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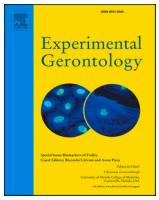

Effects of resistance exercise and whey protein supplementation on cognitive function in older men: secondary analysis of a randomised, double-blind, placebo-controlled trial

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ABSTRACT

Purpose: Ageing is associated with cognitive decline. This study investigated the individual and combined effects of resistance exercise (RE) and whey protein supplementation (PRO) on cognitive function in older men.
Methods: In a pooled-groups analysis, 36 older men (age: 67 ± 4 years) were randomised to either RE (2 x/week; $n = 18$) or no exercise (NE; $n = 18$), and either PRO (2 × 25 g/d whey protein isolate; $n = 18$) or control (CON, 2 × 23.75 g maltodextrin/d; $n = 18$). A sub-analysis was also conducted between RE + CON ($n = 9$) and RE + PRO ($n = 9$). At baseline and 12 weeks, participants completed a battery of neuropsychological tests (CANTAB; Cambridge Cognition, UK) and neurobiological, inflammatory, salivary cortisol and insulin sensitivity biomarkers were quantified.
Results: PRO improved executive function z-score (+0.31 ± 0.08) greater than CON (+0.06 ± 0.08, $P = 0.03$) and there was a trend towards improved global cognitive function ($P = 0.053$). RE and RE + PRO did not improve any cognitive function domains ($p \geq 0.07$). RE decreased tumor necrosis factor-alpha ($P = 0.02$) and interleukin-6 ($P = 0.048$) concentrations compared to NE, but changes in biomarkers did not correlate with changes in cognitive domains. Muscle strength ($r = 0.34$, $P = 0.045$) and physical function ($\rho = 0.35$–0.51, $P < 0.05$) outcomes positively correlated with cognitive function domains at baseline, but only Δskeletal muscle index correlated with Δepisodic memory ($r = 0.34$, $P = 0.046$) following the intervention.
Conclusion: In older men, PRO improved cognitive function, most notably executive functioning. RE did not improve any cognitive function domains but did decrease biomarkers of systemic inflammation. No synergistic effects were observed.

Abbreviations: 1RM, one repetition maximum; 6MWT, 6-min walk test; BDNF, brain-derived neurotrophic factor; CON, control group; CRP, C-reactive protein; DMS, delayed matching to sample; HOMAR-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor 1; IL-10, interleukin-10; IL-6, interleukin-6; MOT, motor screening task; MMSE, mini-mental state examination; MTT, multitasking test; NE, no exercise; PAL, Paired Associates Learning; PRO, whey protein supplementation group; QUICKI, quantitative insulin sensitivity check index; RE, resistance exercise; RTI, reaction time; SMI, skeletal muscle index; SMM, skeletal muscle mass; SPPB, short physical performance battery; SWM, spatial working memory; TNF-α, tumor necrosis factor-alpha.

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1. Introduction

Ageing is linked to an increased risk of developing long-term conditions including dementia and cognitive decline (Daviglus et al., 2010). According to estimates, there are over 50 million cases of dementia worldwide, and by 2030, that number is predicted to rise to ~82 million cases (World Health Organisation, 2019). These conditions have far-reaching effects on people's lives and finances, including decreased capacity for daily living (Giebel et al., 2015), shorter life expectancies (Mooldijk et al., 2022), and a greater financial and emotional strain on families and carers (Kasper et al., 2015). Meta-analyses suggest that age-related cognitive impairment may be linked to sarcopenia (Chang et al., 2016; Cipolli et al., 2019; Peng et al., 2020), the decline in skeletal muscle mass (SMM), strength, and physical function with age (Cruz-Jentoft et al., 2019). This association may be explained by common pathologies of both conditions, which include decreases in growth hormones, insulin resistance, and chronic systemic inflammation (Chang et al., 2016). Accordingly, strategies that curb sarcopenia may also assist in mitigating age-related declines in cognitive function.

Resistance exercise (RE) is well established as a strategy for reducing the progression and associated effects of sarcopenia (Phillips and Martinson, 2019). Several studies have also reported improvements in cognitive function following RE, including enhanced executive function (Anderson-Hanley et al., 2010; Best et al., 2015; Ikudome et al., 2017; Liu-Ambrose et al., 2010; Yoon et al., 2018), memory (Best et al., 2015; Cassilhas et al., 2007; Coelho-Júnior et al., 2020; Ikudome et al., 2017; Marston et al., 2019), and global cognitive function (Coelho-Júnior et al., 2020; Singh et al., 2014; Smolarek et al., 2016). Increases in circulating concentrations of different neurobiological markers, such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1), as well as decreased cortisol secretion and inflammation, are thought to be associated with exercise-induced improvements in cognitive function (Ahlskog et al., 2011; Cassilhas et al., 2007; Erickson et al., 2011; Liu-Ambrose et al., 2012; Tsai et al., 2014, 2015; Walsh et al., 2016).

In addition to RE, in order to prevent sarcopenia, it is advised that older adults consume higher amounts of dietary protein (Phillips and Martinson, 2019). Research also indicates that consuming more dietary protein may help to postpone the cognitive deterioration that comes with ageing (Coelho-Júnior et al., 2021; van de Rest et al., 2013; Yeh et al., 2022). Increased dietary protein intake in older adults has been reported to acutely improve memory (Kaplan et al., 2001) and in the long-term, improve reaction time, memory, and emotion identification (Charlton et al., 2016; Kita et al., 2019; Lefferts et al., 2020; van der Zwaluw et al., 2014). Protein-induced improvements in cognition are thought to be caused by elevated brain insulin receptor signalling stimulation (Frazier et al., 2019), reduced inflammation and increased IGF-1 (Bordoni et al., 2017; Journel et al., 2012), and increased availability of brain neurotransmitters (van de Rest et al., 2013).

Recent randomised-controlled trials suggest that RE and increased dietary protein intake may interact to suppress age-related cognitive decline, whilst synergistically improving reaction time, executive function, memory, and processing speed compared to RE alone (Bell et al., 2019; Rondanelli et al., 2020). Conversely, others have not observed augmented effects (Formica et al., 2020; Mundell et al., 2022; van de Rest et al., 2014); though, van de Rest et al. (2014) did report a synergistic effect on information processing speed compared to protein supplementation alone. The observed inconsistencies may be attributed to the participants' habitual protein intake, variations in daily dosage, and deviations in dietary protein intake from baseline. In studies that reported null findings (Formica et al., 2020; Mundell et al., 2022; van de Rest et al., 2014), habitual protein intake was sufficient (1.0–1.3 g/kg/d) according to consensus groups (Bauer et al., 2013; Deutz et al., 2014) and was increased during the intervention period by ≤0.3 g/kg/d. Contrastingly, whilst Rondanelli et al. (2020) increased dietary protein intake by a similar magnitude to these studies (0.3 g/kg/d), habitual intake was considerably less (0.8 g/kg/d). Bell et al. (2019) also increased dietary protein intake by a notably greater amount to the aforementioned studies (0.5 g/kg/d; from 1.1 to 1.6 g/kg/d). Therefore, even though further research is needed, it's possible that in older adults who regularly consume sufficient dietary protein (1.0–1.2 g/kg/d), a dose of ~1.6 g/kg/d (with a deviation of ~0.5 g/kg/d) may be required to promote synergistic cognitive effects.

The primary aim of this study was to investigate the individual effects of RE and whey protein supplementation (aimed to increase dietary protein intake by >0.5 g/kg/d (from ~1.0 to ~1.6 g/kg/d)) on cognitive function in older men. Secondary aims were to conduct an exploratory sub analysis to determine synergistic cognitive effects of RE combined with whey protein supplementation and to investigate the individual and combined effects on neurobiological, inflammatory and insulin sensitivity biomarkers, and diurnal salivary cortisol, to explore mechanisms of action. We hypothesised that both RE and whey protein supplementation independently would enhance cognitive function and circulating neurobiological biomarkers, and decrease inflammatory biomarkers, and diurnal salivary cortisol. We also postulated that there would be synergistic effects when interventions were combined.

