Sports Medicine
https://doi.org/10.1007/s40279-021-01434-9

ORIGINAL RESEARCH ARTICLE

High-Protein Plant-Based Diet Versus a Protein-Matched Omnivorous Diet to Support Resistance Training Adaptations: A Comparison Between Habitual Vegans and Omnivores

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Accepted: 1 February 2021

Abstract

Background Acute protein turnover studies suggest lower anabolic response after ingestion of plant vs. animal proteins. However, the effects of an exclusively plant-based protein diet on resistance training-induced adaptations are under investigation.

Objective To investigate the effects of dietary protein source [exclusively plant-based vs. mixed diet] on changes in muscle mass and strength in healthy young men undertaking resistance training.

Methods Nineteen young men who were habitual vegans (VEG 26 ± 5 years; 72.7 ± 7.1 kg, 22.9 ± 2.3 kg/m^2) and nineteen young men who were omnivores (OMN 26 ± 4 years; 73.3 ± 7.8 kg, 23.6 ± 2.3 kg/m^2) undertook a 12-week, twice weekly, supervised resistance training program. Habitual protein intake was assessed at baseline and adjusted to 1.6 g kg^{-1} day^{-1} via supplemental protein (soy for VEG or whey for OMN). Dietary intake was monitored every four weeks during the intervention. Leg lean mass, whole muscle, and muscle fiber cross-sectional area (CSA), as well as leg-press 1RM were assessed before (PRE) and after the intervention (POST).

Results Both groups showed significant (all $p < 0.05$) PRE-to-POST increases in leg lean mass (VEG: 1.2 ± 1.0 kg; OMN: 1.2 ± 0.8 kg), rectus femoris CSA (VEG: 1.0 ± 0.6 cm^2; OMN: 0.9 ± 0.5 cm^2), vastus lateralis CSA (VEG: 2.2 ± 1.1 cm^2; OMN: 2.8 ± 1.0 cm^2), vastus lateralis muscle fiber type I (VEG: 741 ± 323 µm^2; OMN: 677 ± 617 µm^2) and type II CSA (VEG: 921 ± 458 µm^2; OMN: 844 ± 638 µm^2), and leg-press 1RM (VEG: 97 ± 38 kg; OMN: 117 ± 35 kg), with no between-group differences for any of the variables (all $p > 0.05$).

Conclusion A high-protein (~ 1.6 g kg^{-1} day^{-1}), exclusively plant-based diet (plant-based whole foods + soy protein isolate supplementation) is not different than a protein-matched mixed diet (mixed whole foods + whey protein supplementation) in supporting muscle strength and mass accrual, suggesting that protein source does not affect resistance training-induced adaptations in untrained young men consuming adequate amounts of protein.

Clinical Trial Registration NCT03907059. April 8, 2019. Retrospectively registered.

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Published online: 18 February 2021

Key Points

Plant-based diets have become increasingly popular over recent years; however, it is currently unknown whether it confers any advantage/disadvantage over an omnivorous diet in supporting exercise-induced muscle adaptations (i.e., gains in muscle strength and mass). It has been proposed that animal proteins are superior to plant proteins in acutely stimulating muscle protein synthesis, suggesting that a sustained vegan diet may be associated with a suboptimal hypertrophic stimulus. However, longitudinal studies in a real-life scenario confirming this hypothesis remain scant.

We showed that vegans and omnivores had similar anabolic adaptations in response to resistance training, as evidenced by multiple layers of reinforcing evidence, from fiber cross-sectional area to muscle functionality, challenging the notion that plant-based proteins have an inferior ability to induce anabolic responses, and suggesting that a high-protein exclusively plant-based diet (whole foods + supplemental plant protein) may adequately support muscle anabolism as long as an optimal protein ingestion (e.g.; 1.6 g kg^{-1} day^{-1}) is achieved.

1 Introduction

Muscle mass is regulated via the balance between rates of muscle protein synthesis (MPS) and breakdown (MPB) [1]. It has been well established that both resistance exercise and protein ingestion can independently [2–5] or synergistically [6, 7] stimulate MPS, over time yielding muscle hypertrophy [8].

The response of MPS depends on post-prandial availability of essential amino acids [9], in particular leucine, which varies significantly between different protein sources [10–12]. In this respect, plant- and animal-based proteins diverge in their essential amino acid (EAA) content [13–15] and digestibility [16], which impact the subsequent amino acid delivery pattern [17]. Several studies have consistently shown lower acute anabolic responses to plant (e.g., soy or wheat) than animal (e.g., whey or milk) protein, in protein-matched conditions combined [10, 12, 18] or not [10, 11, 18] with resistance exercise.

Despite providing an important physiological basis for the understanding of nutrient-mediated muscle anabolism, evidence from acute studies only partially support the recommendation that animal protein is likely more beneficial to support lean mass accrual [19], as no long-term studies have been conducted to investigate if differences in the acute anabolic response as a function of protein source would actually translate into distinct muscle adaptations. Current data regarding resistance training-induced muscle adaptations to different protein sources are limited to the effects of supplemental protein of different sources to mixed diets [20, 21], with no differences being found between supplementary plant and animal proteins in this context;.

To date, no study has addressed the effects of an exclusive plant-based protein diet on the chronic adaptive response to exercise. Therefore, we conducted a parallel-group study to investigate the impact of dietary protein source (plant-based vs. mixed dietary proteins) on RT-induced changes in muscle mass and strength in healthy young habitual vegans and omnivores under conditions of optimal protein intake (1.6 g kg^{-1} day^{-1}) [21]. Considering that the acute intermittent elevations in MPS in response to, and with persistent practice of, resistance exercise in combination with sufficient protein feeding are considered the major drivers of muscle protein accretion and skeletal muscle hypertrophy [22], and given the acute differences in anabolic responses between plant and animal-based proteins, we hypothesized that an exclusive consumption of plant-based dietary protein would be less effective in supporting RT-induced muscle adaptations than an omnivorous diet.

2 Methods

2.1 Experimental Design

In order to evaluate the effects of dietary protein source on resistance training-induced adaptations, we used vegan individuals as a model for exclusive plant-based dietary protein source consumption and omnivores for the animal-based protein source. Before the commencement of the study, blood samples were collected for assessment of baseline levels of nutrition-related markers. In addition, all participants completed six 24-h dietary recalls for baseline habitual protein intake assessment, which was then individually adjusted to 1.6 g kg^{-1} day^{-1} via protein supplementation during the intervention. Protein intake was monitored throughout the intervention by means of additional 24-h dietary recalls every four weeks. Moreover, before (PRE) and after (POST) the 12-wk intervention, participants were assessed for leg lean mass (DXA), muscle (ultrasound) and fiber (muscle biopsy) cross-sectional area, and lower-limb maximal isotonic strength (leg-press 1RM). Training consisted of a twice-a-week, lower-limb resistance training (RT) program individually supervised by a researcher blinded to treatment in a laboratorial setting. Figure 1 illustrates the experimental design.

