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Synergistic Effects of an 8-Week Resistance Training Program and Creatine Supplementation on Hormonal, Metabolic, Inflammatory, and Body Composition Markers in 30- to 45-Year-Old Male Athletes: Modulation by ACTN3 (R577X)

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Abstract

Background & Objectives: Optimizing metabolic health and physical performance in middle-aged male athletes is critical for long-term health maintenance and athletic sustainability. This study investigated the combined effects of an 8-week strength training (ST) program and creatine supplementation on anabolic hormones, metabolic indicators, inflammatory markers, and lean body mass (LBM) in 30- to 45-year-old male athletes, with particular emphasis on the potential moderating role of the ACTN3 genotype (R577X).

Materials & Methods: A randomized, placebo-controlled, 2 × 2 factorial design was employed, involving 48 recreationally trained male athletes who were randomly assigned to one of four groups: ST plus creatine (ST+C), ST plus placebo (ST+P), creatine supplementation alone, and a non-intervention control group (CON). The 8-week intervention consisted of supervised resistance training sessions and daily supplementation with either creatine or placebo. Venous blood samples were collected before and after the intervention to assess serum testosterone, growth hormone, cortisol, fasting glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), lipid profile parameters, and inflammatory markers, including high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6). ACTN3 genotyping was performed using standard molecular techniques. Data were analyzed using paired t-tests and analysis of covariance (ANCOVA), with inclusion of an **exercise** × creatine interaction term.

Results: The combined ST+C intervention resulted in a significant increase in LBM ($p < 0.01$, Cohen's $d = 0.85$) and a significant improvement in insulin sensitivity, as evidenced by reduced HOMA-IR values ($p < 0.05$, Cohen's $d = 0.62$), compared with all other groups. Creatine supplementation alone demonstrated a non-significant trend toward increased testosterone concentrations ($p = 0.07$). Participants in the ST+P group exhibited significant improvements in LBM and insulin sensitivity; however, these changes were less pronounced than those observed in the ST+C group. No significant between-group differences were observed for cortisol levels or inflammatory markers. ACTN3 genotype did not significantly moderate any of the measured outcomes.

Conclusion: Concurrent strength training and creatine supplementation effectively enhance LBM and insulin sensitivity in middle-aged male athletes. Creatine supplementation appears to confer additional benefits beyond resistance training alone, particularly with respect to anabolic hormonal responses. These findings support the use of combined resistance training and creatine supplementation as an effective strategy for optimizing metabolic health and physical performance in this population.

Keywords: Strength Training, Creatine Supplementation, Middle-Aged Athletes, Metabolic Health, Randomized Trial

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Introduction

Maintaining health and enhancing physical performance are paramount objectives for individuals of all ages, particularly for athletes

navigating the physiological changes associated with midlife (1). Strength training and creatine supplementation represent two of the most effective and widely adopted strategies for increasing muscle mass, improving athletic performance, and promoting overall health (2, 3). However, the combined impact of these interventions on complex systemic metabolic processes, especially in the 30- to 45-year age group, remains insufficiently investigated. The primary outcomes of the present study were defined a priori as changes in lean body mass (LBM), total testosterone, and insulin sensitivity, assessed using the homeostatic model assessment of insulin resistance (HOMA-IR), given their central roles in metabolic and hormonal health and their direct relevance to athletic performance and aging. Secondary outcomes included body fat percentage (BFP), growth hormone (GH), cortisol, a comprehensive lipid profile, glucose, insulin, and inflammatory markers, including high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6). The planned interaction analysis was designed to determine whether the combined effects of resistance training and creatine supplementation exceeded the additive effects of each intervention administered independently.

Strength training induces significant physiological adaptations by stimulating both anabolic and catabolic pathways. Specifically, it enhances muscle protein synthesis, thereby promoting skeletal muscle hypertrophy and increasing basal metabolic rate (4). Concurrently, high-intensity exercise may elevate oxidative stress and inflammatory responses, which can influence metabolic homeostasis and recovery processes (5). In contrast, creatine supplementation primarily exerts its effects by increasing intramuscular phosphocreatine (PCr) stores, thereby facilitating rapid adenosine triphosphate (ATP) resynthesis during short-duration, high-intensity activities (6). Although the ergogenic benefits of creatine are well documented, the underlying mechanisms through which creatine influences systemic metabolic regulation, including insulin sensitivity and lipid metabolism, remain to be fully elucidated (7).

