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Leucine-enriched whey protein supplementation, resistance-based exercise, and cardiometabolic health in older adults: a randomized controlled trial

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Abstract

Background Increasing protein intake (above the Recommended Dietary Amount) alone or with resistance-based exercise is suggested to improve cardiometabolic health; however, randomized controlled trials (RCTs) are needed to confirm this.

Methods The Liverpool Hope University-Sarcopenia Aging Trial (LHU-SAT) was a 16 week RCT (ClinicalTrials.gov Identifier: NCT02912130) of 100 community-dwelling older adults [mean age: 68.73 ± 5.80 years, body mass index: 27.06 ± 5.18 kg/m^2 (52% women)] who were randomized to four independent groups [Control (C), Exercise (E), Exercise + Protein (EP), Protein (P)]. E and EP completed supervised and progressive resistance-based exercise (resistance exercise: two times per week, functional circuit exercise: once per week), while EP and P were supplemented with a leucine-enriched whey protein drink (three times per day) based on individual body weight (0.50 g/kg/meal, 1.50 g/kg/day). Outcome measures including arterial stiffness (pulse wave velocity), fasting plasma/serum biomarkers [glucose/glycated haemoglobin, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein, insulin, resistin, leptin, adiponectin, C-reactive protein, tumour necrosis factor-alpha, interleukin-6, cystatin-C, & ferritin], insulin resistance (HOMA-IR), and kidney function (eGFR) were measured before and after intervention.

Results Total protein intake (habitual diet plus supplementation) increased to 1.55 ± 0.69 g/kg/day in EP and to 1.93 ± 0.72 g/kg/day in P, and remained significantly lower ($P < 0.001$) in unsupplemented groups (E: 1.08 ± 0.33 g/kg/day, C: 1.00 ± 0.26 g/kg/day). At 16 weeks, there was a group-by-time interaction whereby absolute changes in LDL-cholesterol were lower in EP [mean difference: −0.79 mmol/L, 95% confidence interval (CI): −1.29, −0.28, $P = 0.002$] and P (mean difference: −0.76 mmol/L, 95% CI: −1.26, −0.26, $P = 0.003$) vs. C. Serum insulin also showed group-by-time interactions at 16 weeks whereby fold changes were lower in EP (mean difference: −0.40, 95% CI: −0.65, −0.16, $P = 0.001$) and P (mean difference: −0.32, 95% CI: −0.56, −0.08, $P = 0.009$) vs. C, and fold changes in HOMA-IR improved in EP (mean difference: −0.37, 95% CI: −0.64, −0.10, $P = 0.007$) and P (mean difference: −0.27, 95% CI: −0.53, −0.00, $P = 0.048$) vs. C. Serum resistin declined in P only (group-by-time interaction at 16 weeks: $P = 0.009$). No other interactions were observed in outcome measures ($P > 0.05$), and kidney function (eGFR) remained unaltered.

Conclusions Sixteen weeks of leucine-enriched whey protein supplementation alone and combined with resistance-based exercise improved cardiometabolic health markers in older adults.

Keywords Aging; Insulin resistance; Inflammation; Lipoproteins

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Introduction

Advancing age is linked to a decline in cardiometabolic health, with older adults at risk of developing metabolic abnormalities such as insulin resistance, type 2 diabetes, arterial stiffness, and obesity.[1] A systemic increase in pro-inflammatory cytokines also characterizes aging, termed inflammaging.[1,2]

The dysregulation of glucose homeostasis and inflammaging are both associated with the loss of muscle mass and strength,[3,4] with insulin resistance and pro-inflammatory cytokines known to confer negative changes in protein metabolism leading to the loss of muscle mass.[4,5] Evidence largely from short-term studies suggests resistance-based exercise and/or a higher intake of dietary protein [beyond the Recommended Dietary Amount (RDA)] may oppose these deleterious alterations.[6–8]

Of the protein sources available, whey protein (a concentrated form of milk protein) contains bioactive peptides and branched-chain amino acids (notably leucine), which are released in the gastrointestinal tract during hydrolysis.[9,10] The functional properties of whey protein are purported to confer metabolic benefits on lipid metabolism, insulin resistance, and immune function.[7,9,10] For instance, whey protein modulates incretin hormones and is highly insulinotropic, and immunoglobins present in whey protein possesses anti-inflammatory properties.[7,9,10] Epidemiological and interventional studies[9,11,12] support this by showing the benefits of whey protein on markers of cardiometabolic disease in healthy and clinical populations; however, long-term randomized controlled trials (RCTs) are needed to confirm these findings in older populations.

We previously reported on the effects of leucine-enriched whey protein alone or combined with resistance-based exercise on body composition, muscle strength, physical performance, myoelectric muscle fatigue, as well as quality of life[13,14] in the Liverpool Hope University-Sarcopenia Aging Trial (LHU-SAT). In this analysis of LHU-SAT, we reported on secondary outcomes relating to cardiometabolic health and more specifically, blood biomarkers. We posited that increasing total protein intake well-beyond the RDA, by means of a leucine-enriched whey protein supplement, alone or combined with resistance-based exercise would improve lipid profile, estimates of insulin resistance, and immunological markers in community-dwelling older adults.