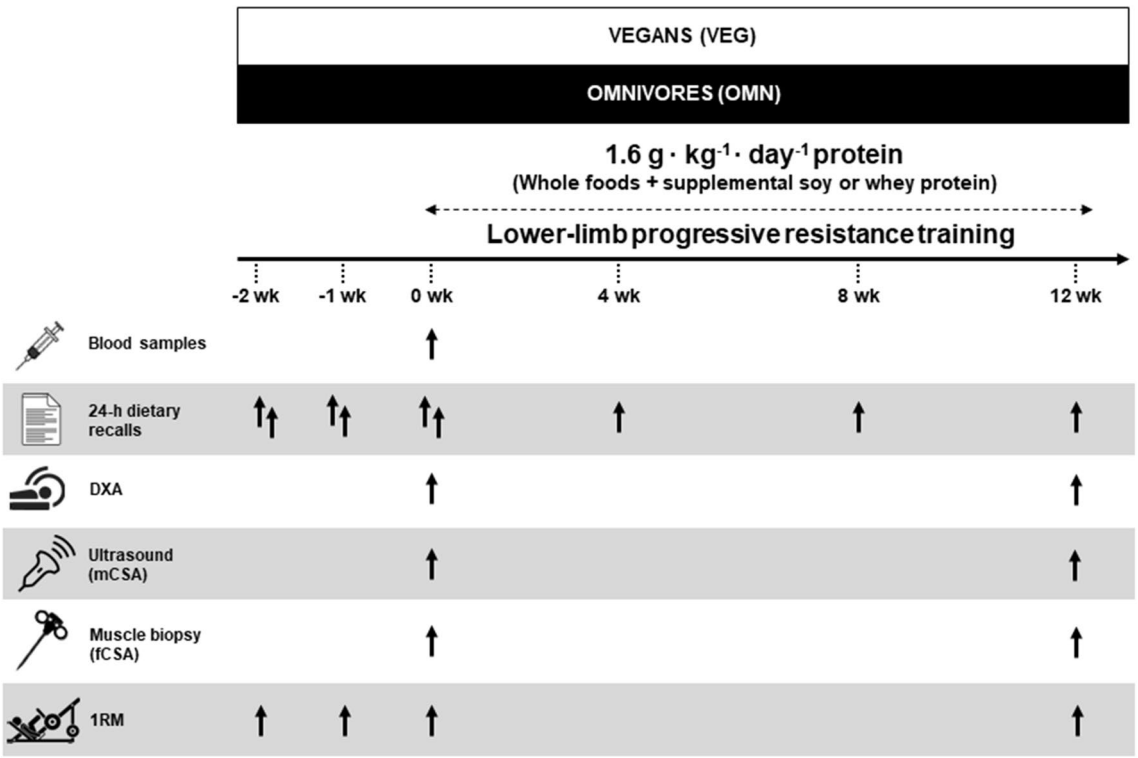

Fig. 1 Experimental design. After baseline assessments, protein intake was individually adjusted to reach 1.6 g kg^{-1} day^{-1} via additional soy or whey protein supplementation to the habitual food intake, which was maintained throughout the intervention until final assessments. *mCSA* muscle cross-sectional area, *fCSA* fiber cross-sectional area, *1RM* maximal isotonic strength

2.2 Participants

Thirty-eight healthy young men aged between 18 and 35 years were recruited to take part in the study by using advertisements on campus and social media (between January 2017 and August 2018). Participants were classified in two groups according to their habitual diets: exclusive plant-based dietary protein consumers—vegans (VEG) or a mixed plant- and animal-based diet—omnivores (OMN). Adherence to either a plant-based or omnivorous diet for at least 1 year prior to the recruitment process was confirmed by a thorough nutritional interview. Additionally, self-reported dietary habits were confirmed by six 24-h dietary recalls on non-consecutive days at baseline. Inclusion criteria were: physically active (according to the International Physical Activity Questionnaire (IPAQ) [23]) but not involved in RT for at least 1 year; absence of any chronic condition that could preclude participation in a RT program or physical testing; habitual protein consumption ≥ 0.8 g kg^{-1} day^{-1}; and adherence to either a plant-based or an omnivorous diet for at least 1 year. The exclusion criteria included prior history of anabolic steroids use, current or previous (≤ 3 m) use of ergogenic or protein-based supplements, current or previous (< 1 year) engagement in energy-restricted diets.

2.3 Blood Samples

Blood samples were collected after a 4-h fast at PRE for total serum protein, ferritin, vitamin B12, and 25-Hydroxyvitamin D (25-(OH)D) assessments. These parameters were evaluated as they are known to be eventually deficient in plant-based-diet consumers [24–26]. Serum was obtained and stored at − 80 °C before analysis. Total serum protein was assessed using colorimetric assays. Ferritin and vitamin B12 were measured by electrochemiluminescence and 25-Hydroxyvitamin D (25-(OH)D) was assessed by chemiluminescence.

2.4 Dietary Assessment

Four weeks prior to the start of the trial, six (4 non-consecutive weekdays and 2 non-consecutive weekend days) 24-h dietary recalls were collected to determine baseline habitual protein intake. Protein intake throughout the trial was monitored via three additional 24-h dietary recalls at weeks 4, 8, and 12. All 24-h dietary recall interviews were collected in-person using the USDA Automated Multiple-Pass Method, a standardized validated method which uses five memory cues (1: quick list; 2: forgotten foods list; 3: time and occasion; 4: detail and review; and 5: final probe) to elicit recall of all possible foods

consumed [27]. Portion size aids were used during each interview by means of food booklets with household measures [28, 29] and real-size food pictures [30].

A trained nutritionist conducted all procedures, and data were analyzed by the same trained professional by means of a specific software (Nutritionist Pro® v.7.3, Axxya Systems, Woodinville, WO, USA). Besides total energy intake (kcal), protein consumption was reported in absolute values (g) relative to energy (%), relative to body weight (g kg^{-1} day^{-1}), and relative to source (i.e. % animal and plant). Total EAA, (g), leucine (g), lysine (g), methionine (g), and branched-chain amino acids (BCAA, g), carbohydrates (g), fat (g), and dietary fiber (g) intake were also estimated and reported. Carbohydrates and fat were also reported relative to energy (%) Additionally, relative per meal protein and estimated leucine intake were reported at PRE and throughout the intervention (weeks 4, 8 and 12).

During the intervention, participants were constantly recommended to keep their habitual dietary intake, and refrain from any other supplement that might influence training performance and/or body composition (e.g., creatine, caffeine, other protein supplements, etc.).

2.5 DXA

Whole-body lean mass, appendicular lean mass, leg lean mass, whole-body fat mass, and whole-body bone mineral content (BMC) were assessed by dual-energy X-ray absorptiometry (DXA) using Hologic QDR 4500A densitometry equipment (Discovery Densitometer, Hologic Inc. Bedford, MA, USA) in the morning after an overnight fast at PRE and POST. Measurements were conducted by a trained investigator blinded to the protocol. Test–retest coefficient of variation for lean-mass DXA assessments is 0.4% in our laboratory.

2.6 Muscle cross-sectional area assessment

Both rectus femoris and vastus lateralis cross-sectional area (mCSA) were assessed at PRE and POST by a B-mode ultrasound with a 7.5-MHz linear-array probe (SonoAce R3, Samsung-Medison, Gangwon-do, South Korea) as previously described [31]. mCSA analyses were performed in a blinded fashion by a single investigator using ImageJ (NIH, USA). Test–retest typical error and coefficient of variation for rectus femoris and vastus lateralis mCSA were 0.01 cm^2 and 0.2% and 0.59 cm^2 and 2.1%, respectively.