The 30- to 45-year age range represents a distinct population of athletes in whom age-related physiological changes, such as a gradual decline in testosterone concentrations, alterations in body composition, including sarcopenia and increased adiposity, and the potential onset of metabolic dysfunction, begin to emerge (8). These individuals are often recreationally trained and seek to maintain physical performance and metabolic health with advancing age, rendering them a particularly relevant population for interventions aimed at mitigating age-associated physiological decline. Furthermore, interindividual variability in response to exercise training and nutritional supplementation can be substantially influenced by genetic factors. The alpha-actinin-3 (ACTN3) gene, which encodes a structural protein expressed predominantly in fast-twitch muscle fibers, has been identified as a key genetic determinant of strength and power-related phenotypes (9, 10). The R577X polymorphism within this gene determines the presence (R allele) or absence (X allele) of functional ACTN3 protein and may therefore modulate an individual's adaptive response to resistance training and supplementation (11, 12).

Although previous investigations have frequently examined the effects of strength training or creatine supplementation in isolation, a notable gap remains in the literature regarding their synergistic effects on metabolic, hormonal, and inflammatory markers, particularly when accounting for the potential moderating influence of ACTN3 genotype. Accordingly, the present study aimed to investigate the combined effects of an 8-week resistance training program and creatine supplementation on testosterone, GH, cortisol, a comprehensive lipid profile, glucose, insulin, HOMA-IR, inflammatory markers (hs-CRP, IL-6), and key indicators of

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body composition in 30- to 45-year-old male athletes. It was hypothesized that the concurrent application of resistance training and creatine supplementation would produce synergistic and more pronounced improvements in these outcomes compared with either intervention alone.

Materials and Methods

Study Design and Participant

This randomized, parallel-group, 2 × 2 factorial, placebo-controlled study employed a pretest–posttest design. Forty-eight healthy, recreationally trained male athletes from Dezful, Iran, aged 30 to 45 years, were recruited for participation. Participants were informed about the nature of the study interventions; however, they were blinded to the specific supplement, creatine or placebo, that they would receive. Participants were randomly allocated to one of four groups, each comprising 12 individuals (n = 12 per group):

1. Combined group (ST+C): Resistance training with creatine supplementation.
2. Training group (ST+P): Resistance training with placebo supplementation.
3. Supplement group (C): Creatine supplementation only, without structured exercise training.
4. Control group (CON): No training or supplementation intervention.

Randomization was performed using a computer-generated allocation sequence by an independent researcher who was not involved in participant recruitment or data collection. Allocation concealment was ensured through the use of opaque, sealed envelopes, which were opened only after participants had met all eligibility criteria and provided written informed consent.

Inclusion and Exclusion Criteria

Inclusion criteria comprised male sex, age between 30 and 45 years, a minimum of one year of regular resistance training experience, defined as engaging in structured strength training two to three times per week, and no use of creatine supplements or anabolic agents during the three months preceding the study. Exclusion criteria included the presence of cardiovascular, renal, or metabolic diseases; use of medications known to affect metabolic or hormonal function; and any condition that could impair adherence to the study protocol.

Intervention Protocols Strength Training Protocol

Participants in the ST+C and ST+P groups completed a supervised 8-week resistance training program, consisting of three training sessions per week. Each session included a 10-minute standardized warm-up, 45 to 60 minutes of circuit-based resistance training, and a 10-minute cool-down period. The training program targeted all major muscle groups through two alternating circuits, designated Circuit A and Circuit B, as presented in Table 1. Circuit A included bench press, barbell row, overhead press, leg press, biceps curl, and triceps extension, whereas Circuit B consisted of squat, deadlift, incline dumbbell press, lat pulldown, calf raise, and abdominal crunches.

Training intensity was prescribed as a percentage of one-repetition maximum (1RM), estimated using the Brzycki equation (13, 14), and was progressively increased over the 8-week period, ranging from 30 to 40 percent of 1RM during the initial phase to 60 to 70 percent of 1RM during the final phase. Repetition tempo was standardized at approximately two seconds for the concentric phase, one second for the isometric pause, and two seconds for the eccentric phase. Participants were instructed to complete each set until reaching two to three repetitions in reserve (RIR) or a rating of perceived exertion (RPE) of 7 to 8 on a 10-point scale. Progressive overload was achieved through systematic increases in training intensity, repetition number, or set volume as individual strength adaptations occurred.

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Table 1. Resistance Training Program Details.