Methods

Study design and participants

LHU-SAT was a 16 week, single-site, RCT (Clinicaltrials.gov identifier: NCT02912130) conducted at the School of Health Sciences, Liverpool Hope University, between September 2016 and March 2018. The trial comprised of four groups: Control (C), Exercise (E), Exercise + Protein (EP), and Protein (P). Recruitment included convenient and snowball sampling, with advertisements in local community and aged care centres, as well as announcements on radio stations, and telephone or email communications detailing study information. The target population was defined with inclusion criteria of men and women aged 60 to 90 years, who reside in the Northwest of England, able to speak and understand English, and willing to consent to participate and follow the study procedures. Exclusion criteria included recent or concurrent participation in other clinical trials, or dietary and/or exercise programmes; participants with self-reported lactose intolerance or uncontrolled diabetes, hypertension, hypotension, and/or psychological and mental illnesses; participants with history of falls or history of osteoporosis; participants with ongoing medical, physical, or hormonal therapies; and patients with major clinical conditions that precluded safe participation in an exercise programme. An exhaustive list of inclusion/exclusion criteria can be found at: https://clinicaltrials.gov/ct2/show/NCT02912130. Eligible participants were block randomized to one of four independent groups by an external advisor not part of the research team nor aware of the participants' identity. All outcome measures were conducted in the morning period after an overnight fast to minimize diurnal variation and were conducted within ±7 days of commencement and completion of the intervention. Ethical approval was granted from the Northwest of England NHS Research Ethics Committee UK (REC Number: 16/NW/0480), and all participants provided written informed consent prior to the commencement of the trial. Primary outcomes of the trial have already been published.[13,14] We report on secondary (cardiometabolic health) outcomes of the trial here following the CONSORT guidelines for reporting on RCTs.

Exercise intervention

E and EP groups completed 16 weeks of a supervised and progressive exercise intervention comprising twice a week resistance exercise and once a week functional circuit exercises on non-consecutive days, prescribed and monitored by qualified exercise scientists (BK and KM). Resistance exercise included leg, chest, calf, and shoulder presses; seated row; back extensions and bicep curl; and two sets to fatigue of each exercise with 3 min break, for 16 weeks, when weight was gradually increased by 2.5 kg for upper and 5 kg for lower body trainings when participants could complete $\geq$ 12 repetitions in both working sets.[13,14] Participants first self-selected a light weight (for warm up) and thereafter selected a moderate weight for the two working sets. Functional circuit exercises comprised 12 bases with 1 min exercise performed at each base, starting with star exercise, followed by wall push up,

battle rope, superman, hip thrust, single led balance, hip hinge, ball throw, lunge, knee plank, box squat, and a mini obstacle course. Exercise adherence was recorded on arrival at the exercise gymnasium reception desk by signing in and average attendance was totalled to give a %. Participants within these two trial groups (E and EP) were advised to abstain from participation in any exercise and physical activity other than what was provided by trial. The non-exercising trial groups (C and P) were instructed to maintain their physical activity behaviour throughout the duration of the study. Any changes to physical activity behaviour were to be reported to the research team.

Leucine-enriched whey protein supplementation

EP and P trial groups were prescribed a vanilla flavoured whey protein isolate supplement enriched with leucine (MyProtein, The HUT Group, Northwich, Cheshire, UK) within a prepared package with quantity calculated based on body weight (1.50 g/kg BW/day or 0.50 g/kg BW/meal whey protein, plus 0.03 g/kg BW L-leucine), together with a shaker bottle (for mixing the supplement with 250 mL water), instructions for consuming the supplements thrice a day for 16 weeks, and a recording sheet handed to researchers on a monthly basis. Compliance with the supplement was calculated by a combination of the recording log and counting unused sachets each month. Participants within these two trial groups (EP and P) were advised to abstain from any protein supplements other than the supplements provided by the trial and to continue consuming their habitual diet. All participants completed a validated 4 day food diary at baseline and endpoint of the trial, to provide a measure of dietary control and ensure that the habitual nutritional intake did not impact the findings. The non-supplemented trial groups (C and E) were instructed to maintain their habitual food intake throughout the duration of the study. Any changes to dietary habits were to be reported to the research team.

Demographics, body-composition, and dietary control

As previously reported,[13,14] participants completed a demographics and health/medical history questionnaire and height (SECA 213 stadiometer) and weight (Tanita MC-180 MA) were measured using standardized laboratory procedures. Body-composition components (lean, fat and bone mass) were evaluated using a multi-frequency bioelectrical impedance device (Maltron Bioscan 920-2, Maltron International, Rayleigh, Essex, UK), and lean (muscle) mass was estimated using a validated equation.[15] Dietary control was monitored using a 4 day food diary (three weekdays & one weekend day), and energy and macronutrient content were analysed using dietary software (Nutritics LTD., Ireland).

Outcome measures

Arterial stiffness

A non-invasive pulse wave analysis was conducted to examine central aortic pressure waveform parameters, and carotid-femoral pulse wave velocity using SphygmoCore XCEL arterial testing device (ATCOR Medical Ltd, New South Wales, Australia). In preparation for this measurement, participant was rested in supine position for 10–15 min. The resting heart rate and branchial systolic and diastolic blood pressure were measured before assessing pulse wave analysis. A femoral cuff was placed over the femoral artery, while a tonometer was simultaneously used to record from the carotid artery. The distance (cm) between the femoral cuff and sternal notch was measured, as a surrogate measure for the aortic arch distance, and used as an intermediate variable for the software of the equipment, to allow calculation of the pulse wave distance, and subsequently pulse wave velocity (further details on this procedure is available in Roche *et al.*[16]).