2. Materials and methods

2.1. Participants and experimental design

Thirty-six older men (67 ± 4 years) participated in this study, which was a secondary pooled analysis of a 12-week randomised, double-blind, placebo-controlled, 4-arm parallel group trial (Griffen et al., 2022a). In the present study, RE ($n = 18$) and no exercise (NE; $n = 18$) groups were pooled and compared to one another, as were whey protein supplementation (PRO; $n = 18$) and control (CON; $n = 18$) groups. To ascertain synergistic effects, an exploratory sub-analysis was carried out between the RE + CON ($n = 9$) and RE + PRO ($n = 9$) groups. Complete descriptions of the eligibility criteria, experimental design, and group pooling for analysis have already been published (Griffen et al., 2022a, 2022b). The Mini-Mental State Examination (MMSE) revealed that none of the participants showed cognitive deficits (score >24) (Folstein et al., 1975). All measurements were taken at baseline and following the 12-week intervention. Ethical approval was granted by Coventry University Ethics Committee (project code: P59723) and the study was registered at clinicaltrials.gov as NCT03299972. All participants provided written informed consent in accordance with the Declaration of Helsinki.

2.2. Exercise training

Full details of the supervised RE intervention (2× per week with >48 h between sessions) have been previously described (Griffen et al., 2022a). Briefly, in each session, participants completed 3 sets of leg press, lateral row, hamstring curl, chest press, leg extension and shoulder press (in that order) on fixed RE machines (Life Fitness, Rosemont, Illinois, USA). Starting at 60 % one repetition maximum (1RM; 10–12 repetitions per set), intensity was progressively raised by ~5–7 % per week for the first four weeks to 80 % 1RM (8 repetitions per set), where it stayed until the end of the intervention. Participants completed each exercise to the point of volitional failure on the last set. When participants were able to execute >12 repetitions on the last set of each exercise and based on 1RM tests conducted every 4 weeks, the intensity was modified accordingly. Participants in the NE group continued their habitual physical activity.

2.3. Nutritional intervention

Full details of the nutritional intervention and rationale behind supplement dosing have been previously described (Griffen et al., 2022a). In summary, participants in the PRO group consumed 25 g of

whey protein isolate (containing ~3 g of leucine and 0.7 g of tryptophan) twice a day, immediately after breakfast and lunch (Instantized BiPRO; Agropur, Quebec, Canada). Participants in the CON group consumed an energy-matched control twice daily at the same times (23.75 g of maltodextrin; Myprotein, Northwich, UK). The nutritional composition of the experimental supplements can be seen in

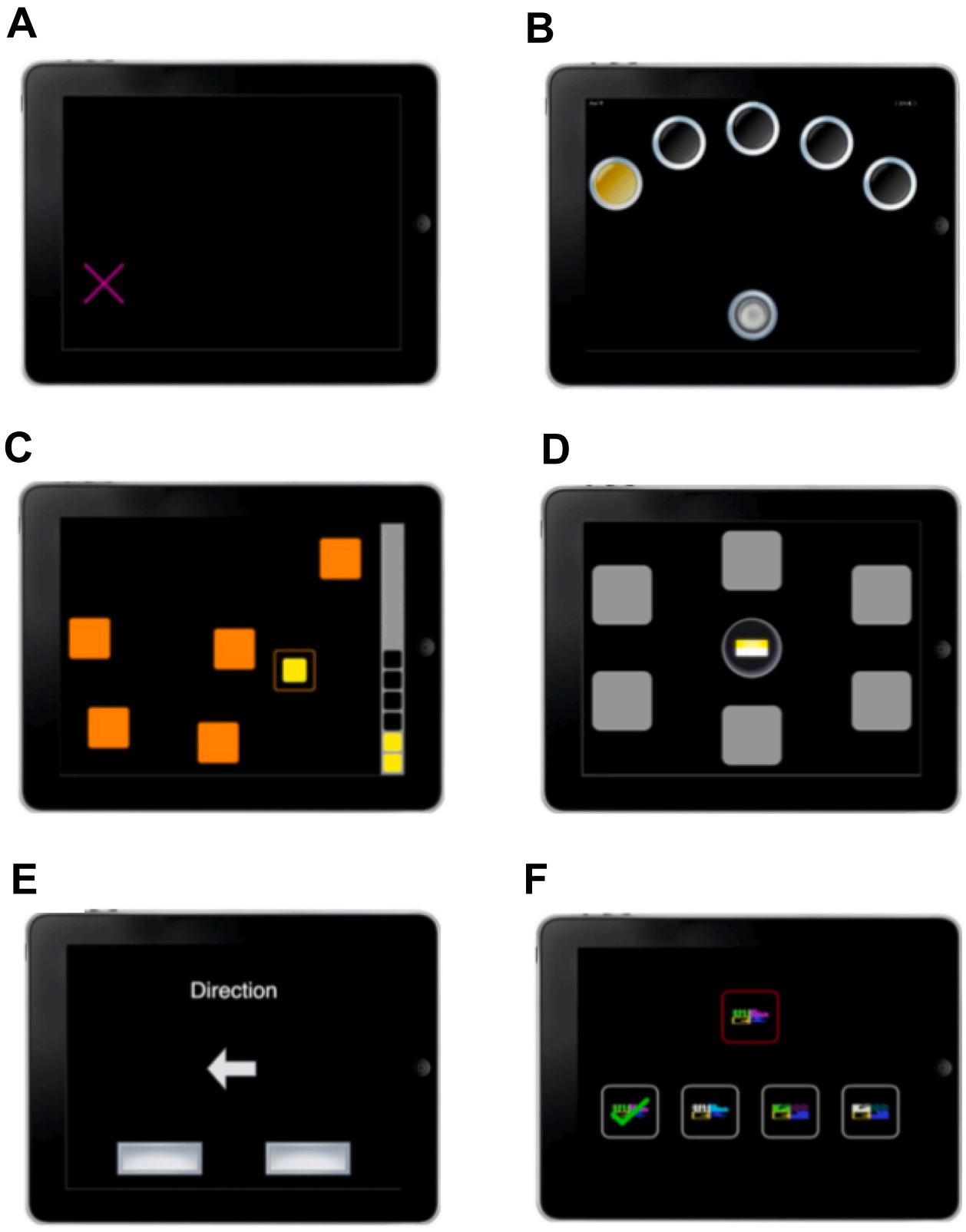

Fig. 1. Visual display of the (A) Motor screening task; (B) Reaction time test; (C) Spatial working memory test; (D) Paired associates learning test; (E) Multitasking test; and (F) Delayed matching to sample test. © Copyright 2018 Cambridge Cognition Limited. All rights reserved.

Supplemental Table 1.

2.4. Dietary intake and habitual physical activity

Full details of the method used to collect dietary intake data has been previously described (Griffen et al., 2022a, 2022b). Briefly, participants recorded their meals for three days—two during the week and one on the weekend—at baseline (before the intervention started) and in weeks 6 and 12. Dietary analysis software was used to examine food records for total energy intake and macronutrient consumption (Nutritics Version 5.097; Nutritics, Dublin, Ireland). Whilst not reported in this study, the method of measurement and results of participants' habitual physical activity have been previously reported in detail (Griffen et al., 2022b).

2.5. Cognitive function

Cognitive function was assessed whilst participants resided in metabolic chambers under highly controlled conditions as previously detailed (Griffen et al., 2022b). Both at baseline and after 12 weeks, cognitive performance was measured at 1000 h to avoid diurnal variance. Post-intervention cognitive testing occurred >72 h following the final RE session and >32 h following ingestion of the final nutritional supplement. Before each assessment, participants had to abstain from alcohol and caffeine for 24 h and consumed a standardised breakfast at 0900 h (see Griffen et al. (2022b) for full details). On a tablet computer (iPad Air 2; Apple Inc., California, USA), participants completed a battery of neuropsychological tests (Cambridge Neuropsychological Test Automated Battery (CANTAB); Cambridge Cognition, Cambridge, UK). According to prior research (Rabbitt and Lowe, 2000; Wild et al., 2008), the CANTAB has been confirmed as a valid and reliable technique for assessing age-related cognitive deficits. The order of cognitive tests was consistent for both visits and participants were familiarised with the CANTAB interface prior to testing.

2.5.1. Motor screening task (MOT)

Touching a flashing cross that was displayed at various points on the screen was required by participants (Fig. 1a). The mean latency (ms) to accurately respond and touch the stimulus was measured during the test.

2.5.2. Reaction time (RTI)

RTI assessed reaction and movement time, and motor and mental response speeds. Holding down a touchscreen button at the bottom of the screen was the instruction given to participants (Fig. 1b). Then, inside one of the five circles, a yellow circle (target stimulus) emerged. Participants had to rapidly release the touchscreen button and tap the yellow circle with the same finger. This was repeated 30 times. Reaction time (median time (ms) taken to release the response button after presentation of the target stimulus (for correct trials only)) and movement time (median time (ms) taken to release the response button and select the target stimulus (for correct trials only)) were assessed as outcome measures.

