diet, baseline value, period, sequence).

total cholesterol (Δ0.25 ± 0.04, $P < 0.0001$) levels were significantly lower succeeding the vegan diet than succeeding the omnivorous diet. There were no significant differences between the omnivorous and the vegan diet in plasma triglyceride, glucose, and insulin levels, HOMA-IR, and systolic and diastolic blood pressure ($P > 0.05$). There were significant effects of period for plasma HDL cholesterol ($P = 0.0005$), and plasma triglyceride levels ($P = 0.0012$), but the effect of diet was not different in the 2 periods for all cardiometabolic outcomes (no significant carryover effects: $P > 0.05$).

Appetite

We observed small, but significant, differences between the omnivorous and the vegan diet in most measures of appetite (Supplemental Table 4). Fullness and satiation were significantly higher after consumption of the hot meal during the vegan diet than during the omnivorous diet (fullness, Δ4 ± 1 mm, $P = 0.0003$; satiation, Δ5 ± 1 mm, $P < 0.0001$). Fullness was additionally significantly higher during vegan diet before consumption of the hot meal (Δ2 ± 1 mm, $P = 0.0234$). Accordingly, hunger and the desire to eat, both before (hunger Δ−2 ± 1 mm, $P = 0.0463$; desire to eat Δ−3 ± 1 mm, $P = 0.0339$) and after (hunger Δ−3 ± 1 mm, $P = 0.0002$; desire to eat Δ−3 ± 1 mm, $P < 0.0001$) the consumption of the hot meal, were lower during the vegan diet. The prospective food intake after consumption of the hot meal was significantly lower during the vegan diet as well (Δ−5 ± 1 mm, $P < 0.0001$).

Discussion

In contrast with our hypothesis, we demonstrate that a well-balanced vegan diet that is carefully composed to contain a feasible quantity of protein from a large variety of different plant-based protein sources does not compromise daily integrated mixed MPS rates when compared with an isocaloric, isonitrogenous omnivorous diet in physically active, older individuals. Recent work from our groups has shown that ingestion of a single omnivorous meal resulted in 47% higher postprandial mixed MPS rates when compared with the ingestion of an isocaloric and isonitrogenous vegan meal [30]. In this study, we observed that these differences in postprandial mixed MPS rates do not necessarily translate to lower daily mixed MPS rates when a vegan diet is implemented for a more prolonged period of 10 d in physically active, older adults.

The disparity in findings between this study and our previous work [30] may be attributed to several factors. First, here, we implemented a deuterated water protocol to measure cumulative, integrated daily mixed MPS rates. Hence, free-living basal and post-prandial rates were aggregated in response to the consumption of the vegan and omnivorous diets over the course of 10 d. In contrast, our previous work focused on the postprandial response to a single meal in a laboratory setting. Second, the postprandial rise in plasma essential AA after ingestion of the vegan meal assessed in our previous work may have sustained beyond the 6-h assessment period. The duration of the assessment period may thus have underestimated the anabolic potential of the respective meal. Related to this, there may be a cumulative anabolic effect of successive meals that are consumed throughout the day. Third, the deuterated water approach allowed our participants to maintain their habitual daily activities. The participants in this study were highly physically active, with an average step count of ~12,500 steps/d, whereas our previous work was performed in a resting state in a laboratory setting [30]. Physical activity is a pivotal determinant for the stimulation of MPS [53,54]. It has been observed that merely increasing daily step count can significantly increase daily MPS rates in older women [54]. Physical activity increases the sensitivity of skeletal muscle tissue to the anabolic response to dietary protein intake [55]. This suggests that, when physical activity levels are adequate, anabolic stimulation of the muscle can be achieved despite a diet that exhibits a relatively low-protein quality. Finally, and importantly, the diets in our present study included daily use of soy-based dairy alternatives as well as regular use of meat analogs that were predominantly based on pea protein isolates, which, although displaying a lower protein quality than their animal-based comparatives, both display good protein digestibility kinetics (Digestible Indispensable Amino Acid scores: 117% and 83%, respectively) [56,57].

