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Effects of creatine supplementation with and without exercise and diet intervention on body composition, cognitive function, and markers of health in middle-aged and older adults

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ABSTRACT

Background: Theoretically, CrM supplementation during a weight loss and exercise intervention may help older individuals promote more optimal changes in body composition, training adaptations, and cognition. To determine whether CrM affects body composition, cognitive function, and/or markers of health in untrained and trained middle-aged and older adults.

Methods: Seventy-three healthy sedentary adults selected whether to participate in a non-exercise or exercise and diet intervention and were then randomized, in a double-blind and counterbalanced manner, to 2 × 5 g/d of maltodextrin placebo (PLA) or CrM. Sixty-four adults aged 45–65 years (54.5 ± 6.2 years, 84.5 ± 21.3 kg; 40 females and 24 males) completed the 12-week intervention and were analyzed. At 0, 6, and 12 weeks, participants had DXA body composition determined, donated fasting blood samples, and completed a battery of cognitive tests and questionnaires. general linear models (GLMs), multivariate and univariate, with repeated measures and mean changes from baseline with 95% confidence intervals, and Chi-squared analyzes were used.

Results: CrM supplementation without exercise training and diet intervention increased lean tissue mass, strength, and muscular endurance while promoting favorable changes in selected blood lipids, HbA1c, and selected markers of cognitive function and memory. Creatine supplementation during an exercise and weight-loss diet intervention increased lean tissue mass, promoted a greater reduction in body fat percentage, and improved muscular strength, endurance, and selected markers of cognitive function and memory. Supplementation was well-tolerated.

Conclusion: CrM supplementation with and without exercise and diet intervention may help middle-aged and older adults maintain muscle mass and strength while favourably affecting selected markers of health and cognitive function. Individual cognitive and biomarker findings should be interpreted as exploratory.

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1. Introduction

Aging is often associated with a progressive increase in adipose tissue and a concomitant decline in skeletal muscle mass, a condition known as sarcopenic obesity [1,2]. This combination has been associated with heightened cardiometabolic risk and functional impairments in older adults [3]. While caloric restriction remains a widely employed strategy to reduce excess body fat, it often leads to concurrent losses in lean tissue, potentially exacerbating sarcopenia and its associated complications [4]. Accordingly, it is recommended that older individuals seeking to reduce fat mass incorporate resistance training and maintain adequate dietary protein intake to preserve lean body mass during weight-loss efforts [5–8].

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Creatine monohydrate (CrM) supplementation has been consistently shown to increase intramuscular phosphocreatine and total creatine stores by approximately 20%–40% when ingested in loading (e.g. 4 × 5 g/day for 5–7 days) and/or long-term low-dose supplementation protocols (e.g. 3–5 g/day) [9–11]. This increase supports enhanced cellular bioenergetics, particularly during high-intensity and short-duration exercise, and has been associated with improvements in strength, muscle accretion, and training adaptations across age groups [12–14]. Given these benefits, CrM supplementation may be a viable strategy to augment resistance-training outcomes and mitigate lean mass loss during energy restriction in older adults.

Emerging evidence also suggests that creatine exerts effects on the central nervous system. The creatine transporter (CRT1), expressed at the blood–brain barrier, facilitates the uptake of creatine into the brain, though passive diffusion also contributes to cerebral creatine transport [15,16]. Studies employing magnetic resonance spectroscopy (MRS) techniques have reported increases in brain creatine and phosphocreatine content by 5% to 15% following CrM supplementation [11,17,18]. However, due to the limited transport capacity across the blood–brain barrier, it has been recommended that higher daily doses (e.g. 10–20 g/day) may be required to elicit appreciable increases in cerebral creatine levels [19,20].

Increased brain creatine availability has been associated with improved ATP resynthesis and buffering capacity, potentially enhancing cognitive performance and resilience to mental fatigue. Several studies have demonstrated that CrM supplementation may support cognitive function, particularly under metabolically demanding conditions or in populations with impaired energy metabolism [21–24]. Moreover, observational data suggest that higher habitual dietary creatine intake is associated with lower circulating levels of neurofilament light chain, a biomarker of neuroaxonal damage [25], better cognitive function [26], and reduced prevalence of depressive symptoms in aging populations [27,28]. Animal studies further support the neuroprotective potential of creatine, with beneficial effects reported following experimentally induced traumatic brain injury [29], spinal cord injury [30], and ischemic stroke [31]. Collectively, these findings suggest that CrM supplementation may have implications beyond musculoskeletal health by supporting neurological integrity and function.

The purpose of this investigation was to assess the effects of twelve weeks of CrM supplementation (2 × 5 g/d) on body composition and cognitive function in healthy, untrained middle-aged and older adults, as well as those engaged in a structured diet and resistance training program aimed at promoting weight loss. We hypothesized that CrM supplementation would favorably influence cognitive performance in non-training individuals and enhance training-induced improvements in body composition and health-related outcomes in the exercise and weight loss group. Primary outcomes included assessments of body composition, training adaptations, and cognitive function, while secondary measures included dietary intake, physical activity, training volume, resting metabolic rate, mood, quality-of-life indices, blood biomarkers, and reported side effects. Individual cognitive-task and biomarkers analysis was prespecified as exploratory rather than separately powered confirmatory endpoints.

2. Methods

2.1. Research design

This study utilized a randomized, double-blind, placebo-controlled, parallel-group design within participant-selected exercise and non-exercise strata, with repeated measures collected over a 12-week intervention period (Figure 1). All experimental procedures were reviewed and approved by the Institutional Review Board (STUDY2024-0233; June 10, 2024) and were conducted in accordance with the ethical standards outlined in the Declaration of Helsinki for research involving human participants. Written informed consent was obtained from all individuals before participation. The first participant was enrolled on July 10, 2024. The trial was submitted to the ISRCTN registry on April 11, 2025, and posted on June 11, 2025 (ID: 83081058). Exercise status and nutritional supplementation were the primary independent variables, while body composition, training adaptations, and cognitive performance were the primary outcome measures. Secondary outcomes included dietary intake, physical activity patterns, training volume, psychological indices, hematologic markers, and reported adverse events.

2.2. Participant recruitment

Healthy, community-dwelling adults aged 45–65 were recruited through targeted advertisements in local media outlets, on social media platforms, and via campus-based communication networks. Interested

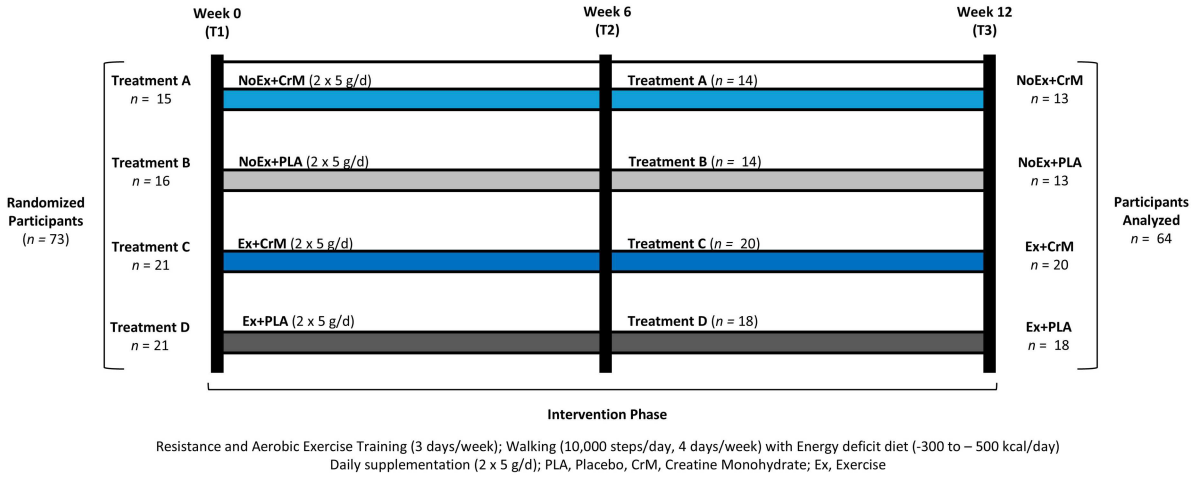

Figure 1. Study experimental design showing participant-selected exercise status and randomized, double-blind supplement assignment. NoEx = no exercise, Ex = exercise, PLA = placebo, CrM = creatine monohydrate, *n* = sample size.

individuals completed an online screening questionnaire, followed by an in-person evaluation to confirm eligibility. Inclusion criteria required participants to be apparently healthy or medically stable males or females within the specified age range, willing to comply with all study procedures and testing visits, and able to abstain from alcohol consumption and non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, and over-the-counter analgesics for at least 48 hours before each testing session. Exclusion criteria included the presence of orthopedic limitations that could interfere with study participation, recent use of weight-loss medications or supplements within two weeks of enrollment, a history of alcohol or substance abuse within the preceding 12 months, current heavy smoking (>1 pack/day within the previous three months), or known hypersensitivity to creatine monohydrate or maltodextrin. The study physician reviewed medical history and screening data to confirm eligibility before enrollment. Before supplement allocation, all participants selected whether they wished to participate in the structured exercise program or remain in the non-exercise condition.

Figure 2 presents the Consolidated Standards of Reporting Trials (CONSORT) flow diagram. A total of 136 individuals responded to recruitment efforts and were initially contacted to assess general eligibility. Of these, 109 completed further screening, and 94 met inclusion criteria and attended a familiarization visit. Fifteen individuals declined participation due to time constraints, two were excluded for clinically elevated blood pressure, two were excluded due to the use of glucagon-like peptide-1 (GLP-1) receptor agonists, one was excluded due to thyroid-related conditions, and one withdrew after initiating a new medication. Seventy-three participants selected exercise or non-exercise participation and were then matched and randomized to PLA or CrM within their selected exercise-status stratum using a counterbalancing approach to minimize baseline differences in age, sex, and body mass index. Group allocation included 16 participants in the no exercise and diet intervention and PLA (NoEx+PLA) group, 15 in the no exercise and diet intervention and CrM (NoEx+CrM) group, 21 in the exercise and diet intervention plus PLA (Ex+PLA) group, and 21 in the exercise and diet intervention plus CrM (Ex+CrM) group. Sixty-six participants completed week 6, and 64 completed week 12 and were included in the repeated-measures analysis. Final group composition included 13 (9 female), 13 (7 female), 18 (10 female), and 20 (14 female) participants in the NoEx+PLA, NoEx+CrM, Ex+PLA, and Ex+CrM groups, respectively.

2.3. Experimental testing overview

An overview of the testing timeline is illustrated in Figure 3. Assessments were conducted at baseline (week 0), mid-intervention (week 6), and post-intervention (week 12). Testing procedures included measurements of anthropometrics, resting metabolic rate, dual-energy x-ray absorptiometry (DXA)-derived body composition, fasting blood biomarkers, physical activity, cognitive performance, light-based reaction testing,

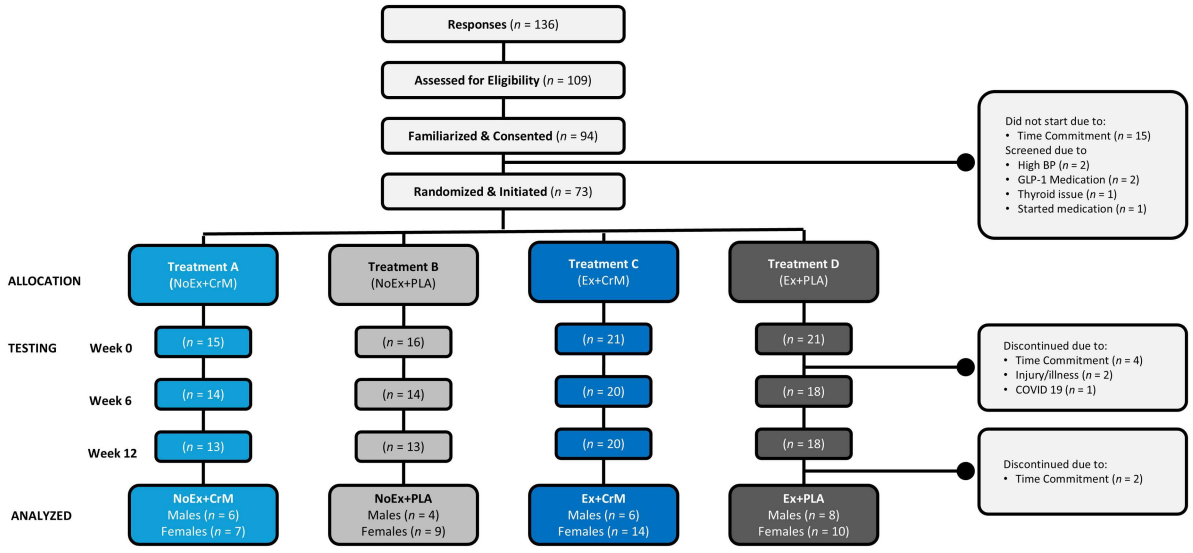

Figure 2. Consolidated standards of reporting trials (CONSORT) diagram for participant recruitment, screening, consent, exercise-status selection, supplement randomization, allocation, testing sessions, completion, and analysis of the treatment groups. NoEx = no exercise, Ex = exercise, PLA = placebo, CrM = creatine monohydrate, *n* = sample size, BP = blood pressure.