2.5.3. Spatial working memory (SWM)

SWM evaluated participants' working memory and strategy skills for remembering and manipulating spatial information. The task given to the participants was to locate a token concealed behind boxes and place it in the column on the right side of the screen (Fig. 1c). There was never a token hidden in the same box twice; therefore, participants weren't supposed to go back to a box that already had a token in it. The test had three stages (6, 8 and 12 boxes). Errors (# a box was reopened in which a token had previously been found) and strategy (# a new search began using the same box started with previously - lower score indicates a high strategy use) were assessed as outcome measures.

2.5.4. Paired associates learning (PAL)

PAL assessed visuospatial episodic memory. To uncover patterns buried therein, boxes were arranged on the screen and opened one at a time in a random order (Fig. 1d). One by one, patterns were then shown in the centre of the screen. It was necessary for participants to touch the box containing the pattern. Total errors (# an incorrect box was chosen) and first attempt memory score (# the correct box was chosen on the first attempt) were assessed as outcome measures.

2.5.5. Multitasking test (MTT)

MTT assessed executive functioning. An arrow was displayed on either the right- or left-hand side of the screen and participants were required to make a right or left response. Participants learnt whether to respond either according to the arrow direction, or according to the side of the screen the arrow appeared, no matter what the direction was. A signal suggesting whether the participant should respond in accordance with the direction or side of the arrow was supplied prior to the presentation of the arrow during the assessment stage (Fig. 1e). Total incorrect (# the incorrect button within the response window was pressed), reaction latency (median latency (ms) of stimulus appearance to pressing the response button – calculated across all correct trials), incongruency cost (difference (ms) between the reaction latency on trials that were congruent versus trials that were incongruent), and multitasking cost (difference (ms) between the reaction latency during trials in which both rules (direction *and* side) were used versus trials in which only a single rule (direction *or* side) was used) were assessed as outcome measures.

2.5.6. Delayed matching to sample (DMS)

DMS measured short-term visual recognition memory. The test measured participants' simultaneous or delayed matching (0, 4 or 12 s) of a complicated visual pattern to four samples (Fig. 1f). Participants were instructed to touch the pattern that matched the sample. The percentage of correct trials (for 0, 4 and 12 s delays individually, and all delays combined) were assessed as outcome measures.

2.5.7. Domain-specific z-scores

Individual cognitive test results were converted into z-scores ((value-mean)/SD) at baseline and after 12 weeks, with the reference population being the mean and SD of the entire sample at baseline (Formica et al., 2020). For the following cognitive domains: working memory, episodic memory, executive function, psychomotor/attention, and global cognitive function, z-scores were clustered into compound scores (see Supplementary Materials for full details). To remain consistent with other cognitive outcomes (i.e., a higher z-score indicating superior cognitive performance), z-scores relating to reaction time and errors where a lower score indicates better performance were reversed (value x − 1).

2.6. Skeletal muscle mass, muscle strength and physical function

Full details of the methods used to measure SMM, muscle strength, and physical function and the between-group differences have been previously been published (Griffen et al., 2022a). Therefore, these outcomes are presented in the present study for the purposes of correlations with cognitive function domains only. Briefly, SMM was estimated by bioelectrical impedance analysis (BIA) utilising Janssen et al. (2000)'s equation. Skeletal muscle index (SMI) was calculated by dividing SMM by height^2. Muscle strength was assessed by 1RM testing of leg press and leg extension and via handgrip strength. Physical function was assessed by the 6-min walk test (6MWT) and the short physical performance battery (SPPB).

2.7. Blood and saliva collection and analysis

Venous blood (~12 mL) was collected at 0815 h following a >10 h supervised overnight fast. Whole blood was drawn into EDTA, heparin and SST vacutainers then centrifuged at 1900 ×*g* for 10 min at 4 °C. Before centrifuging, serum samples were allowed to rest for 30 min to

ensure adequate coagulation. Before analysis, aliquots comprising serum and plasma were kept at −80 °C. A glucose analyser (Biosen C-Line Glucose and Lactate Analyser; EKF Diagnostics, Cardiff, UK) was used to measure plasma glucose. Commercially available enzyme-linked immunosorbent assays (ELISA) were used to quantify serum total BDNF (Item # DNBT00) and IGF-1 (Item # DB100B), and plasma BDNF (Item # DY248), interleukin (IL)-6 (Item # D6050 and HS600C (the latter for high sensitivity)), IL-10 (Item # HS100C), tumor necrosis factor-alpha (TNF-α; Item # HSTA00E), C-reactive protein (CRP; Item # DCRP00) (R&D Systems Inc., Abbington, UK), and insulin (Item # EIA-2935; DRG Instruments GmbH, Marburg, Germany). Using conventional formulae, insulin concentration and fasting plasma glucose were used to determine insulin resistance (homeostatic model assessment of insulin resistance; HOMA-IR) and insulin sensitivity (quantitative insulin sensitivity check index; QUICKI) (Katz et al., 2000; Matthews et al., 1985). In addition to samples collected at 0815 h, venous blood (~7 mL) was also collected following a fasted bout of 30 min moderate intensity step exercise (performed between 0830 and 0900 h at a step rate of 75 steps/min; see Griffen et al., 2022b for full details) at 0900 h and 1230 h to determine the immediate and +3.5 h acute effect of exercise on plasma BDNF. This approach enabled us to comprehensively assess resting BDNF responses to the chronic intervention, but also assess if there was any change in the acute response to a standardised exercise stimulus. We took a more detailed approach to our analysis of plasma BDNF responses as there is strong evidence linking the biomarker to positive changes in cognitive function (Erickson et al., 2011; Szuhany et al., 2015). The CV for plasma glucose was 0.5 %, and the intra-assay CV was 9.5 % for plasma insulin, 8.5 % for serum BDNF, 10.4 % for plasma BDNF, 9.1 % for serum IGF-1, 9.8 % and 11.8 % for plasma IL-6 and IL-6 high sensitivity, respectively, 2.7 % for plasma IL-10, 9.9 % for plasma TNF-α, and 9.9 % for plasma CRP.

Saliva samples were collected whilst participants resided in the metabolic chamber (Griffen et al., 2022b). Using a synthetic swab (Salivette; Sarstedt Nümbrecht, Germany), samples were taken as soon as the participants woke up at 0650 h and again at 0805, 1225, 1700, and 2000 h. Samples were centrifuged at 1900 ×*g* for 2 min and stored at −80 °C until analysis. Samples were analysed for salivary cortisol by ELISA (Item # 1–3002; Salimetrics, Pennsylvania, USA). The intra-assay CV was 9 %. Salivary cortisol data (samples 1–5; 0650–2000 h) were used to calculate multiple indices: i) salivary cortisol area under the curve (AUC; nmol/L × 790 min) using the trapezoidal method; ii) slope (rate of salivary cortisol change from the peak morning (0650 or 0805 h, whichever concentration was highest) to the evening (2000 h)) (Adam et al., 2017); and iii) salivary cortisol concentration at 2000 h.

2.8. Statistical analysis

This was a secondary analysis of our main studies, which were statistically powered to detect changes in muscle strength (Griffen et al., 2022a) and resting metabolic rate (RMR) (Griffen et al., 2022b). As such, a power calculation was not conducted *a priori* for cognitive function outcomes. Nevertheless, this study included $n = 18$ participants per group for RE vs. NE and PRO vs. CON, which are similar group sizes to that in studies reporting cognitive effects of both RE (Anderson-Hanley et al., 2010; Coelho-Júnior et al., 2020; Smolarek et al., 2016; Yoon et al., 2018) and dietary protein (Charlton et al., 2016).