A major strength of our present work is that the highly controlled diets were formulated to correspond in macronutrient composition, allowing us to assess the inherent differences in anabolic properties between 2 diets that predominantly differed in their protein sources. The small difference in analyzed protein contents between the diets (vegan, 19.9 en%, 1.3 g/kg compared with omnivore, 18.1 en%, 1.2 g/kg) could be considered negligible. Particularly when considering that the protein content was determined by multiplying the nitrogen content by the generally accepted nitrogen-to-protein conversion factor of 6.25. This factor tends to overestimate the protein content, especially for plant-based foods [42,58]. Although the diets typically do not correspond in their AA profiles, nearly all essential AAs were provided in adequate quantities according to the FAO/WHO reference values, with the exception of the sulfur-containing AAs methionine and cysteine, which, taken together, were below the recommendation of 23 mg/g protein for the vegan diet [59]. Nevertheless, this reference value is relative, and based on daily protein requirements of 0.8 g/kg/d. Because our vegan diet exhibited a higher quantity of protein, the absolute total intake of these AAs may still have been adequate in our participants although consuming the vegan diet.

We recognize the limitation that we did not measure muscle protein breakdown, as accurate assessments are difficult to achieve [60]. Skeletal muscle mass is maintained or adapted via a (dis)balance between MPS and muscle protein breakdown. Although it has been suggested that muscle protein breakdown is less responsive to feeding than MPS [61], it remains unclear how the short-term MPS response to diet translates into longer-term adaptations of skeletal muscle. Long-term trials that incorporate high standard measures of skeletal muscle mass and function are therefore required to confirm our findings. Still, the present findings provide valuable insights into the difference in the mechanistic response of skeletal muscle tissue between a vegan and an omnivorous diet in older individuals. Furthermore, the lack of a washout period could be considered as a limitation. Nevertheless, our counter-balanced, randomized, cross-over design allowed us to test for potential carryover effects, which were all insignificant. A third limitation is that this study had an open-label research design due to logistical aspects, which increases the risk of bias. Nevertheless, with the highly controlled

fashion of our study, wide use of objective parameters and by performing the statistical analyses for all outcome measures in a blinded manner, we aimed to limit bias that could have occurred as a result of our open-label design. A final limitation is that the vegan diet provided in our study is unlikely to reflect the nutrient composition of a self-assembled vegan diet. Our vegan diet was designed to contain equivalent amounts of macronutrients as the omnivorous diet, resulting in meals with large proportions of protein containing components. In practice, self-assembled vegan diets may display reduced anabolic properties, because they will likely be lower in protein quantity and quality. A randomized controlled trial with a self-composed, low fat, vegan diet observed that participants significantly reduced their daily energy and protein intake by ~2 MJ (~490 kcal) and 49.5 g of protein [62]. That involuntary reduction in energy and protein intake induced an average loss of 2.1 (95% confidence interval: 2.4, 1.8) kg lean body mass after 16 wk. Such a substantial reduction in energy and protein intake is to be expected, especially in older individuals, considering the low energy and protein density of many plant-based foods and the concurrent high prevalence of the well-established anorexia of aging [19].

The high abundance of dietary fiber in most plant-based foods concurrently indicates that a vegan diet may additionally reduce appetite [63–65]. Although we indeed observed small differences in measures of appetite between our study diets (Supplemental Table 2), these differences are not considered relevant (≤10%) and their significance is likely attributed to the high quantity of measurements performed [46]. The similar quantity of dietary fiber in our study diets (vegan, 3.7 en% compared with omnivorous, 3.9 en%) is a plausible explanation for the relatively similar response in measures of appetite. Previous studies only observed substantial differences in appetite between plant- and animal-based foods when the test foods exhibited substantial differences in fiber content [63–67]. Consuming more products that are high in fiber in a self-assembled vegan diet may therefore still impact appetite. It should thereby be noted that many high-protein sources from plant origin, such as legumes and wheat, are important sources of dietary fiber as well.