2.7 Muscle biopsies and fiber cross-sectional area analyses

Muscle biopsies were taken before 1RM testing at PRE and at least 72 h after the final test at POST. Muscle samples were obtained from the *vastus lateralis* of a subsample (VEG $n = 11$ and OMN $n = 11$) through percutaneous muscle biopsy with manual suction. The procedure was performed by a trained physician. Each participant received local anesthesia (2–3 ml of 1% Xylocaine). A total of ~ 100 mg of muscle was extracted from a small incision and was then dissected free from blood and connective tissue. For analyses, muscle samples were prepared as aliquots (20–30 mg), embedded in an optimum cutting temperature (OCT) cut medium, placed perpendicularly to the horizontal surface (cross-sectional orientation of the muscle fiber was verified with the aid of a low-power microscope), quick-frozen in liquid nitrogen-cooled isopentane, and then stored at − 80 °C until analysis. Muscle cross-sections (10 µm-thick) were cut on a cryostat (CM3050; Leica, Nussloch, Germany) with OCT and then mounted on glass slides. For fiber cross-sectional area (fCSA) analyses, muscle samples were brought to room temperature, and fixed in methanol for 10 min, washed (three 10-min washes with phosphate-buffered saline [PBS]), then blocked for 60 min in a blocking buffer solution (containing 1% PBS, 5% bovine serum albumin [BSA] and 0.3% Triton X-100). Following, the slides were incubated with primary antibody (anti-rabbit laminin [1:100, Abcam] and; anti-mouse A4.951 slow isoform [1:75, A4.951 DSHB]) overnight at 4 °C. The next day, slides were again washed (three 10-min washes with PBS) and incubated in appropriate secondary antibody (Alexa Fluor 488 anti-rabbit [1:200, Thermo Fisher Scientific]; Alexa Fluor 568 anti-mouse, [1:1000, Thermo Fisher Scientific]) in the dark for 1 h at room temperature. Samples were then re-washed and cover-slipped. Images were captured with an Olympus BX51 Fluorescence microscope with a magnification of 20×. Type I and type II fCSA quantification were performed using ImageJ software (NIH, USA). A mean of 100 fibers were analyzed per time point per participant by the same investigator in a blinded fashion. Typical error and coefficient of variation between two blinded measurements were 69.3 μm^2 and 2.3%, respectively.

2.8 Lower-limb maximal isotonic strength test (1RM)

Before testing, all participants performed two familiarization sessions separated by at least 72 h. Lower-limb maximal isotonic strength was assessed on an incline leg-press (45°) (Movement Technology, Bruden®, Sao Paulo, Brazil) following recommendations of the American Society of Exercise Physiologists [32]. Test–retest typical error and coefficient of variation for 1RM testing were 5.4 kg and 2.2%, respectively.

2.9 Protein supplementation protocol

Baseline habitual protein values were used to calculate the amount of either soy (SUPRO XT 221D IP®, Solae LLC, DuPont, St. Louis, MO, USA) or whey (THERMAX 690®, Glanbia Nutritionals, Fitchburg, WI, USA) protein supplements needed to meet the 1.6 g kg^{-1} day^{-1} target [21] in both groups. Protein supplementation was individually tailored and delivered (by a researcher not involved in the analyses of any dependent variable) to participants in VEG or OMN groups. Following the principle of the optimal per-meal protein intake for MPS [33, 34], supplementary protein was offered twice daily (training and non-training days), at the lowest protein meals of each participant (in our study, protein supplements were offered at breakfast and evening snack) throughout all the 12-wk experimental intervention. The nutritional composition of both supplements is shown in Online Resource 1. Participants were requested to log day and time of supplement intake to verify compliance.

2.10 Resistance training program

The 12-week, twice-a-week, supervised RT program was comprised of incline leg-press 45º (Movement Technology, Bruden®, Sao Paulo, Brazil) and leg-extension (Movement Technology, Bruden®, Sao Paulo, Brazil) exercises performed on non-consecutive days (Monday and Thursday or Tuesday and Friday). We focused training on lower limbs as the techniques available offered the ability to investigate muscle adaptations at different levels (from DXA to muscle biopsies). All training sessions were performed during the same time of day for each participant, according to availability (either before lunch: ~ 11am or before dinner: ~ 5 pm). In case of missing sessions, they were rescheduled to either Wednesdays or Saturdays (48 h apart from the last training session) to maintain training adherence. Before each training session, participants completed a general warm-up consisted of a 5-min light exercise on a cycle ergometer followed by a specific warm-up comprised of two submaximal sets of leg-press (8 repetitions at 50% and 3 repetitions at 70% of the last training load registered). Training sessions duration varied between 30 and 45 min. Training progression was as follows: weeks 1 to 4: 2 sets of 12–15-repetition maximum (RM) (for each exercise); weeks 5 to 8: 3 sets of 10–12-RM (for each exercise); weeks 8 to 12: 4 sets of 8–10-RM (for each exercise). Resistance was increased whenever the individual performed one or two repetitions over the pre-established number on two consecutive sets [35]. Two minutes of rest were given between sets for all sessions. A trained member of the research team supervised all training sessions. A training log of each exercise session was kept for adherence's monitoring and training volume load calculation (sets × repetitions × resistance for both leg press and leg extension exercises) [36].

2.11 Statistical analyses

Sample size was calculated using mCSA for *vastus lateralis* as the primary outcome of our study. Analyses were run using G*Power® (3.1.9.2) performing a two-way ANOVA with repeated measures (within-between interaction) considering a medium effect size ($f = 0.25$) and setting power to 80% ($\beta = 0.2$) with $\alpha = 0.05$, which yielded an estimate of $n = 17$ per group. There are no current available data comparing the effects of an exclusively plant-based protein source vs a mixed-diet on muscle mass gains to base our calculation off; therefore, we decided to be conservative and estimate differences, if they were present, based on a medium effect size, based on minimal detectable change (MDC) calculations for changes in mCSA assessed by ultrasound technique [31], which is in line with current literature on heterogeneity for changes in mCSA (as assessed by ultrasound) in response to resistance training [37]. We aimed for 23 participants per group due to potential dropouts. All data was normality distributed (assessed using the Shapiro–Wilk test) and there were no missing values at any time point. Baseline characteristics (at PRE), were compared between groups using an independent *t*-test. Effects of dietary protein source on dependent variables were analyzed using a mixed model for repeated measures assuming 'group' (VEG and OMN) and 'time' (PRE and POST) as fixed factors and 'subjects' as a random factor. Whenever a significant F-value was obtained, a post hoc test with Tukey's adjustment was performed for multiple comparison purposes. Additionally, possible between-group differences in PRE to POST absolute changes in 1RM, leg lean mass, muscle and fCSA were tested using *t* tests and confirmed with mixed model analysis. To illustrate the variability in response between groups, data are presented as box-and-whisker plots including the median (lines), interquartile range (boxes), minimum and maximum values (whiskers), and mean (crosses). Dietary intake, relative per-meal protein intake, and per-meal estimated leucine intake throughout the intervention period were assessed by mixed-model for repeated measures assuming 'group' (VEG and OMN) and 'time' (PRE, week 4, week 8 and week 12) as fixed factors, and 'subjects' as random factor. Type I and type II fCSA were assessed by means of a mixed-model analysis with covariance (mixed-model ANCOVA), assuming respective baseline fCSA as covariates. In case of significant *F* value, a Tukey post hoc test was performed. Between-group differences in adherence to the intervention protocol and training volume load throughout the resistance training program were assessed by means of independent *t*-tests. Data were analyzed using the software

SAS® 9.4 (SAS Institute, Inc., Cary, NC, USA), and the level of significance was set at $p < 0.05$.