Statistical analysis was performed using JASP Version 0.15 (https://jasp-stats.org/) and Graphpad Prism Version 10.2.0 (Graphpad Software, San Diego, USA). Data are presented as means ± SD unless otherwise stated and were checked for normality using the Shapiro-Wilk test. Non-normally distributed data were transformed by log transformations. In cases when transformation proved ineffective, non-parametric tests were employed. Baseline characteristics were analysed by independent samples *t*-tests. A mixed-model ANCOVA was conducted on the outcome variables, with time acting as the within-subjects factor, group acting as the between-subjects factor, and corresponding baseline values included as covariates. The dietary intervention group (PRO or CON, for RE vs. NE analyses) or RE participation (RE or NE, for PRO vs. CON analyses) were also included as a covariate. A mixed-model ANCOVA was also used to examine the results for exploratory sub-analyses that compared the RE + CON and RE + PRO groups, and the baseline value was the only covariate included. When analysing data that were not normally distributed, the Scheirer-Ray-Hare two-way ANOVA of ranks test was employed. Correlations were analysed using partial correlation (Pearson's for parametric data and Spearman's rank-order coefficients for non-parametric data). Age and intervention group were controlled for in the baseline and Δbaseline correlations, respectively. A linear mixed model was used to assess the plasma BDNF response to the acute bout of step exercise conducted before and after the intervention. For the mixed model, time and group were coded as fixed effects and participants were coded as random effects. When main effects were identified, post hoc pairwise comparisons were conducted using Fishers LSD test or Tukey's post hoc test, where appropriate. Significance was set at $p < 0.05$.

3. Results

3.1. Participants and adherence

Thirty-nine older men were randomised: 36 completed the study and 3 withdrew (see Supplemental Fig. 1 for participant flow and reasons for withdrawal). Participants were 67 ± 4 years of age, had no cognitive impairments (assessed by the MMSE; 29.4 ± 0.8 points), and attended 15 ± 3 years of full-time education (see Table 1 for baseline characteristics). No differences occurred between the CON and PRO groups for supplement adherence (95 ± 4 % vs. 96 ± 3 %, $P = 0.27$), or between RE groups for RE adherence (98 ± 3 % vs. 98 ± 4 %, $P = 0.89$).

3.2. Dietary intake

Detailed dietary intake data has been previously published (Griffen et al., 2022b). Briefly, protein intake increased (from 1.0 to 1.6 g/kg/d) in the PRO group greater than the CON group at weeks 6 and 12 ($P < 0.001$), and in the RE + PRO group (from 1.0 to 1.6 g/kg/d) greater than the RE + CON group at weeks 6 and 12 ($P < 0.001$). Carbohydrate intake increased in the CON and RE + CON groups greater than the PRO and RE + PRO groups, respectively, at weeks 6 and 12 ($P < 0.001$). No differences in any dietary marker occurred between the RE and NE groups.

3.3. Cognitive function

Scores for each individual cognitive function test and domain-specific z-scores for the CON, PRO, NE, and RE groups can be seen in Table 2. Over the 12-week intervention period, PRO significantly improved executive function z-score compared to the CON group ($P = 0.03$) and there was also a trend for improved global cognitive function ($P = 0.053$). No other significant differences occurred between the PRO and CON groups. RE did not significantly improve any individual cognitive function test score or any domain-specific z-score compared to NE and in fact worsened incongruency cost ($P = 0.02$). No significant differences in any cognitive function outcomes occurred between the RE + CON and RE + PRO groups ($P \geq 0.07$; Table 3).

3.4. Neurobiological, inflammatory, salivary cortisol and insulin sensitivity markers

As also reported elsewhere (Griffen et al., 2022a, 2023b), in the RE group, plasma IL-6 ($P = 0.048$) and TNF-α ($P = 0.02$) significantly decreased over time compared to the NE group and insulin sensitivity (by QUICKI) increased in the PRO group compared to the CON group, which neared statistical significance ($P = 0.06$; Table 4). No other

Table 1
Baseline participant characteristics[a].

	CON	PRO	*P* value	NE	RE	*P* value	RE + CON	RE + PRO	*P* value
n	18	18	–	18	18	–	9	9	–
Age, years	67 ± 4	67 ± 4	0.74	66 ± 5	67 ± 4	0.48	67 ± 4	68 ± 4	0.48
MMSE, points	29.5 ± 0.8	29.3 ± 0.9	0.62	29.5 ± 0.8	29.4 ± 0.9	0.74	29.4 ± 3.7	29.4 ± 3.8	0.67
Education, years	14.6 ± 3.1	14.2 ± 2.8	0.40	14.2 ± 2.7	14.6 ± 3.2	0.70	14.7 ± 1.2	14.4 ± 0.9	0.62
Body mass, kg	78.6 ± 10.7	79.4 ± 10.6	0.81	78.5 ± 9.5	79.5 ± 11.7	0.77	78.2 ± 10.6	80.9 ± 11.2	0.40
BMI, kg/m^2	25.1 ± 2.8	25.8 ± 2.2	0.39	25.0 ± 2.2	25.8 ± 2.7	0.36	25.1 ± 2.2	26.6 ± 2.5	0.08
SMM, kg	26.3 ± 2.6	27.0 ± 2.9	0.43	26.9 ± 1.9	26.4 ± 3.4	0.55	25.9 ± 3.3	26.9 ± 3.8	0.48
SMI, kg/m^2	8.4 ± 0.6	8.8 ± 0.7	**0.04**	8.6 ± 0.5	8.6 ± 0.8	0.83	8.3 ± 0.6	8.9 ± 0.8	0.08
Leg extension 1RM, kg	58 ± 17	59 ± 11	0.79	61 ± 15	56 ± 14	0.28	52 ± 15	59 ± 13	0.30
Leg press 1RM, kg	112 ± 26	112 ± 20	0.91	111 ± 23	112 ± 23	0.93	107 ± 26	118 ± 21	0.49
Handgrip strength, kg	41 ± 8	39 ± 7	0.39	39 ± 6	41 ± 9	0.39	40 ± 12	42 ± 6	0.68
SPPB, points	11.4 ± 0.9	11.6 ± 0.6	0.51	11.6 ± 0.7	11.5 ± 0.8	0.82	11.2 ± 1.0	11.8 ± 0.4	0.09
6MWT, m	633 ± 74	603 ± 65	0.21	627 ± 58	609 ± 82	0.45	627 ± 95	591 ± 77	0.41
Gait speed, m/s	1.12 ± 0.19	1.18 ± 0.14	0.30	1.12 ± 0.19	1.18 ± 0.14	0.28	1.14 ± 0.12	1.22 ± 0.21	0.23
Protein intake, g/kg/d	1.0 ± 0.2	1.0 ± 0.1	0.65	1.0 ± 0.1	1.1 ± 0.2	0.26	1.1 ± 0.1	1.1 ± 0.2	0.12
Step count, steps/d	11,413 ± 2523	12,007 ± 3272	0.55	11,717 ± 3035	11,703 ± 2837	0.99	12,170 ± 3260	11,345 ± 2721	0.58

P values refer to differences between groups analysed by independent samples *t*-test. *P* values in bold indicate statistical significance ($P < 0.05$). 1RM, one repetition maximum; 6MWT, 6-min walk test; BMI, body mass index; MMSE, mini-mental state examination; SMM, skeletal muscle mass; SPPB, short physical performance battery. Education (years) refers to the total amount of full-time education attended from primary school up to postgraduate university level.

[a] Values are means ± SD.

between-group differences occurred (Table 4 and 5). Regarding the plasma BDNF response to an acute bout of moderate intensity exercise (75 steps/min for 30 min), plasma BDNF showed a main effect of time ($P = 0.048$), with resting BDNF being significantly reduced following the intervention period (mean difference of 3511 pg/mL, 95 % confidence interval: 722–6300 pg/mL), but no effect of group ($P = 0.43$) or group-by-time interactions ($P = 0.36$) were observed (Fig. 2), demonstrating that neither 12 weeks of RE nor PRO could mitigate longitudinal decreases in plasma BDNF. There were no further statistically significant time effects observed (all $P > 0.20$), demonstrating that plasma BDNF did not change in response to the acute exercise stress test.

3.5. Correlation analysis

There were no significant correlations (baseline or Δbaseline) observed between any neurobiological, inflammatory, salivary cortisol or insulin sensitivity marker and any domain specific cognitive function z-score. On the other hand, z-scores for cognitive function and sarcopenia outcomes (muscle strength and physical function) reported significant correlations. Baseline data showed a correlation between leg extension 1RM and global cognitive function z-score ($r = 0.34$, $P = 0.045$; Fig. 3a). Additionally, there were significant positive correlations found between baseline SPPB score and psychomotor/attention ($\rho = 0.51$, $P = 0.002$; Fig. 3b) and episodic memory z-scores ($\rho = 0.35$, $P = 0.04$; Fig. 3c), and between 6MWT distance and psychomotor/attention z-score ($r = 0.39$, $P = 0.02$; Fig. 3d). After the intervention, there was a positive correlation ($r = 0.34$, $P = 0.046$; Fig. 3e) between ΔSMI and Δepisodic memory z-score. No correlations were observed for changes in muscle strength or physical function tests and any cognitive function domain-specific z-score.