Plasma and serum blood samples

After fasting overnight, 35 μL capillary whole blood was collected using a lancet, capillary tube/plunger and an assessment kit and injected into the equipment cassette of Alere LDX analyser (Alere Cholestech LDX Analyzer, Cheshire, United Kingdom) to assess plasma glucose, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein (LDL) cholesterol (further details on this procedure is available in Amirabdollahian and Haghighatdoost[17]). A venous blood sample was then collected using a 22 G (or if necessary smaller 23 G) needle and drawn into a 10 mL vacutainer tube (BD Diagnostics, New Jersey, USA). Serum was separated via centrifugation at 1300 rpm for 10 min at 4°C and distributed into six aliquots of 250 μL for each participant and stored at −80°C until used. A biochip array protein analyser Evidence Investigator and Metabolic Syndrome Arrays I and II were used to measure biomarkers using chemiluminescent multiplex immunoassays (Randox Laboratories, Antrim, UK). Metabolic Syndrome Array I was used to measure interleukin-6, tumour necrosis factor-alpha, insulin, leptin, ferritin and resistin, and Metabolic Syndrome Array II was used to examine adiponectin, C-reactive protein, and cystatin-C, following the procedures instructed by the manufacturer. The intra-assay coefficient of variation (4.5% to 9.6%) and inter-assay coefficient of variation (4.9% to 14.0%) were within acceptable ranges. Further details on validation of these assays are reported elsewhere.[18,19]

Estimates of insulin resistance and renal function

Homeostasis model assessment of insulin resistance (HOMA-IR)[20] was calculated using the following validated equation: fasting plasma glucose (mmol/L) × fasting serum insulin (μU/mL)/22.5. Estimated glomerular filtration rate (eGFR)[21] was calculated using the following validated equation: 133 × min (Scys/0.8, 1)$^{-0.499}$ × max (Scys/0.8, 1)$^{-1.328}$ × 0.996^{Age} [×0.932 if female].

Statistical analyses

Baseline characteristics are presented as mean ± standard deviation (±SD), median (interquartile) or frequency with (percentage), as appropriate. Analyses were performed using generalized estimating equations examining the effect of time, group, and group-by-time interaction. This method can account for missing data at random.[22] Residuals were examined visually to evaluate model fit, and some outcomes were transformed using natural logarithm, while for some outcomes, gamma distribution with logarithmic link was used. All outcome measures were adjusted for baseline values in the models. Results are expressed either as mean absolute change in units or fold change with 95% confidence intervals (CI) to interpret the strength of treatment effects between groups. Pearson's correlation was used to evaluate the correlation between changes in fasting biomarkers and body-composition, as well as between changes in biomarkers and compliance to the intervention. All analyses were performed by a biostatistician (SV) using Stata 16.1 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.).

Results

Baseline characteristics and demographics

Three hundred and ninety-four participants completed telephone screening, with 125 participants screened for enrolment at the Health Sciences laboratory. Of those, 123 met randomization criteria and 100 completed the 16 week trial. Mean age of participants was 68.73 ± 5.80 years (52% women), 97 were white, 2 were mixed race, and 1 was of Asian descent. Sixty-four participants lived with their spouse, 28 lived alone and 8 lived with others (family relatives and friends). Employment status ranged: with 84 retired, 5 in full-time employment, 10 in part-time employment, and 1 unemployed. Forty-five had completed full university education, 36 had completed higher occupational training (technical/trade training), 18 had completed only high school education and 1 had completed only primary school education. According to body mass index classifications, 38 were normal weight, 43 were overweight, and 19 were obese, while according to age-specific, sex-specific, and race-specific cut-offs for fat mass (%) a similar number (n = 19) were obese. *Table* 1 shows the baseline characteristics and

Table 1 Baseline characteristics and demographics for each study group

Parameter	Control (n = 31)	Exercise (n = 24)	Exercise + Protein (n = 22)	Protein (n = 23)
Age (years)	68.16 ± 5.85	66.63 ± 3.92	68.59 ± 5.70	71.83 ± 6.51
Sex (women), n (%)	18 (58%)	9 (31%)	13 (59%)	12 (50%)
Ethnicity, White (%)	97%	100%	96%	96%
Body mass index (kg/m^2)	26.24 ± 4.44	28.06 ± 7.21	27.25 ± 4.81	26.92 ± 3.90
Waist/height ratio (cm)	0.53 ± 0.07	0.55 ± 0.10	0.55 ± 0.08	0.54 ± 0.06
BMI, overweight/obese (%)	45%/16%	46%/21%	32%/23%	48%/17%
HbA1c (%)	5.40 (5.20, 5.60)	5.50 (5.25, 5.55)	5.40 (5.30, 5.60)	5.50 (5.30, 5.70)
Plasma glucose (mmol/L)	5.30 (4.87, 5.63)	5.02 (4.67, 5.48)	5.68 (4.16, 6.47)	5.08 (4.28, 5.65)
Systolic BP (mmHg)	140.26 ± 16.32	141.88 ± 19.30	146.77 ± 16.68	148.26 ± 10.42
Diastolic BP (mmHg)	81.48 ± 12.38	83.17 ± 15.71	82.27 ± 8.64	84.39 ± 8.30
Fat mass (%)	31.84 ± 8.35	32.43 ± 10.05	31.66 ± 6.59	33.97 ± 7.72
Fat mass (%), obese, n (%)	5 (16%)	4 (17%)	4 (18%)	5 (22%)
Total lean mass (kg)	24.41 ± 3.88	25.73 ± 5.57	24.38 ± 5.62	24.98 ± 4.58
Appendicular lean mass (kg)	14.46 ± 2.33	15.44 ± 3.05	14.56 ± 3.37	14.31 ± 2.66
Bone mass (kg)	4.16 ± 0.49	4.33 ± 0.67	4.13 ± 0.72	4.15 ± 0.56
Energy intake (kcal/day)	1718.76 ± 330.26	1891.73 ± 395.58	1728.11 ± 359.53	1758.99 ± 348.03
Protein intake (g/kg/day)	1.00 ± 0.26	1.08 ± 0.33	1.16 ± 0.37	0.99 ± 0.24
Protein intake (% total energy)	16.79 ± 3.23	17.23 ± 4.08	17.87 ± 3.36	17.13 ± 4.05
Carbohydrate intake (% total energy)	41.34 ± 9.04	43.87 ± 5.57	39.27 ± 5.94	45.02 ± 7.11
Fat intake (% total energy)	33.48 ± 5.87	35.13 ± 5.08	35.60 ± 6.88	32.78 ± 6.47