3 Results

3.1 Participants

Figure 2 shows the flow of the participants. Three hundred and eight participants were assessed for eligibility. Two hundred and forty did not meet the inclusion criteria and 22 declined to participate after the initial interview. Four participants (two due to lack of time and two due to non-trial-related health issues) withdrew from the study in VEG group. Two participants in OMN group dropped out before the end of baseline assessments and other two (one due to lack of time and one due to non-trial-related health issues) dropped out of the study and were excluded from the analysis. Participants allocated in the VEG group were vegans on average for 3.2 ± 3.0 years (range 1.5–12 years). Table 1 shows baseline characteristics for the participants who completed the trial ($n = 19$ per group). Participants were comparable for age, body weight, height, leg lean mass, and lower-limb maximal isotonic strength.

Table 1 Baseline characteristics of the participants

	VEG ($n = 19$)	OMN ($n = 19$)	p value
Age, y	26 ± 5	26 ± 4	0.73
Body weight, kg	72.7 ± 7.1	73.3 ± 7.8	0.79
Height, cm	178 ± 5	176 ± 6	0.35
BMI, kg/m^2	22.9 ± 2.3	23.6 ± 2.3	0.33
Whole-body lean mass, kg	57.3 ± 5.0	57.3 ± 5.8	0.99
Appendicular lean mass, kg	25.9 ± 2.8	26.1 ± 3.2	0.85
Leg lean mass, kg	18.1 ± 2.2	19.1 ± 2.4	0.82
Whole-body fat mass, kg	12.8 ± 4.8	13.4 ± 3.9	0.68
Whole-body BMC, kg	2.6 ± 0.3	2.7 ± 0.4	0.58
Leg-press 1RM, kg	258 ± 59	261 ± 63	0.86
PAL, min/week	302 ± 127	282 ± 130	0.65
Total serum protein, g/dL	7.5 ± 0.4	7.5 ± 0.3	0.58
Ferritin, ng/mL	140 ± 83	196 ± 121	0.10
Vitamin B12, pg/mL	301 ± 264	408 ± 1371	0.13
25-OH(D), ng/mL[†]	18.0 ± 6.6	24.0 ± 6.5	0.01

Values are represented as means ± SD

VEG vegans, *OMN* omnivores, *BMI* body mass index, *BMC* bone mineral content, *1RM* maximal isotonic strength, *PAL* physical activity level, *25-OH(D)* 25-hydroxyvitamin D

[†]Indicates $p < 0.05$ for between-group differences when compared at PRE (independent *t* test at PRE)

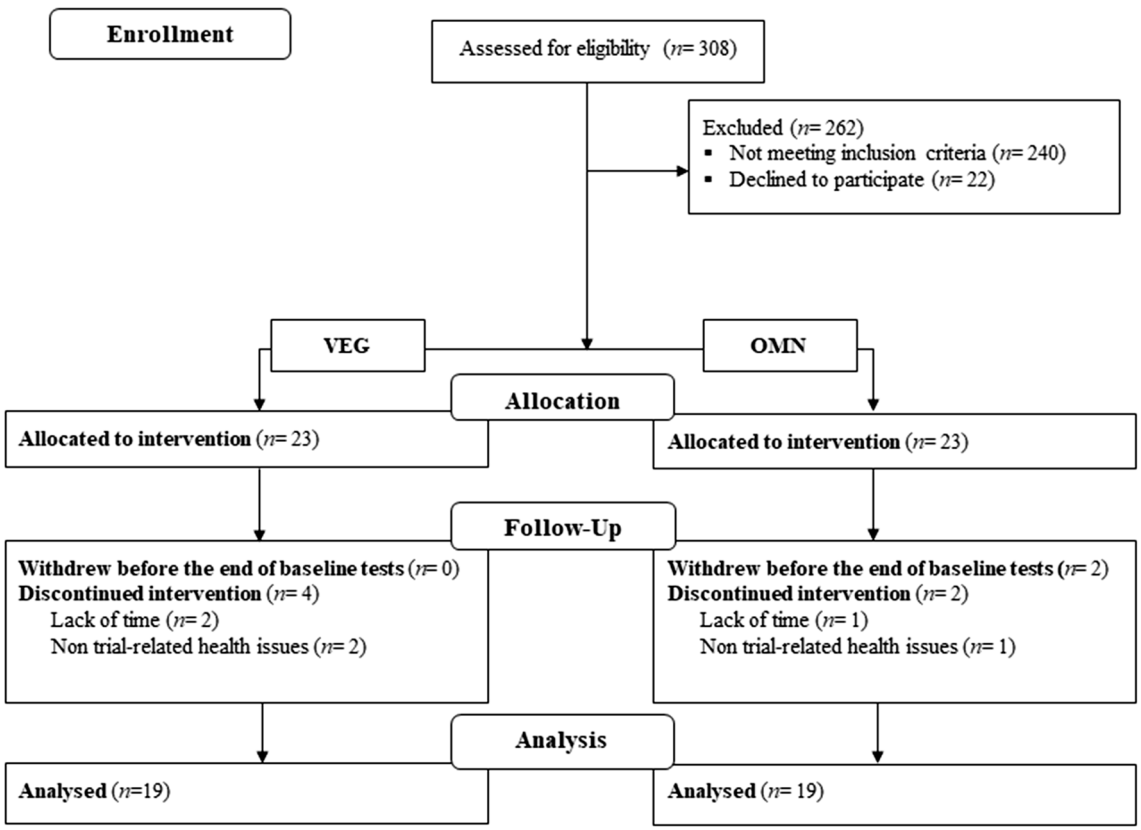

Fig. 2 CONSORT Flow diagram

3.2 Dietary Assessments

Both groups achieved the targeted protein intake (i.e., 1.6 g kg^{-1} day^{-1}) via supplemental protein to their habitual dietary food sources. Habitual dietary protein intake remained stable throughout the intervention for both groups (both $p > 0.05$). Supplemental protein was 0.79 ± 0.21 g kg^{-1} day^{-1} for VEG and 0.52 ± 0.19 g kg^{-1} day^{-1} for OMN (in absolute values, VEG: 58 ± 17 g and OMN: 39 ± 17 g). Relative and total protein intake increased similarly from baseline in both groups (all $p < 0.05$ when comparing PRE values with weeks 4, 8, and 12), remaining stable throughout the trial (no within- or between-group differences at weeks 4, 8, and 12 after post-hoc analysis, all $p > 0.05$). Estimated intakes of EAA, leucine, lysine, methionine, and BCAA were also significantly increased from baseline in both groups (all $p < 0.0001$), remaining stable throughout the trial with no within-group differences at weeks 4, 8, and 12 (all $p > 0.05$). There was a main effect of group for EAA, leucine, lysine, methionine, and BCAA throughout the trial (OMN > VEG; all $p < 0.0001$). Additional dietary information is found in Table 2.

Table 3 describes per-meal relative protein and leucine intake. Relative protein intake was significantly increased from baseline in both groups at all meals (all $p < 0.05$ for the main effect of time), with neither within-group difference at weeks 4, 8 and 12 (all $p > 0.05$) nor group-by-time interaction ($p > 0.05$) being found. Overall, VEG had a slightly lower relative protein intake at lunch than OMN ($p < 0.05$ for the main effect of the group). Regarding per-meal leucine intake, we found a main effect of time for breakfast, dinner, and evening snack intakes, with increased leucine content values from PRE in both groups (all $p < 0.05$). There were main effects of the group for leucine content in lunch and dinner meals, with greater leucine content in OMN than VEG (both $p < 0.05$).