4. Discussion

This study investigated the individual and combined effects of 12 weeks of RE and whey protein supplementation on cognitive function in older men and explored potential mechanisms of action. The main findings were: **i)** whey protein supplementation significantly improved executive function compared to a carbohydrate control; **ii)** RE did not improve any domain of cognitive function compared to no exercise, with processing speed (incongruency cost) worsening; **iii)** no synergistic effects of RE combined with whey protein supplementation were observed; **iv)** outcomes related to sarcopenia (muscle strength and physical function) positively correlated with several cognitive function domains at baseline; however, only changes in SMI and episodic memory correlated at 12 weeks; and **v)** whilst RE significantly reduced systemic inflammation, no significant between-group changes in other biomarkers occurred and changes did not correlate with changes in cognitive function.

4.1. Whey protein supplementation

Whey protein supplementation significantly improved executive function compared to a carbohydrate control. The improvement in executive function following increased dietary protein intake agrees with previous work (Muth and Park, 2021). Whilst no significant between-group differences were observed for working memory or global cognitive function, clinically relevant (>0.5 SD (Crichton et al., 2012)) within-group increases were observed in the whey protein supplementation group, suggesting that although not statistically significant compared to a carbohydrate control, these improvements may be clinically meaningful. Previous acute (Kaplan et al., 2001) and longitudinal studies (Charlton et al., 2016; Kita et al., 2019; Lefferts et al., 2020; van der Zwaluw et al., 2014) are consistent with the clinically significant improvement in working memory; however, cognitive benefits have not been observed by others (Bell et al., 2019; Moran et al., 2018; Zajac et al., 2019). Inconsistencies may be explained by variations in the particular study groups, the length of the intervention, and the protein dosage utilised. In support, two studies (Bell et al., 2019; Zajac et al., 2019) that did not find benefits from higher dietary protein intake examined the effects over a period of 6–8 weeks, which, according to earlier research, was probably insufficient to stimulate longitudinal changes in cognitive function (Vellas et al., 2008). Furthermore, only 8 g of whey protein was present in the multi-ingredient supplement that Moran et al. (2018) examined, whilst dietary intake data from study participants by van der Zwaluw et al. (2014) showed an increase in protein intake of 0.4 g/kg/d (from 1.0 to 1.4 g/kg/d). These deviations in the amount of protein consumed were lower than that of the present study (0.6 g/kg/d; 1.0–1.6 g/kg/d). The dose of protein supplemented in these studies may therefore have been insufficient to yield changes in cognitive function; though, no dose-response study has yet to be conducted to determine the optimal daily intake of protein to improve cognitive function in older adults. This should therefore be examined in future work.

4.2. Resistance exercise

Previously, we have shown that RE significantly increased muscle strength and physical function in this cohort (Griffen et al., 2022a);

Table 2
Cognitive function test scores for the control, whey protein supplementation, no exercise and resistance exercise groups at baseline and 12 weeks[a].

	CON		PRO		*P* value	NE		RE		*P* value
	Baseline	12 weeks	Baseline	12 weeks		Baseline	12 weeks	Baseline	12 weeks	
Motor screening task										
Mean latency, ms	848.3 ± 154.8	835.1 ± 137.3	820.0 ± 135.4	744.8 ± 136.4	0.07	835.5 ± 104	804.9 ± 102	839.6 ± 172.1	752.6 ± 165.9	0.12
Reaction time										
Reaction time, ms	383.3 ± 46.5	391.9 ± 48.6	390.5 ± 35.9	397.2 ± 38.9	0.91	375.4 ± 42.8	383.2 ± 42.4	393.9 ± 36.6	397.7 ± 45.1	0.78
Movement time, ms	295.1 ± 62.3	297.7 ± 43.8	308.1 ± 60.0	302.4 ± 50.1	0.99	313.3 ± 63.8	303.1 ± 61.5	287.9 ± 50.9	293.8 ± 51.1	0.93
Spatial working memory										
Errors (all boxes), *n*	15.0 ± 7.3	11.1 ± 8.0	15.2 ± 8.6	12.4 ± 9.4	0.62	14.8 ± 8.6	10.4 ± 8.6	15.3 ± 7.6	13.2 ± 8.8	0.32
Errors (6 boxes), *n*	3.6 ± 2.7	2.8 ± 2.9	3.5 ± 2.8	2.2 ± 2.7	0.49	3.8 ± 3.3	2.2 ± 2.7	4.1 ± 2.9	3.4 ± 3.3	0.25
Errors (8 boxes), *n*	10.1 ± 5.1	6.4 ± 5.0	9.8 ± 6.6	8.6 ± 6.3	0.21	10.4 ± 6.1	7.4 ± 5.9	10.5 ± 5.7	8.8 ± 5.9	0.45
Errors (12 boxes), *n*	32.9 ± 8.6	31.8 ± 9.0	34.3 ± 13.9	27.8 ± 13.6	0.19	37.1 ± 11.7	28.3 ± 10.9	32.2 ± 11.6	31.9 ± 11.5	0.11
Strategy score	8.2 ± 2.7	7.0 ± 2.8	7.9 ± 3.2	6.6 ± 3.2	0.76	7.8 ± 2.6	6.4 ± 3.1	8.6 ± 3.1	7.7 ± 2.9	0.32
Paired associates learning										
Total errors, *n*	20.8 ± 13.0	16.9 ± 14.5	20.2 ± 15.3	13.1 ± 9.6	0.36	15.9 ± 11.2	16.5 ± 12.9	24.9 ± 14.6	16.5 ± 12.9	0.27
First attempt memory score, *n*	11.3 ± 3.6	12.1 ± 3.9	11.6 ± 4.3	12.9 ± 4.3	0.59	13.0 ± 3.1	12.1 ± 4.1	10.3 ± 4.2	12.1 ± 4.3	0.22
Multitasking test										
Total incorrect, *n*	2.3 ± 1.6	2.1 ± 1.9	3.6 ± 4.2	1.7 ± 1.8	0.39	3.4 ± 3.5	1.9 ± 1.9	3.1 ± 3.9	2.1 ± 1.9	0.67
Reaction latency, ms	712.0 ± 111.6	734.5 ± 91.9	726.7 ± 117.2	722.6 ± 108.7	0.41	733.1 ± 91.1	729.5 ± 78.6	706.8 ± 126.1	725.2 ± 114.1	0.60
Incongruency cost, ms	79.1 ± 70.5	97.1 ± 60.2	83.9 ± 50.8	80.6 ± 55.9	0.29	84.2 ± 73.4	75.0 ± 55.8	87.6 ± 41.1	107.0 ± 52.6	**0.02**
Multitasking cost, ms	277.3 ± 116.6	212.9 ± 95.4	287.3 ± 173.5	213.3 ± 102.0	0.87	271.8 ± 169.8	211.2 ± 111.0	297.9 ± 152.1	225.9 ± 101.1	0.93
Delayed matching to sample										
% correct (all delays)	87.0 ± 7.4	85.0 ± 11.2	82.3 ± 9.7	84.0 ± 10.6	0.97	83.3 ± 8.9	86.7 ± 8.5	85.5 ± 8.9	82.6 ± 12.1	0.21
% correct (0 s delay)	90.0 ± 10.3	87.5 ± 16.1	88.2 ± 14.2	87.1 ± 19.9	0.79	83.2 ± 14.1	90.0 ± 15.7	93.3 ± 9.7	83.3 ± 19.7	0.82
% correct (4 s delay)	87.5 ± 16.1	83.7 ± 15.0	80.0 ± 23.4	87.1 ± 15.7	0.62	83.3 ± 18.4	85.6 ± 16.5	82.2 ± 21.6	85.6 ± 13.4	0.91
% correct (12 s delay)	84.4 ± 11.0	84.4 ± 17.6	80.0 ± 16.8	78.9 ± 17.5	0.44	83.3 ± 14.1	84.4 ± 17.6	81.1 ± 14.5	78.9 ± 17.5	0.40
Domain specific z-scores										
Psychomotor/attention	0.04 ± 0.62	0.05 ± 0.71	−0.05 ± 0.68	0.14 ± 0.64	0.16	0.01 ± 0.62	0.08 ± 0.66	−0.03 ± 0.70	0.11 ± 0.70	0.63
Executive function	0.09 ± 0.47	0.11 ± 0.49	−0.16 ± 0.83	0.18 ± 0.69	**0.03**	0.03 ± 0.66	0.30 ± 0.49	−0.11 ± 0.71	−0.01 ± 0.65	0.07
Episodic memory	−0.04 ± 0.90	0.03 ± 0.92	−0.02 ± 0.96	0.31 ± 0.92	0.32	0.31 ± 0.78	0.18 ± 0.95	−0.36 ± 1.04	0.17 ± 0.96	0.14
Working memory	0.10 ± 1.67	0.69 ± 1.51	−0.11 ± 2.49	1.16 ± 2.28	0.28	−0.34 ± 2.20	1.29 ± 1.87	0.32 ± 1.98	0.56 ± 1.96	0.06
Global cognitive function	0.05 ± 0.73	0.21 ± 0.66	−0.09 ± 0.91	0.48 ± 0.80	0.053	0.01 ± 0.81	0.46 ± 0.68	−0.04 ± 0.84	0.24 ± 0.79	0.27

P values refer to group-by-time interaction. *P* values in bold indicate statistical significance ($P < 0.05$).
[a] Values are means ± SD.