Weeks	Frequency/week	Repetitions	Intensity (% 1RM)	Rest between exercises (s)	Rest between circuits (min)
1-2	3	15-20	30-40%	40-60	3-5
3-4	3	12-15	40-50%	40-60	3-5
5-6	3	10-12	50-60%	40-60	3-5
7-8	3	8-10	60-70%	40-60	3-5

Supplementation Protocol

Participants in the ST+C and C groups received creatine monohydrate powder. The supplementation protocol consisted of a 7-day loading phase, during which participants consumed 20 g of creatine per day divided into four 5 g doses, followed by a 7-week maintenance phase of 5 g per day. Participants in the ST+P and CON groups received a maltodextrin placebo that was identical in appearance, texture, and dosing schedule. The study employed a single-blind supplementation design, whereby participants and the researchers responsible for supplement administration were unaware of group allocation. To evaluate the effectiveness of blinding, participants were asked at the conclusion of the study to indicate whether they believed they had received creatine or placebo. No significant differences were observed between groups in the accuracy of these guesses, indicating successful maintenance of blinding throughout the intervention period.

While an intensity range of 30 to 70 percent of 1RM might be considered relatively low for maximizing hypertrophy in highly trained individuals, this program was specifically designed for recreationally trained, middle-aged men. The lower initial training intensity facilitated proper movement execution and neuromuscular adaptation, with a progressive increase toward moderate intensities that are effective for promoting strength gains and metabolic improvements while simultaneously minimizing injury risk in this population (13). Furthermore, the application of RIR and RPE guidelines ensured that participants were consistently challenged within each training set despite the conservative intensity progression. Training adherence was monitored by qualified supervisors present at every training session, with attendance systematically recorded. Supplement adherence was assessed through daily self-report diaries in which participants documented supplement intake, complemented by weekly verification of remaining supplement quantities. Adherence to both training and supplementation protocols exceeded 90 percent across all intervention groups, indicating high compliance. Participants were instructed to maintain their habitual dietary intake, sleep routines, and physical activity levels outside of the supervised training sessions. Although strict dietary control was not implemented in order to enhance ecological validity and reflect real-world conditions, participants were advised to avoid substantial changes in diet or lifestyle during the study period. Specifically, they were instructed to standardize hydration status and to refrain from excessive protein consumption beyond their usual intake for 24 hours before bioelectrical impedance analysis (BIA) assessments, in order to minimize acute fluid-related variability.

Measurements

All outcome measures were obtained 48 hours before the initiation and 48 hours after the completion of the 8-week intervention period.

Anthropometry and Body Composition

Body weight was measured to the nearest 0.1 kg using a calibrated digital scale (Seca, Germany), and height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body composition variables, including body fat

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percentage and LBM, were assessed using a bioelectrical impedance analysis device (InBody IOI 353, South Korea). Prior to BIA assessment, participants were required to fast for 12 hours, abstain from strenuous physical activity for 24 hours, and avoid alcohol and caffeine consumption for 24 hours. Participants were also instructed to empty their bladder and bowel immediately before testing. Hydration status was not directly controlled beyond these standardized instructions, which represents a recognized limitation of BIA methodology, particularly in the context of creatine supplementation.

Biochemical and Hormonal Assays

Venous blood samples (10 mL) were collected by a trained phlebotomist from the antecubital vein between 07:00 and 09:00 h following a 12-hour overnight fast. Participants were instructed to abstain from strenuous physical activity for 48 hours and from alcohol and caffeine consumption for 24 hours prior to blood sampling to minimize acute confounding influences. Serum was separated by centrifugation at 3000 revolutions per minute for 10 minutes within 30 minutes of collection and subsequently stored at −80°C until biochemical analysis.

Serum concentrations of total testosterone, GH, cortisol, insulin, hs-CRP, and IL-6 were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (IBL International GmbH, Hamburg, Germany for testosterone and GH; DRG International, Inc., Springfield, NJ, USA for cortisol and insulin; R&D Systems, Minneapolis, MN, USA for hs-CRP and IL-6). The intra-assay and inter-assay coefficients of variation for all assays were below 5 percent and 8 percent, respectively. Fasting glucose concentrations and lipid profile parameters, including total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides, were determined using standard enzymatic colorimetric methods with an automated analyzer (BT-3000 Plus, Biotecnica Instruments S.p.A., Rome, Italy).

Insulin resistance was estimated using the HOMA-IR, calculated as follows:

HOMA-IR = (fasting glucose [mmol/L] × fasting insulin [μU/mL]) / 22.5. Fasting glucose values expressed in mg/dL were converted to mmol/L by dividing by 18.018 prior to HOMA-IR computation.

ACTN3 Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a standard commercial extraction kit (Qiagen DNeasy Blood and Tissue Kit, Hilden, Germany). The R577X polymorphism of the ACTN3 gene was genotyped using polymerase chain reaction (PCR) followed by direct sequencing. The primer sequences used were as follows: forward **5′-**TCTTGACAGTGAGAGTTGGG-3′ and reverse 5′-GGTCGTTTGGTTGGTTTCTC-3′**.** PCR amplification conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension step at 72°C for 7 minutes. Amplified PCR products were purified and sequenced using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Genotyping quality control procedures included duplicate genotyping of randomly selected samples and verification against known reference standards. Genotype distributions were tested for Hardy–Weinberg equilibrium (HWE) using a chi-square test ($P > 0.05$), indicating no significant deviation from expected population frequencies.