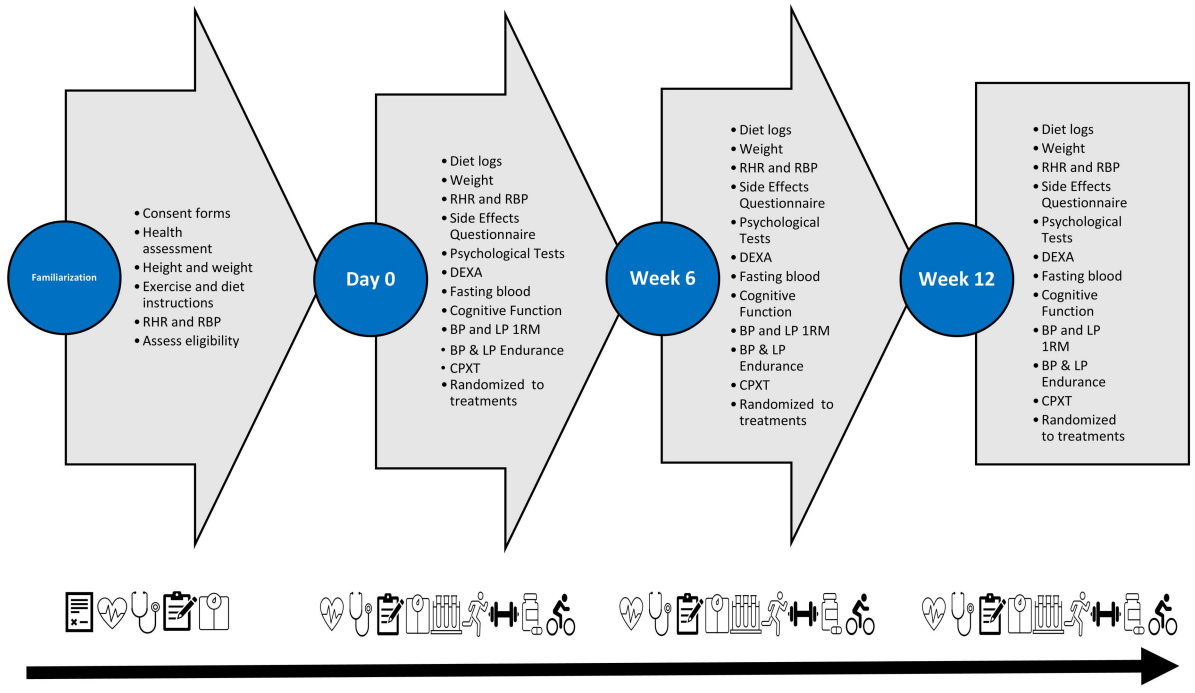

Figure 3. Testing order and timeline. RHR = resting heart rate, RBP = resting blood pressure, DEXA = dual-energy X-ray absorptiometry, BP = bench press, LP = leg press, 1RM = one repetition maximum, CPXT = cardiopulmonary exercise test.

muscular strength and endurance, cardiopulmonary exercise capacity, and psychological health indices. All testing sessions were standardized with respect to time of day, fasting status, and pre-assessment activity restrictions to ensure consistency across visits.

2.4. Familiarization

Participants completed a familiarization session before baseline testing to review study procedures, provide informed consent, and undergo preliminary anthropometric, vital sign, and memory assessments. During

this visit, participants completed practice trials of the computerized cognitive battery and light-tracking reaction task to reduce learning effects during formal testing. Instruction and demonstration of one-repetition maximum strength testing, muscular endurance assessments, and maximal cardiorespiratory exercise testing were also provided. Participants received standardized guidance for documenting their dietary intake over 4 days using either a smartphone-based nutrition-tracking application or written food records.

2.5. Randomization and supplementation

Within each participant-selected exercise-status stratum, participants were matched by age, height, body mass, body mass index (BMI), and sex and randomly assigned in a counterbalanced manner to CrM or PLA. Placebo supplements consisted of maltodextrin (DE 20; Don Xiao, Dongxiao Co., China), whereas the active supplement was creatine monohydrate (Creavitalis® Special Grade; Alzchem Trostberg GmbH, Trostberg, Germany). Supplements were packaged in identical, coded 5 g foil sachets to maintain blinding. Participants and study personnel remained blinded to supplement assignment until the database was finalized; exercise status was necessarily apparent.

Participants were instructed to consume two servings daily by dissolving the powder in water or a flavored beverage, with one dose taken in the morning and one in the evening. Compliance was monitored using biweekly supplement logs and verification of returned empty sachets at weeks 6 and 12.

2.6. Exercise intervention

Participants assigned to the exercise conditions completed a supervised resistance and aerobic training program three times per week for 12 weeks. Resistance training sessions emphasized whole-body movements using machine-based, free-weight, and bodyweight exercises. Training loads were progressively increased at 1–2-week intervals, with participants performing three sets of 10 repetitions per exercise and resting 90–120 seconds between sets. Aerobic exercise consisted of approximately 20 minutes of continuous activity at 60%–80% of heart rate reserve, performed on a stationary bicycle, treadmill, or outdoors walking/jogging. Exercise intensity was monitored using wearable heart rate sensors (Polar Electro Inc., Bethpage, NY, USA). Training duration, distance, and average heart rate were recorded for each aerobic session, and workloads were adjusted as needed to maintain target intensity throughout the intervention. Program adherence was defined as completion of at least 70% of prescribed sessions (≥25 of 36), a benchmark used in previous literature [32,33]. Participants were also asked to accumulate 10,000 steps/d on non-training days to maintain total physical activity. Steps were tracked via smartwatches, their accompanying smartphones, or clip-on pedometers (BATAUU, Shenzhen, China). Participants recorded steps in daily step logs collected at each testing session.

2.7. Diet intervention

All participants followed a diet plan aligned with the American Heart Association's macronutrient recommendations (55% carbohydrate, 15% protein, 30% fat) to induce a modest energy deficit of approximately 300–500 kcal/day. Daily energy intake targets ranged from 1200 to 1600 kcal/day and were individualized based on the baseline resting metabolic rate, with a minimum intake of 1200 kcal/day. Dietary intake was recorded using a smartphone-based application (MyFitnessPal Inc., Baltimore, MD, USA) [34] and reviewed for compliance.

2.8. Procedures

2.8.1. Participant characteristics and resting measures

Participant height and body mass were determined using a calibrated Health-O-Meter Professional 500KL digital scale (Pelstar LLC, Alsip, IL, USA). Resting heart rate (HR) and blood pressure (BP) were assessed after 5 minutes of seated rest using an automated monitor and a sphygmomanometer (Connex ProBP 3400, Welch Allyn Inc., Skaneateles Falls, NY, USA), following previously described methods [35].

2.8.2. Dietary intake

Nutritional intake was tracked via MyFitnessPal (MyFitnessPal Inc., Baltimore, MD, USA), a validated dietary tracking application [36]. Participants recorded food and fluid intake over four consecutive days, including three weekdays and one weekend day. Four-day food logs were analyzed at baseline, week six, and week twelve to quantify caloric and macronutrient intake for compliance and analysis. Research staff calculated average energy, carbohydrate, protein, and fat intakes at each assessment point for subsequent statistical analysis.

2.8.3. Physical activity and exercise volume

Daily physical activity was quantified using the International Physical Activity Questionnaire–Long Form (IPAQ-LF), a validated self-report tool assessing frequency, intensity, and duration of movement across occupational, household, transport, and recreational domains [37]. Exercise training logs were used to document weight lifted and repetitions per set, enabling calculation of training volume (load × reps) by upper and lower body across weeks 0–6 and 6–12. Step count on non-training days was recorded via wearable devices and averaged over the period [38,39].

2.8.4. Resting energy expenditure

Resting metabolic rate (RMR) was assessed in a controlled environment using a TrueOne® 2400 metabolic measurement system (ParvoMedics Inc., Sandy, UT, USA). Prior to each session, the system was calibrated for flow and gas analysis using a 3.0-liter syringe (Series 5530; Hans Rudolph Inc., Shawnee, KS, USA) and a certified reference gas mixture containing known concentrations of O_2 and CO_2. During testing, participants were positioned supine with hips and knees flexed on a padded cushion to promote relaxation and minimize muscular activity. After a 10-minute acclimatization period, data were collected continuously for 20–30 minutes. The last five consecutive minutes meeting the criteria of <5% coefficient of variation and $FeCO_2$ between 1.0% and 1.2% were averaged for analysis. Substrate utilization was estimated using respiratory exchange ratio (RER) values based on methods previously documented with CVs of 5.3% and ICC of 0.92 in similar populations [32,40–45].

2.8.5. Body composition assessment

Whole-body composition, excluding cranial regions, was determined using a calibrated Hologic Discovery W dual-energy X-ray absorptiometry (DXA) scanner with APEX software (version 4.5.3; Hologic Inc., Marlborough, MA, USA). Scans provided quantitative data on total and regional lean mass, fat mass, bone mineral content, and visceral adipose tissue. The device was calibrated before each testing session according to manufacturer guidelines using a standardized phantom. Test-retest precision from our laboratory revealed coefficients of variation (CVs) of 0.31%–0.45% for bone mineral content, total body mass, and lean tissue mass, with an average intraclass correlation coefficient (ICC) of 0.98, indicating high reliability [46].

2.8.6. Muscular strength and endurance testing

Participants performed upper- and lower-body maximal strength testing using conventional bench press (BP) and leg press (LP) equipment (Nebula Fitness, Versailles, OH, USA). Following a standardized warm-up, 1RM assessments were performed using incremental loading strategies and 3-minute rest intervals between attempts [47]. Proper technique was emphasized, and repetitions were terminated when full range of motion could not be maintained. After determining 1RM, participants rested for 5 minutes, then completed a muscular endurance test at 70% of their 1RM by performing repetitions to volitional fatigue. This approach aligns with prior testing methodologies in resistance training research [47].

2.8.7. Peak aerobic capacity assessment

Peak oxygen uptake (VO_2peak) was evaluated using a modified Bruce treadmill protocol until volitional fatigue [48]. Participants completed the test on a TrackMaster TMX425 motorized treadmill (Full Vision Inc., Newton, KS, USA), while expired gases were collected and analyzed using the ParvoMedics TrueOne® 2400 metabolic system. Electrocardiographic monitoring was performed using a 12-lead CardioCard® ECG system

(Nasiff Associates, Brewerton, NY, USA). Subjective exertion was recorded at each stage using the Borg 6–20 Rating of Perceived Exertion (RPE) scale [49].

2.8.8. Cognitive function testing

Cognitive functioning was evaluated using a suite of tests from the computerized mental performance assessment system (COMPASS, version 6.0) cognitive battery (Northumbria University, Newcastle upon Tyne, UK), a validated system employed in numerous peer-reviewed investigations [50,51]. The battery included a series of tasks designed to evaluate key domains of cognition, including episodic memory, sustained attention, working memory, and cognitive control. Specific assessments included immediate and delayed word recall, word recognition, picture recognition, choice reaction, the Corsi block tapping task, the digit vigilance test, and the stroop color-word task. During the Immediate and Delayed Word Recall tests, participants were asked to recall and record a list of words both immediately after presentation and after a delay, evaluating verbal episodic memory accuracy [51]. The Word and Picture Recognition tasks assessed familiarity-based memory by measuring the proportion of correctly identified items from previously shown lists [50]. The choice reaction time test evaluates how effectively individuals can identify and respond correctly to stimulus and assess cognitive processing and decision-making speed [52]. The Digit Vigilance task gauged attentional control and sustained concentration through the identification of target digits among distractors while recording both reaction times and error rates [53]. The Corsi Block Tapping Test involved reproducing sequences of illuminated spatial targets to measure visuospatial short-term memory and attention span [54]. Cognitive flexibility and selective attention were evaluated via the Stroop task, where participants had to report the color of the font used to display incongruent color words, requiring suppression of automatic responses [55]. These computerized tasks have demonstrated robust psychometric properties and sensitivity to cognitive changes following interventions [56–58]. Accuracy, response time, and task completion rates were recorded. The COMPASS tasks were computer-administered and automatically scored, and participants and study personnel remained blinded to supplement assignment during testing.

Perceptual-cognitive processing speed and visual tracking capacity were further assessed using the NeuroTracker Pro system, integrated with NeurotrackerX software version 1.28.10 (Montreal, QC, Canada), according to established methods [59–61]. Each participant completed three sessions of three CORE modules, with each module comprising 20×8-second trials. A 3D digital light processing projector (Optoma Corp., New Taipei City, Taiwan) was used to display the stimulus. Participants viewed the test using BOBLOV JX-30 active shutter 3D glasses (Shenzhen Technology Co., Ltd., Shenzhen, China) and interacted with the program via a Zephyrus GX501 gaming laptop (ASUSTeK Computer Inc., Taipei, Taiwan) connected to a Logitech G PRO wireless gaming mouse (Logitech Europe S.A., Lausanne, Switzerland). Intra-individual consistency for NeuroTracker performance has previously been shown with a test–retest coefficient of variation of 6.5% [60,61].