Table 3
Cognitive function test scores for the resistance exercise + control and resistance exercise + whey protein supplementation groups at baseline and 12 weeks[a].

	RE + CON		RE + PRO		*P* value
	Baseline	12 weeks	Baseline	12 weeks	
Motor screening task					
Mean latency, ms	902.7 ± 579.0	810.9 ± 511.1	800.8 ± 441.0	720.2 ± 471.9	0.95
Reaction time					
Reaction time, ms	400.0 ± 42.4	402.4 ± 55.4	388.3 ± 27.6	392.9 ± 28.5	0.81
Movement time, ms	282.3 ± 48.0	308.7 ± 40.1	293.5 ± 56.1	279.1 ± 31.5	0.17
Spatial working memory					
Errors (all boxes), *n*	16.3 ± 9.3	12.9 ± 7.3	14.2 ± 7.8	13.4 ± 10.2	0.68
Errors (6 boxes), *n*	4.8 ± 2.8	4.0 ± 2.8	3.3 ± 3.0	2.8 ± 3.6	0.71
Errors (8 boxes), *n*	10.8 ± 4.5	8.0 ± 4.8	10.2 ± 6.6	9.6 ± 6.9	0.58
Errors (12 boxes), *n*	31.8 ± 6.8	31.7 ± 8.5	32.7 ± 15.3	32.1 ± 14.1	0.87
Strategy score	8.6 ± 3.1	8.3 ± 2.3	8.7 ± 3.0	7.1 ± 3.3	0.38
Paired associates learning					
Total errors, *n*	24.9 ± 11.0	21.1 ± 15.6	24.9 ± 17.7	11.9 ± 7.5	0.14
First attempt memory score, *n*	9.9 ± 2.8	11.3 ± 4.5	10.7 ± 5.1	12.8 ± 4.2	0.71
Multitasking test					
Total incorrect, *n*	3.9 ± 5.0	2.3 ± 1.7	4.1 ± 5.7	1.8 ± 1.8	0.38
Reaction latency, ms	698.2 ± 133.2	737.5 ± 108.1	715.4 ± 117.0	713.1 ± 119.4	0.41
Incongruency cost, ms	82.6 ± 39.3	116.3 ± 49.8	92.1 ± 44.4	98.6 ± 18.6	0.53
Multitasking cost, ms	324.8 ± 121.9	218.3 ± 98.7	270.9 ± 48.9	233.5 ± 55.8	0.34
Delayed matching to sample					
% correct (all delays)	86.6 ± 8.2	80.7 ± 12.7	84.4 ± 9.3	84.4 ± 11.1	0.53
% correct (0 s delay)	91.1 ± 9.9	82.2 ± 19.8	95.6 ± 8.7	84.4 ± 19.5	0.84
% correct (4 s delay)	84.4 ± 15.8	82.2 ± 11.3	80.0 ± 26.4	88.9 ± 14.4	0.41
% correct (12 s delay)	84.4 ± 8.2	77.8 ± 17.5	77.8 ± 18.6	80.0 ± 17.4	0.41
Domain specific z-scores					
Psychomotor/attention	−0.14 ± 0.68	−0.11 ± 0.79	0.09 ± 0.72	0.33 ± 0.48	0.25
Executive function	0.03 ± 0.37	−0.07 ± 0.45	−0.24 ± 0.93	0.06 ± 0.81	0.07
Episodic memory	−0.41 ± 0.76	−0.09 ± 1.10	−0.32 ± 1.26	0.43 ± 0.78	0.07
Working memory	0.29 ± 1.41	0.58 ± 1.36	0.37 ± 2.46	0.54 ± 2.46	0.93
Global cognitive function	−0.06 ± 0.62	0.08 ± 0.62	−0.03 ± 1.02	0.33 ± 0.93	0.22

P values refer to group-by-time interaction.
[a] Values are means ± SD.

however, in the current study, these RE-induced improvements in muscle function did not translate to improvements in cognitive function. Additionally, there was a significant worsening in incongruency cost—the time needed to interpret contradicting information. These findings are in contrast with several studies that observed cognitive improvements following RE in older adults (Cassilhas et al., 2007; Coelho-Junior et al., 2022; Ikudome et al., 2017; van de Rest et al., 2014; Yoon et al., 2018). Other studies are, however, in agreement with the present findings, reporting no improvements in either processing speed (Anderson-Hanley et al., 2010; Bell et al., 2019) or working memory (Liu-Ambrose et al., 2010). Disparities may be at least in part explained by the intensity of RE employed. Studies that have demonstrated improvements in working memory (Ikudome et al., 2017) and processing speed (van de Rest et al., 2014; Yoon et al., 2018) employed an intervention of moderate intensity RE (≤70 % 1RM). The present study and others that did not observe improvements employed a higher (≥80 % 1RM) RE intensity (Bell et al., 2019; Liu-Ambrose et al., 2010). Herold et al. (2019)'s systematic review also suggests that moderate-intensity RE is likely superior than high-intensity RE in terms of enhancing working memory and processing speed. A meta-analysis by Wilke et al. (2019), which found that moderate-intensity RE produced larger effect sizes in healthy people than high-intensity RE supports this. Furthermore, we have also recently published data demonstrating that the RE intervention employed in this study decreased participants' habitual activity (Griffen et al., 2022b), hypothesised to be due to a compensatory effect of increased RMR to maintain energy balance. Reduced physical activity has been linked with cognitive decline (Cunningham et al., 2020); therefore, it is possible that the decrease in habitual physical activity in the RE group may have offset any cognitive effects of the RE intervention. Nevertheless, when the results of this larger study are considered together (Griffen, 2020), it appears that high intensity RE (~80 % 1RM) may be more beneficial for muscle strength, and physical and metabolic function (Griffen et al., 2022a, 2022b, 2023b) as opposed to cognitive function.

4.3. Resistance exercise combined with whey protein supplementation

Here we report no statistically significant synergistic effects of RE combined with whey protein supplementation on domains of cognitive function. Although, it must be emphasised that this was an exploratory sub analysis which was underpowered to detect between-group differences. Greater increases in cognitive function domain scores overall were observed following RE combined with whey protein supplementation compared to RE and a carbohydrate control; however, based on the RE and no exercise comparisons reported in this study, these increases were likely driven by the whey protein supplementation and not an interaction effect. The lack of statistically significant synergistic effects agrees with published data in older adults (Formica et al., 2020; Mundell et al., 2022; van de Rest et al., 2014). Bell et al. (2019), on the other hand, found that multimodal exercise (RE + high intensity interval

Table 4
Fasting neurobiological, inflammatory, insulin sensitivity and salivary cortisol markers for the control, whey protein supplementation, no exercise and resistance exercise groups at baseline and 12 weeks[a].