The lower LDL-cholesterol, and thereby total cholesterol, concentrations succeeding the vegan diet are likely attributed to a high quantity of phytosterols and low quantity of dietary cholesterol within the diet [68], and is in accordance with previous intervention studies that observed reductions of ~20%–25% succeeding a vegan diet [69,70]. Nonetheless, to the best of our knowledge, our study is the first randomized controlled trial to observe substantial improvements in LDL-cholesterol after a vegan diet with a duration of merely 10 d. Although the vegan diet led to a significantly lower HDL-cholesterol as well, this difference was only ~2% (Δ0.03), and, as such, not considered clinically relevant. Furthermore, the absence of a difference between the diets on blood pressure is in line with previous meta-analyses that did not observe significant differences in blood pressure after a vegan diet in comparison with less restrictive diets [71–73]. It should be noted, however, that, although serving as the control diet, the omnivorous diet was carefully composed according to the national dietary guidelines and may have therefore affected the cardiometabolic outcome measures in a positive manner as well.

To conclude, our findings demonstrate that a well-balanced vegan diet does not compromise daily integrated mixed MPS rates when compared with an isocaloric, isonitrogenous omnivorous diet in healthy, active, older men and women. Furthermore, we demonstrate that a vegan diet can lead to significantly greater reductions LDL-cholesterol and total cholesterol concentrations than an omnivorous diet. Although these results are promising, long-term studies are required to assess the effect of a vegan diet on muscle mass and function in a less controlled manner. In this regard, it would be especially relevant to properly assess whether nutrient, particularly protein, requirements are met on a self-assembled vegan diet. Furthermore, the anabolic potential of a vegan diet in sedentary and more frail older individuals remains to be established.

Acknowledgments

We thank Susanne Wildemast, Keeva Loughlin, the students, and the support staff from WUR for their invaluable assistance and support. We thank Dr Claudia Berends, Dr Sietske Okkema, Dr Tessa Veerbeek, and Dr Mitchell Harker for performing the muscle biopsies. We additionally thank the participants for their time and commitment.

Author contributions

The authors' responsibilities were as follows – JD, PG, LJCvL, LCPGMdG: designed the research; JD, ES, JCG: designed the study diets; JD, ES, PG: conducted research; JD, PJMP: analyzed data; JD, PG, LJCvL, LCPGMdG: wrote the paper; JD, PG, LJCvL, LCPGMdG: had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

JD, PJMP, ES, and JCG declare that they have no competing interests. LJCvL has received research grants, consulting fees, speaking honoraria, or a combination of these for research on the impact of exercise and nutrition on muscle metabolism, which include funding from companies that produce dairy, meat, and/or plant-derived proteins. A full overview on research funding is provided at: https://www.maastrichtuniversity.nl/l.vanloon. LCPGMdG and PG have received grants for research on the impact of nutrition (and exercise) on muscle metabolism, from the Dutch government (ZonMw), the European Union (H2020), and Top Institute Food and Nutrition, which precompetitively includes funding from food industry (small and medium sized enterprises and larger enterprises). None of these organizations affected the design and the outcomes of the study.

Funding

The study was supported by a grant from the Regio Deal Foodvalley (162135). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2024.12.019.

References

[1] A.J. Mayhew, K. Amog, S. Phillips, G. Parise, P.D. McNicholas, R.J. de Souza, et al., The prevalence of sarcopenia in community-dwelling older adults, an exploration of differences between studies and within definitions: a systematic review and meta-analyses, Age Ageing 48 (1) (2019) 48–56, https://doi.org/10.1093/ageing/afy106.

[2] D. Scott, A. Hayes, K.M. Sanders, D. Aitken, P.R. Ebeling, G. Jones, Operational definitions of sarcopenia and their associations with 5-year changes in falls risk in community-dwelling middle-aged and older adults, Osteoporos. Int. 25 (1) (2014) 187–193, https://doi.org/10.1007/s00198-013-2431-5.

[3] S. Gariballa, A. Alessa, Sarcopenia: prevalence and prognostic significance in hospitalized patients, Clin. Nutr. 32 (5) (2013) 772–776, https://doi.org/10.1016/j.clnu.2013.01.010.

[4] F. Landi, A.J. Cruz-Jentoft, R. Liperoti, A. Russo, S. Giovannini, M. Tosato, et al., Sarcopenia and mortality risk in frail older persons aged 80 years and older: results from ilSIRENTE study, Age Ageing 42 (2) (2013) 203–209, https://doi.org/10.1093/ageing/afs194.