2.8.9. Psychological and mood assessments

Participants completed the 65-item Profile of Mood States (POMS) questionnaire, a widely recognized instrument for assessing transient and fluctuating mood states [62,63]. The scale includes six subscales measuring key mood dimensions: confusion, anger, fatigue, depression, tension, and vigor. A composite total mood disturbance score (TMDS) was calculated by summing the negative mood subscale scores and subtracting the vigor score. This measure has consistently demonstrated reliability and validity in exercise and clinical psychology contexts.

2.8.10. Memory perceptions

To assess age-associated memory impairments (AAMI), participants completed the Mini-Mental State Examination (MMSE), the Memory Complaint Questionnaire (MAC-Q), and Memory Complaint Scale (MCS). The MMSE is a structured 30-point screening tool commonly used to identify potential cognitive deficits, with score interpretations ranging from mild to severe impairment [64]. Its psychometric properties include test–retest reliability coefficients typically exceeding 0.85 [65]. The MAC-Q, a self-report questionnaire, was administered to assess subjective concerns about memory function; scores ≥ 25 indicate

perceived memory complaints [66]. The MCS is a subjective rating of memory complaints associated with depression and cognitive decline [66–68]. Higher scores indicate greater memory issues.

2.8.11. Health-related quality of life

Participants' perceived quality of life was assessed using the SF-36 Health Survey, version 2 (SF-36v2; Rand Health Care, Santa Monica, CA, USA), which evaluates eight health domains across physical and mental health spectrums [69]. The instrument yields both domain-specific and composite summary scores and has been extensively validated in diverse populations. Reported test-retest reliability ranges between 0.81 and 0.95, depending on the domain assessed [70].

2.8.12. Blood collection and laboratory analysis

At each collection time point, approximately 20 mL of venous blood was drawn from the antecubital vein using standard aseptic techniques. Blood was collected into two 7.5 mL serum separator tubes (SST; BD Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and one 3.5 mL EDTA tube (BD Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Following collection, all tubes were maintained upright at room temperature for approximately 15 minutes to allow clotting and stabilization. The samples were then centrifuged at 3000 rpm for 10 minutes at 4 °C using a Heraeus MegaFuge 40 R refrigerated centrifuge (Thermo Fisher Scientific Inc., West Palm Beach, FL, USA). Post-centrifugation, serum was separated from one SST tube and aliquoted into pre-labeled polypropylene microcentrifuge tubes (VWR International, Radnor, PA, USA) for biobanking at −80 °C until further biochemical analysis. The remaining SST and EDTA tubes were prepared and transported on ice to a certified clinical laboratory (Clinical Pathology Laboratory, Austin, TX, USA) for complete blood count with differential (CBC) and comprehensive metabolic panel (CMP) analyzes.

2.8.13. Adverse event monitoring

Participant-reported side effects were recorded using a validated subjective questionnaire adapted from prior supplementation trials [71–73]. The questionnaire captured the presence, severity, and frequency of common side effects, including dizziness, headache, tachycardia, heart palpitations, dyspnea, nervousness, and blurred vision. Responses were evaluated using a 5-point Likert-type scale. The instrument's psychometric properties have shown coefficients of variation (C_V) ranging from 1% to 3% and intraclass correlation coefficients (ICC) ranging from 0.60 to 0.88.

2.8.14. Statistical analysis

All statistical procedures were performed using IBM SPSS Statistics version 31.0 (IBM Corporation, Armonk, NY, USA), following methods previously described in detail [70] and consistent with previously established protocols for evaluating intervention effects in randomized controlled trials [74]. Sample size estimates were informed by several considerations. First, our prior studies examining nutritional interventions on cognitive function indicated that approximately 12 to 20 participants per group were generally sufficient to detect significant treatment effects in cognitive function tests evaluated [40,57,58,75–82]. Second, we reviewed prior investigations reporting significant cognitive responses to creatine supplementation [83,84], and found that most of these trials enrolled between 8 and 20 participants per group. We also considered reported means, standard deviations, and statistically significant mean differences from related studies to estimate power, assuming 80% power, variability of approximately 5% to 10% relative to the mean and expected improvements of 5% to 10% in primary outcomes. The present study included 64 completers, with 13–20 participants per group, which is larger than most prior creatine studies assessing these outcomes. Individual cognitive-task outcomes were exploratory and were not separately powered as confirmatory efficacy endpoints.

A mixed-design general linear model (GLM) with repeated measures was employed to examine differences over time between groups. Assumptions of normality and sphericity were assessed using Shapiro–Wilk and Mauchly's tests, respectively. If Mauchly's test indicated a violation of sphericity, the Greenhouse-Geisser correction was applied to adjust the degrees of freedom and control for Type I error rate inflation [85,86]. In addition to Wilks' Lambda multivariate tests, planned simple-effects and pairwise comparisons of means were conducted using Fisher's least significant difference (LSD) post hoc analysis,

with significance thresholds set at $p \leq 0.05$. No across-outcome multiplicity adjustment was prespecified because the individual cognitive tasks and biomarker variables represented distinct exploratory outcomes rather than a single confirmatory endpoint family or hierarchical decision rule. All outcomes are reported and interpreted in conjunction with the multivariate tests, effect sizes, and 95% CIs. Trends approaching significance were noted for values between $p > 0.05$ and ≤ 0.10. Effect sizes were calculated using partial eta squared (η_p^2), interpreted as small ($\eta_p^2 = 0.01$), medium ($\eta_p^2 = 0.06$), or large ($\eta_p^2 \geq 0.14$), in accordance with Cohen's guidelines [87]. Clinical relevance of findings was assessed by examining mean changes from baseline with 95% confidence intervals (CIs), allowing interpretation of the potential magnitude of treatment effects [88]. All data were reported as mean ± standard deviation (SD) or mean percent change from baseline (mean [95% CI]). The repeated-measures GLM included the 64 participants who completed the 12-week intervention. Missing observations among completers were minimal, generally involving 0–4 isolated values for only a few variables. When necessary, missing numerical observations were replaced primarily using the participant's prior observed value; series means were used in limited instances, and missing ordinal survey responses were replaced using the most frequently observed response [79,80]. No complete follow-up record was imputed for a participant who discontinued [89,90].

This statistical approach comprehensively assesses differences among groups while reducing the likelihood of type II error, enabling researchers to determine whether additional research is warranted [91–93]. Additionally, it provides an assessment of the clinical significance of findings [88,91–96]. Scholar GPT 5.6 (San Francisco, CA USA) was used to assist with conducting a literature search and to suggests how to suggest how to phrase methods to reduce similarity to our other publications. We also used iThenticate Plagiarism Detection Software (Turnitin, LLC, Oakland, CA USA) to identify similarity to other publications (including our own publications) and reduce similarity as much as feasible.

3. Results

3.1. Participant demographics

As shown in Table S1, the participants were 54.5 ± 6.2 years, 84.5 ± 21.3 kg, 66.3 ± 3.6 in tall, had a BMI of 29.93 ± 6.1 kg/m^2, 36.8% ± 8.5% fat, and had a resting heart rate of 66.4 ± 9.0 bpm, systolic blood pressure of 117.7 ± 6.4 mmHg, a diastolic blood pressure of 76.3 ± 8.0 mmHg, and VO_2peak of 36.8 ± 8.5 mL/kg/min. Multivariate Wilk's Lambda analysis revealed no statistically significant group ($p = 0.856$, $\eta_p^2 = 0.116$, medium effect), a significant sex effect ($p < 0.001$, $\eta_p^2 = 0.854$, large effect), and no statistically significant group × sex ($p = 0.837$, $\eta_p^2 = 0.116$, medium effect) within-subject effects. As expected, height ($p < 0.001$, $\eta_p^2 = 0.487$, large effect), weight ($p < 0.001$, $\eta_p^2 = 0.487$, large effect), and percent body fat $p < 0.001$, $\eta_p^2 = 0.487$, large effect) statistically significantly differed between sexes. However, no statistically significant group × sex effects were observed in demographic variables.

3.2. Diet and physical activity

3.2.1. Energy and macronutrient intake

Table S2 presents energy and macronutrient intake data expressed in absolute (g/d) and relative terms (g/kg/d). Multivariate Wilk's Lambda showed no statistically significant time ($p = 0.569$, $\eta_p^2 = 0.063$, medium effect) or group × time ($p = 0.961$, $\eta_p^2 = 0.045$, small effect) within-subject effects. Univariate analysis found no statistically significant time effects. Energy and carbohydrate intake in the NoEx+CrM group was significantly greater than in the remaining groups. On average, participants consumed 1527 kcals/d [1437, 1617] (19.1 kcals/kg/d [17.7, 20.5]), consisting of 155.4 g/d [143, 168] (1.97 g/kg/d [1.8, 2.1]) of carbohydrate, 74.3 g/d [69, 79] (0.94 g/kg/d [0.85, 1.03) protein, and 66.1 g/d [62, 71] (0.82 g/kg/d [0.75, 0.89]) fat. Energy intake, carbohydrate intake, and fat intake generally decreased in the exercise and diet groups, whereas they were maintained in the no-exercise or no-diet groups.

3.2.2. Resting energy expenditure

Table S3 shows resting energy expenditure results. Multivariate Wilk's Lambda showed no statistically significant time ($p = 0.408$, $\eta_p^2 = 0.035$) or group × time ($p = 0.591$, $\eta_p^2 = 0.045$) within-subject effects.

Univariate analysis revealed no statistically significant group, time, or group × time interaction effects for resting energy expenditure, respiratory quotient, carbohydrate oxidation, or fat oxidation.

3.2.3. Training volume

Multivariate Wilk's Lambda analysis of training volume data (Table S4) revealed statistically significant time ($p < 0.001$, $\eta_p^2 = 0.663$) and group × time ($p < 0.001$, $\eta_p^2 = 0.431$) within-subject effects for the exercise groups. Univariate analysis revealed time effects ($p < 0.001$) in walking steps/day, lower body, upper body, and total lifting volume. No statistically significant interaction effects were observed between steps/d and walking during total lifting volume.

3.2.4. Physical activity

Table S5 shows the IPAQ-LF results. The multivariate Wilk's Lambda analysis showed no time ($p = 0.710$, $\eta_p^2 = 0.193$, large effect) or group × time ($p = 0.567$, $\eta_p^2 = 0.208$, large effect) within-subject effects. Likewise, univariate analysis found no statistically significant group, time, or interaction effects in IPAQ-LF questions. Pairwise comparison revealed some differences among groups with the exercise groups generally showing greater engagement in moderate and vigorous physical activity.

3.3. Primary outcomes

3.3.1. Body composition

Table S6 displays body composition results. Multivariate Wilk's Lambda analysis revealed a statistically significant effect for time ($p < 0.001$, $\eta_p^2 = 0.162$, large effect) and group × time effect ($p = 0.037$, $\eta_p^2 = 0.073$, medium effect). Univariate analysis revealed statistically significant time effects for all variables, with statistically significant interaction effects seen in fat mass ($p = 0.007$, $\eta_p^2 = 0.139$), fat-free mass ($p = 0.016$, $\eta_p^2 = 0.123$), percent body fat ($p = 0.001$, $\eta_p^2 = 0.174$), and approaching statistical significance for total scanned mass ($p = 0.052$, $\eta_p^2 = 0.107$). Pairwise comparison revealed that body composition was more favorably changed in the exercise groups. Analysis of mean changes from baseline (Figure 4) revealed that participants in the exercise and diet intervention lost more weight, fat mass, and body fat than those in the non-exercise groups. Participants in the CrM supplemented groups gained fat-free mass compared to the NoEx+PLA and Ex+PLA groups, while percentage body fat loss was statistically significantly greater in the Ex+CrM than NoEx+PLA, NoEx+CrM and Ex+PLA groups. No statistically significant multivariate or univariate group, time, or group × time effects or pairwise comparisons were observed among groups in bone mineral content, area, or density (see Table S7).