Statistical Analysis

All statistical analyses were performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro–Wilk test. Baseline characteristics were compared across groups using one-way analysis of variance (ANOVA)**.** Within-group pre-intervention and post-intervention

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comparisons were conducted using paired-samples t-tests. Between-group differences at post-intervention were analyzed using analysis of covariance (ANCOVA), with baseline values included as covariates. The ANCOVA models explicitly incorporated training × creatine supplementation interaction terms to evaluate potential synergistic effects. Separate ANCOVA models were constructed for each dependent variable. Effect sizes were calculated for all statistically significant outcomes, with Cohen's d reported for within-group comparisons and partial eta-squared (ηp^2) reported for ANCOVA results. Effect sizes were interpreted as small (0.2 for Cohen's d; 0.01 for ηp^2), medium (0.5 for Cohen's d; 0.06 for ηp^2), or large (0.8 for Cohen's d; 0.14 for ηp^2), and all were accompanied by 95 percent confidence intervals. Statistical significance was established at $p < 0.05$. An a priori power analysis, based on the detection of a large effect size ($f = 0.40$) for LBM across four groups with 12 participants per group, assuming a pre–post correlation of 0.5, yielded an estimated statistical power of approximately 0.82 at an alpha level of 0.05. This calculation was based on the study's primary outcome variable, LBM.

Results

Baseline Characteristics

There were no significant differences among the four groups at baseline with respect to age, body weight, height, body mass index (BMI), BFP, or LBM ($p > 0.05$ for all variables), indicating successful randomization and baseline comparability.

Changes in Body Composition

After the 8-week intervention, analysis of covariance (ANCOVA) revealed significant between-group differences for changes in body fat percentage and LBM ($p < 0.001$ for both outcomes, $\eta p^2 > 0.30$). The ST+C group demonstrated the greatest reduction in body fat percentage (−1.7 percent, Cohen's d = 1.13, 95 percent confidence interval (CI): −2.0 to −1.4) and the largest increase in LBM (+2.9 kg, Cohen's d = 1.35, 95 percent CI: +2.5 to +3.3). The ST+P group also exhibited statistically significant improvements; however, these changes were less pronounced (body fat percentage: −1.0 percent, Cohen's d = 0.63, 95 percent CI: −1.3 to −0.7; LBM: +1.7 kg, Cohen's d = 0.82, 95 percent

Table 2. Changes in Body Composition from Pre- to Post-Intervention (Mean ± SD).

Variable	Group	Pre-Intervention	Post-Intervention	Within-Group P-value	Cohen's d (95% CI)	Between-Group P-value (ANCOVA)	Partial ηp^2
BFP	ST+C	18.2 ± 1.5	16.5 ± 1.3	<0.001	1.13 (0.75, 1.51)	<0.001	0.34
	ST+P	18.5 ± 1.6	17.5 ± 1.4	0.003	0.63 (0.28, 0.98)		
	C	18.0 ± 1.4	17.8 ± 1.3	0.321	0.13 (-0.24, 0.50)		
	CON	18.3 ± 1.5	18.4 ± 1.6	0.654	-0.06 (-0.42, 0.30)		
LBM (kg)	ST+C	64.2 ± 4.8	67.1 ± 5.0	<0.001	1.35 (0.95, 1.75)	<0.001	0.38
	ST+P	63.8 ± 4.5	65.5 ± 4.7	0.001	0.82 (0.45, 1.19)		
	C	64.5 ± 4.9	64.9 ± 5.0	0.187	0.16 (-0.21, 0.53)		
	CON	64.0 ± 4.7	63.9 ± 4.6	0.789	-0.04 (-0.40, 0.32)		

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CI: +1.4 to +2.0). No significant changes were observed in either the creatine-only (C) group or the control (CON) group (Table 2).

The training × creatine supplementation interaction term was statistically significant for both body fat percentage ($F(1,43) = 7.21$, $p = 0.011$, $\eta p^2 = 0.14$) and LBM ($F(1,43) = 8.55$, $p = 0.005$, $\eta p^2 = 0.16$), indicating a synergistic effect of resistance training and creatine supplementation.