Table 2 Dietary intake at baseline and during weeks 4, 8, and 12 of the intervention

	PRE		Week 4		Week 8		Week 12		Group by time *p* value
	VEG	OMN	VEG	OMN	VEG	OMN	VEG	OMN	
Energy, kcal day^{-1}	2251 ± 414	2120 ± 244	2324 ± 472	2057 ± 379	2378 ± 429	2197 ± 479	2359 ± 394	2271 ± 430	0.61
Protein g kg^{-1} day^{-1}#	0.91 ± 0.19	1.18 ± 0.19†	1.68 ± 0.19	1.70 ± 0.15	1.68 ± 0.19	1.73 ± 0.12	1.66 ± 0.21	1.70 ± 0.16	< 0.001
Protein, g day^{-1}#	65 ± 11	86 ± 13†	122 ± 12	124 ± 12	122 ± 13	127 ± 13	120 ± 18	124 ± 17	< 0.001
Protein, % of energy#	12 ± 2	16 ± 2†	22 ± 5	25 ± 5	21 ± 4	24 ± 4	21 ± 5	22 ± 4	0.14
Animal protein, g day^{-1} (%)*	–	58 ± 9 (67)†	–	99 ± 16 (79)	–	100 ± 14 (79)	–	97 ± 17 (78)	< 0.001
Plant protein, g day^{-1} (%)*	65 ± 11 (100)	28 ± 5 (33)†	122 ± 12 (100)	25 ± 9 (21)	122 ± 13 (100)	27 ± 9 (21)	120 ± 18 (100)	27 ± 9 (22)	< 0.001
EAA, g day^{-1}*#	21 ± 4	33 ± 5†	42 ± 4	54 ± 7	43 ± 5	55 ± 7	43 ± 7	53 ± 8	0.89
Leucine, g day^{-1}*#	5 ± 1	7 ± 1†	9 ± 1	11 ± 2	9 ± 1.0	11 ± 2	9 ± 1	11 ± 2	0.95
Lysine, g day^{-1}*#	3 ± 1	6 ± 1†	6 ± 1	11 ± 2	7 ± 1	11 ± 1	7 ± 1	10 ± 1	0.004
Methionine, g day^{-1}*#	1 ± 0	2 ± 0†	2 ± 0	3 ± 0	2 ± 0	3 ± 0	2 ± 0	3 ± 0	0.07
BCAA, g day^{-1}*#	10 ± 2	15 ± 2†	20 ± 2	24 ± 3	20 ± 2	25 ± 3	20 ± 3	24 ± 4	0.91
CHO, g day^{-1}*	365 ± 73	262 ± 38†	360 ± 97	216 ± 62	349 ± 87	243 ± 79	342 ± 75	272 ± 60	0.02
CHO, % of energy*	63 ± 5	49 ± 4†	62 ± 7	42 ± 8	58 ± 7	44 ± 6	58 ± 7	48 ± 5	0.0005
Fat, g day^{-1}*	62 ± 20	80 ± 14†	53 ± 20	76 ± 25	63 ± 21	79 ± 20	64 ± 24	79 ± 17	0.71
Fat, % of energy*	24 ± 6	33 ± 4†	20 ± 7	33 ± 7	24 ± 6	32 ± 5	24 ± 7	32 ± 5	0.12
Dietary fiber, g day^{-1}*	39 ± 10	18 ± 5†	40 ± 16	15 ± 7	39 ± 14	19 ± 10	38 ± 13	19 ± 10	0.18

Data are expressed as mean ± SD

VEG vegans, *OMN* omnivores, *CHO* carbohydrates, *EAA* essential amino acids, *BCAA* branched-chain amino acids

†Indicates $p < 0.05$ for between-group differences when compared at PRE (independent *t* test at PRE)

#Indicates $p < 0.05$ for the main effect of time (when comparing PRE values with weeks 4, 8, and 12)

*Indicates $p < 0.05$ for the main effect of group

Table 3 Per-meal relative protein intake (g kg^{-1}) and leucine intake (g) at baseline and during weeks 4, 8, and 12 of the intervention

	PRE		Week 4		Week 8		Week 12		Group by time *p* value
	VEG	OMN	VEG	OMN	VEG	OMN	VEG	OMN	
Protein intake									
Breakfast, g $kg^{-1\#}$	0.15 ± 0.06	0.17 ± 0.06	0.38 ± 0.20	0.30 ± 0.18	0.38 ± 0.19	0.30 ± 0.20	0.32 ± 0.27	0.33 ± 0.24	0.26
Lunch, g kg^{-1}* #	0.36 ± 0.09	0.47 ± 0.10†	0.47 ± 0.14	0.59 ± 0.18	0.43 ± 0.13	0.58 ± 0.14	0.47 ± 0.16	0.51 ± 0.11	0.13
Dinner, g $kg^{-1\#}$	0.27 ± 0.08	0.39 ± 0.11†	0.44 ± 0.17	0.48 ± 0.11	0.47 ± 0.20	0.52 ± 0.18	0.41 ± 0.18	0.50 ± 0.15	0.79
Evening snack, g $kg^{-1\#}$	0.03 ± 0.04	0.07 ± 0.07	0.39 ± 0.27	0.33 ± 0.17	0.40 ± 0.26	0.33 ± 0.25	0.46 ± 0.29	0.36 ± 0.22	0.82
Leucine intake									
Breakfast, g $day^{-1\#}$	1 ± 0	1 ± 0†	2 ± 1	2 ± 2	2 ± 1	2 ± 2	2 ± 2	2 ± 2	0.54
Lunch, g day^{-1}*	2 ± 0	3 ± 0†	2 ± 1	3 ± 1	2 ± 1	3 ± 1	3 ± 1	3 ± 2	0.08
Dinner, g day^{-1}*#	1 ± 0	2 ± 1†	2 ± 1	3 ± 1	3 ± 1	3 ± 1	2 ± 1	3 ± 1	0.94
Evening snack, g $day^{-1\#}$	0 ± 0	0 ± 0	2 ± 2	3 ± 1	2 ± 2	3 ± 2	3 ± 2	3 ± 2	0.98

Data are expressed as mean ± SD

VEG vegans, *OMN* omnivores; Estimated leucine intake values were rounded to whole numbers for precision purposes

†Indicates $p < 0.05$ for between-group differences when compared at PRE (independent *t* test at PRE)

#Indicates $p < 0.05$ for the main effect of time (when comparing PRE values with weeks 4, 8, and 12)

*Indicates $p < 0.05$ for the main effect of group

Importantly, there were no group-by-time interactions for leucine content in any of the meals (all $p > 0.05$).

3.3 DXA

3.3.1 Leg Lean Mass

We observed a main effect of time for leg lean mass ($p < 0.0001$). VEG increased from 18.9 ± 2.2 to 20.1 ± 2.2 kg, and OMN from 19.1 ± 2.4 to 20.3 ± 2.7 kg (all $p < 0.0001$ for within-group comparisons), with no group-by-time interaction ($p = 0.94$). Additionally, absolute changes were also not significantly different between groups ($p = 0.99$) (Fig. 3a, b).

Similarly, a main effect of time was observed for appendicular lean mass, whole-body lean mass, and body weight (all $p < 0.0001$), with no between group differences at any time point (all $p > 0.05$). No within- or between-group differences were observed for whole-body fat mass or bone mineral content (all $p > 0.05$) (Online Resource 2).

