BMJ Nutrition, Prevention & Health

Randomised, double-blind, placebo-controlled study to evaluate the effect on human strength and endurance after resistance training and supplementation of *Vicia faba* protein hydrolysate compared with placebo

Niamh Máire Mohan , Nora Khaldi, Brian Keogh, Andy Franklyn Miller

To cite: Mohan NM, Khaldi N, Keogh B, *et al*. Randomised, double-blind, placebo-controlled study to evaluate the effect on human strength and endurance after resistance training and supplementation of *Vicia faba* protein hydrolysate compared with placebo. *BMJ Nutrition, Prevention & Health* 2025;**0**. doi:10.1136/bmjnph-2024-001050

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjnph-2024-001050).

Nuritas Limited, Joshua Dawson House, Dawson Street, Dublin, Ireland

Correspondence to
Dr Niamh Máire Mohan;
mohan.niamh@nuritas.com

Received 30 August 2024
Accepted 7 March 2025

ABSTRACT

Objective To assess the effects of a *Vicia faba* protein hydrolysate (VFH) on muscular strength and endurance when combined with resistance training for 56 days compared with exercise alone in a mixed population.

Design A double-blind placebo-controlled parallel trial.

Participants 72 healthy men and women aged 19–40, stratified in a 1:1 ratio by gender. Participants were excluded if they performed >3 hours of resistance training per week in the 6 months prior.

Setting Ontario, Canada. Study performed by KGK Science from August 2023 to January 2024.

Intervention VFH or silica microcrystalline cellulose is given in five capsules daily for 56 days.

Main outcome measures Primary outcome: Leg strength *via* one-repetition maximum for bilateral leg extension. Secondary outcomes: Muscular endurance *via* repetitions to exhaustion, body composition *via* dual-energy X-ray absorptiometry, plasma biomarkers *via* ELISA, quality of life *via* short form survey (SF-36) questionnaire.

Results Intergroup analysis revealed a significantly greater increase in leg strength compared with placebo at day 28 (p=0.045) and 56 (p=0.05), respectively. Significantly enhanced muscular endurance was also observed from days 0 to 56 with a difference of 2.2 times in the change in repetitions performed from baseline (p=0.022) and a 21.6% increase compared with the placebo. Significant changes in bone mineral content were reported between groups (p=0.032) with a mean increase of 0.7% gained in the VFH group. The improvements in performance were supported by myokine analysis where VFH was shown to modulate a range of biomarkers associated with glucose homeostasis, bone formation, mitochondrial and metabolic function. Quantitative physical strength gains were consistent with qualitative data which showed significantly improved changes in self-assessed health.

Conclusions VFH supplementation demonstrated significant improvements in muscular strength, endurance and bone mineral content when compared with placebo. These low-dose, peptide-induced improvements enhance the effects of exercise for musculoskeletal health and have the potential to influence all-cause mortality *via* muscular strength.

Trial registration number NCT05946746.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ As a population, muscle loss is a hallmark of ageing, leading to loss of mobility and general health. Resistance training and protein supplementation are known to increase muscle strength and mass, which are correlated with a reduction in all-cause mortality.

WHAT THIS STUDY ADDS

⇒ Regardless of protein intake, supplementation with *Vicia faba* protein hydrolysate (VFH) enhances gains in strength and muscular energy greater than resistance training alone. VFH also added to bone mineral content substantially compared with placebo.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The low dose of peptides within VFH target positive changes in muscle strength, without additional protein intake. This raises the possibility of a significant impact on mobility and all-cause mortality by adding to everyday food, getting more from what we consume.

INTRODUCTION

Stress, poor work–life balance, declining economic wealth and intake of highly processed foods mean that only one in four adults meets physical activity guidelines.[1] This lifestyle is associated with a 50% increased risk of cardiovascular disease, 48%–53% increase in anxiety and 12% increase in type 2 diabetes.[2] Current WHO guidelines recommend two resistance training sessions weekly.[3] This correlates with a meta-analysis

demonstrating a 27% reduction in all-cause mortality with modest increases in strength at only 60 min of training per week.[4] Resistance training aids in the prevention and management of type 2 diabetes by reducing visceral fat, lowering glycated haemoglobin (HbA1C) and upregulating glucose transporter type 4,[5] alongside other benefits, such as reducing blood pressure, modulating triglycerides, promoting bone development[6] and ameliorating inflammation.[5]

In parallel, the UN predicts that the elderly population will reach 2 billion by 2050, and that global ageing will bring new challenges for health and economics.[7] The loss of muscular strength and resultant sarcopenia are significant risk factors for morbidity and mortality. Muscle loss occurs at a rate of 3%–4% per year after age 75 and is considered one of the most physically avoidable aspects of ageing.[8]

In gaining muscle, sufficient dietary protein is needed to offset muscular catabolism.[9] The recommended intake is 0.8 g/kg-bw for non-exercising adults and 1.8–2.0 g/kg-bw for athletes,[10] with recent findings suggesting no upper limit on absorption.[11] Plant protein is considered as having lower digestibility and poorer essential branched-chain amino acid (BCAA) profile compared with those from animal sources.[12] BCAAs, such as leucine, trigger muscle anabolism, in part, through activation of mammalian target of rapamycin complex 1 (mTORC1) in muscle cells which promotes protein synthesis by activating ribosomal protein S6 kinase, stimulating hypertrophy.[13] During digestion, proteins become hydrolysed into smaller peptide fragments which are well established as having critical cell signalling roles in muscle regulation.[14] While peptides have shown limited oral bioavailability, membrane impermeability and poor stability *in vivo*,[15] artificially intelligent (AI) predictive technologies are helping to overcome these limitations and identify sequences with improved half-lives and gastrointestinal survivability.[16] Peptides can be absorbed in their intact form and elicit numerous direct pathway augmenting effects.[14]

Vicia faba protein hydrolysate (VFH) is a generally regarded as safe supplement containing a network of peptides, previously shown to target mTOR, muscle atrophy genes Atrogin-1 and Murf-1 and inflammation.[17] VFH has also been shown to modulate muscle biomarkers of homeostasis after an exercise insult, reducing myostatin and fibroblast growth factor 21 (FGF-21) and transiently increasing exerkines such as irisin and interleukin-15 (IL-15) to active synthesis pathways.[18] Our hypothesis is that VFH, independent of protein intake, will support strength gains and muscular endurance improvements due to upregulation of peptide-driven mechanisms. To test our hypothesis, we conducted an 8-week randomised double-blind placebo-controlled trial to assess the effects of VFH supplementation when combined with resistance training on the enhancement of strength and endurance in young, healthy, untrained men and women.

MATERIALS AND METHODS

Subjects

115 healthy, untrained men and women (19–40 years) were screened. 72 met inclusion/exclusion criteria (table 1), were randomised in a 1:1 ratio and stratified by sex to the VFH or placebo groups. Inclusion/exclusion criteria were assessed again before each clinic visit by the clinic staff. A sample size of 72 participants achieved 80% power (with up to 20% dropout) to detect an effect size of 0.75 (calculated using R V.4.3.2 Statistical Software) with a two-sided significant level of 0.05 using the two-sample t-test on the primary outcome.