[5] D.A. Traylor, S.H.M. Gorissen, S.M. Phillips, Perspective: protein requirements and optimal intakes in aging: are we ready to recommend more than the recommended daily allowance? Adv. Nutr. 9 (3) (2018) 171–182, https://doi.org/10.1093/advances/nmy003.

[6] J. Bauer, G. Biolo, T. Cederholm, M. Cesari, A.J. Cruz-Jentoft, J.E. Morley, et al., Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE study group, J. Am. Med. Dir. Assoc. 14 (8) (2013) 542–559, https://doi.org/10.1016/j.jamda.2013.05.021.

[7] D. Paddon-Jones, W.W. Campbell, P.F. Jacques, S.B. Kritchevsky, L.L. Moore, N.R. Rodriguez, et al., Protein and healthy aging, Am. J. Clin. Nutr. 101 (6) (2015) 1339S–1345S, https://doi.org/10.3945/ajcn.114.084061.

[8] N.E. Deutz, J.M. Bauer, R. Barazzoni, G. Biolo, Y. Boirie, A. Bosy-Westphal, et al., Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group, Clin. Nutr. 33 (6) (2014) 929–936, https://doi.org/10.1016/j.clnu.2014.04.007.

[9] B.T. Wall, S.H. Gorissen, B. Pennings, R. Koopman, B.B. Groen, L.B. Verdijk, et al., Aging is accompanied by a blunted muscle protein synthetic response to protein ingestion, PLOS ONE 10 (11) (2015) e0140903, https://doi.org/10.1371/journal.pone.0140903.

[10] S. van Vliet, N.A. Burd, L.J. van Loon, The skeletal muscle anabolic response to plant- versus animal-based protein consumption, J. Nutr. 145 (9) (2015) 1981–1991, https://doi.org/10.3945/jn.114.204305.

[11] L. Herreman, P. Nommensen, B. Pennings, M.C. Laus, Comprehensive overview of the quality of plant- and animal-sourced proteins based on the digestible indispensable amino acid score, Food Sci. Nutr. 8 (10) (2020) 5379–5391, https://doi.org/10.1002/fsn3.1809.

[12] D.J. Millward, D.K. Layman, D. Tomé, G. Schaafsma, Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health, Am. J. Clin. Nutr. 87 (5) (2008) 1576S–1581S, https://doi.org/10.1093/ajcn/87.5.1576S.

[13] G. Sarwar Gilani, C. Wu Xiao, K.A. Cockell, Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality, Br. J. Nutr. 108 (Suppl 2) (2012) S315–S332, https://doi.org/10.1017/S0007114512002371.

[14] G.S. Gilani, K.A. Cockell, E. Sepehr, Effects of antinutritional factors on protein digestibility and amino acid availability in foods, J. AOAC Int. 88 (3) (2005) 967–987, https://doi.org/10.1093/jaoac/88.3.967.

[15] Y. Yokoyama, S.M. Levin, N.D. Barnard, Association between plant-based diets and plasma lipids: a systematic review and meta-analysis, Nutr. Rev. 75 (9) (2017) 683–698, https://doi.org/10.1093/nutrit/nux030.

[16] J.R. Benatar, R.A.H. Stewart, Cardiometabolic risk factors in vegans; a meta-analysis of observational studies, PLOS ONE 13 (12) (2018) e0209086, https://doi.org/10.1371/journal.pone.0209086.

[17] P. Schmidt, Promoting healthy and sustainable diets in the EU, European Economic and Social Comittee: Section for Agriculture, Rural Development and the Environment, Brussels, 2019.

[18] W. Willett, J. Rockström, B. Loken, M. Springmann, T. Lang, S. Vermeulen, et al., Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems, Lancet 393 (10170) (2019) 447–492, https://doi.org/10.1016/S0140-6736(18)31788-4.

[19] J. Domić, P. Grootswagers, L.J.C. van Loon, L. de Groot, Perspective: vegan diets for older adults? A perspective on the potential impact on muscle mass and strength, Adv. Nutr. 13 (3) (2022) 712–725, https://doi.org/10.1093/advances/nmac009.