3.4 Muscle CSA

There was a main effect of time for both rectus femoris and vastus lateralis mCSA (all $p < 0.0001$). For VEG, rectus femoris mCSA increased from 8.6 ± 1.6 to 9.6 ± 1.6 cm^2, and for OMN, the increase was from 8.7 ± 2.1 to 9.4 ± 2.2 cm^2

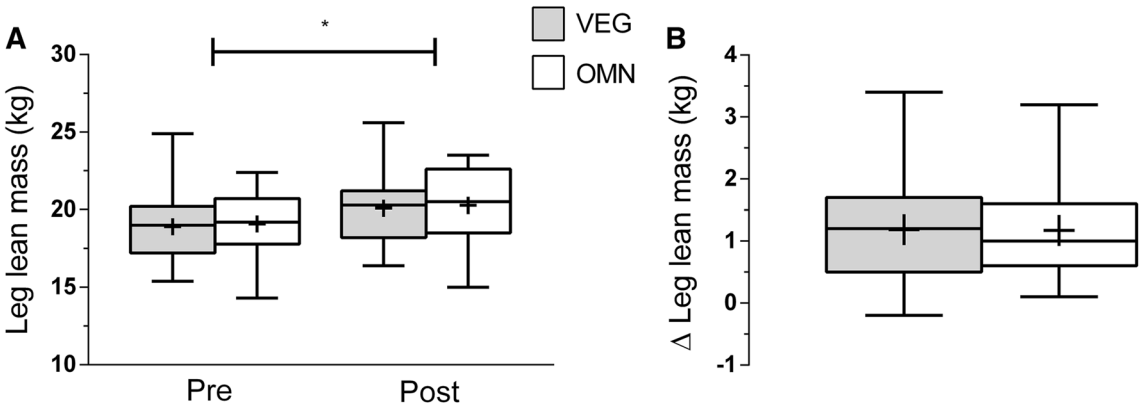

Fig. 3 Panel A shows leg lean mass before (PRE) and after (POST) intervention in groups VEG and OMN. Panel B shows delta change (Δ) (PRE-to-POST intervention) in leg lean mass in groups VEG and OMN. Values are presented as median (lines) with interquartile range (boxes), minimum and maximum (whiskers), and mean (+). *Indicates significantly different from PRE ($p < 0.0001$ for the main effect of time). *VEG* exclusive plant-based dietary protein consumers, *OMN* animal-based dietary protein consumers

(all $p < 0.0001$ for within-group comparisons). Similarly, increases in mCSA for vastus lateralis were as follows: VEG from 21.6 ± 3.1 to 23.8 ± 3.6 cm^2, and OMN from 22.1 ± 4.1 to 24.9 ± 4.4 cm^2 (all $p < 0.0001$ for within-group comparisons), with no group-by-time interactions ($p = 0.67$ and $p = 0.14$, respectively). Furthermore, between-group absolute changes were not significantly different (all $p > 0.05$) (Fig. 4a–d).

3.5 Fiber CSA

There was a main effect of time for type I fCSA ($p < 0.0001$). We have ran ANCOVA analysis for type I fCSA and no influence of PRE values on the statistical model was detected ($p = 0.76$). Also, no group-by-time interaction was observed ($p = 0.77$). VEG increased from 3750 ± 648 to 4491 ± 641 µm^2, $p = 0.0004$; and OMN from 4444 ± 908 to 5121 ± 1216 µm^2, $p = 0.001$ (within-group comparisons). Additionally, delta changes in type I fCSA were not significantly different between groups ($p = 0.77$). We observed a significant between-group difference for type II fCSA at PRE ($p = 0.02$); however, ANCOVA analysis detected no influence of PRE values on the statistical model ($p = 0.82$). Both groups significantly increased fCSA for type II fibers across time (main effect of time, $p < 0.0001$). VEG increased from 3844 ± 571 to 4765 ± 431 µm^2, $p = 0.0001$ and OMN from 4663 ± 960 to 5507 ± 1301 µm^2, $p = 0.0003$ (within-group comparisons). No group-by-time interaction was found ($p = 0.75$). Additionally, pre-to-post changes were not significantly different between VEG and OMN ($p = 0.75$) (Fig. 4e–h).

Fiber type distribution was similar across groups, with no within- or between-group differences (all $p > 0.05$) (VEG type I: PRE = $54 \pm 12\%$, POST = $53 \pm 15\%$; OMN type I: PRE $49 \pm 5\%$, POST = $48 \pm 9\%$ and VEG type II: PRE = $46 \pm 12\%$, POST = $47 \pm 15\%$; OMN type II: PRE = $51 \pm 5\%$, POST = $52 \pm 8\%$).

3.6 Lower-Limb Maximal Isotonic Strength

There was a main effect of time for leg-press 1RM ($p < 0.0001$). Both VEG (from 258 ± 59 to 354 ± 81 kg, $p < 0.0001$) and OMN (from 261 ± 63 to 383 ± 74 kg, $p < 0.0001$) significantly increased their 1RM. No group-by-time interaction was found ($p = 0.10$), and no between-group difference was detected in 1RM absolute changes ($p = 0.11$) (Fig. 5a, b).

3.7 Adherence, Adverse Effects, and Training Volume Load

Adherence to training was excellent with VEG completing $94 \pm 6\%$ of all prescribed training sessions and OMN: $95 \pm 6\%$ of all prescribed training sessions ($p > 0.05$, for between-group comparison). Self-reported protein supplementation was also excellent (VEG: $94 \pm 6\%$; OMN: $96 \pm 3\%$) and was similar between the two groups ($p > 0.05$). Additionally, self-reported protein supplementation was double-checked by the research staff via logging the containers returned by the subjects. No adverse effects of either training or supplementation were reported throughout the trial.

Training volume load was similar between VEG and OMN either per exercise (leg press: VEG = 177 ± 54 and OMN = 186 ± 43 10^3 kg, leg extension: VEG = 52 ± 13 and OMN = 58 ± 14 10^3 kg; all $p > 0.05$) or combining leg-press and leg-extension exercises (VEG: 229 ± 63 and OMN: 244 ± 52 10^3 kg; $p = 0.43$).

4 Discussion

In the current study, we compared the effects of an exclusive consumption of plant-based dietary protein *vs.* an omnivorous diet on RT-induced muscle adaptations following a 12-week lower-limb RT program in young individuals under an optimal protein intake (1.6 g kg^{-1} day^{-1}) combining whole foods + supplemental protein (either soy or whey protein isolates). We observed no significant differences in increases in leg lean mass, mCSA (rectus femoris and vastus lateralis), fCSA (type I and type II muscle fiber), and muscle strength following diet and resistance training between the two groups, regardless of dietary protein source.

Previous studies have compared the effects of supplemental protein of different sources (plant *vs.* animal) on muscle mass with contrasting results [38–44]. However, these studies investigated the addition of either a whey or soy protein supplement to an omnivorous diet, which does not provide an answer to the question of how exclusively plant *vs.* omnivorous diets impact muscle adaptations with RT [38–42]. In some studies, dietary protein intake was not controlled throughout the intervention [41, 42] or at baseline [38], which prevents a definitive conclusion. In addition, two studies required participants to refrain from meat consumption during the trial [43, 44], which still would have allowed the possibility of consumption of eggs and dairy. Collectively, these studies [38–44] do not provide a complete understanding of the role protein source plays in mediating muscle anabolism in response to exercise.