Trial design

This randomised, double-blind, placebo-controlled study investigated the effects of VFH and resistance training on muscle strength and endurance when supplemented for 8 weeks in a mixed population.

After inclusion, participants were assigned to the VFH or placebo group using block randomisation and subsequently enrolled. Prior to day 0, a mandatory exercise familiarisation session was conducted. Baseline strength, vitals, anthropometrics, dual-energy X-ray absorptiometry (DXA) and physical activity diaries were recorded on day 0, prior to supplementation, then again on days 28 and 56. Pre-exercise and post-exercise venous blood samples were collected on days 0 and 56. Blood was collected in EDTA tubes, which were inverted eight times and centrifuged (15 min at 1200 g at 4°C). Plasma was transferred to cryovials and stored at −80°C. Participant self-reported health-related quality of life was recorded at days 0, 28 and 56 using the RAND SF-36 questionnaire.[19] The study assessment schedule is further detailed in online supplemental file 1.

Food diaries

Participants used Libro (https://www.nutritics.com/en/product/libro/), a food diary application to record consumption, calculate daily calories and macronutrient intake. Food and drink intake was self-reported *via* the app for 3 days in the week prior to each study visit.

Supplementation regime

VFH is a fava bean (*Vicia faba*) protein hydrolysate, containing a patented network of peptides. Both groups performed the same resistance training programme, and silicified microcrystalline cellulose (MCC) was supplemented in the placebo group. All capsules were identical to retain blinding. In line with dosing regimens where VFH was previously investigated, participants were instructed to consume five capsules (2.4 g of VFH) with their first meal for 56 days.[18]

Resistance exercise protocol

Both groups were asked to complete three sessions of resistance training per week targeting all major muscle groups (online supplemental file 2), consistent with American College of Sports Medicine guidelines.[20] Exercises were

Table 1 Subject inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
► Men and women between 19 and 40 years of age, inclusive ► Body mass index between 18.5 and 29.9 kg/m^2, inclusive ► Women not of childbearing potential must have a negative baseline pregnancy test and agree to use a medically approved method of birth control ► Participant is capable and willing to perform the recommended physical training and exercise regimen 3×/week ► Motivated to comply with exercise guidelines as assessed by a Self-Motivation Questionnaire ► Self-reported stable body weight for the last 3 months ► Agrees to refrain from taking NSAIDs and alcohol consumption for 24 and 48 hours, respectively, prior to each clinic visit ► Agrees to avoid vigorous exercise for 48 hours prior to the exercise challenge ► Agrees to maintain current lifestyle habits as much as possible ► Willingness to complete questionnaires, records and diaries associated with the study and to complete all study assessments ► Provide voluntary, written and informed consent to participate in the study ► Healthy as determined by medical history, physical examination, ECG and vital signs	► Individuals who are pregnant, breastfeeding or planning to become pregnant during the study ► Known allergy, sensitivity or intolerance to fava bean or MCC ► Has participated in structured resistance/power exercise training for >3 hours/week in the 6 months ► Currently experiencing any medical condition (eg, orthopaedic injury, surgery, neuromuscular problems, musculoskeletal injury and/or disease) that may interfere with the ability to undergo physical strength testing during the study ► Metal implants or other physical characteristics/limitations that may affect DXA scan results ► Participants who have followed a specific diet (eg, ketogenic, paleo, high-protein, vegetarian) or have had a change of diet within 30 days ► Have a history of being diagnosed with phenylketonuria or another disease affecting amino acid metabolism ► Significant cardiovascular event in the past 6 months ► Unstable hypertension ► Self-reported confirmation of current or pre-existing thyroid condition. ► Current or history of significant diseases of the gastrointestinal tract ► Current unstable significant psychiatric condition (eg, clinical depression, eating disorders) and/or sleep disorders ► Currently have cancer ► Type I or type II diabetes ► Current unstable diagnosis with kidney and/or liver diseases ► Current use of any prescribed or over-the-counter medications and/or supplements that may affect muscle mass, muscle strength or metabolism ► Alcohol intake average >2 standard drinks per day ► Alcohol or drug abuse within the last 12 months ► Regular use of tobacco, vapes or nicotine products within 6 months of study start ► Blood donation 30 days prior to screening, during the study or a planned donation within 30 days of the last study appointment ► Participation in other clinical research studies 30 days prior to baseline ► Individuals who are unable to give informed consent ► Any other condition, chronic disease, or lifestyle factor, that, may adversely affect the participant's ability to complete the study

DXA, dual-energy X-ray absorptiometry; MCC, microcrystalline cellulose; NSAIDs, non-steroidal anti-inflammatory drugs.

completed to failure, and compliance was determined *via* logging in physical activity diaries.

Strength measurements

Leg and upper body muscle strength was assessed using one repetition maximum (1RM) test for bilateral leg extension and bench press, respectively, and was defined as the maximum weight lifted for a single repetition with correct form. Participants performed a general warm-up followed by stretching and a specific warm-up for leg extension. At baseline, the individual 1RM test began based on an estimated 1RM, previously determined during a familiarisation session. The highest weight correctly executed was recorded as the baseline 1RM. At subsequent study visits, the 1RM recorded at the previous visit was used as a starting point.

Endurance measurements

Muscle endurance was assessed by participants completing bilateral leg extensions or bench press to failure (defined as not being able to complete another full repetition) at 80% of their 1RM at baseline.

Dual-energy X-ray

Body composition was measured using DXA (GE Lunar Prodigy Advance DXA Scanner) and converted into body fat and muscle mass percentage for assessment along with bone mineral content (BMC). A single, whole-body scan was conducted at days 0 and 56 and included Lunar Prodigy Advance Maximum Scan Area (long×transverse) with total body measurements: 197.5×60 cm measurement field.

Luminex ELISA

Myokine levels in plasma were measured *via* magnetic bead MILLIPLEX multiplex immunoassays (HMYOMAG-56K Merck, Darmstadt, Germany) on a MAGPIX system (Luminex xMAP-technology). The assay was performed according to the manufacturer's instructions and analysed using the xPONENT data analysis software (v.3.1.7; Luminex Corporation) and a 5-parameter logistic-curve fit.

Growth differentiation factor-15 ELISA

Growth differentiation factor-15 (GDF-15) was quantified in human plasma samples using a human GDF-15 DuoSet ELISA according to the manufacturer's instructions (Bio-techne), read using a ClarioStar plate reader (BMG Biotech) and analysed using Mars Software V3.31.