BMI; body mass index; BP, blood pressure; HbA1c, glycated haemoglobin.
No significant differences in baseline characteristics ($P > 0.05$; P values not shown in accordance with CONSORT guidelines for reporting RCTs). Note, some baseline data have been reported in previous analyses.[13,14] Values represent mean ± SD, median (IQR: 25th, 75th percentile) or number (n) with proportions. Overweight and obesity are based on BMI classifications (underweight: <18.5 kg/m^2; normal weight: 18.5–24.9 kg/m^2; overweight: 25–29.9 kg/m^2; obese: ≥30 kg/m^2).[23] Proportions of obesity using fat mass (%) are based on age-specific, sex-specific, and race-specific cut-offs for men (White: >31%, Asian/African American: >29%) and women (White: >43%, Asian/African American: >41%).[24]

demographics of participants in four study groups, with no statistical differences observed ($P > 0.05$). Note, some baseline characteristics have been reported in previous analyses of LHU-SAT.[13,14]

Adherence, dropouts, and adverse events

Compliance with the exercise intervention was high in EP (mean %: 78.46 ± 9.57) and E (mean %: 77.13 ± 9.70), while compliance to the supplement was lower in EP (mean %: 43.37 ± 14.78) and P (mean %: 74.27 ± 25.29). Total protein intake (habitual diet plus supplementation) increased from 1.16 ± 0.37 at baseline to 1.55 ± 0.69 g/kg/day in EP during the trial and from 0.99 ± 0.24 at baseline to 1.93 ± 0.72 g/kg/day in P during the trial meeting our target, with total protein intake remaining significantly lower ($P < 0.001$) in unsupplemented groups (E: 1.08 ± 0.33 g/kg/day; C: 1.00 ± 0.26 g/kg/day). As previously reported,[13,14] total energy, fat, and carbohydrate intake (grammes and % of total energy intake) did not differ at baseline ($P > 0.05$). Regarding dropouts, 3 participants failed to return for follow-up testing, 2 dropped out due to disinterest in the study, and 1 due to return-to-work priorities. Adverse events were minor: 2 participants dropped out due to musculoskeletal issues (muscle soreness: $n = 1$; shoulder pain: $n = 1$) related to the exercise intervention and 15 dropped out due to issues with the supplement (undesirable taste: $n = 10$; gastrointestinal discomfort: $n = 5$). Adverse events did not require medical attention and resolved after cessation of the intervention (confirmed by investigator follow-up telephone calls). A flow chart of this information is available in a previous report of LHU-SAT.[13]

Effects of intervention on plasma glucose/HbA1c, lipoproteins, and arterial stiffness

Across time, LDL-cholesterol increased in C and declined in EP and P leading to significant group-by-time interactions at 16 weeks whereby absolute changes in LDL-cholesterol were lower in EP (mean net difference: −0.79 mmol/L, 95% CI: −1.29, −0.28, $P = 0.002$) and P (mean net difference: −0.76 mmol/L, 95% CI: −1.26, −0.26, $P = 0.003$) vs. C. No group-by-time interactions were observed for total cholesterol, HDL-cholesterol and arterial stiffness (*Table* 2, $P > 0.05$), nor were there any effects of time or group-by-time interactions for glucose and glycated haemoglobin (HbA1c) (data not shown as preliminary reported in Kirk *et al.*[14]).

Effects of intervention on serum biomarkers

Across time, insulin declined in EP and P leading to significant group-by-time interactions at 16 weeks whereby fold changes

Table 2 Effect of intervention on plasma lipoproteins and arterial stiffness

Parameter	*n*	Control	*n*	Exercise	*n*	Exercise + Protein	*n*	Protein	Interaction (*P* value)
Total cholesterol									
Baseline (mmol/L)	31	5.30 (4.87, 5.63)	24	5.02 (4.67, 5.48)	21	5.68 (4.16, 6.47)	23	5.08 (4.28, 5.65)	
Δ 16 weeks (units)	30	0.22 (−0.14, 0.58)	23	−0.00 (−0.41, 0.41)	22	−0.29 (−0.72, 0.14)	23	−0.32 (−0.73, 0.09)	0.163
HDL-cholesterol									
Baseline (mmol/L)	31	1.53 (1.38, 1.90)	24	1.40 (1.20, 1.85)	19	1.43 (1.14, 2.06)	22	1.50 (1.08, 1.94)	
Δ 16 weeks (units)	30	0.01 (−0.11, 0.14)	23	0.08 (−0.07, 0.22)	19	0.05 (−0.11, 0.22)	22	0.10 (−0.05, 0.25)	0.847
LDL-cholesterol									
Baseline (mmol/L)	31	2.89 (2.49, 3.46)	22	2.99 (2.41, 3.37)	18	3.04 (2.04, 3.80)	21	2.66 (2.37, 3.65)	
Δ 16 weeks (units)	28	0.33 (0.02, 0.64)	22	−0.04 (−0.40, 0.32)	19	**−0.46 (−0.86, −0.06)**	19	**−0.43 (−0.82, −0.04)**	**0.004**
Arterial stiffness									
Baseline (m/s)	31	8.80 (7.90, 10.00)	24	8.55 (7.75, 9.55)	19	8.50 (7.50, 9.70)	23	10.00 (8.10, 11.30)	
Δ 16 weeks (fold)	31	1.00 (0.90, 1.12)	23	1.04 (0.91, 1.17)	22	0.97 (0.85, 1.11)	22	1.07 (0.94, 1.21)	0.758

HDL, high-density lipoproteins; LDL, low-density lipoproteins.
All outcomes were adjusted for baseline values in the models. Values represent median (IQR) at baseline. Mean delta (Δ) change at 16 weeks is expressed in units or as fold values with 95% CI. Significant between-group values bolded ($P < .05$).