Changes in Hormonal Markers

Significant between-group differences were observed for serum testosterone, GH, and cortisol concentrations (testosterone: $p < 0.001$, $\eta p^2 = 0.42$; GH: $p < 0.001$, $\eta p^2 = 0.39$; cortisol: $p = 0.045$, $\eta p^2 = 0.09$). The ST+C group experienced the most substantial increases in testosterone (+130.3 ng/dL, Cohen's d = 2.45, 95 percent CI: 1.95 to 2.95) and GH (+1.1 ng/mL, Cohen's d = 2.75, 95 percent CI: 2.22 to 3.28), along with a significant reduction in cortisol levels (−3.5 µg/dL, Cohen's d = −0.98, 95 percent CI: −1.38 to −0.58).

The ST+P group also demonstrated significant increases in testosterone (+65.1 ng/dL, Cohen's d = 1.25, 95 percent CI: 0.85 to 1.65) and GH (+0.4 ng/mL, Cohen's d = 1.00, 95 percent CI: 0.62 to 1.38); however, these increases were significantly smaller than those observed in the ST+C group (Table 3). Cortisol concentrations in the ST+P group did not change significantly ($p = 0.187$).

The training × creatine interaction effect was significant for testosterone ($F(1,43) = 9.87$, $p = 0.003$, $\eta p^2 = 0.19$) and GH ($F(1,43) = 8.92$, $p = 0.004$, $\eta p^2 = 0.17$), further supporting a synergistic hormonal response. In contrast, the interaction term for cortisol was not statistically significant ($F(1,43) = 1.50$, $p = 0.228$, $\eta p^2 = 0.03$).

Changes in Metabolic and Inflammatory Markers

Detailed changes in metabolic and inflammatory markers are presented in Table 4. Both resistance training groups (ST+C and ST+P) exhibited significant improvements in insulin sensitivity, as evidenced by reductions in fasting glucose, insulin, and HOMA-IR, as well as significant decreases in inflammatory markers, including hs-CRP and IL-6. These improvements were consistently greater in the ST+C group (all $p < 0.001$, large effect sizes).

Notably, the ST+C group was the only group to demonstrate significant improvements across the entire lipid profile ($p < 0.05$ for all lipid parameters, with medium to large effect sizes), including significant reductions in total cholesterol, LDL-C, and triglycerides, along with a significant increase in HDL-C. The ST+P group showed significant reductions in total cholesterol and LDL-C; however, no significant changes were observed for HDL-C or triglycerides.

Table 3. Changes in Hormonal Markers from Pre- to Post-Intervention (Mean ± SD).

Variable	Group	Pre-Intervention	Post-Intervention	P-value (Within)	Cohen's d (95% CI)	P-value (ANCOVA)	Partial ηp^2
Testosterone (ng/dL)	ST+C	550.2 ± 45.1	680.5 ± 58.3	<0.001	2.45 (1.95, 2.95)	<0.001	0.42
	ST+P	549.5 ± 46.0	614.6 ± 52.1	<0.001	1.25 (0.85, 1.65)		
	C	548.0 ± 46.5	555.2 ± 47.0	0.289	0.15 (-0.21, 0.51)		
	CON	547.5 ± 47.8	542.9 ± 46.9	0.543	-0.10 (-0.46, 0.26)		
Growth Hormone (ng/mL)	ST+C	1.8 ± 0.3	2.9 ± 0.4	<0.001	2.75 (2.22, 3.28)	<0.001	0.39
	ST+P	1.9 ± 0.4	2.3 ± 0.3	0.004	1.00 (0.62, 1.38)		
	C	1.8 ± 0.3	1.8 ± 0.3	0.912	0.00 (-0.36, 0.36)		
	CON	1.9 ± 0.3	1.8 ± 0.3	0.765	-0.10 (-0.46, 0.26)		
Cortisol (µg/dL)	ST+C	15.2 ± 1.5	11.7 ± 1.3	<0.001	-0.98 (-1.38, -0.58)	0.045	0.09
	ST+P	15.0 ± 1.4	14.5 ± 1.2	0.187	-0.36 (-0.72, 0.00)		
	C	15.5 ± 1.6	15.6 ± 1.5	0.801	0.06 (-0.30, 0.42)		
	CON	15.3 ± 1.5	15.4 ± 1.6	0.710	0.06 (-0.30, 0.42)		

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Table 4. Changes in Metabolic and Inflammatory Markers from Pre- to Post-Intervention (Mean ± SD).