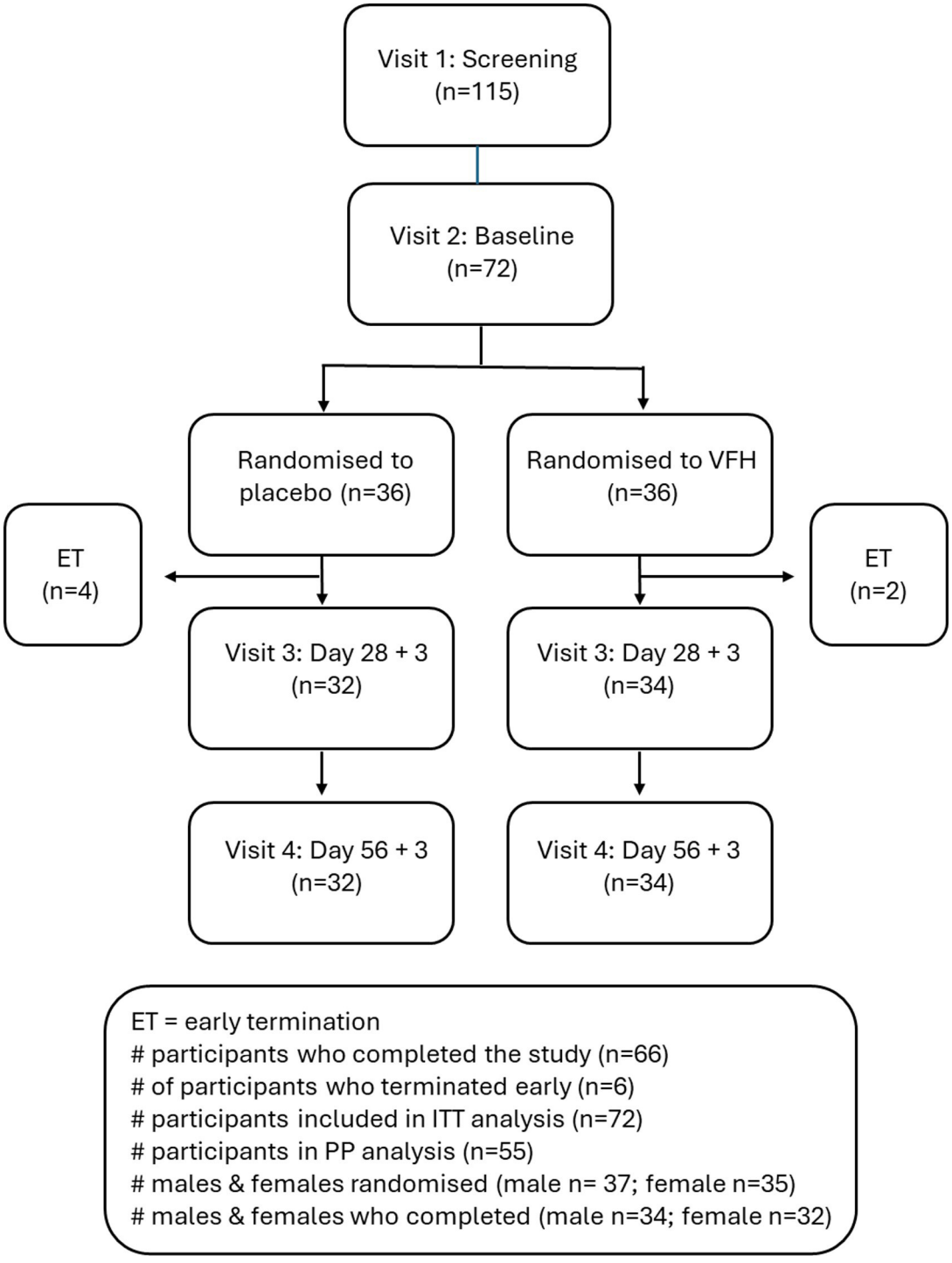

Figure 1 CONSORT flow diagram of the study. CONSORT, Consolidated Standards of Reporting Trials; ITT, intention to treat; PP, per protocol; VFH, *Vicia faba* hydrolysate.

Data analysis and statistics

Summary statistics are presented as the mean, SD, SEM, median, minimum and maximum for continuous demographic and baseline characteristic variables. Coefficient of variation is presented for the immunoassays. Categorical demographic and baseline variables are summarised as frequencies and percentages. Data are represented graphically using error bar plots and boxplots highlighting the median, IQR, maximum and minimum values. Statistical tests were performed one-sided given the nature of the study whereby positive change is anticipated given the inclusion of resistance exercise in both groups and interpreted using a 5% level of significance. Cohen's d effect sizes were calculated for between-group differences. Effect sizes were classified as small (d<0.2), medium (d>0.21, d<0.5), or large (d>0.51) and were calculated as; effect size = mean (VFH) − mean (MCC) / averaged SD.

Continuous variables were evaluated for normality using the Shapiro-Wilk's test. Where appropriate, an ROUT outlier analysis was performed with a 1% threshold. In cases where normality assumptions were satisfied, between-group differences were calculated using independent T-tests; otherwise, Mann-Whitney U tests were performed. Where appropriate, post-intervention differences were adjusted for using analysis of covariance (ANCOVA) analysis where normality assumptions were met, and rank transformed ANCOVA where assumptions were not met. Within-group differences were assessed using paired T-tests if the outcome was normally distributed, and using the Wilcoxon signed rank test otherwise. The Fisher's exact test was used for the comparison of categorical variable distribution between study arms. P values within-group for categorical variables were calculated using Bhapkar's test. A motivational questionnaire was conducted prior to baseline (online supplemental file 1) to assess participants' commitment to comply with study procedures. As such, missing data in the analysis of primary and secondary endpoints was minimal and, therefore, no imputation or sensitivity analysis was performed. GraphPad Prism V.9.1.0 was used to analyse data. The results presented below refer to the per protocol data set only given their adherence to the study protocol. Adjudication of adherence was performed blinded.

Table 2 Demographic information for the per protocol population

Variable	Placebo (n=28)	VFH (n=27)	P value between groups
Age (years)			
Mean±SD	31.00±6.27	32.56±5.90	0.347
Median (Min to Max)	32.00 (19.00 to 41.00)	32.00 (21.00 to 40.00)	
Weight (kg)			
Mean±SD	70.71±8.72	75.63±13.15	0.111
Median (Min to Max)	69.80 (53.80 to 86.60)	75.90 (56.50 to 105.80)	
Height (cm)			
Mean±SD	171.57±9.60	173.70±10.26	0.430
Median (Min to Max)	171.50 (152.00 to 191.00)	174.00 (152.00 to 191.00)	
BMI (kg/m^2)			
Mean±SD	23.98±1.83	24.93±2.71	0.135
Median (Min to Max)	24.10 (20.10 to 27.90)	24.60 (19.80 to 29.60)	
Gender (n, %)			
Women	14 (50.00)	11 (40.74)	0.591
Men	14 (50.00)	16 (59.26)	
Race (n, %)			
African American/black	1 (3.57)	0 (0.00)	0.720
Central American	1 (3.57)	1 (3.70)	
Eastern European White	3 (10.71)	6 (22.22)	
Middle Eastern	2 (7.14)	0 (0.00)	
South American	6 (21.43)	8 (29.63)	
South Asian	1 (3.57)	0 (0.00)	
South-East Asian	2 (7.14)	1 (3.70)	
Western European White	12 (42.86)	11 (40.74)	

BMI, body mass index; Max, maximum; Min, minimum; VFH, *Vicia faba* hydrolysate.