Table S8 shows visceral adipose tissue (VAT) results. Multivariate Wilk's Lambda analysis revealed a statistically significant effect for time ($p < 0.001$, $\eta_p^2 = 0.177$, large effect) and with an interaction effect approaching significance ($p = 0.072$, $\eta_p^2 = 0.076$, medium effect). Univariate analysis revealed statistically significant interaction effects for gynoid fat mass ($p < 0.002$). Pairwise comparison revealed that visceral adipose tissue markers were statistically significantly decreased in the exercise groups. Figure 5 shows

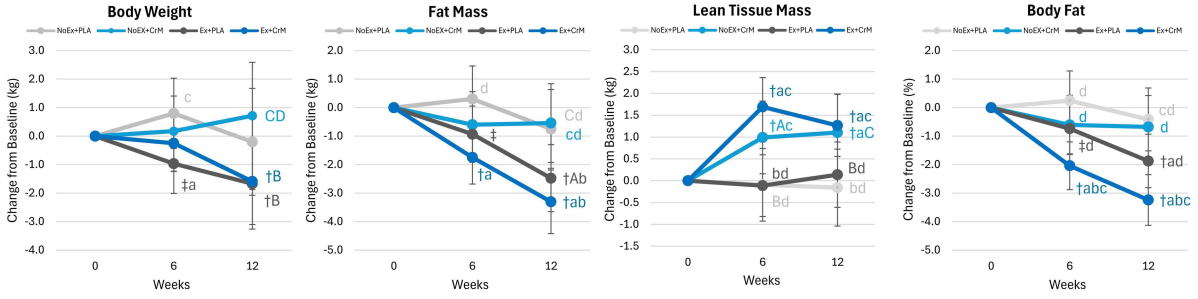

Figure 4. Changes in body weight and composition. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx+CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

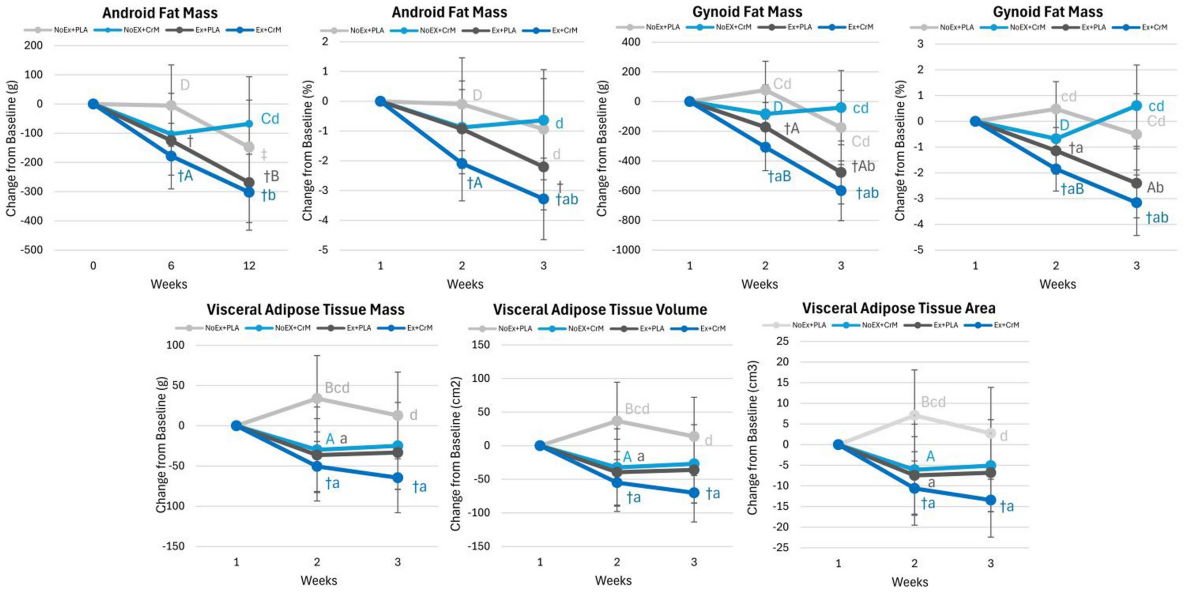

Figure 5. Changes in visceral adipose tissue-related variables. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx+CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

mean changes from baseline values. Android fat, gynoid fat, VAT mass, VAT volume, and VAT area values statistically significantly decreased in the exercise groups, with changes in the Ex+CrM group consistently significantly lower than the NoEx+PLA group. Interestingly, VAT changes with NoEx+CrM were not statistically significantly different from Ex+PLA values.

3.3.2. Training adaptations

Table S9 presents training-related variables. Multivariate analysis revealed statistically significant time ($p < 0.001$, $\eta_p^2 = 0.264$) and group × time ($p < 0.001$, $\eta_p^2 = 0.127$) within-subject effects. Univariate analysis revealed statistically significant interaction effects in leg press 1RM ($p = 0.001$, $\eta_p^2 = 0.271$, large effect) and bench press 1RM ($p = 0.003$, $\eta_p^2 = 0.175$, large effect), with leg press lifting volume ($p = 0.075$, $\eta_p^2 = 0.092$, medium effect) and treadmill time to finish ($p = 0.062$, $\eta_p^2 = 0.095$, medium effect) approaching statistical significance. Figure 6 shows the mean percentage changes in training adaptation. Changes were generally greater with exercise training and CrM supplementation. Interestingly, some statistically significant benefits were observed in the NoEx+CrM group compared to NoEx+PLA group in 1RM bench press performance.

3.3.3. Cognitive function

3.3.3.1. Word recall. Table S10 displays the results of the word recall assessment. Multivariate Wilk's Lambda showed no statistically significant time ($p = 0.062$, $\eta_p^2 = 0.061$, medium effect) or group × time ($p = 0.352$, $\eta_p^2 = 0.053$, small effect) within-subject effects. The univariate analysis revealed time effects for delayed recalled correct responses ($p = 0.014$, $\eta_p^2 = 0.072$, medium effect) with no other time or interaction effects observed in the remaining variables. Analysis of mean changes from baseline (Figure 7) shows that the number of delayed recalled correct responses was statistically significantly higher after 6-weeks in the NoEx+CrM group compared to placebo groups.

3.3.3.2. Word recognition. Table S11 displays the results of the word recognition assessment. Multivariate Wilk's Lambda analysis revealed no time ($p = 0.147$, $\eta_p^2 = 0.070$, medium effect) or group × time ($p = 0.133$, $\eta_p^2 = 0.062$, medium effect) within-subject effects. The univariate analysis revealed statistically significant interaction effects in Yes reaction time ($p = 0.037$, $\eta_p^2 = 0.112$, medium effect) with correct reaction time approaching statistical significance ($p = 0.071$, $\eta_p^2 = 0.095$, medium effect). Percent changes from baseline with 95% CIs are

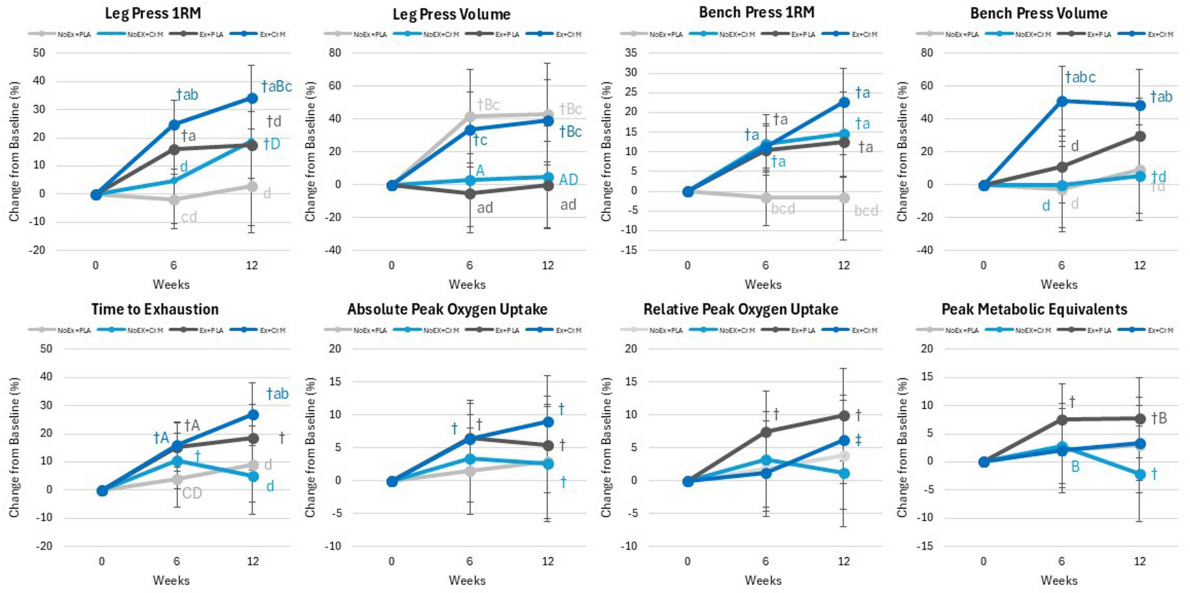

Figure 6. Training adaptation results. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

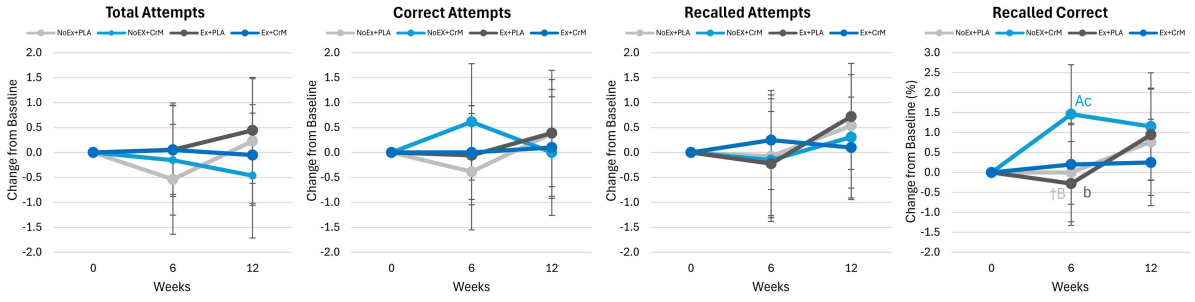

Figure 7. Results for the word recall assessment. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

shown in Figure 8. This analysis shows that participants supplementing their diet with CrM were generally able to recognize more correct words, with evidence of an increase in correct, Yes, and No reaction times in the NoEx +CrM or Ex+PLA groups, while better maintained in the NoEx+PLA and Ex+CrM groups.

3.3.3.3. Picture recognition test. Table S12 presents the results of the picture recognition test. Multivariate Wilk's Lambda showed no time ($p = 0.459$, $\eta_p^2 = 0.104$, medium effect) or group × time ($p = 0.908$, $\eta_p^2 = 0.057$, small effect) within-subject effects. The univariate analysis revealed no statistically significant time or group × time effects. However, the Yes reaction time interaction approached statistical significance ($p = 0.081$, $\eta_p^2 = 0.089$, medium effect) with reaction times higher in the Ex+PLA group compared to the NoEx+PLA and Ex +CrM groups. Analysis of mean changes from baseline did not reveal statistically significant improvements in picture recognition correct responses or reaction time (see Figure 9).

3.3.3.4. Choice reaction time test. Table S13 displays choice reaction time test results. Multivariate analysis showed no time ($p = 0.705$, $\eta_p^2 = 0.016$, small effect) effects while group × time effects approached significance ($p = 0.061$, $\eta_p^2 = 0.074$, medium effect). Univariate analysis revealed a statistically significant interaction in the percentage of correct scores identified ($p = 0.038$, $\eta_p^2 = 0.105$, medium effect) with no time or interaction

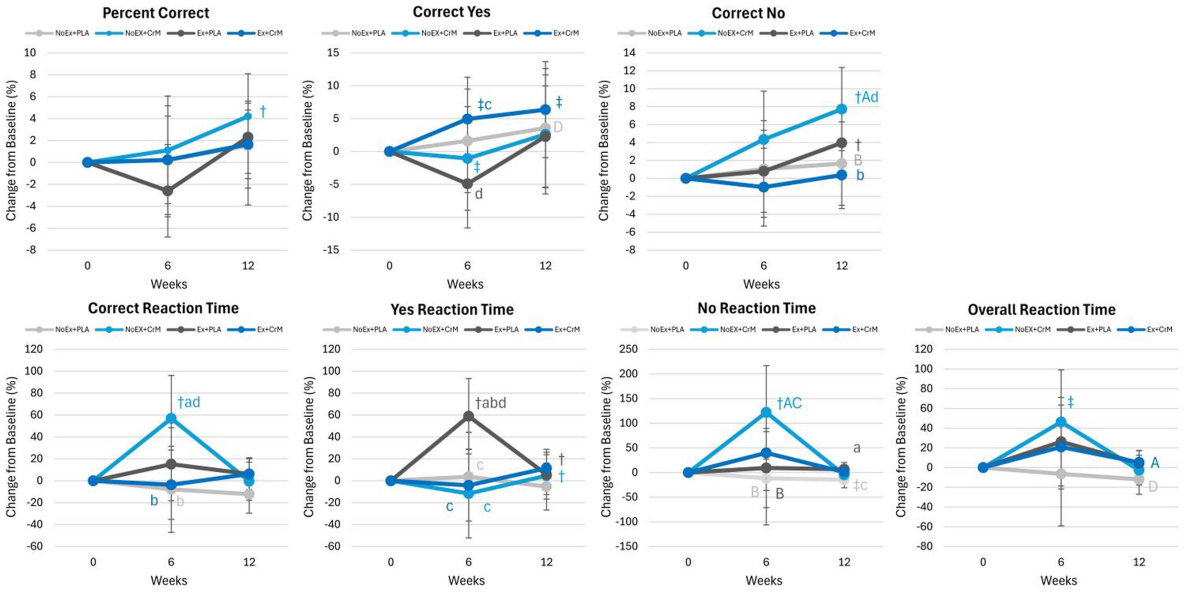

Figure 8. Results for the word recognition assessment. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

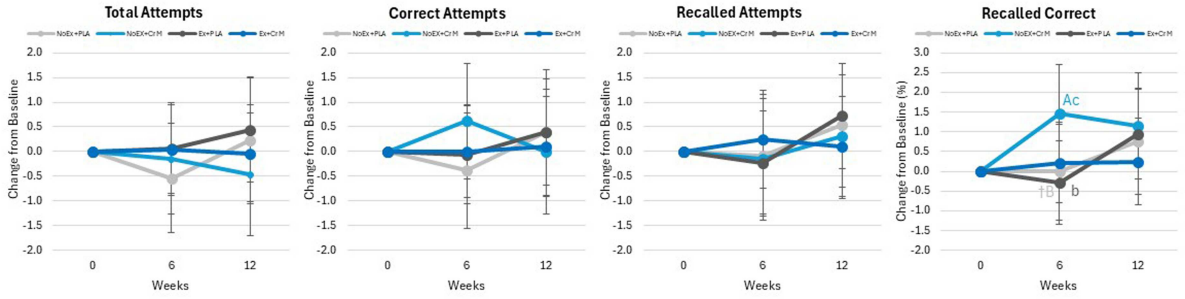

Figure 9. Results for the picture recall assessment. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

effects observed in overall reaction time or correct reaction time. Mean change from baseline analysis revealed that the percentage of correct choices were greater in the NoEx+PLA group, while reaction time at week 12 tended to be faster with Ex+CrM (−143 ms [−312. 26], $p = 0.095$) compared to NoEx+PLA (see Figure 10).