	CON		PRO		*P* value	NE		RE		*P* value
	Baseline	12 weeks	Baseline	12 weeks		Baseline	12 weeks	Baseline	12 weeks	
Serum total BDNF[b], pg/mL	24.8 ± 8.7	24.2 ± 11.2	24.8 ± 4.1	25.4 ± 7.2	0.74	24.5 ± 6.5	23.2 ± 8.1	25.8 ± 4.0	26.4 ± 9.6	0.24
Serum IGF-1[b], ng/mL	150 ± 74	127 ± 61	118 ± 27	105 ± 35	0.97	143 ± 79	123 ± 67	129 ± 46	109 ± 34	0.61
Plasma IL-6[b], pg/mL	4.1 ± 3.0	4.1 ± 3.2	4.9 ± 4.3	4.3 ± 3.5	0.59	4.4 ± 3.1	4.9 ± 3.7	4.6 ± 4.3	3.6 ± 3.1	**0.048**
Plasma IL-10[b], pg/mL	6.6 ± 2.7	6.5 ± 2.2	6.9 ± 2.4	6.2 ± 2.3	0.45	6.2 ± 1.9	6.2 ± 2.1	7.4 ± 3.1	6.6 ± 2.3	0.67
Plasma TNF-α[b], pg/mL	3.3 ± 1.3	2.8 ± 0.7	2.7 ± 1.1	2.7 ± 0.8	0.89	2.8 ± 1.0	3.0 ± 0.8	3.2 ± 1.4	2.5 ± 0.7	**0.02**
Plasma CRP[b], ng/mL	1.6 ± 1.5	1.3 ± 0.8	1.8 ± 1.2	1.5 ± 1.1	0.78	1.9 ± 1.6	1.4 ± 1.3	1.5 ± 1.1	1.4 ± 0.8	0.52
Salivary cortisol AUC[c], nmol/L × 790 min	4827 ± 2292	4837 ± 1886	4108 ± 1298	4539 ± 1184	0.90	4078 ± 1128	4449 ± 1314	4814 ± 4905	4904 ± 1748	0.90
Salivary cortisol (2000 h)[c], nmol/L	2.1 ± 1.6	1.9 ± 1.7	2.0 ± 1.6	2.4 ± 1.7	0.28	2.0 ± 1.3	2.0 ± 1.6	2.1 ± 1.9	2.2 ± 1.9	0.99
Salivary cortisol slope[c], nmol/L	11.1 ± 5.5	12.4 ± 6.8	9.7 ± 3.4	15.3 ± 8.9	0.18	10.1 ± 2.8	11.4 ± 5.9	10.5 ± 5.8	16.3 ± 9.1	0.08
HOMA-IR[b]	2.7 ± 1.5	2.3 ± 1.7	2.5 ± 1.3	1.8 ± 1.4	0.24	2.7 ± 1.3	2.4 ± 1.6	2.3 ± 1.6	1.7 ± 0.9	0.21
QUICKI[b]	0.34 ± 0.03	0.35 ± 0.03	0.35 ± 0.04	0.37 ± 0.05	0.06	0.33 ± 0.04	0.35 ± 0.05	0.35 ± 0.03	0.36 ± 0.03	0.85

P values refer to group-by-time interaction. *P* values in bold indicate statistical significance ($P < 0.05$). AUC, area under the curve; BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor 1; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor-alpha; QUICKI, quantitative insulin-sensitivity check index.

[a] Values are means ± SD.

[b] CON ($n = 16$), PRO ($n = 18$), NE ($n = 17$), RE ($n = 17$) due to missing data.

[c] CON ($n = 16$), PRO ($n = 17$), NE ($n = 16$), RE ($n = 17$) due to missing data.

Fig. 2. Plasma BDNF responses at rest (0830 h) and in response to acute aerobic exercise (immediately post at 0900 h and + 3.5 h post at 1230 h), before and after 12 weeks of resistance exercise (RE) or no exercise (NE) (Panel A) and whey protein supplementation (PRO) or control (CON) (Panel B). # = significant main effect of time ($P < 0.05$). Symbols represent individual participants.

training) in conjunction with twice-daily ingestion of a multi-ingredient supplement based on whey protein, had more cognitive effects than multimodal exercise plus a control supplement. More recently, Rondanelli et al. (2020) showed that performance on the Trail Making Test, which measures executive functioning, processing speed, visual search speed, and mental flexibility, was enhanced by physical rehabilitation (including muscle strengthening, balance, and gait exercises) plus twice-daily consumption of a leucine- and vitamin D-enriched whey protein supplement. Noteworthy features of these studies, however, included multimodal exercise programmes, vitamin D-containing whey protein supplements, and omega-3 polyunsaturated fatty acids in the Bell et al. (2019) experimental supplement. These ingredients have previously been shown to attenuate cognitive decline (Balion et al., 2012; Karr et al., 2011). As a result, these methodological differences may explain disparities between studies. Also, due to the methodological approach applied, it is unknown in the aforementioned studies (Bell et al., 2019; Rondanelli et al., 2020) whether the RE and whey protein *per se,* or the synergism of exercise modalities and/or ingredients in these supplements, contributed to the synergistic effects. Further research with a larger sample size to the sub analysis of the present study, employing solely a RE training programme and supplementing only whey protein

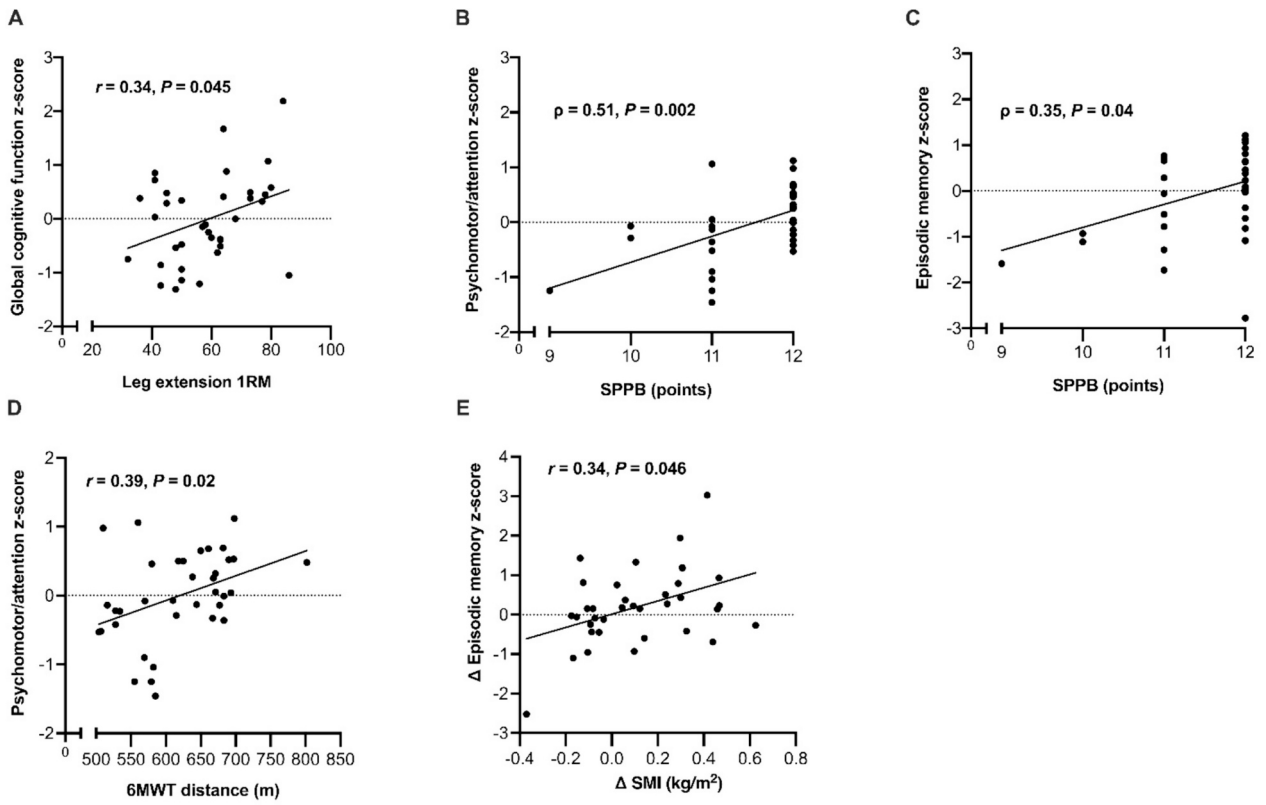

Fig. 3. Baseline correlations between (A) leg extension 1RM and global cognitive function z-score; (B) SPPB score and psychomotor/attention z-score; (C) SPPB score and episodic memory z-score; and (D) 6MWT distance and psychomotor/attention z-score. Panel E shows the correlation between ΔSMI and Δepisodic memory z-score following the intervention. 1RM, one repetition maximum; 6MWT, 6-min walk test; SMI, skeletal muscle index; SPPB, short physical performance battery.

without additional ingredients, is therefore required to confirm whether increased dietary protein augments RE-induced improvements in cognitive function.