Table 3 Protein intake as assessed by food records from baseline at day 28 and day 56 for the PP population (n=55)

Timepoint	Placebo	VFH	P value between groups estimated difference (95% CI)
Protein (g) baseline (day 0)			
Mean±SD	79.76±25.84	91.88±19.80	0.060*
Median (Min to Max)	73.05 (34.04 to 147.97)	92.73 (61.88 to 136.3)	−12.12 (−24.77 to −0.518)
Protein (g) day 28			
Mean±SD	84.23±25.86	86.95±24.14	0.688†
Median (Min to Max)	86.06 (39.22 to 132.51)	88.03 (37.83 to 132.13)	−2.72 (−16.25 to 10.80)
Protein (g) day 56			
Mean±SD	83.85±29.25	91.13±25.65	0.334†
Median (Min to Max)	81.01 (20.56 to 140.18)	94.85 (43.92 to 140.01)	−7.28 (−22.28 to 7.72)

*P values between groups were calculated using Wilcoxon rank sum test.
†P values between groups were calculated using an independent t-test.
Max, maximum; Min, minimum; PP, per protocol; VFH, *Vicia faba* hydrolysate.

RESULTS

Trial design and population allocation

72 participants were included in the intention-to-treat analysis, with 36 randomised in each group. 17 participants were excluded from the per-protocol (PP) population due to failure to comply (n=6), poor exercise compliance (n=8), out-of-window study visit (n=2) and non-compliance to 1RM procedure (n=1) (online supplemental file 3). The PP population included 55 participants (30 men and 25 women), n=27 in the VFH group and n=28 in the placebo group (figure 1). There were no significant between-group differences in demographic characteristics (table 2).

Food diaries

Macronutrient intake was assessed from Libro app data. The greater proportion of men in the VFH group (9.26%) contributes to the slightly greater intake of protein throughout the study compared with placebo; however, no significant difference in protein intake was observed between groups (table 3). Intake of fat and carbohydrate did not differ between the groups (online supplemental file 4).

Strength

The VFH group had 2.35 kg and 2.04 kg greater increases in 1RM leg muscle strength compared with placebo from baseline at days 28 (p=0.096) and 56 (p=0.125), respectively. Intergroup analysis at day 56 showed that the VFH group had a significantly greater increase in 1RM leg muscle strength compared with placebo (p=0.05, Cohen's d=0.43) with 83.90 kg and 74.74 kg lifted, respectively (figure 2, online supplemental file 5). Consistent with the findings at day 56, significant differences in leg strength were observed between groups at day 28 in the mixed population, whereby the placebo group had a mean of 68.99 kg vs the VFH group who averaged 78.45 kg on the 1RM test (p=0.045, Cohen's d=0.45) indicating a moderate effect size and meaningful effect. While non-significant, iAUC analysis of the strength data indicates a 24.5% increase in the change in strength between groups (p=0.140, Cohen's d=0.35) (online supplemental file 6).

The effects of an 8-week resistance exercise programme alone on strength can be observed in the within-group analysis of the placebo group where participants had an increase of 6.55 kg and 12.30 kg from baseline at days 28 and 56, respectively (p<0.001). Similarly, strength gains

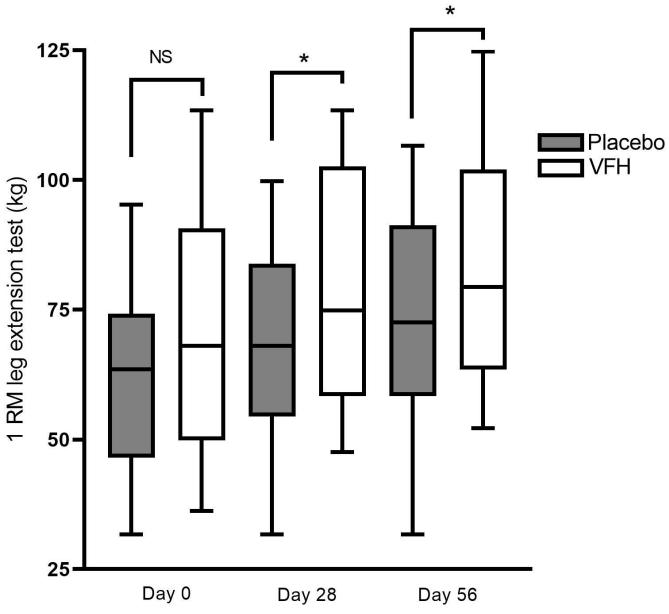

Figure 2 VFH in combination with resistance exercise increases leg muscle strength assessed *via* 1RM for bilateral leg extension. No differences were observed at day 0, whereas significant differences were observed between VFH and Placebo groups at days 28 and 56. (Box and whisker plots, horizontal line represents the median, boxes denote the IQR, bars indicate the maximum and minimum values, one-tailed independent t-test at day 56 p=0.05, Cohen's d=0.43 and day 28 p=0.045, Cohen's d=0.45, *p<0.05). 1RM, one repetition maximum; VFH, *Vicia faba* hydrolysate.