Previous data show greater acute muscle protein synthetic responses after whey *vs.* soy protein ingestion in combination with resistance training [10, 12, 18], which has been, at least partially, attributed to the fact that plant-based protein is more directed towards oxidation instead of MPS [18], and with greater splanchnic nitrogen retention [17, 45]. These differences in MPS efficiency are thought to be related to digestibility and EAA content [14, 15, 45], particularly

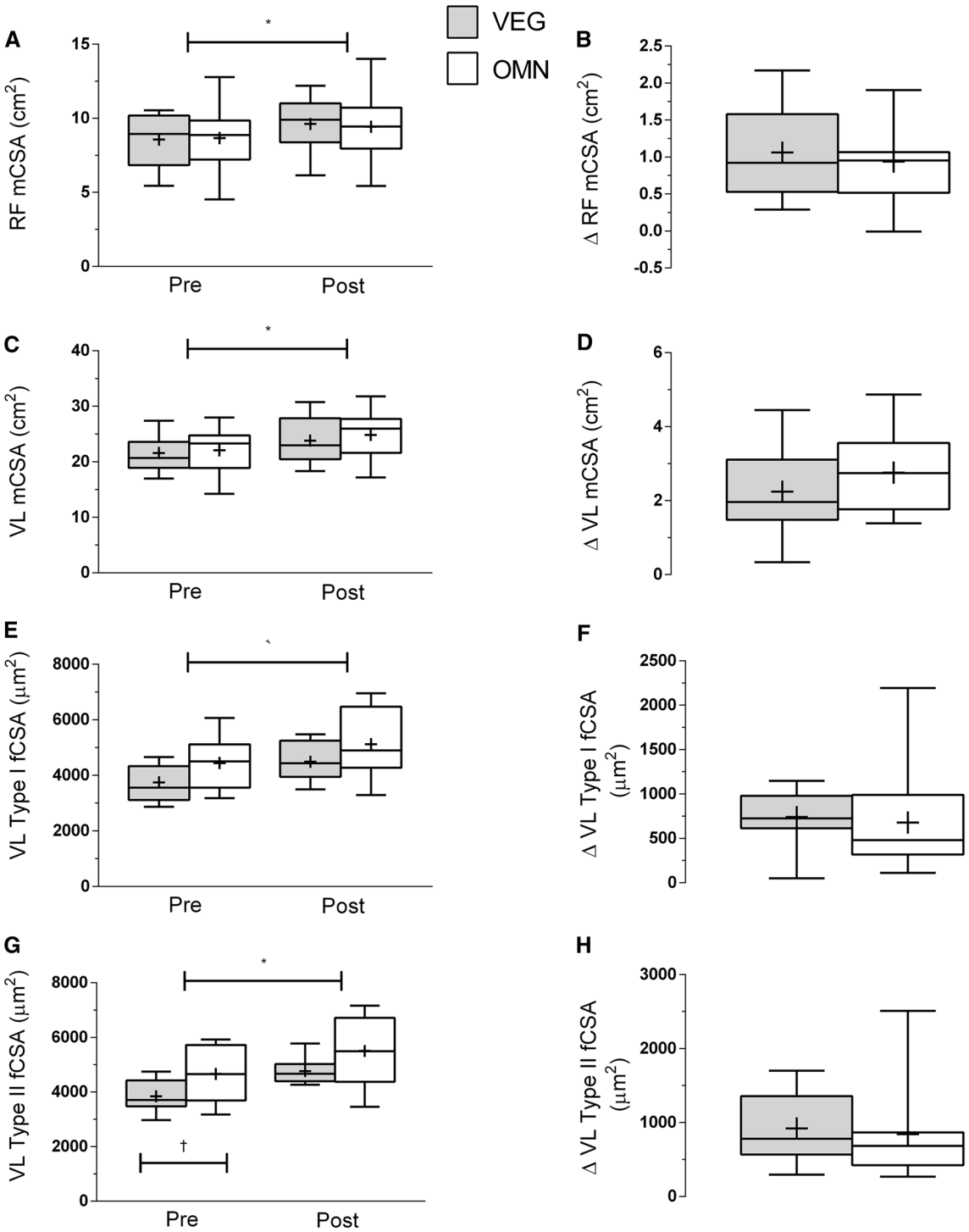

Fig. 4 **a**, **c** Show rectus femoris and vastus lateralis muscle cross-sectional area (mCSA) before (PRE) and after (POST) intervention in groups VEG and OMN. **b**, **d** Show delta changes (Δ) (PRE-to-POST intervention) in rectus femoris and vastus lateralis mCSA in groups VEG and OMN. **e**, **g** Show vastus lateralis muscle fiber cross-sectional area (fCSA) for type I and type II fibers before (PRE) and after (POST) intervention in groups VEG and OMN. **f**, **h** Show delta changes (Δ) (PRE-to-POST intervention) in vastus lateralis muscle fiber cross-sectional area (fCSA) for type I and type II fibers in groups VEG and OMN. Values are presented as median (lines) with interquartile range (boxes), minimum and maximum (whiskers), and mean (+). †Indicates between-group difference at PRE ($p < 0.05$); *Indicates significantly different from PRE ($p < 0.0001$ for the main effect of time). *VEG* exclusive plant-based dietary protein consumers, *OMN* animal-based dietary protein consumers, *RF* rectus femoris, *VL* vastus lateralis

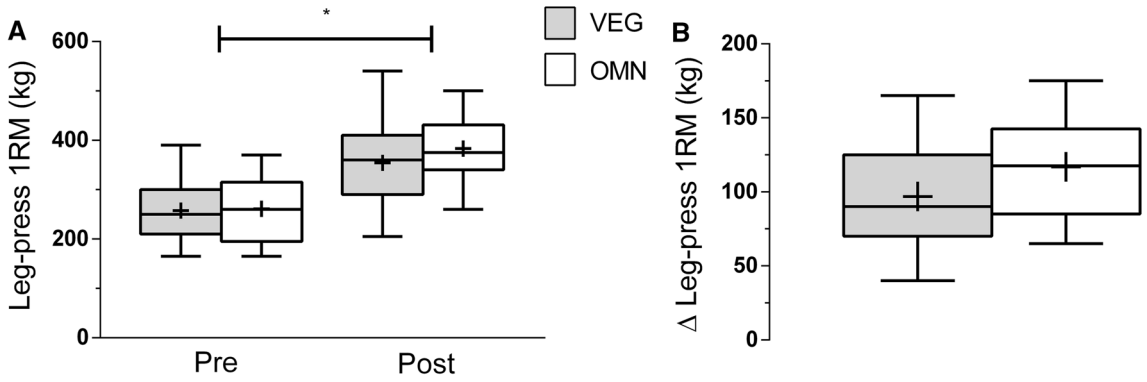

Fig. 5 **a** Shows lower-limb 1RM before (PRE) and after (POST) intervention in groups VEG and OMN. **b** Shows delta change (Δ) (PRE-to-POST intervention) in lower-limb 1RM in groups VEG and OMN. Values are presented as median (lines) with interquartile range (boxes), minimum and maximum (whiskers), and mean (+). *Indicates significantly different from PRE ($p < 0.0001$ for the main effect of time). *1RM* one-repetition-maximum, *VEG* exclusive plant-based dietary protein consumers, *OMN* animal-based dietary protein consumers

leucine [13–15], as increased plasma and/or intramuscular leucine has been shown to be a primary driver of protein-induced MPS stimulation [46–48]. If these differences between animal- and plant-based proteins in acutely stimulating MPS persisted across time (e.g., weeks to months), one may hypothesize that the anabolic response in individuals consuming an exclusive plant-based vs. a mixed protein diet would be inferior. Despite recent meta-analyses indicating that such a difference is not readily apparent [20, 21], these data refer to the effects of supplemental protein to a mixed diet, and not to an exclusive dietary protein source consumption. The present study was designed to test this hypothesis and our main findings challenge the notion that an exclusive plant-based diet is less efficient than an omnivorous diet to support muscle anabolic adaptations to chronic RT.