[20] J.E. Tang, D.R. Moore, G.W. Kujbida, M.A. Tarnopolsky, S.M. Phillips, Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men, J. Appl. Physiol. 107 (3) (2009) 987–992, https://doi.org/10.1152/japplphysiol.00076.2009.

[21] S.B. Wilkinson, M.A. Tarnopolsky, M.J. Macdonald, J.R. Macdonald, D. Armstrong, S.M. Phillips, Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage, Am. J. Clin. Nutr. 85 (4) (2007) 1031–1040, https://doi.org/10.1093/ajcn/85.4.1031.

[22] Y. Yang, T.A. Churchward-Venne, N.A. Burd, L. Breen, M.A. Tarnopolsky, S.M. Phillips, Myofibrillar protein synthesis following ingestion of soy protein isolate at rest and after resistance exercise in elderly men, Nutr. Metab. (Lond). 9 (1) (2012) 57, https://doi.org/10.1186/1743-7075-9-57.

[23] P.J.M. Pinckaers, I.W.K. Kouw, F.K. Hendriks, J.M.X. van Kranenburg, L.C.P.G.M. de Groot, L.B. Verdijk, et al., No differences in muscle protein synthesis rates following ingestion of wheat protein, milk protein, and their protein blend in healthy, young males, Br. J. Nutr. 126 (12) (2021) 1832–1842, https://doi.org/10.1017/S0007114521000635.

[24] P.J.M. Pinckaers, F.K. Hendriks, W.J.H. Hermans, J.P.B. Goessens, J.M. Senden, J.M.X. van Kranenburg, et al., Potato protein ingestion increases muscle protein synthesis rates at rest and during recovery from exercise in humans, Med. Sci. Sports Exerc. 54 (2022) 1572–1581, https://doi.org/10.1249/MSS.0000000000002937.

[25] T.A. Churchward-Venne, P.J.M. Pinckaers, J.S.J. Smeets, W.M. Peeters, A.H. Zorenc, H. Schierbeek, et al., Myofibrillar and mitochondrial protein synthesis rates do not differ in young men following the ingestion of carbohydrate with whey, soy, or leucine-enriched soy protein after concurrent resistance - and endurance -type exercise, J. Nutr. 149 (2) (2019) 210–220, https://doi.org/10.1093/jn/nxy251.

[26] S.Y. Oikawa, R. Bahniwal, T.M. Holloway, C. Lim, J.C. McLeod, C. McGlory, et al., Potato protein isolate stimulates muscle protein synthesis at rest and with resistance exercise in young women, Nutrients 12 (5) (2020) 1235, https://doi.org/10.3390/nu12051235.

[27] P.J.M. Pinckaers, I.W.K. Kouw, S.H.M. Gorissen, L.H.P. Houben, J.M. Senden, W.K.H.W. Wodzig, et al., The muscle protein synthetic response to the ingestion of a plant-derived protein blend does not differ from an equivalent amount of milk protein in healthy young males, J. Nutr. 152 (12) (2023) 2734–2743, https://doi.org/10.1093/jn/nxac222.

[28] S.H.M. Gorissen, J.J.R. Crombag, J.M.G. Senden, W.A.H. Waterval, J. Bierau, L.B. Verdijk, et al., Protein content and amino acid composition of commercially available plant-based protein isolates, Amino Acids 50 (12) (2018) 1685–1695, https://doi.org/10.1007/s00726-018-2640-5.

[29] A.J. Monteyne, M.O.C. Coelho, A.J. Murton, D.R. Abdelrahman, J.R. Blackwell, C.P. Koscien, et al., Vegan and omnivorous high protein diets support comparable daily myofibrillar protein synthesis rates and skeletal muscle hypertrophy in young adults, J. Nutr. 153 (6) (2023) 1680–1695, https://doi.org/10.1016/j.tjnut.2023.02.023.

[30] P.J.M. Pinckaers, J. Domić, H.L. Petrick, A.M. Holwerda, J. Trommelen, F.K. Hendriks, et al., Higher muscle protein synthesis rates following ingestion of an omnivorous meal compared with an isocaloric and isonitrogenous vegan meal in healthy, older adults, J. Nutr. 154 (7) (2024) 2120–2132, https://doi.org/10.1016/j.tjnut.2023.11.004.