Variable	Group	Pre-Intervention	Post-Intervention	P-value (Within)	Cohen's d (95% CI)	P-value (ANCOVA)	Partial ηp²
Glucose (mg/dL)	ST+C	95.1 ± 5.2	85.5 ± 4.8	<0.001	-1.81 (-2.25, -1.37)	<0.001	0.45
	ST+P	94.8 ± 5.0	90.2 ± 4.5	0.002	-0.92 (-1.30, -0.54)		
	C	95.5 ± 5.3	95.0 ± 5.1	0.587	-0.10 (-0.46, 0.26)		
	CON	95.0 ± 5.1	95.6 ± 5.4	0.432	0.12 (-0.24, 0.48)		
Insulin (μU/mL)	ST+C	10.5 ± 1.2	7.8 ± 1.0	<0.001	-2.25 (-2.69, -1.81)	<0.001	0.50
	ST+P	10.4 ± 1.1	9.1 ± 1.0	0.005	-1.18 (-1.56, -0.80)		
	C	10.6 ± 1.3	10.5 ± 1.2	0.710	-0.08 (-0.44, 0.28)		
	CON	10.5 ± 1.2	10.7 ± 1.3	0.554	0.16 (-0.20, 0.52)		
HOMA-IR	ST+C	2.48 ± 0.30	1.65 ± 0.25	<0.001	-2.93 (-3.48, -2.38)	<0.001	0.55
	ST+P	2.45 ± 0.28	2.10 ± 0.22	0.003	-1.42 (-1.83, -1.01)		
	C	2.50 ± 0.31	2.48 ± 0.30	0.687	-0.07 (-0.43, 0.29)		
	CON	2.49 ± 0.30	2.55 ± 0.32	0.415	0.20 (-0.16, 0.56)		
Total Cholesterol (mg/dL)	ST+C	198.5 ± 15.2	165.2 ± 12.8	<0.001	-2.20 (-2.64, -1.76)	<0.001	0.38
	ST+P	197.8 ± 14.9	181.5 ± 13.5	0.003	-1.09 (-1.47, -0.71)		
	C	199.1 ± 15.5	198.0 ± 15.0	0.587	-0.07 (-0.43, 0.29)		
	CON	198.9 ± 15.1	200.1 ± 15.3	0.412	0.08 (-0.28, 0.44)		
HDL-C (mg/dL)	ST+C	42.5 ± 4.1	51.8 ± 4.5	<0.001	2.15 (1.72, 2.58)	<0.001	0.41
	ST+P	42.1 ± 4.0	44.5 ± 4.2	0.065	0.57 (0.20, 0.94)		
	C	42.7 ± 4.2	42.9 ± 4.1	0.812	0.05 (-0.31, 0.41)		
	CON	42.3 ± 4.0	42.0 ± 3.9	0.760	-0.07 (-0.43, 0.29)		
LDL-C (mg/dL)	ST+C	120.3 ± 11.5	95.7 ± 9.8	<0.001	-2.22 (-2.66, -1.78)	<0.001	0.40
	ST+P	119.8 ± 11.2	109.1 ± 10.5	0.005	-0.95 (-1.33, -0.57)		
	C	120.5 ± 11.7	119.9 ± 11.4	0.655	-0.05 (-0.41, 0.31)		
	CON	120.1 ± 11.3	121.0 ± 11.6	0.389	0.08 (-0.28, 0.44)		
Triglycerides (mg/dL)	ST+C	145.1 ± 18.7	105.8 ± 14.3	<0.001	-2.20 (-2.64, -1.76)	<0.001	0.43
	ST+P	146.0 ± 19.1	135.5 ± 17.8	0.058	-0.55 (-0.92, -0.18)		
	C	144.5 ± 18.5	143.9 ± 18.2	0.721	-0.03 (-0.39, 0.33)		
	CON	145.8 ± 19.0	147.0 ± 19.5	0.519	0.06 (-0.30, 0.42)		
hs-CRP (mg/L)	ST+C	2.8 ± 0.5	1.5 ± 0.3	<0.001	-2.60 (-3.11, -2.09)	<0.001	0.48
	ST+P	2.7 ± 0.4	2.1 ± 0.3	0.001	-1.50 (-1.92, -1.08)		
	C	2.9 ± 0.5	2.8 ± 0.5	0.612	-0.10 (-0.46, 0.26)		
	CON	2.8 ± 0.5	2.9 ± 0.5	0.750	0.08 (-0.28, 0.44)		
IL-6 (pg/mL)	ST+C	3.5 ± 0.6	2.0 ± 0.4	<0.001	-2.50 (-2.99, -2.01)	<0.001	0.46
	ST+P	3.4 ± 0.5	2.7 ± 0.4	0.003	-1.27 (-1.66, -0.88)		
	C	3.5 ± 0.6	3.5 ± 0.6	0.680	-0.08 (-0.44, 0.28)		
	CON	3.5 ± 0.6	3.6 ± 0.6	0.590	0.08 (-0.28, 0.44)		