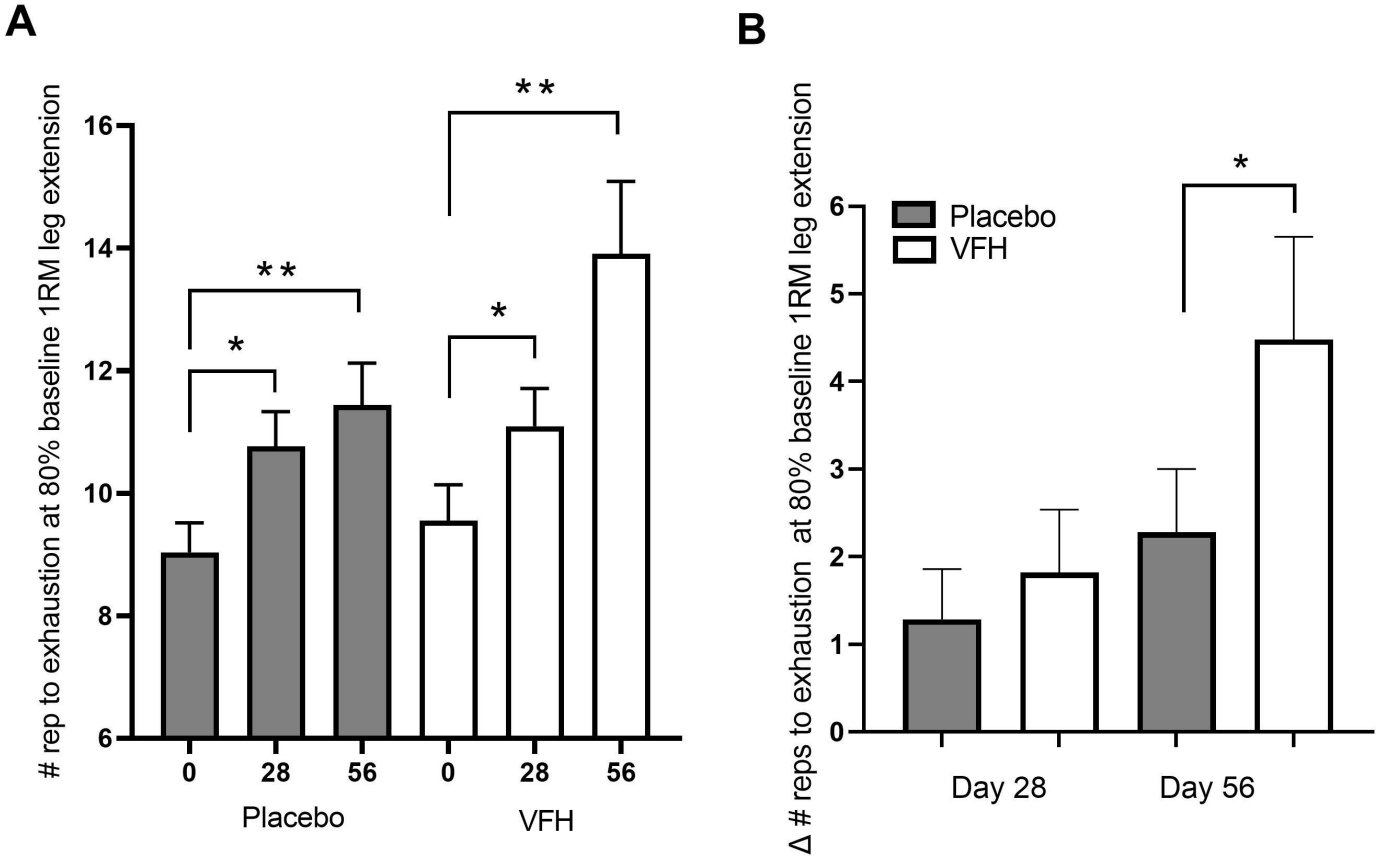

Figure 3 VFH in combination with resistance exercise significantly increases muscular endurance as measured by bilateral leg extension. (A) Both groups increase the number of repetitions to exhaustion at 80% of the 1RM test on the bilateral leg extension (mean±SEM, placebo; p=0.037 and p=0.004 VFH p=0.020 and p=0.001 at day 28 and day 56, respectively, Wilcoxon signed rank test). While non-significant, there is a 21.6% greater improvement in the VFH group at day 56, Cohen's d=0.54. (B) VFH supplementation increases the change in repetitions from baseline at day 28 and day 56 compared with placebo (mean±SEM p=0.022, Cohen's d=0.47 ANCOVA with rank transformation, *p<0.05, **p<0.01). ANCOVA, analysis of covariance; 1RM, one repetition maximum; VFH, *Vicia faba* hydrolysate.

were consistent, but greater in magnitude for the VFH group with increases of 8.9 kg and 14.36 kg in leg strength from baseline at days 28 and 56, respectively (p<0.001).

Changes in upper body muscle strength as assessed by bench press 1RM test from baseline at day 28 (p=0.883, Cohen's d=0.02) and 56 (p=0.775, Cohen's d=0.01) are shown in online supplemental file 7. No significant changes and very small effect sizes were observed, suggesting performance improved over time but remained consistent between both groups throughout the trial.

Muscular endurance

Endurance was significantly improved in both the VFH and placebo group from baseline to day 28 (p=0.020 and p=0.037, respectively) and again from baseline to the end of the study period (p=0.001 and p=0.004, respectively, figure 3A). While not significant (p=0.106), the large effect size (Cohen's d=0.54) suggests the 21.6% difference between the VFH and placebo groups from baseline at day 56 may be meaningful and warrants further investigation with a larger sample size. Participants supplemented with VFH had significant increases of 1.82 and 4.48 repetitions completed at 80% of baseline 1RM to failure for leg extension from baseline at days 28 and 56, while those on placebo had significant increases of 1.29 and 2.28 repetitions, respectively. Adjusting to baseline, the number of repetitions from baseline at day 56 in the VFH group were significantly greater than the placebo group (estimated difference of 2.2 in ranks of repetitions; p=0.022, ANCOVA with rank transformation, Cohen's d=0.47, figure 3B).

Changes in upper body endurance at day 28 (p=0.205, Cohen's d=0.46) and 56 (p=0.199, Cohen's d=0.51) are shown in online supplemental file 7. The moderate size of effect suggests that the 2.63 rep difference performed by the end of the study may indicate a substantial, yet non-statistically significant impact on endurance.

Body composition, BMC and anthropometrics.

A summary of changes from baseline and day 56 in fat mass (g/%), muscle mass (g/%) and android to gynoid fat ratio are shown in online supplemental file 8. There were no significant between-group differences observed.

BMC was assessed using a single, whole-body DXA scan and analysed as an exploratory endpoint. The VFH group

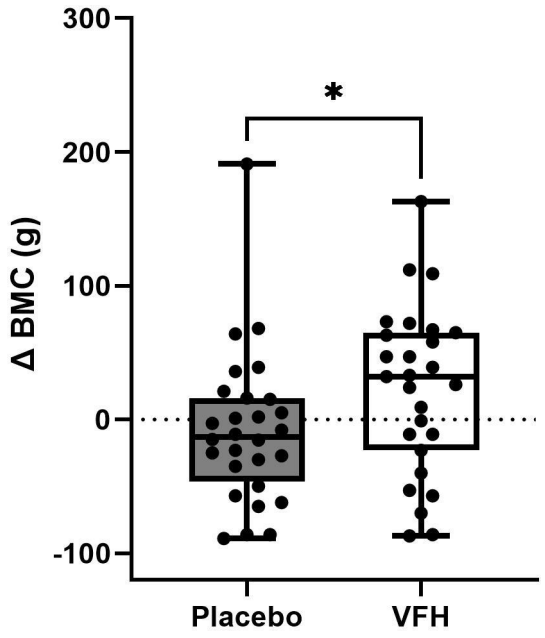

Figure 4 VFH supplementation in combination with resistance exercise augments BMC compared with placebo. Change in BMC was assessed using whole body DXA from baseline to day 56 between VFH and placebo groups. (Box and whisker plots, horizontal line represents the median, boxes denote the inter-quartile range, bars indicate the maximum and minimum values and dots represent individual participants, one-tailed Mann-Whitney U test at day 56, p=0.032, Cohen's d=0.50 *p<0.05). BMC, bone mineral content; DXA, dual-energy X-ray absorptiometry; VFH, *Vicia faba* hydrolysate.

had significantly greater BMC compared with the placebo group at baseline (p=0.037) and at day 56 (p=0.026) (figure 4). However, adjusting for baseline differences, significant changes in BMC with a large, meaningful effect size were observed between groups (p=0.032, Cohen's d=0.50) from baseline to day 56, whereby participants supplemented with VFH had an increase of 22.2 g in BMC (p=0.077), while those on placebo had a mean decrease of 8.2 g (p=0.187).

Changes in anthropometrics (arm, chest, thigh and waist circumference) are shown in online supplemental file 9. No significant between-group differences were observed.

Myokine analysis

Myokines were analysed in plasma collected on days 0 and 56, pre-exercise and post-exercise. Data are expressed as a fold change from baseline to day 56, in the absence of exercise. Significant fold-increases in follistatin-like 1 (FSTL-1), fractalkine, oncostatin, fatty acid binding protein-3 (FABP3), interleukin-6 (IL-6), osteocrin and apelin were observed along with a significant decrease in GDF-15 (figure 5). No significant changes in brain-derived neurotrophic factor, erythropoietin, FGF-21, interleukin-15 (IL-15), irisin, myostatin or osteonectin were observed.