4.4. Mechanisms of action

Potential mechanisms through which RE and increased dietary protein intake are purported to improve cognitive function include, but are not limited to, increases in various growth and neurotrophic factors, decreases in inflammation and cortisol secretion, and improved insulin sensitivity (Ahlskog et al., 2011; Camfield et al., 2011; Liu-Ambrose et al., 2012; Tsai et al., 2015; Walsh et al., 2016). Meta-analyses also suggest a relationship between sarcopenia outcomes (SMM, strength and function) and cognitive function (Chang et al., 2016; Cipolli et al., 2019; Peng et al., 2020), likely explained by common pathologies of both conditions. Interestingly, baseline correlation data from the present study coincides with these meta-analyses, with significant correlations observed between muscle strength and physical function outcomes and domains of cognitive function. However, whilst fat-free mass, muscle strength and physical function outcomes were improved in this cohort, particularly following RE but also gait speed following whey protein supplementation, as previously reported (Griffen et al., 2022a), changes in most of these outcomes did not correlate with changes in cognitive function domain scores, and only a significant positive correlation was observed between changes in SMI and episodic memory. This finding highlights the importance of maintenance of SMM for cognitive health. Furthermore, whilst systemic inflammation decreased (Griffen et al., 2022a) and insulin sensitivity (derived from QUICKI) increased (Griffen et al., 2023b) following RE and whey protein supplementation, respectively, RE failed to improve cognitive function and whilst whey protein supplementation did improve executive function, no changes in biomarkers correlated with cognitive function domains. No between-group differences in fasting BDNF or IGF-1, or salivary cortisol indicies also occurred, and in fact, BDNF decreased over the 12-week intervention period following both RE and whey protein supplementation. Consequently, the findings of this study imply mechanistic changes in cognitive function in this cohort were not due to changes in the aforementioned purported mechanisms. Instead, as amplified brain insulin receptor signalling stimulation (Frazier et al., 2019) and increased brain neurotransmitter availability (van de Rest et al., 2013) have been purported to contribute to protein-induced improvements in cognitive function, we cannot rule out these mechanisms contributed to improved cognitive function following whey protein supplementation. However, due to not measuring these outcomes in this study, this cannot be confirmed. Analysis of these outcomes should therefore be prioritised in future work.

4.5. Strengths and limitations

Our study has several strengths, including the comprehensive assessment of multiple domains of cognitive function under highly controlled conditions in a population of older men and measurement of a wide array of biomarkers to explore potential mechanisms of action, including the comprehensive measurement of the acute-chronic-acute effects on BDNF, which, to our knowledge, has not been investigated previously following RE and whey protein supplementation. Due to the robustness of our methods, the results presented in this study reliably add to the current evidence base. Limitations of this study include investigation of relatively healthy participants who already had high levels of physical and cognitive functioning prior to recruitment and were generally well educated. Further limitations include the use of pooled data for the main analyses, which may have impacted the individual effects of RE and whey protein supplementation; the small sample size to determine between-group cognitive effects, particularly in our

Table 5
Fasting neurobiological, inflammatory, insulin sensitivity and salivary cortisol markers for the resistance exercise + control and resistance exercise + whey protein supplementation groups at baseline and 12 weeks[a].

	RE + CON		RE + PRO		*P* value
	Baseline	12 weeks	Baseline	12 weeks	
Serum total BDNF[b], pg/mL	22.5 ± 8.2	28.6 ± 12.4	26.6 ± 3.3	25.2 ± 5.7	0.51
Serum IGF-1[b], ng/mL	137 ± 45	119 ± 34	118 ± 45	100 ± 30	0.63
Plasma IL-6[b], pg/mL	3.2 ± 2.5	2.4 ± 1.7	5.8 ± 5.4	4.6 ± 3.6	0.24
Plasma IL-10[b], pg/mL	7.0 ± 10.2	6.1 ± 6.5	7.7 ± 8.4	7.0 ± 7.2	0.52
Plasma TNF-α[b], pg/mL	3.4 ± 1.7	2.7 ± 0.8	3.0 ± 1.2	2.4 ± 0.6	0.74
Plasma CRP[b], ng/mL	0.8 ± 0.6	0.8 ± 0.6	2.0 ± 1.2	1.8 ± 0.6	0.23
Salivary cortisol AUC[b], nmol/L × 790 min	5588 ± 2751	5326 ± 2200	4127 ± 1761	4530 ± 1242	0.72
Salivary cortisol (2000 h)[b], nmol/L	2.2 ± 1.7	1.8 ± 2.0	2.1 ± 2.1	2.6 ± 1.8	0.32
Salivary cortisol slope[b], nmol/L	12.5 ± 7.1	15.7 ± 7.6	8.8 ± 4.2	16.9 ± 10.5	0.49
HOMA-IR[b]	2.9 ± 2.0	2.4 ± 1.7	2.2 ± 1.2	1.6 ± 0.9	0.41
QUICKI[b]	0.33 ± 0.03	0.35 ± 0.03	0.35 ± 0.03	0.37 ± 0.03	0.77

P values refer to group-by-time interaction. AUC, area under the curve; BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor 1; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor-alpha; QUICKI, quantitative insulin-sensitivity check index.

[a] Values are means ± SD.

[b] RE + CON ($n = 8$) due to missing data from one participant.

exploratory sub analysis investigation of synergistic effects; and the relatively short intervention period of 12 weeks.

4.6. Theoretical and practical implications, applications, and recommendations for future research

The results of this study have important theoretical and practical implications and applications. From a theoretical perspective, the results further build the evidence base for a role of dietary protein intake (at a dose of ~1.6 g/kg/d incorporating high-quality protein sources such as whey protein) as a nutritional strategy to slow cognitive decline with ageing. Consequently, the findings provide preliminary evidence to allow researchers to investigate such an intervention on a larger scale which may have a significant impact on future dietary guidelines. Additionally, as the mechanisms of action for improved cognitive function following whey protein supplementation could not be confirmed in the present study, this study highlights a research gap to allow researchers to investigate to further build the evidence base. As no effect of high intensity RE on cognitive function was observed in the present study, hypothesised to be due to reduced habitual physical activity, this contributes significantly to the literature that there may be a fine balance of RE intensity to maximise overall health in older age (e.g., physical and cognitive function), which should be examined in future work.

From a practical perspective, these findings provide evidence to healthcare professionals and the general population that consuming a high protein diet (~1.6 g/kg/d), focussing on increased intakes at breakfast and lunch where intake is suboptimal in older adults (Griffen et al., 2022a), is feasible in healthy older adults and may promote improved cognitive health, particularly executive functioning. Therefore, such an intervention, or recommendations to increase protein intakes at these meals, should be promoted and may be a beneficial strategy to curb cognitive decline with age.

To further build the evidence base, additional research is warranted including a larger sample size over a longer duration of ≥6 months with an emphasis on dose to determine optimum thresholds of RE and whey protein supplementation to provide improved cognitive outcomes. As previously mentioned, additional research to determine mechanisms of action is also warranted. Moreover, over recent years, there has been a rapidly increasing global interest in reducing animal-derived products and increasing plant-based food consumption, for various reasons, including sustainability, religious/cultural, animal welfare, health, and personal preference reasons (Delsoglio et al., 2023; Griffen et al., 2023a, 2023c). However, whilst the effects of plant-based proteins on cognitive function are beginning to be investigated, the effects are relatively unexplored and further research is warranted.

5. Conclusion

Twice daily ingestion of whey protein supplementation for 12 weeks is an effective stimulus to elicit improvements in cognitive function, specifically executive function. This data reinforces the importance of dietary protein to support healthy ageing. Twice weekly RE, at an intensity of 80 % 1RM, had no positive effects on cognitive function and no synergistic effects occurred. Finally, changes in cognitive function domains did not correlate with changes in neurobiological, inflammatory, or insulin sensitivity markers but change in SMI correlated with change in episodic memory. This emphasises the importance of maintenance of SMM with age.

CRediT authorship contribution statement

Corbin Griffen: Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tom Cullen:** Writing – review & editing, Formal analysis, Data curation. **John Hattersley:** Writing – review & editing, Supervision, Conceptualization. **Martin O. Weickert:** Writing – review & editing. **Alexander Dallaway:** Writing – review & editing. **Michael Duncan:** Writing – review & editing, Supervision. **Derek Renshaw:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The whey protein supplement used in this study (Instantized BiPRO) was supplied by Agropur, Quebec, Canada. Agropur provided the supplement free of charge but had no involvement in data collection or analysis of this study. The authors declare no other conflicts of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2024.112477.

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