The training × creatine interaction term was statistically significant for glucose ($F(1,43) = 10.50$, $p = 0.002$, $\eta p^2 = 0.20$), insulin ($F(1,43) = 12.80$, $p = 0.001$, $\eta p^2 = 0.23$), HOMA-IR ($F(1,43) = 14.50$, $p < 0.001$, $\eta p^2 = 0.25$), total cholesterol ($F(1,43) = 7.80$, $p = 0.008$, $\eta p^2 = 0.15$), HDL-C ($F(1,43) = 8.10$, $p = 0.007$, $\eta p^2 = 0.16$), LDL-C ($F(1,43) = 7.50$, $p = 0.010$, $\eta p^2 = 0.14$), triglycerides ($F(1,43) = 8.90$, $p = 0.004$, $\eta p^2 = 0.17$), hs-CRP ($F(1,43) = 9.20$, $p = 0.004$, $\eta p^2 = 0.18$), and IL-6 ($F(1,43) = 8.70$, $p = 0.005$, $\eta p^2 = 0.17$), providing robust evidence for synergistic metabolic and anti-inflammatory effects.

Influence of ACTN3 Genotype

The distribution of ACTN3 genotypes was RR (45.8 percent, n = 22), RX (41.7 percent, n = 20),

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and XX (12.5 percent, n = 6). Subgroup analyses indicated that participants carrying the R allele (RR and RX genotypes) in both the ST+C and ST+P groups exhibited significantly greater improvements in LBM, testosterone, and GH compared with participants with the XX genotype ($p < 0.05$, $\eta p^2 = 0.15$ to 0.25). This genotype-related advantage was most pronounced in the ST+C group ($p < 0.01$, $\eta p^2 = 0.28$ to 0.35), suggesting that creatine supplementation augmented training responsiveness in individuals genetically predisposed to strength and power performance. Nevertheless, due to the limited sample size of the XX subgroup (n = 6), these findings should be interpreted as exploratory.

Adherence, Adverse Events, and Data Handling

As reported in the Methods section, adherence to both the resistance training and supplementation protocols was high, exceeding 90 percent across all intervention groups. No serious adverse events were reported by any participant during the 8-week intervention period. All collected data were included in the final analyses using an intention-to-treat approach, and no missing values were observed for either primary or secondary outcome variables. Assumptions underlying ANCOVA, including normality of residuals and homogeneity of regression slopes, were examined visually and confirmed for all analyses.

Discussion

This study demonstrates that the combination of an 8-week strength training program and creatine supplementation produces significant synergistic benefits for body composition, anabolic hormone status, and metabolic health in male athletes aged 30 to 45 years.

The greater increase in LBM and reduction in BFP observed in the ST+C group are consistent with the existing literature. Creatine supplementation enhances the quality and volume of training sessions by increasing ATP availability, thereby providing a stronger stimulus for muscle protein synthesis and hypertrophy (3, 4, 6). The present findings confirm that the beneficial effects of creatine on body composition are maximized when supplementation is combined with an adequate strength training stimulus.

The most striking finding of the present study was the pronounced synergistic effect on anabolic hormone responses. Although strength training alone resulted in significant increases in testosterone and GH, consistent with the findings of Kraemer et al. (8), the addition of creatine supplementation more than doubled the magnitude of the testosterone response and significantly potentiated the GH increase. These findings suggest that creatine does not directly elevate circulating hormone concentrations; rather, it facilitates greater training intensity and volume, which subsequently elicits a stronger physiological stimulus for anabolic hormone secretion (14). Nevertheless, it must be acknowledged that GH secretion follows a pulsatile pattern, and a single time-point assessment may not fully reflect overall hormonal dynamics. Moreover, the concurrent reduction in cortisol levels in the ST+C group indicates an improved anabolic-to-catabolic hormonal balance, thereby creating a more favorable internal environment for muscle hypertrophy and recovery.

The observed metabolic improvements, particularly enhanced insulin sensitivity and a more favorable lipid profile in the ST+C group, are also of considerable clinical relevance. Increased skeletal muscle mass functions as a major site for glucose uptake, while resistance exercise independently enhances insulin signaling pathways (1). The additional metabolic benefit associated with creatine supplementation may be attributable to its role in maintaining cellular energy homeostasis. Furthermore, given the well-established bidirectional association between chronic low-grade inflammation

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and metabolic dysfunction, including insulin resistance and dyslipidemia, as well as the reported anti-inflammatory properties of creatine (15), the significant reductions in hs-CRP and IL-6 observed in the ST+C group provide a plausible mechanistic explanation for the enhanced metabolic adaptations. Although numerous studies have reported beneficial or neutral effects of creatine on hormonal and metabolic markers, some investigations have demonstrated null effects on specific endocrine or lipid outcomes, particularly in untrained individuals or when alternative dosing protocols were employed (e.g., relevant references should be included here). Such variability highlights the critical influence of participant characteristics, training status, and methodological design on the physiological response to creatine supplementation.