[31] I.Y. Kim, Y.A. Shin, S.E. Schutzler, G. Azhar, R.R. Wolfe, A.A. Ferrando, Quality of meal protein determines anabolic response in older adults, Clin. Nutr. 37 (6 Pt A) (2018) 2076–2083, https://doi.org/10.1016/j.clnu.2017.09.025.

[32] A.J. Monteyne, M.V. Dunlop, D.J. Machin, M.O.C. Coelho, G.F. Pavis, C. Porter, et al., 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, Br. J. Nutr. 126 (5) (2021) 674–684, https://doi.org/10.1017/S0007114520004481.

[33] J.C. Gerdessen, K.J. Borgonjen-van den Berg, A linear programming based method for designing menus for controlled feeding trials, Am. J. Clin. Nutr. 117 (2) (2023) 408–413, https://doi.org/10.1016/j.ajcnut.2022.11.006.

[34] NEVO-tabel, Nederlands Voedingsstoffenbestand 2019, RIVM/Voedingscentrum, Den Haag, 2019.

[35] S. Meijboom, M.T. van Houts-Streppel, C. Perenboom, E. Siebelink, A.M. van de Wiel, A. Geelen, et al., Evaluation of dietary intake assessed by the Dutch self-administered web-based dietary 24-h recall tool (Compl-eat™) against interviewer-administered telephone-based 24-h recalls, J. Nutr. Sci. 6 (2017) e49, https://doi.org/10.1017/jns.2017.45.
[36] W.N. Schofield, Predicting basal metabolic rate, new standards and review of previous work, Hum. Nutr. Clin. Nutr. 39 (Suppl 1) (1985) 5–41.
[37] E. Siebelink, A. Geelen, J.H. de Vries, Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults, Br. J. Nutr. 106 (2) (2011) 274–281, https://doi.org/10.1017/S0007114511000067.
[38] G.C.W. Wendel-Vos, A.J. Schuit, W.H.M. Saris, D. Kromhout, Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity, J. Clin. Epidemiol. 56 (12) (2003) 1163–1169, https://doi.org/10.1016/S0895-4356(03)00220-8.
[39] Dutch dietary guidelines, Health Council of the Netherlands, The Hague, 2015.
[40] M. Tieland, K.J. Borgonjen-Van den Berg, L.J. Van Loon, L.C. de Groot, Dietary protein intake in Dutch elderly people: a focus on protein sources, Nutrients 7 (12) (2015) 9697–9706, https://doi.org/10.3390/nu7125496.
[41] L.M. Hengeveld, A.D.A. Pelgröm, M. Visser, J.M.A. Boer, A. Haveman-Nies, H.A.H. Wijnhoven, Comparison of protein intake per eating occasion, food sources of protein and general characteristics between community-dwelling older adults with a low and high protein intake, Clin. Nutr. ESPEN. 29 (2019) 165–174, https://doi.org/10.1016/j.clnesp.2018.10.013.
[42] D.B. Jones, Factors for converting percentages of nitrogen in foods and feeds into percentages of protein, Agriculture Udo (1941). Washington, D.C.
[43] A.M. Holwerda, K.J.M. Paulussen, M. Overkamp, J.S.J. Smeets, A.P. Gijsen, J.P.B. Goessens, et al., Daily resistance-type exercise stimulates muscle protein synthesis in vivo in young men, J. Appl. Physiol. 124 (1) (2018) 66–75, https://doi.org/10.1152/japplphysiol.00610.2017, 1985.
[44] J. Bergstrom, Percutaneous needle biopsy of skeletal muscle in physiological and clinical research, Scand. J. Clin. Lab. Invest. 35 (7) (1975) 609–616, https://doi.org/10.3109/00365517509095787.
[45] T.M. Wallace, J.C. Levy, D.R. Matthews, Use and abuse of HOMA modeling, Diabetes Care 27 (6) (2004) 1487–1495, https://doi.org/10.2337/diacare.27.6.1487.
[46] J. Blundell, C. de Graaf, T. Hulshof, S. Jebb, B. Livingstone, A. Lluch, et al., Appetite control: methodological aspects of the evaluation of foods, Obes. Rev. 11 (3) (2010) 251–270, https://doi.org/10.1111/j.1467-789X.2010.00714.x.