Potential differences in EAA availability between the two diets may have been mitigated in our study, as a mixture of several plant-based food sources (as seen in VEG) is thought to enhance dietary EAA profile, which may ensure not only a higher MPS response than that of a single plant source [14] but also a more closely resembling MPS response to an animal protein ingestion. In fact, a commonly observed combination such as grains (typically lower in lysine and higher in methionine) and beans (typically lower in methionine but higher in lysine), for example, may provide a more "complete protein" for plant-based protein consumers [14], which may have contributed positively to MPS over time, adequately supporting morphological and functional changes in muscle tissue in response to training. Additionally, the soy protein isolate provided to our participants via supplementation not only contributed to increased total EAA, leucine, lysine, and methionine intake during the intervention, but is also free from antinutritional factors [49, 50] and has a high protein-quality score [51], and hence, may have been a factor in improving availability of protein-derived amino acids for MPS.

Interestingly, the absence of significance in muscle adaptations between groups was observed in the presence of a higher intake of total EAA, leucine, lysine, methionine, and BCAA in OMN, irrespective of comparable total and relative protein intakes. Nonetheless, despite the slight between-group differences, the absolute intake of these amino acids in VEG was actually high (e.g.; ~ 9 g day^{-1} for leucine intake), and, thus, likely enough to maximally stimulate muscle anabolism, especially when considering the ceiling effect in the anabolic response to protein intake [52, 53], which ultimately mitigates any effect of dietary protein source.

Despite our results demonstrating similar effects between an exclusive plant- and an animal-based protein diet on muscle adaptations, it is important to take caution when extrapolating these findings. In order for the VEG group to meet the targeted protein intake, ~ 58 g day^{-1} of supplementary soy protein was necessary (vs. ~ 41 g day^{-1} in OMN), meaning that achieving 1.6 g kg^{-1} day^{-1} of protein from mostly whole foods might be challenging since it would require the consumption of a significantly greater amount of food, with a consequent increase in energy intake. Additionally, this increased whole-food intake could be accompanied by increased amounts of antinutritional factors, possibly causing, among other effects, a decrease in dietary protein digestibility [49]. One may speculate that this potential decreased protein digestibility may impact long-term muscle adaptations, warranting further clinical trials dedicated to this specific research question. Therefore, our results are confined to a vegan diet with a relatively large supplemental plant protein isolate intake as a practical way to achieve adequate total intake (1.6 g kg^{-1} day^{-1}) when dietary protein is obtained exclusively from plant sources.

No previous research, so far as we are aware, has compared the effects of protein source ingestion between exclusively plant-based protein consumers vs. omnivores in response to exercise. Additionally, our results are strengthened by muscle-based assessments of hypertrophy at both macroscopic (by DXA scans and ultrasound imaging) and microscopic levels (by muscle biopsy). However, this study is not without limitations. The lack of randomization can be seen as a bias in our results; importantly, we opted for this study design as we considered it the most effective way to address our research question without compromising the results due to any possible physiological effect from an acute and abrupt change in the dietary pattern (e.g.; abruptly changing the dietary habit of omnivores to an exclusively plant-based diet). Submitting an omnivore to a plant-based diet would bring about a residual effect of their previous history of animal protein consuming diet. Additionally, other known issues deriving from this, such as a decrease in energy and whole-food protein intake as well as changes in body weight [43] could constitute major confounding factors to our results. Second, even though beyond the scope of the study, no mechanisms underpinning the anabolic responses were assessed. Importantly, our fCSA data refers to a subsample of individuals within our study. Importantly, we have run the calculations using MDCs for each variable based on available literature [54–56], and the recruited sample size offered enough power on all variables. Despite the consistency in fCSA increases across groups, future studies with more representative sample sizes are imperative to confirm our findings. Our study focused on lower limbs; however, there is no evidence, as far as we know, that is available to suggest that upper-limb muscles would respond differently. In addition, we relied on 24-h dietary recalls to estimate dietary intake (i.e., protein and amino acids) which may be prone to systematic errors, such as over- or under-reporting dietary intake from real life, or precision level when quantifying individual amino acids, thus requiring caution when interpreting the absolute numbers presented herein. Nonetheless, we aimed to reduce these errors by increasing the number of 24-h dietary recalls at baseline to capture the dietary intake variation on a daily basis. Lastly, even though participants were constantly contacted throughout the experiment to assure compliance with the protocol, we did not assess physical activity level at POST, thus posing a limitation in this respect. Future research investigating the effects of dietary protein source on muscle adaptations in a different population, training status, and clinical conditions are warranted, especially those such as the elderly who may have anabolic resistance [57, 58], for whom dietary protein source may play a larger role in sustaining muscle mass.

5 Conclusion

In conclusion, a high-protein (1.6 g kg^{-1} day^{-1}), exclusively plant-based diet (plant-based whole foods + soy protein isolate supplementation) is as efficacious in supporting muscle strength and mass accrual in untrained young men who are habitually vegan as a protein content-matched mixed diet (mixed whole foods + whey protein supplementation) in untrained young men who are habitually omnivorous. This suggests that dietary protein source does not affect resistance training-induced adaptations in untrained young men, provided adequate amounts of protein are consumed.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40279-021-01434-9.

Acknowledgements We appreciate DUPONT for donating both whey and soy protein supplements. We also thank Liliam Takayama (Bone Metabolism Laboratory, Rheumatology Division, University of São Paulo) for DXA assessments and the study subjects for participating in this study.

Compliance with Ethical Standards

Funding This work was supported by Sao Paulo Research Foundation (FAPESP) (Grant no. 2016/22083–3).

Conflict of interest SMP reports personal fees from Enhanced Recovery, equity from Exerkine, personal fees from Dairy Farmers of Canada, personal fees from US National Dairy Export Council, grants from Alliance for Potato Research and Education, grants from US National Dairy Council, outside the submitted work; In addition, SMP has a patent 3052324 issued to Exerkine, and a patent 16/182891 pending to Exerkine. VHL, BG, IL, SG, ALF, LARC, RMRP, GGA and HR declare that they have no competing interests.

Ethics approval This study was performed in line with the principles of the 1964 Helsinki Declaration. Approval was obtained from the local Ethical Review Board (No. 54014116.9.0000.5391).

Consent to participate Each participant provided written consent after being informed of the purpose of the study, experimental procedures, and potential risks.

Consent for publication Not applicable.

Availability of data and materials All data supporting the results of this study may be made available from the corresponding author on reasonable request.

Author contributions The authors' contributions were as follows: Designed research: HR, SMP, BG, RMRP, GGA, and VHL; Conducted research: VHL, IL, SG, ALF, and LARC; Provided essential materials: RMRP; Analyzed data/Statistical analysis: VHL, HR, SMP, BG, SG, and GGA; Wrote paper: HR, SMP, BG, GGA, and VHL; Primary responsibility for final content: HR. All authors: read and approved the manuscript.

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