Table 3 Effect of intervention of serum biomarkers, insulin resistance (HOMA-IR), and kidney function (eGFR)

Parameter	*n*	Control	*n*	Exercise	*n*	Exercise + Protein	*n*	Protein	Interaction (*P* value)
Insulin									
Baseline (μU/mL)	29	7.10 (4.72, 10.51)	23	6.74 (4.21, 17.40)	21	6.23 (4.02, 11.61)	23	8.66 (5.75, 12.71)	
Δ 16 weeks (fold)	29	1.15 (0.98, 1.35)	22	1.14 (0.95, 1.37)	22	**0.77 (0.64, 0.92)**	23	**0.84 (0.70, 1.00)**	**0.001**
HOMA-IR									
Baseline	26	1.78 (1.19, 2.61)	22	1.62 (0.93, 3.09)	21	1.54 (0.95, 3.88)	22	2.10 (1.37, 3.06)	
Δ 16 weeks (fold)	29	1.09 (0.92, 1.31)	21	1.15 (0.94, 1.40)	22	**0.76 (0.62, 0.92)**	22	**0.84 (0.69, 1.02)**	**0.006**
CRP									
Baseline (μg/mL)	29	0.97 (0.82, 1.79)	23	0.76 (0.58, 2.04)	22	0.96 (0.54, 1.51)	22	0.85 (0.63, 1.56)	
Δ 16 weeks (fold)	28	0.75 (0.45, 1.23)	22	1.07 (0.61, 1.89)	21	0.80 (0.45, 1.42)	22	0.95 (0.54, 1.69)	0.785
IL-6									
Baseline (pg/mL)	29	2.37 (1.85, 2.88)	23	0.83 (0.63, 1.57)	21	1.12 (0.64, 1.90)	23	2.11 (0.89, 3.81)	
Δ 16 weeks (fold)	29	1.24 (0.78, 1.98)	22	0.88 (0.52, 1.50)	22	1.18 (0.68, 2.02)	23	0.91 (0.54, 1.53)	0.712
TNF-α									
Baseline (pg/mL)	29	7.02 (6.16, 7.67)	23	6.11 (5.25, 6.96)	21	5.79 (4.92, 6.13)	23	6.55 (4.88, 7.71)	
Δ 16 weeks (fold)	29	0.95 (0.85, 1.06)	22	0.94 (0.83, 1.07)	22	0.92 (0.81, 1.04)	23	0.95 (0.84, 1.08)	0.969
Adiponectin									
Baseline (μg/mL)	28	5.34 (3.49, 7.65)	22	3.38 (2.58, 6.41)	22	4.86 (1.67, 10.04)	22	3.22 (2.83, 8.02)	
Δ 16 weeks (fold)	28	1.04 (0.83, 1.30)	22	0.81 (0.62, 1.04)	22	1.09 (0.85, 1.41)	22	1.24 (0.96, 1.60)	0.120
Leptin									
Baseline (ng/mL)	29	2.05 (1.51, 9.12)	23	2.82 (1.54, 4.29)	21	2.89 (1.97, 5.21)	23	3.24 (1.38, 7.76)	
Δ 16 weeks (fold)	29	1.05 (0.66, 1.67)	21	1.02 (0.59, 1.76)	22	0.77 (0.45, 1.33)	23	1.53 (0.90, 2.58)	0.364
Resistin									
Baseline (ng/mL)	29	3.36 (2.82, 4.56)	23	2.59 (1.52, 3.84)	21	2.29 (1.78, 2.68)	23	3.75 (2.38, 5.92)	
Δ 16 weeks (fold)	29	0.96 (0.81, 1.14)	22	0.93 (0.76, 1.13)	22	1.06 (0.86, 1.30)	23	**0.67 (0.55, 0.82)**	**0.009**
Ferritin									
Baseline (ng/mL)	27	138 (80, 224)	22	110 (68, 185)	20	72 (18, 154)	21	154 (104, 270)	
Δ 16 weeks (fold)	27	0.89 (0.74, 1.07)	20	0.86 (0.70, 1.06)	22	0.85 (0.69, 1.04)	22	0.86 (0.71, 1.06)	0.985
Cystatin-C									
Baseline (μg/mL)	29	1.07 (0.91, 1.26)	23	0.93 (0.80, 1.00)	21	0.82 (0.70, 1.05)	23	1.08 (0.91, 1.32)	
Δ 16 weeks (fold)	29	0.96 (0.84, 1.10)	22	1.16 (0.99, 1.35)	22	1.19 (1.02, 1.41)	23	1.03 (0.88, 1.21)	0.161
eGFR									
Baseline (ml/min/1.73 m^2)	29	68.9 (52.4, 83.3)	23	83.3 (70.2, 98.8)	21	91.4 (66.6, 104.1)	23	60.9 (49.9, 81.7)	
Δ 16 weeks (fold)	29	1.04 (0.90, 1.21)	22	0.87 (0.74, 1.03)	22	0.84 (0.71, 1.00)	23	0.98 (0.83, 1.16)	0.209

CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostatic model assessment of insulin resistance; IL-6, interleukin-6; TNF-α, tumour necrosis factor-alpha.