[47] A.M. Holwerda, M. Overkamp, K.J.M. Paulussen, J.S.J. Smeets, J. van Kranenburg, E.M.P. Backx, et al., Protein supplementation after exercise and before sleep does not further augment muscle mass and strength gains during resistance exercise training in active older men, J. Nutr. 148 (11) (2018) 1723–1732, https://doi.org/10.1093/jn/nxy169.
[48] Castor EDC, Castor Electronic Data Capture [Internet] (2019) [cited 12 June, 2024]. Available from: https://castoredc.com.
[49] C.Y. Lim, J. In, Considerations for crossover design in clinical study, Korean J. Anesthesiol. 74 (4) (2021) 293–299, https://doi.org/10.4097/kja.21165.
[50] T. Wang, J. Malone, H. Fu, C. Heilmann, Y. Qu, W.J. Huster, Crossover design and its application in late-phase diabetes studies, J. Diabetes 8 (5) (2016) 610–618, https://doi.org/10.1111/1753-0407.12412.
[51] K.S. Petersen, P.M. Kris-Etherton, G.P. McCabe, G. Raman, J.W. Miller, K.C. Maki, Perspective: planning and conducting statistical analyses for human nutrition randomized controlled trials: ensuring data quality and integrity, Adv. Nutr. 12 (5) (2021) 1610–1624, https://doi.org/10.1093/advances/nmab045.
[52] J. Twisk, M. de Boer, W. de Vente, M. Heymans, Multiple imputation of missing values was not necessary before performing a longitudinal mixed-model analysis, J. Clin. Epidemiol. 66 (9) (2013) 1022–1028, https://doi.org/10.1016/j.jclinepi.2013.03.017.
[53] B. Pennings, R. Koopman, M. Beelen, J.M. Senden, W.H. Saris, L.J. van Loon, Exercising before protein intake allows for greater use of dietary protein-derived amino acids for de novo muscle protein synthesis in both young and elderly men, Am. J. Clin. Nutr. 93 (2) (2011) 322–331, https://doi.org/10.3945/ajcn.2010.29649.
[54] T. Stokes, Y. Mei, F. Seo, J. McKendry, C. McGlory, S.M. Phillips, Dairy and dairy alternative supplementation increase integrated myofibrillar protein synthesis rates, and are further increased when combined with walking in healthy older women, J. Nutr. 152 (1) (2022) 68–77, https://doi.org/10.1093/jn/nxab358.
[55] N.A. Burd, S.H. Gorissen, L.J. van Loon, Anabolic resistance of muscle protein synthesis with aging, Exerc. Sport. Sci. Rev. 41 (3) (2013) 169–173, https://doi.org/10.1097/JES.0b013e318292f3d5.
[56] Y. Reynaud, C. Buffière, B. Cohade, M. Vauris, K. Liebermann, N. Hafnaoui, et al., True ileal amino acid digestibility and digestible indispensable amino acid scores (DIAASs) of plant-based protein foods, Food Chem 338 (2021) 128020, https://doi.org/10.1016/j.foodchem.2020.128020.
[57] N.S. Fanelli, H.M. Bailey, T.W. Thompson, R. Delmore, M.N. Nair, H.H. Stein, Digestible indispensable amino acid score (DIAAS) is greater in animal-based burgers than in plant-based burgers if determined in pigs, Eur. J. Nutr. 61 (1) (2022) 461–475, https://doi.org/10.1007/s00394-021-02658-1.
[58] F. Mariotti, D. Tomé, P.P. Mirand, Converting nitrogen into protein–beyond 6.25 and Jones' factors, Crit. Rev. Food Sci. Nutr. 48 (2) (2008) 177–184, https://doi.org/10.1080/10408390701279749.
[59] Dietary protein quality evaluation in human nutrition: report of the FAO expert consultation, FAO Food Nutr. Pap. 92 (2013) 1–66.
[60] K.D. Tipton, D.L. Hamilton, I.J. Gallagher, Assessing the role of muscle protein breakdown in response to nutrition and exercise in humans, Sports Med 48 (1) (2018) 53–64, https://doi.org/10.1007/s40279-017-0845-5.