DISCUSSION

VFH and resistance training significantly improved leg strength and muscular endurance in healthy untrained men and women over 56 days. While leg strength improved in both groups, significantly greater gains were observed in those supplemented with VFH. Changes in physical performance were supported by the modulation of myokines associated with metabolic and muscle function. Clinical and functional observations were supported by qualitative questionnaires, showing an improved self-assessed change in health (online supplemental file 10). VFH has been shown previously to support muscle recovery and repair.[18 21] Given the effects of VFH peptides on the phosphorylation of S6, reduction in atrophy-associated genes and inflammation,[18] we hypothesised that VFH may not only improve recovery,[21] but also enhance performance.

Resistance training-induced strength gains can be attributed to muscular adaptation, characterised by hypertrophy and neural adaptations which stimulate agonistic muscles and increase peak force.[22] The exercise programme significantly improved leg strength in both groups (p<0.001). This is consistent with published findings describing the effects of an 8- week training programme on significant increases in 1RM tests.[23] However, the VFH group showed additive effects on strength which were significantly greater compared with placebo (day 28; p=0.045, day 56; 0.05, respectively). Gains may be supported by myokine analysis whereby significant fold increases were observed in IL-6, which, given its role in the promotion of satellite cell proliferation and differentiation, may have contributed to hypertrophy and physical conditioning. Upper body strength improved in both groups; however, no significant difference was observed with VFH supplementation. This may be explained by biomechanical differences and muscle mass disparity whereby quadriceps have greater potential for hypertrophy compared with upper body muscles.

While resistance training alone has the potential to induce neuromuscular adaptive responses,[24] protein supplementation augments the effects of exercise.[9] Interestingly, Davies *et al*[25] investigated the effects of intact *Vicia faba* protein (0.33 g/kg^{-1} or approx. 23 g/day) on FSR in an untrained mixed population versus placebo and concluded no significant difference. By contrast, at 20 g/day, VFH has been shown to increase FSR over those seen with milk protein concentrate during remobilisation, indicating a signalling peptide effect as opposed to a protein effect.[21] Macronutrient recording showed no augmentation or difference in protein (VFH=1.21 g/kg-bw and placebo=1.13 g/kg-bw), fat or carbohydrate intake between groups throughout the trial. Despite this, a 24.5% (p=0.140) increase in the change in strength between groups was observed. Similar increases have been reported for creatine supplementation, with changes in leg strength of 25% reported in men[26] and 28% in women.[27] Contrastingly, Syrotuik *et al*[28] describe an 8% increase under similar trial conditions in a trained

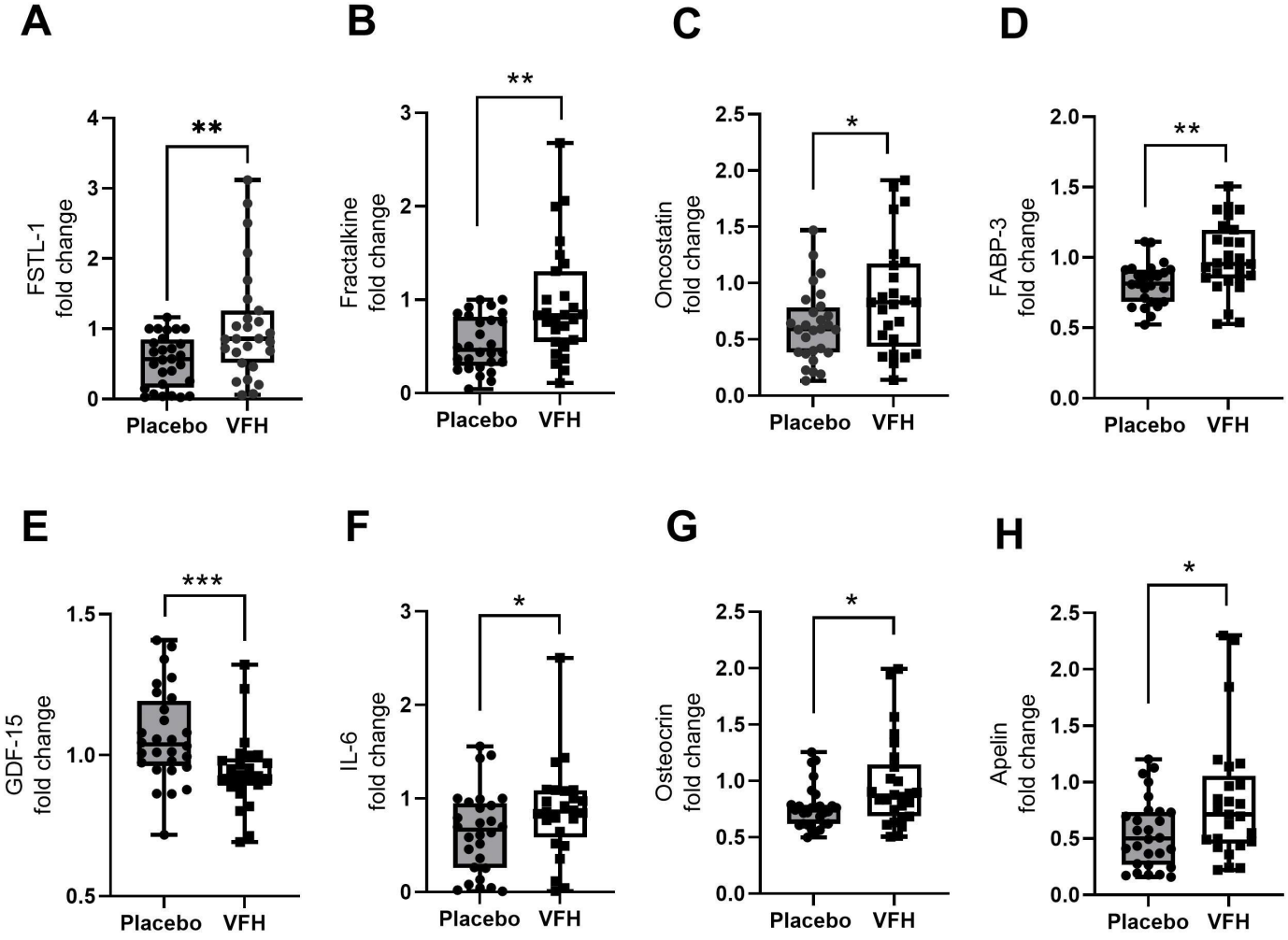

Figure 5 VFH supplementation in combination with resistance exercise alters plasma myokine concentrations after 8 weeks compared with exercise alone. Data are represented as the fold change in analyte concentration from baseline to day 56 prior to the strength test *via* box and whisker plots. The horizontal line represents the median, boxes denote the IQR, bars indicate the maximum and minimum values and dots represent individual participants. Effect of VFH supplementation compared with the placebo on (A) Follistatin-like 1 (FSTL-1) (p=0.002, Cohen's d=0.89, (CV) 0.75, 0.68), (B) Fractalkine (p=0.002, Cohen's d=0.94, (CV) 0.63, 0.53), (C) Oncostatin (p=0.015, Cohen's d=0.62, (CV) 0.57, 0.52), (D) Fatty acid binding protein-3 (FABP3) (p=0.001, Cohen's d=0.91, (CV) 0.25, 0.18), (E) growth differentiation factor-15 (GDF-15) (p<0.001, Cohen's d=0.85, (CV) 0.14, 0.15) (F) Interleukin-6 (IL-6) (p=0.039, Cohen's d=0.44 (CV) 0.58, 0.69), (G) Osteocrin (p=0.012, Cohen's d=0.70, (CV) 0.40, 0.25), (H) Apelin (p=0.021, Cohen's d=0.67, (CV) 0.67, 0.56). Oncostatin and FABP3 were analysed by one-tailed independent T-tests. All other myokines were analysed by Mann-Whitney U test. *p<0.05, **p<0.01, ***p<0.001. Indicates significant fold change from baseline values pre-exercise with VFH supplementation. CV, coefficient of variation; VFH, *Vicia faba* hydrolysate.