All outcomes were adjusted for baseline values in the models. Values represent median (IQR) at baseline. Mean delta (Δ) change is expressed in units or as fold values with 95% CI. Significant between-group values bolded ($P < .05$).

in insulin were lower in EP (mean difference: −0.40, 95% CI: −0.65, −0.16, *P* = 0.001) and P (mean difference: −0.32, 95% CI: −0.56, −0.08, *P* = 0.009) vs. C, and in EP (mean difference: −0.39, 95% CI: −0.13, −0.66, *P* = 0.003) and P (mean difference: −0.31, 95% CI: −0.05, −0.56, *P* = 0.019) vs. E. Resistin also declined across time in P leading to significant group-by-time interactions at 16 weeks, whereby fold changes in resistin were lower in P vs. EP (mean difference: −0.45, 95% CI: −0.17, −0.73, *P* = 0.002) and vs. E (mean difference: −0.32, 95% CI: −0.04, −0.60, *P* = 0.024) and C (mean difference: −0.36, 95% CI: −0.62, −0.10, *P* = 0.007). No group-by-time interactions were observed for C-reactive protein,

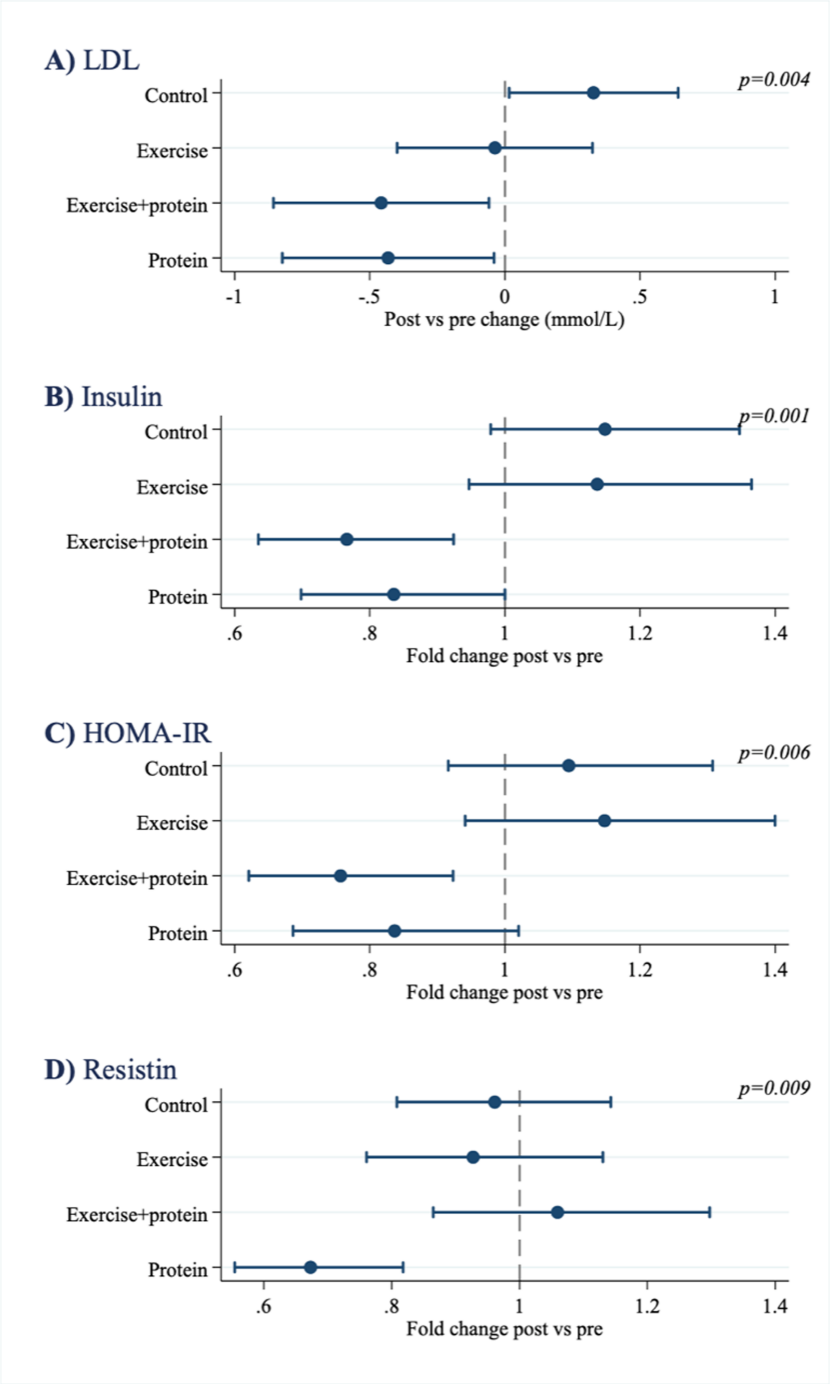

Figure 1 Effect of intervention on (A) LDL-cholesterol, (B) insulin, (C) HOMA-IR, and (D) Resistin. Mean delta (Δ) change is expressed in units or as fold values with 95% CI. Significant *P* value represents group-by-time interaction. HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein.

interleukin-6, tumour necrosis factor-alpha, adiponectin, leptin, ferritin, or cystatin-C (*Table* 3, $P > 0.05$).

Effects of intervention on insulin resistance and renal function

There was a significant group-by-time interaction whereby fold changes in HOMA-IR improved in EP (mean difference: −0.37, 95% CI: −0.64, −0.10, $P = 0.007$) and P (mean difference: −0.27, 95% CI: −0.53, −0.00, $P = 0.048$) vs. C, and in EP (mean difference: −0.42, 95% CI: −0.14, −0.70, $P = 0.004$) and P (mean difference: −0.32, 95% CI: −0.04, −0.60, $P = 0.027$) vs. E (*Figure* 1). No group-by-time interactions were observed for eGFR (*Table* 3, $P > 0.05$).