Finally, the interaction between training responses and the ACTN3 genotype offers important insight into the emerging field of personalized sports nutrition. The observation that individuals carrying the R allele exhibited more pronounced adaptations, particularly in response to creatine supplementation, supports the gene–environment interaction framework. Given that the ACTN3 protein plays a pivotal role in fast-twitch muscle fiber function, and that these fibers rely heavily on the phosphocreatine (PCr) energy system, it is biologically plausible that creatine supplementation preferentially benefits individuals with a genetic predisposition toward strength- and power-oriented phenotypes (9, 11).

Conclusion

An 8-week intervention combining strength training with creatine supplementation elicits a robust synergistic effect, leading to significant enhancements in anabolic hormone profiles, including increased testosterone and GH concentrations alongside reduced cortisol levels, improved body composition characterized by increased LBM and decreased BFP, and comprehensive metabolic benefits, such as improved insulin sensitivity, a favorable lipid profile, and reduced systemic inflammation, in middle-aged male athletes. These adaptations were substantially greater than those achieved through strength training alone. In addition, the ACTN3 genotype appears to play a meaningful modulatory role in these physiological responses, thereby supporting the development of genetically informed training and nutritional interventions. Collectively, these findings provide strong empirical support for practitioners to recommend the combined implementation of resistance training and creatine supplementation to optimize performance and health outcomes in trained middle-aged male athletes. The large effect sizes observed further underscore the clinical and practical relevance of this intervention.

Study Limitations

Despite the significant findings, several limitations should be acknowledged. First, the relatively small sample size, with 12 participants per group, and the restriction to middle-aged male athletes limit the generalizability of the results to other populations, including women, older adults, and sedentary individuals. Genotype-based subgroup analyses, although informative, were conducted on even smaller samples, which may have reduced statistical power to detect subtle gene-specific effects, particularly for the XX genotype (n = 6). Second, although the 8-week intervention duration is common in exercise science research, it may not fully capture longer-term physiological adaptations. Third, while participants were instructed to maintain their habitual diets, the absence of strict control over macronutrient and micronutrient intake, as well as sleep patterns, may have introduced additional variability. Fourth, the lack of direct measurements of muscle protein synthesis and the absence of comprehensive hormonal profiling, such as repeated blood

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sampling to assess GH pulsatility, limit the depth of mechanistic interpretation. Finally, although BIA was used for practical reasons, this method is less precise than gold-standard techniques such as dual-energy X-ray absorptiometry (DEXA) and may be influenced by fluctuations in body water content, which can occur with creatine supplementation (a relevant reference should be added here). Although hydration was standardized before BIA assessments, direct measurements of total body water, intracellular water, and extracellular water were not performed. In addition, the inflammatory marker panel was limited, and a broader assessment could have provided a more comprehensive understanding of the anti-inflammatory effects.

Clinical Applications

1. Personalized Training and Supplementation: The findings highlight the potential for individualized intervention strategies, indicating that individuals carrying the ACTN3 R allele may derive greater anabolic and metabolic benefits from combined strength training and creatine supplementation. Genetic screening may therefore inform more precise exercise and nutritional prescriptions.

2. Health Promotion in Middle-Aged Men: This combined intervention represents an effective strategy for mitigating age-related declines in skeletal muscle mass and metabolic function, which are key determinants of long-term health and disease prevention, including sarcopenia, type 2 diabetes, and cardiovascular disease.

3. Enhanced Athletic Performance and Recovery: Coaches and athletes may exploit these synergistic adaptations to optimize resistance training programs, resulting in superior gains in strength, power, and body composition, alongside improved recovery and attenuated inflammatory responses.

4. Comprehensive Metabolic Benefits: The favorable effects on lipid profiles, insulin sensitivity, and inflammatory markers suggest that this combined approach may contribute to the prevention or management of metabolic syndrome components in physically active middle-aged individuals.

5. Safety and Practicality: The absence of serious adverse events supports the safety and feasibility of creatine supplementation when combined with supervised resistance training, reinforcing its applicability across a wide range of athletic populations.

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Conflict of Interest

The authors declare no conflicts of interest.

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Ethical Consideration

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Islamic Azad University, Dezful Branch. All participants provided written informed consent prior to participation.

Code of Ethices

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Author Contributions

Mohammad Amiri and Saeed Tanvorsaz contributed equally to all stages of the research process, including conceptualization, study design, data interpretation, manuscript drafting, and critical revision. Gholamreza Bahramfar and Ali Sepehriyan were primarily responsible for

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data collection, methodological implementation, data validation, and source management.

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