3.3.3.5. Digit vigilance task test. Table S14 presents digit vigilance test results. Multivariate Wilk's Lambda showed no time ($p = 0.109$, $\eta_p^2 = 0.043$, small effect) or group × time ($p = 0.869$, $\eta_p^2 = 0.031$, small effect) within-subject effects. Univariate analysis found no time, group, or group × time effects for the digit vigilance variables. Figure 11 shows that overall reaction times increased in the Ex+PLA and Ex+CrM groups while being unchanged in the NoEx+PLA and NoEx+CrM groups. The number of false alarms identified tended to increase in the Ex +CrM group.

3.3.3.6. Stroop color-word task test. Table S15 shows the results of the Stroop color-word task test results. Multivariate Wilk's Lambda showed no time ($p = 0.205$, $\eta_p^2 = 0.084$, medium effect) effects while the group × time interaction approached statistical significance ($p = 0.084$, $\eta_p^2 = 0.084$, medium effect). Univariate analysis found that the percentage of words correctly identified interacted ($p = 0.051$, $\eta_p^2 = 0.098$, medium

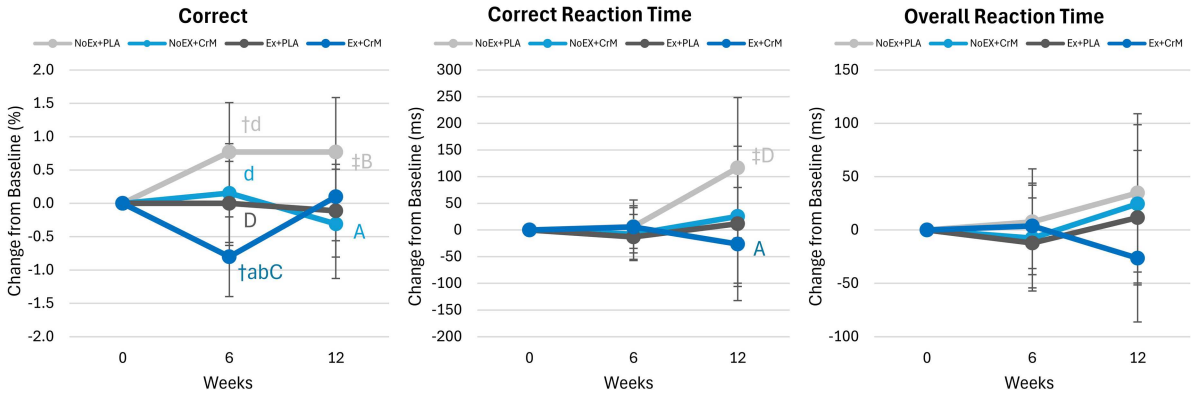

Figure 10. Results for the choice reaction time test. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

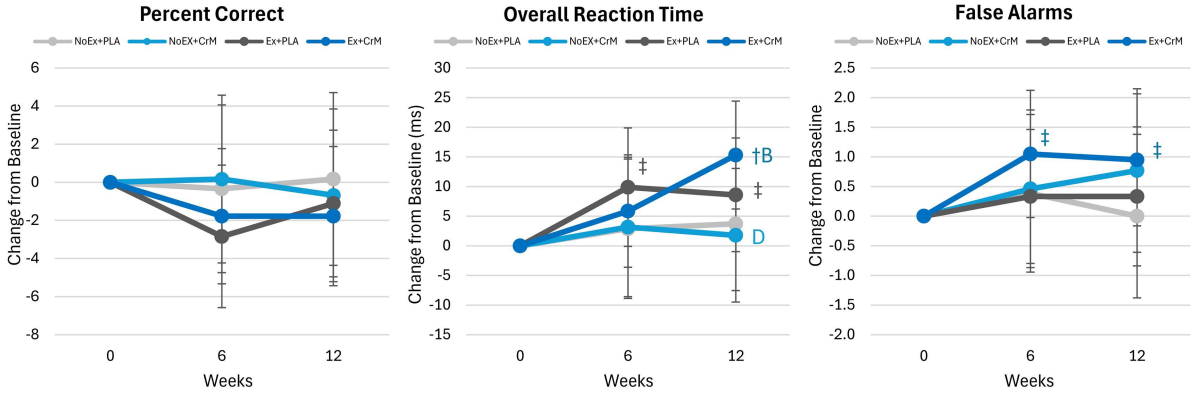

Figure 11. Results for the digit vigilance task test. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

effect) with no other time or interaction effects observed. Mean change analysis from baseline (Figure 12) did not identify evidence that CrM improved cognitive function on this test.

3.3.3.7. Corsi block task test. Table S16 shows the results of the Corsi Block assessment. Although some differences were observed among groups in baseline values that became statistically non-significant during the course of the study, no statistically significant time ($p < 0.833$, $\eta_p^2 = 0.003$, small effect) or group × time ($p = 0.764$, $\eta_p^2 = 0.026$, small effect) within-subject effects were observed among groups. Additionally, no statistically significant differences were observed in mean change from baseline analysis (see Figure 13).

3.3.3.8. Light reaction test. Average and best scores on the light reaction test are presented in Tables S17 and S18, respectively. No statistically significant multivariate or univariate effects were observed in move time, start speed, targets correctly identified, consistency score, total scores, total time, fastest successful trial, lowest missed trial, or reaction time scores.

3.4. Secondary outcomes

3.4.1. Profile of mood states

Profile of Mood States responses are shown in Table S19. Multivariate Wilk's Lambda showed no statistically significant time ($p = 0.135$, $\eta_p^2 = 0.071$, medium effect) or group × time ($p = 0.372$, $\eta_p^2 = 0.052$, small effect)

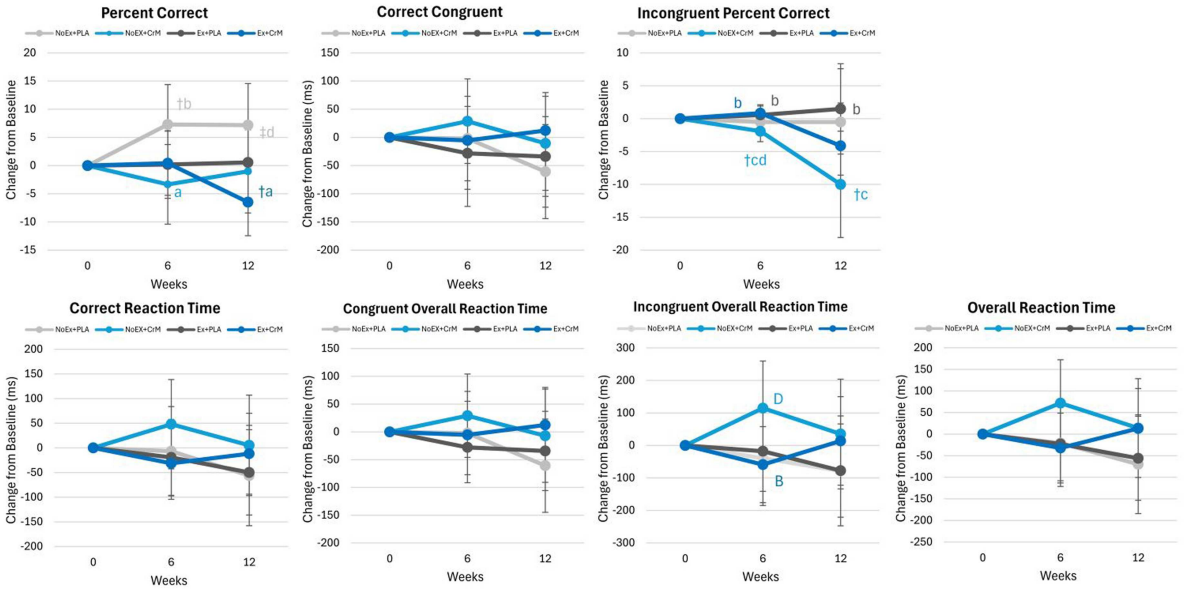

Figure 12. Results for the stroop color-word task test. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

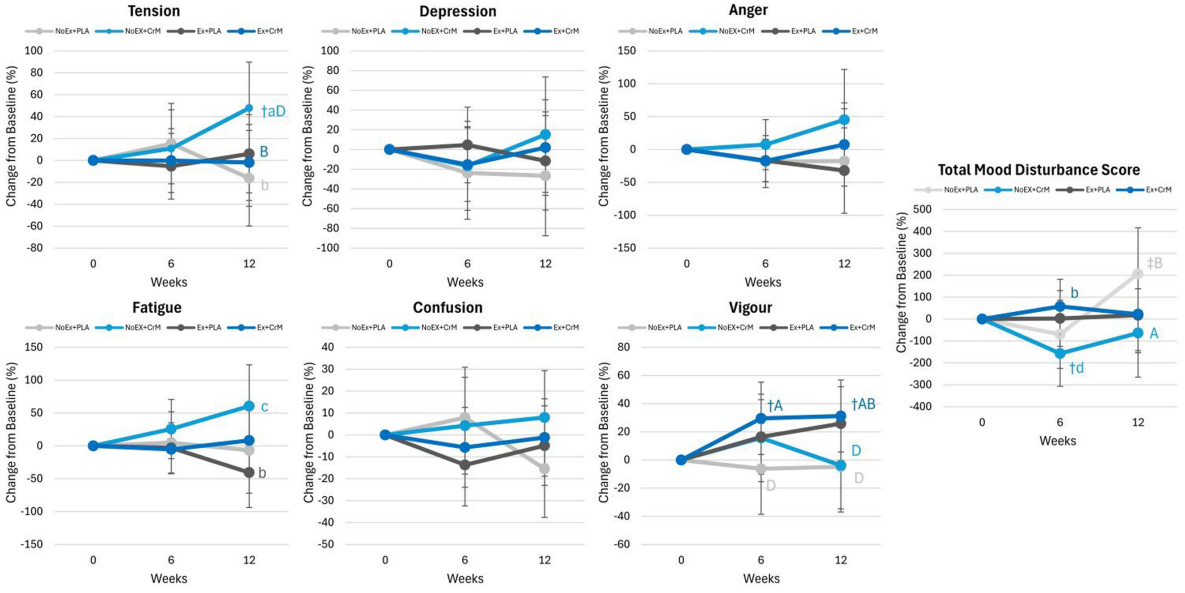

Figure 13. Results for the Corsi block task test. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

within-subject effects. Univariate analysis revealed that confusion ratings decreased over time ($p = 0.029$, $\eta_p^2 = 0.059$, medium effect) while fatigue scores tended to decrease ($p < 0.095$, $\eta_p^2 = 0.039$, small effect). However, no significant interaction effects were observed. Figure 14 shows mean percent changes from baseline analysis. Tension scores were statistically significantly higher in the NoEx+CrM compared to NoEx +PLA responses after 12 weeks, while vigor scores in the Ex+CrM group increased from baseline and tended

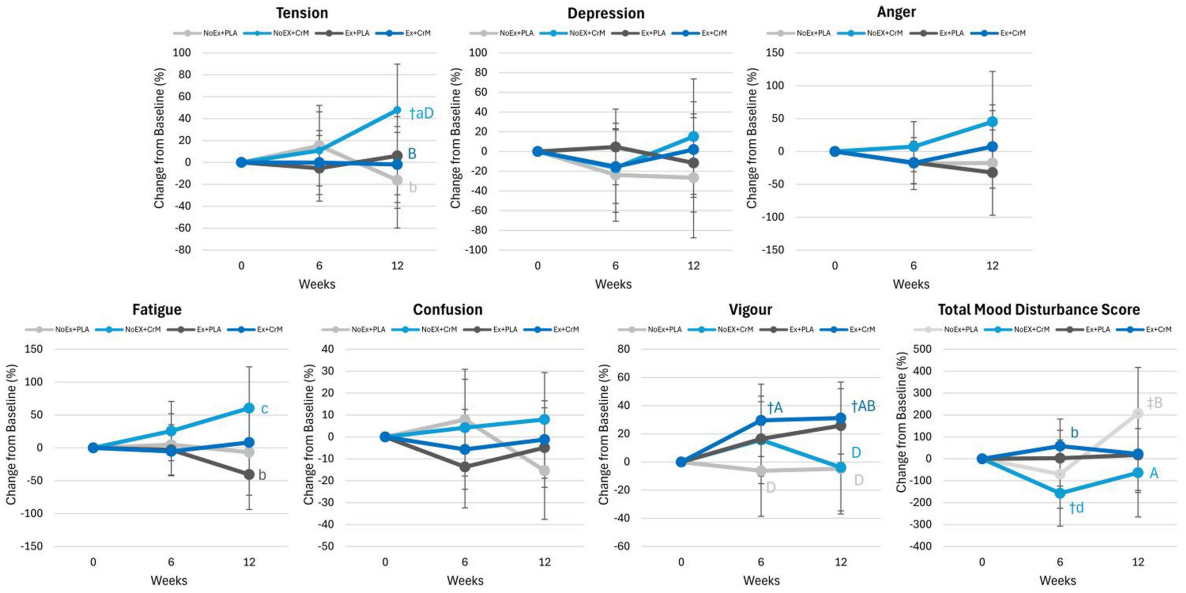

Figure 14. Results for the profile of mood states test. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

to be higher after 6 and 12 weeks of diet and training than NoEx+PLA responses and the NoEx+CrM group after 12 weeks.