Correlations between changes in fasting biomarkers and body composition

There were no correlations between changes in LDL-cholesterol, HOMA-IR, or resistin and body-composition components [total lean mass, appendicular lean mass or fat mass (%)] in EP and P nor in these groups combined ($P > 0.05$; Supporting Information, *Figures* S1–S9).

Dose–response relationship between leucine-enriched whey protein supplementation and changes in fasting biomarkers

Exploratory analysis revealed no dose–response relationship between compliance with protein supplementation and changes in LDL-cholesterol, insulin, HOMA-IR, or resistin in EP and P nor in these groups combined ($P > 0.05$; *Table* 4).

Discussion

We report that 16 weeks of leucine-enriched whey protein supplementation alone and combined with resistance-based exercise improved cardiometabolic health markers in older adults. More specifically, increasing total protein intake (1.7 ± 0.7 g/kg/day, average of EP and P groups) by means of supplementation reduced LDL-cholesterol and serum insulin, and improved estimates of insulin resistance (HOMA-IR) when compared with exercise or control groups alone. Furthermore, an even greater total protein intake (~1.9 g/kg/day) in the absence of exercise conferred beneficial reductions in serum resistin when compared with all other interventional groups.

Increasing protein intake beyond the RDA has been suggested as a promising strategy to enhance cardiometabolic health.[9] A recent meta-analysis demonstrated beneficial effects of higher vs. lower protein diets on LDL-cholesterol and HOMA-IR (but not fasting glucose/HbA1c or blood pressure) in middle-aged and older adults with type 2 diabetes,[11] and another meta-analysis[12] evaluating the specific effects of whey protein in healthy/metabolically impaired adults (18–80 years) showed reductions in fasting insulin, HOMA-IR, and LDL-cholesterol (but not HDL-cholesterol or plasma glucose), which corroborates our findings. In the meta-analysis of the latter study,[12] an interaction was observed between study duration and changes in fasting insulin, whereby longer duration studies were more likely to show beneficial effects of whey protein on this biomarker.

Individual RCTs (not included in the above reviews) lend support to our findings. Following 12 weeks of whey protein supplementation (~55 g/day; 30% of total energy intake), LDL-cholesterol and serum insulin (but not plasma glucose) declined, and HOMA-IR improved when compared with isoenergetic amounts of carbohydrates in overweight/obese middle-aged adults.[25] Similar to our findings, these improvements were independent of body composition changes (lean and fat mass). More recently, 13 weeks of leucine-enriched whey protein supplementation (increasing total protein intake to 1.15 g/kg/day) combined with vitamin D and resistance exercise in older adults with obesity and type 2 diabetes reduced serum insulin and improved HOMA-IR (with no between-group differences in plasma glucose) when compared with isocaloric placebo (0.82 g/kg/day) plus resistance exercise.[26] It should be noted this study included a hypocaloric diet and the observed changes in biomarkers occurred alongside increases in lean mass and reductions in fat mass in the supplementation group,[26] which may have been the underlying mechanism for improvements in insulin resistance.

Other RCTs in older adults have revealed conflicting findings. After 24 weeks, Miller *et al*.[27] showed no greater improvements of whey protein supplementation (increasing

Table 4 Dose–response relationship between compliance with the supplement and changes in the outcome

Parameter	*n*	Exercise + Protein (*r*)	*n*	Protein (*r*)	*n*	Combined (*r*)
LDL-cholesterol	17	0.08	17	0.19	34	0.15
Insulin	23	−0.12	21	0.05	44	0.03
HOMA-IR	21	−0.12	21	0.13	42	0.06
Resistin	21	−0.25	23	0.11	44	−0.22

HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein.
No significant relationship was observed ($P > 0.05$).

total protein intake to ~1.4 g/kg/day (average of training/non-training days) plus vitamin D and resistance exercise on HbA1c or HOMA2-IR when compared with resistance exercise alone in overweight/obese older adults with type 2 diabetes. Huang *et al*.[28] demonstrated reductions in visceral fat in older men randomized to receive 1.3 g/kg/day of protein (0.7 g/kg/day via packaged meals; 0.5 g/kg/day whey/casein supplement) vs. 0.8 g/kg/day (control) for 24 weeks but observed no changes in serum biomarkers (fasting glucose, fasting insulin, HOMA-IR, leptin, adiponectin, interleukin-6, or C-reactive protein). Another trial[29] found no changes in LDL-cholesterol, plasma glucose, serum insulin, or HOMA-IR in response to a 12 week milk protein supplement (~0.33 g/kg/day added to habitual diet) either alone or as an adjuvant to resistance-based exercise in older adults, although the dose of whey protein (and leucine) was markedly lower than our supplement and the sample size < 50% of our trial.

In our study, the mechanisms by which leucine-enriched whey protein isolate supplementation induced beneficial changes in LDL-cholesterol, fasting insulin, and HOMA-IR likely relates to the composite of the supplement (and particularly the high concentration of leucine ~3–5 g per drink). Recent commentary[9,10] of pre-clinical and human work suggests whey protein exerts benefits on lipid metabolism and insulin resistance via interconnected pathways. Indeed, whey protein contains bioactive peptides and amino acids such as immunoglobulins, isoleucine, leucine, valine, glutamine, lactoferrin, and lactalbumin. During hydrolysis of whey protein, the release of branched-chain amino acids and bioactive peptides inhibits dipeptidyl peptidase-IV and subsequently decreases degradation of gastric emptying incretin hormones (gastric inhibitory peptide and glucagon-like polypeptide-1), which act in concert to promote insulin secretion.[10] These acute insulinotropic effects of whey protein are suggested to translate into benefits on insulin resistance over long-term supplementation protocols,[9] as we observed here.