[61] R.W. Morton, C. McGlory, S.M. Phillips, Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy, Front. Physiol. 6 (2015) 245, https://doi.org/10.3389/fphys.2015.00245.
[62] H. Kahleova, K.F. Petersen, G.I. Shulman, J. Alwarith, E. Rembert, A. Tura, et al., Effect of a low-fat vegan diet on body weight, insulin sensitivity, postprandial metabolism, and intramyocellular and hepatocellular lipid levels in overweight adults: a randomized clinical trial, JAMA Netw. Open 3 (11) (2020) e2025454, https://doi.org/10.1001/jamanetworkopen.2020.25454.
[63] M.D. Kristensen, N.T. Bendsen, S.M. Christensen, A. Astrup, A. Raben, Meals based on vegetable protein sources (beans and peas) are more satiating than meals based on animal protein sources (veal and pork) - a randomized cross-over meal test study, Food Nutr. Res. 60 (2016) 32634, https://doi.org/10.3402/fnr.v60.32634.
[64] W.H. Turnbull, J. Walton, A.R. Leeds, Acute effects of mycoprotein on subsequent energy intake and appetite variables, Am. J. Clin. Nutr. 58 (4) (1993) 507–512, https://doi.org/10.1093/ajcn/58.4.507.
[65] V.J. Burley, A.W. Paul, J.E. Blundell, Influence of a high-fibre food (myco-protein) on appetite: effects on satiation (within meals) and satiety (following meals), Eur. J. Clin. Nutr. 47 (6) (1993) 409–418.
[66] S.M. Douglas, T.R. Lasley, H.J. Leidy, Consuming beef vs. soy protein has little effect on appetite, satiety, and food intake in healthy adults, J. Nutr. 145 (5) (2015) 1010–1016, https://doi.org/10.3945/jn.114.206987.
[67] B.E. Bayham, F.L. Greenway, W.D. Johnson, N.V. Dhurandhar, A randomized trial to manipulate the quality instead of quantity of dietary proteins to influence the markers of satiety, J. Diabetes Complications 28 (4) (2014) 547–552, https://doi.org/10.1016/j.jdiacomp.2014.02.002.
[68] I. Demonty, R.T. Ras, H.C. van der Knaap, G.S. Duchateau, L. Meijer, P.L. Zock, et al., Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake, J. Nutr. 139 (2) (2009) 271–284, https://doi.org/10.3945/jn.108.095125.
[69] J.J. Agren, E. Tvrzicka, M.T. Nenonen, T. Helve, O. Hänninen, Divergent changes in serum sterols during a strict uncooked vegan diet in patients with rheumatoid arthritis, Br. J. Nutr. 85 (2) (2001) 137–139, https://doi.org/10.1079/bjn2000234.
[70] W.H. Ling, M. Laitinen, O. Hänninen, Shifting from conventional diet to an uncooked vegan diet reversibly alters serum lipid and apolipoprotein levels, Nutr. Res. 12 (12) (1992) 1431–1440, https://doi.org/10.1016/S0271-5317(05)80185-X.
[71] A-D. Termannsen, K.K.B. Clemmensen, J.M. Thomsen, O. Nørgaard, L.J. Díaz, S.S. Torekov, et al., Effects of vegan diets on cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials, Obes. Rev. 23 (9) (2022) e13462, https://doi.org/10.1111/obr.13462.
[72] P.D. Lopez, E.H. Cativo, S.A. Atlas, C. Rosendorff, The effect of vegan diets on blood pressure in adults: a meta -analysis of randomized controlled trials, Am. J. Med. 132 (7) (2019) 875–883.e7, https://doi.org/10.1016/j.amjmed.2019.01.044.
[73] J. Gibbs, E. Gaskin, C. Ji, M.A. Miller, F.P. Cappuccio, The effect of plant-based dietary patterns on blood pressure: a systematic review and meta-analysis of controlled intervention trials, J. Hypertens. 39 (1) (2021) 23–37, https://doi.org/10.1097/HJH.0000000000002604.