3.4.2. Memory perceptions

Tables S20, S22, and S21 display the MMSE, MAC-Q, and MCS results, respectively. Participants had a baseline MMSE total score of 28.4 ± 0.8, MAC-Q total score of 23.9 ± 3.8, and MCS total score of 0.83 ± 0.6, indicating they did not have dementia, cognitive deficit, or memory complaints. MMSE scores increased from baseline in the Ex+CrM group and were statistically significantly higher than the no exercise groups after 12 weeks (see Figure 15). Individuals in the CrM groups remembered names, purchased items, and high school events to a greater degree than those in the placebo groups on the MAC-Q assessment (see Figure 16). Conversely, forgetfulness scores on the MCS test were generally higher than placebo or Ex+CrM scores in the NoEx+CrM group (see Figure 17).

3.4.3. Quality of life

Table S23 presents SF-36 Quality of Life data. Chi squared analysis revealed differences among groups at week 6 "As a result of any emotional problems, cut down on time spent on work or other activities" ($\chi^2 = 0.015$) and "accomplishing less than I would like" ($\chi^2 = 0.036$) with ratings approaching statistical significance among groups in "As a result of physical health, had difficulty performing work or other activities" ($\chi 2 = 0.097$), "As a result of any emotional problems, did work or other activities less carefully than usual" ($\chi^2 = 0.073$), and "How much time do you feel tired" ($\chi^2 = 0.079$). Ratings generally improved with exercise and CrM supplementation compared to the non-exercise and placebo groups.

3.4.4. Blood markers

Tables S24 to S27 show CBC, markers of catabolism and enzymes, electrolytes, and blood lipids, respectively. Multivariate analysis revealed no interaction effects among CBC, markers of catabolism, enzymes, electrolytes, or blood lipids. Mean change analysis revealed that participants in the NoEx+CrM group experienced more favorable changes in triglycerides, HDL cholesterol, VLDL cholesterol, and the cholesterol-to-HDL ratio than those in the NoEx+PLA group (see Figure 18). Although some time and

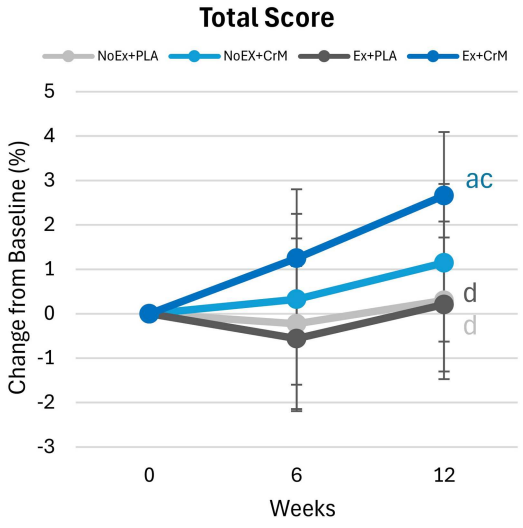

Figure 15. Results for the mini-mental state examination (MMSE) test. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

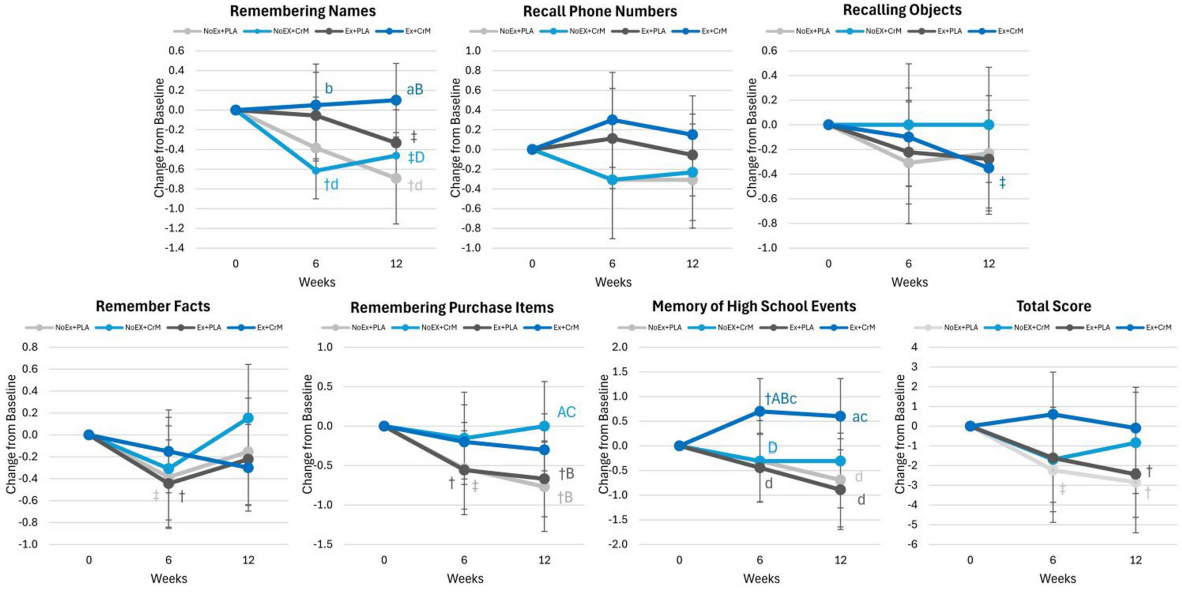

Figure 16. Results for the memory complaint questionnaire (MAC-Q) test. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

pairwise differences were observed in some variables, all values remained well within normal clinical ranges with no consistent pattern of effects among groups.

Table S28 markers of interest related to creatine supplementation (i.e. creatinine, homocysteine, estimated glomerular filtration rate (eGFR), glucose, and glycosylated hemoglobin (HbA1c). Multivariate Wilk's Lambda showed significant time ($p < 0.001$, $\eta_p^2 = 0.131$, medium effect) and group × time ($p = 0.003$, $\eta_p^2 = 0.088$, medium effect) within-subject effects. Univariate interaction effects were seen in serum creatinine ($p < 0.001$, $\eta_p^2 = 0.207$, large effect), creatinine-calculated eGFR ($p = 0.002$, $\eta_p^2 = 0.120$, medium effect), and HbA1c ($p = 0.011$, $\eta_p^2 = 0.076$, medium effect). Changes from baseline are shown in Figure 19.

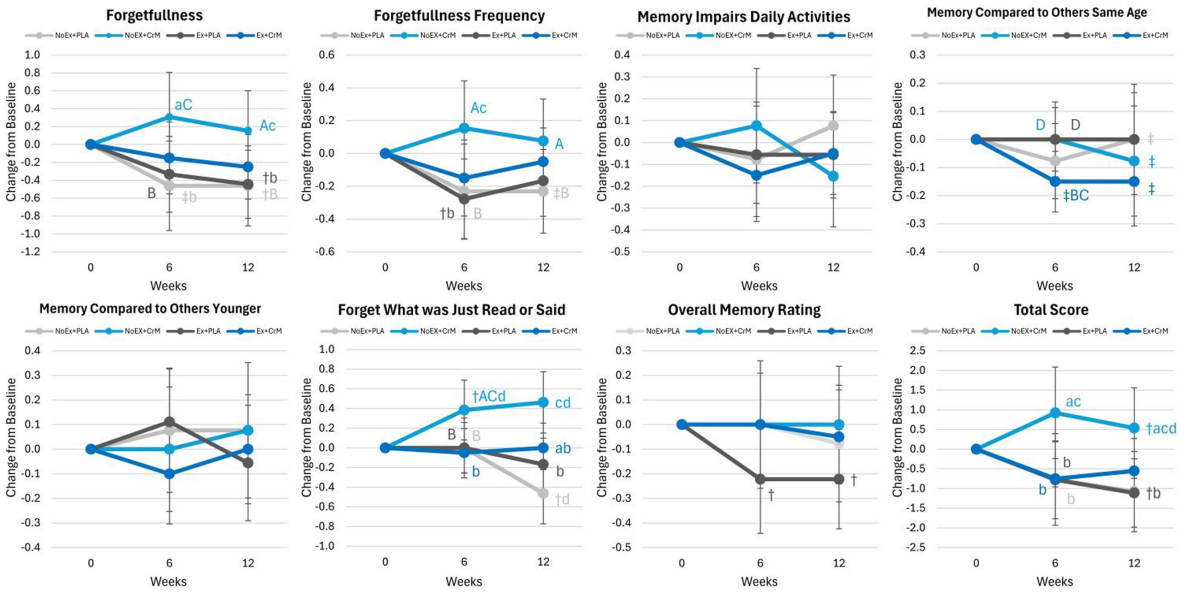

Figure 17. Results for the memory complaint scale (MCS) test. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

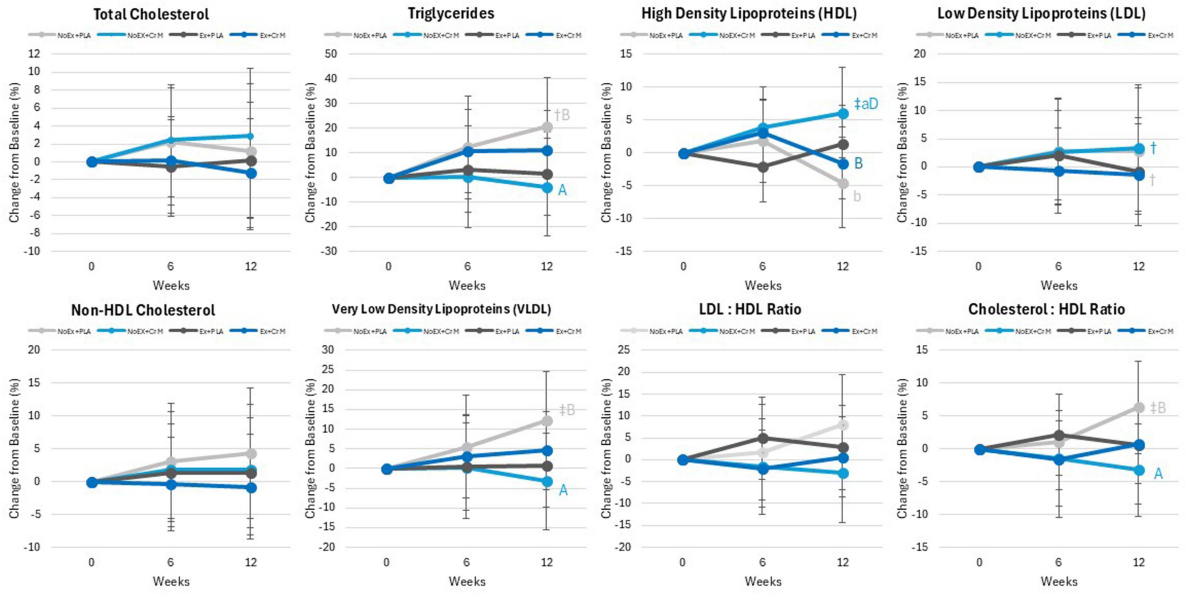

Figure 18. Blood lipid panel results. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx + CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex+PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex+CrM.

As expected, creatinine increased in the NoEx+CrM and Ex+CrM groups, with a greater increase in the exercise group. However, this change was small (<0.15 mg/dL). Since eGFR is calculated from creatinine values and influenced by exercise and muscle mass, eGFR declined within the CrM groups, although remaining within normal clinical limits (>60 mL/min/1.73 m^2). CrM supplementation did not affect homocysteine levels. Interestingly, HbA1c values decreased with exercise training in the Ex+PLA and Ex+CrM

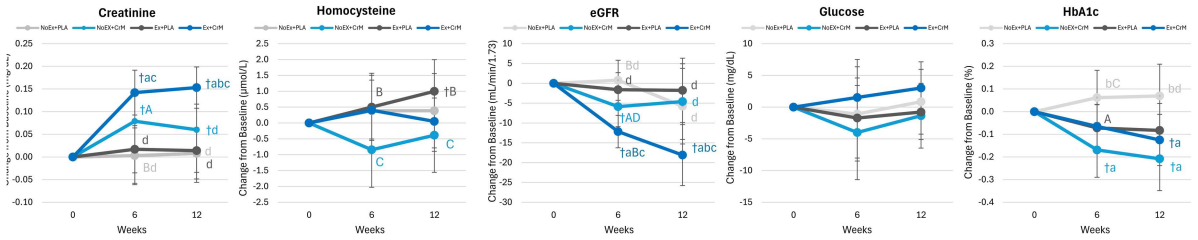

Figure 19. Results for creatinine, renal function, homocysteine, and glucose homeostasis serum markers. † = $p \leq 0.05$ difference from baseline (‡ = $p \geq 0.05$–$p < 0.10$), a = $p \leq 0.05$ difference (A = $p \geq 0.05$–$p < 0.10$) from NoEx+PLA, b = $p \leq 0.05$ difference (B = $p \geq 0.05$–$p < 0.10$) from NoEx+CrM, c = $p \leq 0.05$ difference (C = $p \geq 0.05$–$p < 0.10$) from Ex +PLA, d = $p \leq 0.05$ difference (D = $p \geq 0.05$–$p < 0.10$) from Ex + CrM.

groups as well as in the NoEx+CrM group. The improvement in HbA1c was observed despite no changes in fasting blood glucose levels.