An interesting observation in our trial was the significant reduction in resistin that occurred in the protein group alone. Given that total protein intake increased to a higher degree in P vs. EP, we explored the possibility of a dose–repose relationship between protein compliance and changes in this biomarker but observed no correlation. Thus, the mechanisms as well as the clinical significance of this finding is unclear and requires further investigation as this adipokine is linked to lipid metabolism, insulin resistance, and chronic inflammation during aging.[30]

In line with experimental studies and meta-analyses of older adults who are free of renal impairment,[31–34] increasing protein intake well-beyond the RDA for 16 weeks did not exert beneficial nor harmful effects on kidney function (eGFR) or whole-body bone mass (data not shown here). The inclusion of a marker of renal function is a novelty of our study given that only 4/13 of RCTs reported on this outcome at follow-up as highlighted in a recent meta-analysis[11] comparing the effects of high vs. low protein diets on cardiometabolic health.

Independent of protein supplementation, we observed no change in lipids or immunological markers in response to 16 weeks of exercise which we suspect is due to the lower intensity of the intervention with self-selected moderate loads and subjective intensities used during resistance and functional exercise classes, respectively. Three recent RCTs[35–37] demonstrated improvements in lipid and/or immunological profiles following 12–19 weeks of higher intensity resistance and/or aerobic exercise using objective measures of exercise intensity in middle-aged and older adults. Thus, our findings do not disregard the established benefits of specific exercise regimens for cardiometabolic health.

To our knowledge, we have performed the largest RCT on this topic with four (Control, Exercise, Exercise + Protein, and Protein) independent groups in community-dwelling older adults and revealed novel findings regarding cardiometabolic risk factors. This was achieved by prescribing whey protein and leucine at recommended dosages,[38] at each meal and by individual body-weight, a major strength of the present trial. However, some limitations should be noted. First, the secondary outcomes reported here were based on cardiometabolic health, with the initial power calculation based on primary outcomes focusing on musculoskeletal health. Second, the RCT was not placebo-controlled which increases the risk of bias, although as Miller *et al*.[27] highlighted the inclusion of a common isoenergetic carbohydrate placebo, such as maltodextrin, has the potential to influence glycaemic control. Third, we were not able to include changes in energy and macronutrient (fat and carbohydrate) intake as a covariate in the main analyses due to the number of missing food diaries relative to blood biomarkers at post-intervention in some groups (n = 11/24, ~46% of the exercise group), although we do not believe this significantly impacted findings given there was no difference in energy or macronutrient intake at baseline (from n = 81 food diaries) and all participants were instructured to maintain their habitual dietary intake throughout the trial. In support, a very recent meta-analysis (mean age: 46 ± 10 years)[39] on the topic which excluded RCTs with very-low to low fat or carbohydrate intake to limit confounding, found that a higher vs. lower protein diet improved lipid profile and reduced serum insulin without affecting glycaemic control (glucose or HbA1c) as we reported here. Fourth, we did not use the gold standard euglycemic-hyperinsulinemic clamp to measure insulin resistance; however, given our sample size this would have been impractical and laborious with this method requiring intravenous infusions. Finally, our method of evaluating body-composition was carried out using segmental bioimpedance analysis. Including more sensitive measures of muscle mass using computed tomography or magnetic resonance imaging would have enhanced the precision of our exploratory correlation analysis. By including these imaging techniques, the effect of the intervention(s) on intra/

intermuscular fat infiltration and its relationship with insulin resistance could have also been explored. Future RCTs should consider these aspects during study design.

To conclude, we report that 16 weeks of leucine-enriched whey protein supplementation alone and combined with resistance-based exercise reduced LDL-cholesterol and serum insulin, and improved insulin resistance in community-dwelling older adults. In addition, protein supplementation alone (in the absence of exercise) conferred positive decreases in the adipokine resistin. RCTs are now needed to confirm these findings using gold standard dietary control methods in community-dwelling older adults, and these trials should include both risk factors for cardiovascular disease and cardiovascular events as primary and secondary outcomes. Elucidating this information will enable the efficacy and safety of higher vs. lower protein diets on cardiometabolic health to be further evaluated.

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Ethical statement

The authors certify that they comply with the ethical guidelines for publishing in the *Journal of Cachexia, Sarcopenia and Muscle*: update 2019.[40]

Author contributions

All authors contributed to the study design, interpretation of data, drafting and critical appraisal of the manuscript, and approved the final version.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Correlations between changes in LDL-cholesterol and total lean mass in A) Exercise+Protein, B) Protein and C) Combined.

Figure S2. Correlations between changes in LDL-cholesterol and appendicular lean mass in A) Exercise+Protein, B) Protein and C) Combined.

Figure S3. Correlations between changes in LDL-cholesterol and fat mass (%) in A) Exercise+Protein, B) Protein and C) Combined.

Figure S4. Correlations between changes in HOMA-IR and total lean mass in A) Exercise+Protein, B) Protein and C) Combined.

Figure S5. Correlations between changes in HOMA-IR and appendicular lean mass in A) Exercise+Protein, B) Protein and C) Combined.

Figure S6. Correlations between changes in HOMA-IR and fat mass (%) in A) Exercise+Protein, B) Protein and C) Combined.

Figure S7. Correlations between changes in resistin and total lean mass in A) Exercise+Protein, B) Protein and C) Combined.

Figure S8. Correlations between changes in resistin and appendicular lean mass in A) Exercise+Protein, B) Protein and C) Combined.

Figure S9. Correlations between changes in resistin and fat mass (%) in A) Exercise+Protein, B) Protein and C) Combined.

Conflicts of interest

G.D. holds a research grant from TSI Pharmaceuticals and has received consultancy fees from TSI Pharmaceuticals and Abbott Australia. B.K. is currently supported by a post-doctoral research fellowship from TSI Pharmaceuticals. O.K. holds a research grant from Vifor Pharma.

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