population, highlighting the impact of neuromuscular adaptation, muscle recruitment and synchronisation on strength.[24]

Strength improvements in the VFH group were accompanied by improvements in muscular endurance with 2 times and 1.7 times the change in repetitions performed on the leg extension and on the bench press, respectively, compared with placebo. While the training programme was designed primarily for strength, 87% of the VFH group experienced enhanced leg endurance vs 69% in the placebo group with a large effect size (Cohen's d=0.54) suggesting sustained efficacy of VFH, and a role in recovery. This is further supported by the significant fold increases and large effect sizes in myokines including FSTL-1, which increases energy through lipolysis during exercise;[29] fractalkine, which regulates beta cell function;[30] apelin, which helps modulate atrophy;[30] and FABP3, which supports mitochondrial function.[31] Significant fold decreases were observed in GDF-15, a stress-related marker of ageing, associated with sarcopenia and mitochondrial myopathy.[32] Plomgaard *et al*[33] have described the role of GDF-15 in metabolic signalling and shown that energy deprivation is linked with prolonged elevation in plasma, suggesting that the reduction induced by VFH may be supportive of enhanced endurance and recovery. Supplements including caffeine and sodium bicarbonate have been shown to enhance endurance; however, side effects, such as gastrointestinal issues, have limited their use and overshadowed the benefits.[34] Contrastingly, no adverse events were reported with 2.4 g/day of VFH

indicating good tolerability during long-term use. Early strength gains often result first from neural efficiency, followed by increases in muscle mass which drive additional strength changes after neuromuscular adaptations plateau.[24] The strength improvements, muscular co-ordination and force generation in the VFH group may have, therefore, contributed to enhanced strength in the absence of increased mass or changes in circumference measurements. The training programme may have also influenced strength over hypertrophy, with more progressive overloads, higher repetitions and recovery time often required for growth.[35] Other potential confounding factors may have included fitness and body composition, variability in adaptation to exercise, weight selection and hormonal responses.

VFH may also impact bone health as the physiological interactions from muscular tension on skeletal-tendon-bone during exercise affect both contraction and bone formation.[36] The 22 g increase in BMC in the VFH group equates to an increase of approximately 0.7%. Resistance training-induced changes in BMC and bone mineral density tend to be more pronounced in young men, with increases of 2.7%–7.7% previously described,[37] versus more modest changes of up to 1.6% in young women.[38] Myokine analysis supports a possible role in maintaining bone health, whereby significant increases were observed in oncostatin, which regulates bone and muscle maintenance and regeneration[39] and osteocrin, which supports bone formation.[40] Given the short timeframe and inclusion of healthy young women, the change in BMC may be interesting. However, given the variability associated with single whole body DXA, the lack of structural detail on bone microarchitecture, bone function quality and turnover, additional work on the effects of VFH on bone formation and mineralisation is warranted.

The study outcomes were affected by the inclusion of untrained participants, which increased the capacity for strength gains in both groups. In addition, while a mixed-gender population best represents a real-world environment and supports generalisability of the findings, the inclusion of both sexes contributed to increased variability and could be described as a limitation of the study. Stratification based on strength and mass may have offset these differences. Future studies investigating the effect of VFH on BMC and FSR at the proposed dose in a mixed population, as well as on neural and localised muscular adaptations, assessed by changes in peak force and cross-sectional area of muscles, are warranted.

In conclusion, VFH was shown to significantly enhance the effects of resistance exercise and induce sustained improvements in strength performance, endurance, physical conditioning and BMC. As VFH contains only 1.6 g of protein within the supplement and participants in that group consumed 1.21 g/kg-bw, or an average of 91.88 g protein/day, the contribution of the protein content from VFH was less than 1.74%, which would not explain the findings. Therefore, the bioactive peptides within the VFH matrix offer the ability to use VFH as a supplement to target positive changes in strength, without augmenting total protein intake. This raises the possibility of a significant impact on all-cause mortality and longevity, getting more out of existing nutrition and providing a positive benefit to foods containing peptides.

Acknowledgements The authors would like to thank KGK science for conducting the study, Alfonso Blanco and the Conway Institute for the use of their core facilities and finally, Audrey Wall and Mark Gaffney for useful discussion.

Contributors Conceptualisation, NMM, BK, AFM and NK; methodology, NMM, BK and AFM; formal analysis, NMM and BK; writing—original draft preparation, NMM; writing—review and editing, NMM, BK and AFM; visualisation, NMM and BK; supervision, NK, BK and AFM. All authors have read and agreed to the published version of the manuscript. AFM acted as guarantor. An AI platform called 'Magnifier' was used in the identification of the bioactive peptides within the plant-based ingredient described in this publication. This platform is proprietary to Nuritas. This platform has been described and published previously as the AI technology discovery engine for all of Nuritas ingredient discoveries. I am happy to provide links to all of Nuritas publications describing Magnifier.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests All coauthors employed by Nuritas. The project was financially supported by Nuritas. Nuritas is responsible for the study design, data analysis, decision to publish and preparation of the manuscript. KGK is responsible for conducting the study, data collection and analysis.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This trial was conducted in compliance with the Declaration of Helsinki and ethical approval was granted by the Advarra Institutional Review Board 23NLCFP01. The trial was carried out and data were collected by KGK Science, Ontario, Canada.

Provenance and peer review Not commissioned; externally peer reviewed by Mrs. Alice Benskin, The Vicarage Reading Road, UK.

Data availability statement Data are available on reasonable request.