3.4.5. Side effects

Table S29 presents changes in resting heart rate, blood pressure, and waist and hip circumferences. Multivariate Wilk's Lambda showed statistically significant time ($p = 0.051$, $\eta_p^2 = 0.085$) and group × time ($p = 0.582$, $\eta_p^2 = 0.048$) within-subject effects. Similarly, no statistically significant interaction effects were observed from univariate analysis. Participants involved in exercise training observed a statistically significant reduction in waist and hip circumferences with statistically significant differences among groups. Systolic and diastolic blood pressure decreased to a greater extent in the Ex+CrM group compared to the Ex +PLA group. The frequency and severity of self-reported side effects are displayed in Table S30. The chi-squared analysis showed no differences between groups in the frequency or severity of dizziness, headaches, tachycardia, heart palpitations, dyspnea, blurred vision, or other complaints. Reported side effects were infrequent, rated as minimal to slight, similar across groups, and often dissipated during the study. No participant withdrew from the study because of issues with the supplement.

4. Discussion

The purpose of this study was to determine whether CrM supplementation (2 × 5 g/d for 12 weeks) would promote more optimal changes in body composition and cognitive function in healthy, older adults beginning an exercise and diet program designed to maintain muscle mass while promoting fat loss. We hypothesized that CrM supplementation would promote greater improvements in body composition, training adaptations, and health-related outcomes in older individuals. Additionally, CrM supplementation would enhance measures of cognitive function with and without exercise participation. The main findings of this study are that CrM supplementation during exercise and a diet-induced weight-loss program increased lean tissue mass and promoted a greater reduction in body fat percentage than in participants in the NoEx+PLA and Ex+PLA groups. Additionally, CrM supplementation without exercise training increased lean tissue mass and 1RM strength. We also found that CrM supplementation, with or without exercise, positively affected several markers of cognitive function. These findings support contentions that CrM supplementation can help older individuals maintain muscle mass, strength, and cognitive function. The following discusses these findings in more detail and provides recommendations about future research.

4.1. Primary outcomes

4.1.1. Body composition

Weight loss is typically associated with a reduction in fat and muscle mass and a decrease in resting energy expenditure [97–100]. Resistance training and increasing dietary protein intake have been recommended to help individuals maintain muscle mass and resting energy expenditure during weight loss [97–101]. Creatine supplementation has been consistently reported to increase muscle mass in response to resistance-exercise training in younger and older populations [102–106]. Theoretically, creatine

supplementation during an exercise program that includes resistance training and a hypo-energetic diet designed to promote weight loss may help maintain muscle mass and promote more favorable changes in body composition.

In the present study, we evaluated the effects of 12 weeks of CrM supplementation (10 g/d) on DXA-determined body composition in older individuals initiating an exercise program (resistance and cardiovascular training) while reducing energy intake by 300–500 kcal/d to promote weight loss. Although this dosage is higher than typical low-dose supplementation or maintenance dosages [107], it follows recent recommendations that higher daily doses may be needed to improve cognitive function [16,20,26,108]. We found that CrM supplementation during a resistance-training exercise and diet-induced weight loss program not only maintained but increased lean tissue mass (1.69 kg [1.0,2.4], 1.27 kg [0.56, 1.98]) compared to no change in the NoEx+PLA (−0.09 kg [−0.9, 0.7], −0.16 kg [0.56, 1.98]) and Ex+PLA (−0.11 kg [−0.8, 0.6], 0.14 kg [−0.6, 0.9]) groups after 6 and 12 weeks, respectively. This finding may have important implications for individuals trying to maintain or increase lean tissue mass while dieting or as they age. Interestingly, CrM supplementation without participating in a structured exercise program or diet also increased lean tissue mass (0.99 kg [0.2, 1.8], 1.1 kg [0.2, 2.0]), and this increase was statistically significantly greater than in the PLA groups with and without exercise. This finding is consistent with a recent meta-analysis by Ashtary-Larky and colleagues [109] who found that creatine supplementation without diet intervention increased fat-free mass in trained (1.82 kg) and untrained populations (1.23 kg) in a dose-related manner. These findings were observed despite maintaining a relatively low dietary protein intake (74.3 g/d [69,79]; 0.94 g/kg/d [0.85, 1.03]) for individuals involved in resistance training. Additional research should evaluate co-ingesting CrM with protein while maintaining a hypo-energetic diet during resistance training on changes in body composition in active older individuals.

As expected, exercise training and dieting resulted in a statistically significant reduction in whole-body, android, and gynoid fat mass in the Ex+PLA and Ex+CrM groups. However, changes in percentage body fat in the Ex+CrM group (−2.04% [−2.9, −1.2], −3.24% [−4.1, −2.4]) were significantly greater than the NoEx +PLA (0.25% [−0.8, 1.2], −0.42% [−1.5, 0.7]), NoEx+CrM (−0.61% [−1.6, 0.4], −0.42% [−1.8, 0.4]), and Ex+PLA groups (−0.73% [−1.6, 0.1], −1.87% [−2.8, −0.9]). These findings indicate that CrM supplementation during resistance exercise and a diet-induced weight-loss program can promote a greater reduction in body fat percentage by increasing lean tissue mass and reducing fat mass. Although some studies report that creatine supplementation without diet intervention can promote a modest amount of fat loss during training [110], we believe that this is the first study to show that CrM supplementation during an exercise- and diet-induced weight-loss program promotes greater reductions in body fat percentage than exercise and diet alone.

4.1.2. Training adaptations

Resistance and endurance exercise increase muscular strength, endurance, and aerobic capacity [33,97]. Creatine supplementation during training has been reported to increase maximal strength, muscle endurance, and anaerobic capacity with variable effects on peak aerobic capacity [105,110]. In the present study, we found that creatine supplementation without training increased 1RM leg press and bench press, with no statistically significant effects on aerobic capacity. Creatine supplementation with resistance and endurance exercise training resulted in statistically significantly greater gains in 1RM leg press, 1RM bench press, and time to exhaustion during the incremental maximal exercise test, with no statistically significant differences in aerobic capacity. These findings support the contention that CrM supplementation can improve strength and endurance in untrained and trained populations, with greater benefits when combined with exercise training. Results may have important implications for older adults attempting to maintain strength and functional capacity as they age [108].

4.1.3. Cognitive function

Creatine deficiency syndromes resulting from enzyme or transporter gene defects are characterized by markedly reduced muscle and brain creatine concentrations that impair normal cognitive and neuromuscular development in affected children [111]. Clinical evidence further indicates that early diagnosis and therapeutic intervention in children with guanidinoacetate methyltransferase (GAMT) and L-arginine:glycine amidinotransferase (AGAT) deficiencies using high-dose creatine monohydrate supplementation (approximately

0.35–1.1 g/kg/day) increases brain and skeletal muscle creatine content and is associated with normalization or substantial improvement in cognitive and psychomotor development [112–115].

In healthy individuals, magnetic resonance spectroscopy studies demonstrate that oral creatine supplementation can increase brain creatine content. Dechent and colleagues [11] reported that a single 20-g dose of creatine monohydrate increased brain creatine concentrations by approximately 3.1%–7.7%, while repeated supplementation (4 × 5 g/day for 4 weeks) increased total brain creatine content by 4.7%–14.6% across multiple brain regions. Consistent with these findings, Lyoo et al. [17] observed that creatine supplementation (0.3 g/kg/day for 7 days followed by 0.03 g/kg/day for 7 days) increased the ratios of brain creatine to *N*-acetyl aspartate (NAA) and creatine to choline by 8.1% and 9.3%, respectively, while also increasing phosphocreatine (PCr), *β*-nucleoside triphosphate, and inorganic phosphate peaks by 3.1%–9.8%. Similarly, Pan and colleagues [18] reported that short-term creatine supplementation (20 g/day for 7 days) increased the PCr/ATP ratio by approximately 5.2%, particularly in brain regions with lower baseline creatine and PCr concentrations. Additional investigations indicate that short-term creatine supplementation (20 g/day or ~0.3 g/kg/day for 7 days) can increase brain creatine or PCr content by approximately 3.9% in children [116], 6.4% in adolescent females [117], and between 5.9% [24] and 9.2% [23] in adults. However, several studies have reported no statistically significant effects [118,119]. Longer-term, lower-dose supplementation protocols (2–5 g/day for 8–24 weeks) have generally reported increases in brain creatine content ranging from approximately 4%–9% [117,120,121].

Beyond alterations in brain energetics, creatine supplementation has been reported to improve selected measures of cognitive performance across a range of populations and experimental conditions [22,23,83,116,122–130], including during sleep deprivation [126–128,131,132]. For example, McMorris et al. [133] reported that creatine supplementation (4 × 5 g/day for 7 days) improved forward number recall and picture recall in older men, suggesting enhancements in spatial and long-term memory. The same research group also observed that creatine supplementation (20 g/day for 7 days) attenuated declines in random movement generation, choice reaction time, static balance, and mood state during cognitively demanding conditions [127]. Rae and Broer [134] further demonstrated that six weeks of creatine supplementation (5 g/day) improved working memory (backward digit span) and performance on Raven's Advanced Progressive Matrices in vegetarian and vegan adults, tasks that place high demands on processing speed and cognitive capacity. In contrast, several controlled trials have reported no statistically significant effects of creatine supplementation on cognitive outcomes [119,135,136], indicating that cognitive responses to creatine may be population-, task-, and context-dependent.

Since only about 20% of creatine crosses the blood–brain barrier, it has been suggested that higher daily doses of CrM may be needed to increase and maintain elevated brain creatine content and thereby more consistently affect cognitive function measures [16,104,108,137]. This dosage is more in line with recommended dosages used in clinical populations (i.e. 10–30 g/d) to improve muscle, neurological, and/or brain function [104,138,139]. In the present study, we evaluated the effects of 12 weeks of CrM supplementation (10 g/d), with and without exercise and diet intervention, on a comprehensive battery of cognitive tests in healthy adults. We found evidence that CrM supplementation without exercise improved: the number of delayed recalled correct responses on Word Recall Test suggesting improved short-term memory [75]; the percent of Words and No words correctly identified on the Word Recognition test suggesting an improvement in episodic memory; and, had faster reaction times in identifying the number of pictures correctly recalled on the Picture Recognition Test indicative of improved memory and processing speed [51,140]. With exercise training, there was evidence that participants in the Ex+CrM group identified more YES words correctly with faster reaction times on the Picture Recall Test suggesting improved memory and process speed [141]; and had faster reaction times in the Choice Reaction Time test, suggesting an improvement in cognitive processing speed and decision-making. Conversely, creatine supplementation had no effects on the Stroop Color-Word test which assesses attention and processing skills [142], the Corsi Block Task Test [143], which is a measure of attention and vigilance, or the Neurotracker Light Reaction Test which assesses attention, working memory, information processing speed, and executive functions. Additionally, the number of false alarms (inaccurate selections) in the Ex+CrM group tended to be higher than the NoEx +PLA group suggesting a greater lapse in attention [144]. Although results are not entirely consistent, results provide additional evidence that CrM supplementation, with and without exercise training, can affect episodic memory, cognitive processing, attention, executive function, and reaction time in middle-

aged and older adults. These task-specific findings are exploratory and should be confirmed in studies designed and powered for the individual cognitive outcomes.

4.2. Secondary outcomes

4.2.1. Mood state

Given the proposed role of creatine in supporting cerebral energy metabolism, several investigations have examined the effects of creatine monohydrate (CrM) supplementation on mood state using the Profile of Mood States (POMS) questionnaire. McMorris and colleagues [128] reported that CrM supplementation (4×5 g/day for 7 days) during a period of sleep deprivation statistically significantly improved vigor and reduced fatigue scores following 24 hours of wakefulness. However, in a subsequent study employing a longer duration of sleep deprivation (36 hours), the same research group observed no statistically significant differences in overall mood state following CrM supplementation using a similar dosing protocol (4×5 g/day for 7 days) [127]. These findings are consistent with other controlled trials indicating that short-term CrM supplementation at higher doses (20–24 g/day for 5 days) does not statistically significantly alter mood states as assessed by the POMS under non-sleep-deprived conditions [145,146]. In the present study, we found that changes in tension scores after 12 weeks were higher in the NoEx+CrM group compared to NoEx+PLA group and tended to be higher than the Ex+CrM groups. Creatine supplementation with training promoted statistically significant increases in vigor scores and lower total mood disturbance scores than the NoEx+PLA group. These findings support contentions that creatine supplementation when combined with exercise may improve mood states.