ORCID iD

Niamh Máire Mohan http://orcid.org/0009-0007-1406-6229

REFERENCES

1 Centers for Disease Control and Prevention. Physical activity guidelines for Americans 2008-2018. Trends meet 2008 phys act guidel 2008—2018. 2018. Available: https://www.cdc.gov/physicalactivity/downloads/trends-in-the-prevalence-of-physical-activity-508.pdf [Accessed 15 Jul 2024].
2 Lane MM, Gamage E, Du S, *et al*. Ultra-processed food exposure and adverse health outcomes: umbrella review of epidemiological meta-analyses. *BMJ* 2024;384:e077310.
3 Bull FC, Al-Ansari SS, Biddle S, *et al*. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med* 2020;54:1451–62.
4 Momma H, Kawakami R, Honda T, *et al*. Muscle-strengthening activities are associated with lower risk and mortality in major non-

communicable diseases: a systematic review and meta-analysis of cohort studies. *Br J Sports Med* 2022;56:755–63.
5 Flack KD, Davy KP, Hulver MW, *et al*. Aging, resistance training, and diabetes prevention. *J Aging Res* 2010;2011:127315.
6 Wolff I, van Croonenborg JJ, Kemper HC, *et al*. The effect of exercise training programs on bone mass: a meta-analysis of published controlled trials in pre- and postmenopausal women. *Osteoporos Int* 1999;9:1–12.
7 United Nations. World population ageing 2017 - highlights. New York, 2017.
8 Mitchell WK, Williams J, Atherton P, *et al*. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Front Physiol* 2012;3:260.
9 Morton RW, Murphy KT, McKellar SR, *et al*. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *Br J Sports Med* 2018;52:376–84.
10 Wu G. Dietary protein intake and human health. *Food Funct* 2016;7:1251–65.
11 Trommelen J, van Lieshout GAA, Nyakayiru J, *et al*. The anabolic response to protein ingestion during recovery from exercise has no upper limit in magnitude and duration in vivo in humans. *Cell Reports Medicine* 2023;4:101324.
12 Berrazaga I, Micard V, Gueugneau M, *et al*. The Role of the Anabolic Properties of Plant- versus Animal-Based Protein Sources in Supporting Muscle Mass Maintenance: A Critical Review. *Nutrients* 2019;11:1825.
13 Dodd KM, Tee AR. Leucine and mTORC1: a complex relationship. *Am J Physiol Endocrinol Metab* 2012;302:E1329–42.
14 König D, Kohl J, Jerger S, *et al*. Potential Relevance of Bioactive Peptides in Sports Nutrition. *Nutrients* 2021;13:3997.
15 Wang L, Wang N, Zhang W, *et al*. Therapeutic peptides: current applications and future directions. *Sig Transduct Target Ther* 2022;7:48.
16 Corrochano AR, Cal R, Kennedy K, *et al*. Characterising the efficacy and bioavailability of bioactive peptides identified for attenuating muscle atrophy within a *Vicia faba*-derived functional ingredient. *Curr Res Food Sci* 2021;4:224–32.
17 Cal R, Davis H, Kerr A, *et al*. Preclinical Evaluation of a Food-Derived Functional Ingredient to Address Skeletal Muscle Atrophy. *Nutrients* 2020;12:2274.
18 Kerr A, Hart L, Davis H, *et al*. Improved Strength Recovery and Reduced Fatigue with Suppressed Plasma Myostatin Following Supplementation of a Vicia faba Hydrolysate, in a Healthy Male Population. *Nutrients* 2023;15:986.
19 RAND. 36-item short form survey (SF-36). RAND med outcomes study 2024. Available: https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form/scoring.html#:~:text=Note [Accessed 29 Jun 2024].
20 American College of Sports Medicine. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 2009;41:687–708.
21 Weijzen MEG, Holwerda AM, Jetten GHJ, *et al*. Vicia faba Peptide Network Supplementation Does Not Differ From Milk Protein in Modulating Changes in Muscle Size During Short-Term Immobilization and Subsequent Remobilization, but Increases Muscle Protein Synthesis Rates During Remobilization in Healthy Young Men. *J Nutr* 2023;153:1718–29.
22 Sale DG. Neural adaptation to resistance training. *Med Sci Sports Exerc* 1988;20:S135–45.
23 Díaz I, Márquez-Alvarez C, Mohino F, *et al*. Combined Alkyl and Sulfonic Acid Functionalization of MCM-41-Type Silica. *J Catal* 2000;193:283–94.
24 Hughes DC, Ellefsen S, Baar K. Adaptations to Endurance and Strength Training. *Cold Spring Harb Perspect Med* 2018;8:a029769.
25 Davies RW, Kozior M, Lynch AE, *et al*. The Effect of Fava Bean (*Vicia faba* L.) Protein Ingestion on Myofibrillar Protein Synthesis at Rest and after Resistance Exercise in Healthy, Young Men and Women: A Randomised Control Trial. *Nutrients* 2022;14:3688.
26 Willoughby DS, Rosene J. Effects of oral creatine and resistance training on myosin heavy chain expression. *Med Sci Sports Exerc* 2001;33:1674–81.
27 Vandenberghe K, Goris M, Van Hecke P, *et al*. Long-term creatine intake is beneficial to muscle performance during resistance training. *J Appl Physiol (1985)* 1997;83:2055–63.
28 Syrotuik DG, Bell GJ, Burnham R, *et al*. Absolute and Relative Strength Performance Following Creatine Monohydrate Supplementation Combined With Periodized Resistance Training. *J Strength Cond Res* 2000;14:182.
29 Nam JS, Park S-J, Ahn CW, *et al*. Follistatin-like 1 is a myokine regulating lipid mobilization during endurance exercise and recovery. *Obesity (Silver Spring)* 2024;32:352–62.
30 Chow LS, Gerszten RE, Taylor JM, *et al*. Exerkines in health, resilience and disease. *Nat Rev Endocrinol* 2022;18:273–89.
31 Zhuang L, Li C, Chen Q, *et al*. Fatty acid-binding protein 3 contributes to ischemic heart injury by regulating cardiac myocyte apoptosis and MAPK pathways. *Am J Physiol Heart Circ Physiol* 2019;316:H971–84.
32 De Paepe B. The Cytokine Growth Differentiation Factor-15 and Skeletal Muscle Health: Portrait of an Emerging Widely Applicable Disease Biomarker. *Int J Mol Sci* 2022;23:13180.
33 Plomgaard P, Hansen JS, Townsend LK, *et al*. GDF15 is an exercise-induced hepatokine regulated by glucagon and insulin in humans. *Front Endocrinol (Lausanne)* 2022;13:1037948.
34 Saunders B, Sale C, Harris RC, *et al*. Sodium bicarbonate and high-intensity-cycling capacity: variability in responses. *Int J Sports Physiol Perform* 2014;9:627–32.
35 Dankel SJ, Mattocks KT, Jessee MB, *et al*. Frequency: The Overlooked Resistance Training Variable for Inducing Muscle Hypertrophy? *Sports Med* 2017;47:799–805.
36 McLeod M, Breen L, Hamilton DL, *et al*. Live strong and prosper: the importance of skeletal muscle strength for healthy ageing. *Biogerontology* 2016;17:497–510.
37 Almstedt HC, Canepa JA, Ramirez DA, *et al*. Changes in bone mineral density in response to 24 weeks of resistance training in college-age men and women. *J Strength Cond Res* 2011;25:1098–103.
38 Blimkie CJ, Rice S, Webber CE, *et al*. Effects of resistance training on bone mineral content and density in adolescent females. *Can J Physiol Pharmacol* 1996;74:1025–33.
39 Sims NA, Lévesque J-P. Oncostatin M: Dual Regulator of the Skeletal and Hematopoietic Systems. *Curr Osteoporos Rep* 2024;22:80–95.
40 Watanabe-Takano H, Ochi H, Chiba A, *et al*. Mechanical load regulates bone growth via periosteal Osteocrin. *Cell Rep* 2021;36:109380.