4.2.2. Memory perceptions

The Mini-Mental State Examination (MMSE), the Memory Complaint Questionnaire (MAC-Q), and Memory Complaint Scale (MCS) are often used to assess cognitive decline as individuals age. Alves and coworkers [147] reported that 24 weeks of creatine supplementation (20 g/d for 5 days and 5 g/d thereafter) with and without resistance training did not affect MMSE scores in older women. More recently, in non-blinded, single-arm study, Smith et al. [148] evaluated the effects of eight weeks of creatine supplementation (20 g/d) in patients with Alzheimer's disease. The researchers found that brain creatine content increased by 11% which was associated with an improvement on several cognitive tests (i.e. global and fluid composites, List Sorting, Oral Reading, and Flanker tests). However, these authors reported no effects on MMSE scores (21.6 ± 4.4 to 21.0 ± 5.6, $p = 0.33$). To our knowledge, no studies have investigated the impact of creatine supplementation on MAC-Q and MCS scores.

In the present study, we used the MMSE, MAC-Q, and MCS questionnaires to verify that the participants did not have evidence of memory or cognitive decline. Additionally, we were interested to see if CrM supplementation would affect these measures. We found that CrM supplementation with exercise and diet intervention promoted a significantly greater increase in total MMSE scores than those in the placebo groups. Additionally, there was evidence that participants consuming CrM remembered names, purchased items, and high school events to a greater degree than those in the PLA groups on the MAC-Q assessment. Conversely, several variables related to forgetfulness and memory were higher with CrM supplementation, and the EX+PLA group was the only group to show a statistically significant reduction in total memory scores, although not statistically significantly different from the other groups. Obviously, more research is needed to assess the impact of CrM supplementation on memory perception in individuals with and without cognitive impairment.

4.2.3. Quality of life

Given the established effects of creatine supplementation on muscular strength and functional performance, several investigations have examined whether these physiological adaptations translate into improvements in health-related quality of life. These outcomes have been evaluated primarily in clinical populations [149–155], as well as in older adults [14,156], where enhancements in strength, functional capacity, and physical performance may be particularly relevant to daily living and overall well-being. Collectively, these studies suggest that improvements in physical functional capacity associated with creatine monohydrate (CrM) supplementation are often accompanied by favorable perceptions of quality of life. For example, Amiri and colleagues [156] reported that CrM supplementation (0.1 g/kg/day) during a 10-week resistance

training program resulted in approximately double the magnitude of strength gains and was associated with concomitant improvements in quality-of-life scores in older adults. Consistent with these observations, the present findings indicate that CrM supplementation may influence selected markers of quality of life, with ratings improving in response to exercise and CrM supplementation compared with non-exercising and placebo conditions. Obviously, more research is needed to assess how creatine supplementation may affect QOL as we age.

4.2.4. Health markers

We evaluated a comprehensive panel of whole and serum clinical markers as well as several health markers of interest, including blood lipids, glucose, and HbA1c, creatinine, eGFR, and homocysteine. Additionally, participants completed a questionnaire on the frequency and severity of side effects. While most markers were not affected and multivariate analysis of the lipid panel did not reveal a statistically significant group × time interaction, we observed a modest improvement in blood lipids (i.e. triglycerides, HDL cholesterol, VLDL cholesterol, cholesterol-to-HDL ratio) in the non-exercise and diet intervention group taking CrM compared to participants taking placebos when evaluating mean changes from baseline. This included a statistically significant increase in HDL cholesterol in the NoEx+CrM group compared with the Ex +PLA group. While not all creatine supplementation studies have reported benefits on lipid panels, several studies support the present findings. For example, Earnest and colleagues [157] reported that creatine supplementation (20 g/d for 5 days) statistically significantly decreased triglycerides, total cholesterol, and VLDL cholesterol levels in individuals with elevated cholesterol (>200 mg/dL). Kreider et al. [103] reported that creatine supplementation (16 g/d for 28 days) during off-season college football training increased HDL-cholesterol while promoting more favorable changes in VLDL and the ratio of total cholesterol to HDL-cholesterol. Arciero and associates [158] reported that 12 weeks of creatine supplementation (6 g/d) during a resistance training program decreased total cholesterol in healthy adults. More recently, Clarke and coworkers [159] found that creatine supplementation (5 g/d for 28 days) significantly decreased triglycerides in older adults maintaining normal physical activity levels. This group subsequently reported that older adults supplementing their diet with creatine (5 g/d for 28 days) while participating in a structured exercise program decreased triglyceride levels. The individual and change-from-baseline analyzes nevertheless suggest that creatine supplementation may favorably affect selected lipid measures; these exploratory findings require confirmation.

As expected, HbA1c levels decreased following exercise training and weight loss, indicating improved glucose homeostasis. Since glucose uptake into muscle is sodium, glucose, and insulin-dependent [107], there has been interest in whether creatine supplementation affects glucose storage, insulin sensitivity, and HbA1c levels, particularly in individuals with type 2 diabetes mellitus participating in an exercise program. Initial studies that assessed how creatine supplementation affects rehabilitation after immobilization reported that creatine supplementation during immobilization (20 g/d), early rehabilitation (15 g/d), and late rehabilitation (5 g/d) preserved muscle GLUT-4 levels during immobilization and promoted a 40% increase in GLUT-4 during rehabilitation, suggesting enhanced insulin-related glucose uptake and muscle glucose transport [160,161]. Gualano and coworkers [162] reported that creatine supplementation (5 g/d for 12 weeks) improved insulin sensitivity without affecting HbA1c in adults with type 2 diabetes who maintained normal physical activity levels. In a follow-up study, Gualano et al. [163] reported that creatine supplementation (5 g/d for 12 weeks) decreased HbA1c values and improved the postprandial glycemic response to a glucose challenge in adults with type 2 diabetes participating in a structured exercise program. In the current study, we found that HbA1c levels decreased in the group consuming 10 g/d of CrM for 12 weeks with no exercise or diet intervention. Additionally, CrM supplementation during training and diet intervention promoted similar changes as the Ex+PLA group, which were significantly lower than the NoEx+PLA group. These findings provide additional evidence that CrM supplementation may affect glucose homeostasis. More research is needed to evaluate the role of CrM supplementation, with and without exercise and diet intervention (e.g. low glycemic, higher protein diet), on glucose control. These findings should be considered exploratory and do not establish a generalized glycemic effect.

Consistent with Kreider and colleagues [138] recent comprehensive safety analysis of 685 clinical trials on creatine supplementation in younger and older healthy and clinical populations, we found that CrM supplementation was well tolerated and did not promote clinically significant changes in health markers

or self-reported side effects As expected, CrM supplementation (10 g/d for 12 weeks) without exercise led to a small but statistically significant increase in serum creatinine levels (6%–8% or <0.1 mg/dL). Exercise training and gains in muscle mass promote a greater degradation of muscle creatine to creatinine [107]. Consequently, individuals participating in a structured exercise program typically have higher creatinine levels, particularly when taking CrM during training [138]. In the present study, CrM supplementation during a structured resistance exercise program increased creatinine levels by 16%–18% (<0.16 mg/dL). However, values remained less than 1.0 mg/dL, which is well within normal clinical values. Creatinine-calculated eGFR decreased by 5%–7% in the NoEx+CrM group and tended to differ from the NoEx+PLA group after 6 weeks, but not 12 weeks. When combined with exercise training and weight loss, the increase in creatinine values promoted a 14% to 20% decrease in eGFR despite remaining within normal clinical ranges (i.e. >60 (mL/min/1.73 m^2). It is important that clinicians do not misinterpret this finding as prior research using more accurate renal function measurements has clearly shown that CrM supplementation has no negative effect on renal function [164]. In fact, a recent epidemiological study reported that adults with higher dietary creatine intake (>2 g/d) had no statistically significant difference in the incidence of renal failure compared to individuals consuming lower dietary creatine (<1 g/d) [165]. CrM supplementation has also been used in patients with chronic kidney disease and during dialysis as a way to preserve homocysteine levels [166–168]. Collectively, present findings indicate that CrM supplementation in older individuals is well-tolerated and may result in some improvement in health markers whether participating in an exercise program or not. Cystatin C and directly measured GFR were not assessed; therefore, the creatinine-derived eGFR changes should not be interpreted as evidence of reduced renal filtration.

4.3. Strengths, limitations, and future directions

The strength of this study is that we comprehensively evaluated the effects of higher daily doses of CrM (10 g/d), with and without a structured exercise and diet program designed to promote weight loss, on body composition, training adaptations, markers of health, and several cognitive function tests in healthy older women and men. We believe this is also the first study that we are aware of that evaluated the effects of CrM supplementation during an exercise and diet intervention designed to promote weight and fat loss. Moreover, a strength of this study was that we evaluated the effects of CrM supplementation in participants not participating in a structured exercise and diet intervention. This study is limited by the amount and length of supplementation, the population studied, exercise and diet compliance, and the primary and secondary measures evaluated. It is also possible that there may have been synergistic and/or additive benefits of the exercise and supplement interventions while dieting on the dependent variables evaluated. Moreover, exercise participation was self-selected, whereas supplement assignment within each exercise-status stratum was randomized and double-blind. The repeated-measures analyzes were based on the 64 participants who completed 12 weeks; missing observations among completers were exceptionally rare and were replaced primarily using the prior observed value. Residual practice effects from repeated cognitive testing cannot be excluded, although familiarization was provided and group × time interactions were evaluated. Because multiple distinct exploratory cognitive and biomarker outcomes were analyzed without an across-outcome adjustment, individual findings should be interpreted by outcome and confirmed in future studies. Creatinine-based eGFR is influenced by creatine intake and muscle mass, and cystatin C or directly measured GFR was not available. Future research should evaluate whether increasing dietary protein intake with CrM supplementation may promote more effective weight loss in this population. Additionally, to examine whether CrM supplementation may help slow age-related sarcopenia and/or maintain muscle mass during pharmacological weight loss intervention programs [169,170]. Finally, additional research should evaluate the effects of increasing creatine dosages on brain creatine content and cognitive function in older individuals who are beginning to experience perceived memory issues.

5. Conclusion

Creatine supplementation (10 g/d) without exercise training and diet intervention increased lean tissue mass, strength and muscular endurance, promoted favorable changes in selected blood-lipid measures, reduced HbA1c levels, and improved selected markers of cognitive function and memory in middle-aged

and older adults. Creatine supplementation during an exercise and weight-loss diet intervention in middle-aged and older adults promoted a greater reduction in body fat percentage, gains in muscular strength and endurance, and improved selected markers of cognitive function and memory. These findings may have important implications for middle-aged and older individuals seeking to reduce body fat while maintaining muscle mass and cognitive function. The supplementation intervention was well-tolerated. However, the individual cognitive and metabolic findings are exploratory and require confirmation. Additional research should evaluate the effects of CrM supplementation in active aging and clinical populations who may benefit.

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All authors have read and agreed to the published version of the manuscript.

Author contributions

CRediT: **Richard B. Kreider:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing.

Disclosure statement

R.B.K. has conducted industry-sponsored research on creatine, received financial support for presenting at conferences, and has served as an expert witness throughout his career. He serves as Chair of the Scientific Advisory Board for AlzChem (a company that makes creatine monohydrate), is a co-founder of the non-profit International Society of Sports Nutrition (ISSN), and a member of the scientific advisory boards for Oath Nutrition and Trace Minerals. J.C., Y.L., G.L.K, H.L., K.B., N.R., I.B., J.K., B.D., D.E.G., R.J.S., and C.J.R. report no conflicts of interest.

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Data availability statement

Data and statistical analyzes are available for non-commercial scientific inquiry and/or educational if request and use does not violate IRB restrictions and/or research agreement terms.

Informed consent statement

All participants provided signed written consent to participate in this study.

Ethics approval

This study was conducted with approval by Texas A&M University's Institutional Review Board (STUDY2024-0233; approved June 10, 2024, The first participant was enrolled on July 10, 2024, and the study was submitted to ISRCTN on April 11, 2025 and posted on June 11, 2025 (ISRCTN #83081058).

Consent for publication

All authors have reviewed and approved the publication.

Abbreviations

AAMI	Associated memory impairment (AAMI)
AGAT	L-arginine: glycine amidino-transferase
ANOVA	Analysis of Variance
COMPASS	Computerized Mental Performance Assessment
CI	Confidence interval
CONSORT	Consolidated Standards of Reporting Trials
CrM	Creatine monohydrate
CRT1	Creatine transporter 1
DXA	Dual-energy X-ray absorptiometry
eGFR	Estimated glomerular filtration rate
GLM	General Linear Model
GMAT	Guanidinoacetate *N*-methyltransferase
HbA1c	Glycosylated hemoglobin
IPAQ-LF	International Physical Activity Questionnaire Long-Form
LSD	Least Significant Difference
MAC-Q	Memory Complaint Questionnaire
MCS	Memory Complaint Scale
MMSE	Mini-Mental State Examination
NSAIDS	Non-steroidal anti-inflammatory drugs
PCr	Phosphocreatine
PLA	Placebo
POMS	Profile of Mood States
QOL	Quality of life
SPSS	Statistical Package for the Social Sciences
SD	Standard deviation
TMDS	Total mood disturbance score

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