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Article

Urolithin A and Creatine as A Dual Mitochondrial–Bioenergetic Intervention against Exercise-Induced Skeletal Muscle Injury in Rats

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Abstract: Background: Eccentric-biased exercise can produce delayed force loss, membrane leakage, redox imbalance, inflammatory signaling and transient mitochondrial dysfunction. Urolithin A (UA) promotes mitochondrial quality control, whereas creatine strengthens the creatine–phosphocreatine energy-buffering system. Methods: Seventy-two adult Wistar rats (36 male, 36 female) were allocated to sedentary control (SED-CON), sedentary UA plus creatine (SED-UA+Cr), exercise control (EX-CON), exercise plus UA (EX-UA), exercise plus creatine (EX-Cr), or exercise plus UA and creatine (EX-UA+Cr) groups (n=12/group). UA was administered orally at 25 mg/kg/day and creatine monohydrate was incorporated into diet at 2% (w/w) for 28 days before a single 90-min downhill run (16 m/min, −16°); supplementation continued through 48 h of recovery. Grip force, serum creatine kinase (CK) and lactate dehydrogenase (LDH) were assessed serially. At 48 h, skeletal muscle ATP, total creatine, lactate, glycogen, citrate synthase, respiratory-chain Complex I/II activities, redox markers, cytokines and mitochondrial quality-control/biogenesis transcripts were quantified. Results: Downhill running reduced 48-h grip force to 77.0 ± 3.8% of baseline in EX-CON. Recovery was greater with UA (88.5 ± 4.7%), creatine (90.9 ± 3.7%) and UA+creatine (96.1 ± 6.2%); the combination differed from EX-CON by 19.1 percentage points (95% CI 14.7–23.6, P<0.0001). CK AUC0–48h fell from 85,149 ± 4,901 to 40,027 ± 2,776 U·h/L with the combination (P<0.0001). Compared with EX-CON, EX-UA+Cr preserved ATP (4.46 ± 0.38 vs 2.84 ± 0.24 nmol/mg wet tissue), increased total muscle creatine (42.75 ± 3.18 vs 29.25 ± 2.75 μmol/g), restored Complex I activity (99.2 ± 8.6 vs 66.6 ± 8.3 nmol/min/mg protein), lowered malondialdehyde and pro-inflammatory cytokines, increased IL-10, and produced the largest increases in Pink1, Park2, Ppargc1a, Tfam and Nrf1 transcript abundance. Grip-force recovery correlated positively with ATP (Spearman ϱ=0.76, P<0.0001) and inversely with CK AUC (ϱ=−0.78, P<0.0001). Conclusion: UA and creatine provided complementary protection against eccentric-biased exercise injury, linking faster functional recovery with preservation of muscle energy status, mitochondrial enzyme activity, redox balance and inflammatory control. The combination consistently produced the strongest recovery phenotype, although formal interaction effects were outcome-dependent.

Keywords: urolithin A; creatine; eccentric exercise; skeletal muscle; mitochondrial quality control; bioenergetics; PINK1; Parkin; PGC-1α

Introduction

Skeletal muscle exposed to unfamiliar or high-volume eccentric work develops a characteristic injury–recovery response that includes immediate and delayed force loss, transient elevation of circulating muscle enzymes, perturbation of calcium homeostasis, redox imbalance, inflammatory signaling and changes in mitochondrial function[1][2][3][4]. Downhill running is an established rodent model because the locomotor muscles perform a large eccentric component while exercise intensity, decline, duration and recovery time can be standardized. Functional force recovery and biochemical injury markers are complementary measures; neither should be interpreted as an interchangeable surrogate for the other.

Mitochondria are central to recovery from eccentric-biased stress. Damaged or inefficient organelles can increase oxidant pressure and constrain ATP supply at a time when ion transport, membrane repair, proteostasis and contractile recovery remain energy demanding. Exercise also regulates mitochondrial biogenesis and quality-control pathways involving PGC-1α, NRF1, TFAM, PINK1 and Parkin[5][6][7]. Consequently, an intervention that improves mitochondrial quality without simultaneously supporting rapid high-energy phosphate buffering may not fully normalize short-term muscle function.

Urolithin A is a gut-microbiome-derived metabolite formed from ellagitannin-related precursors. Ryu et al. identified UA as a mitophagy-inducing compound and reported improved muscle function in rodent models[8]. Subsequent human studies found changes in muscle endurance, strength, inflammatory markers and mitochondrial-associated molecular signatures after oral UA supplementation[9][10][11][12][13]. In highly trained distance runners, four weeks of UA lowered post-exercise CK exposure and altered mitochondrial- and inflammation-related skeletal-muscle pathways, although performance effects were not uniformly significant. These findings support UA as a candidate recovery intervention but also indicate that mitochondrial remodeling alone does not guarantee complete functional restoration.

Creatine acts through a distinct energetic mechanism. The creatine–phosphocreatine system buffers ATP turnover and transports high-energy phosphate between sites of production and utilization[14][15][16]. Creatine monohydrate has an extensive safety and performance literature [17] and rodent studies have shown that dietary creatine can increase tissue creatine and attenuate selected oxidative-stress responses to acute exercise[18][19]. Human evidence regarding exercise-induced muscle damage is heterogeneous: some studies and meta-analyses support faster functional recovery, whereas others report little effect on circulating injury markers[20][21[22]. This inconsistency is biologically plausible because creatine primarily increases phosphagen availability and is not a direct substitute for mitochondrial quality control.

The present study therefore examined UA and creatine as mechanistically complementary interventions in the same controlled downhill-running model. The primary endpoint was recovery of body-mass-normalized hindlimb grip force 48 h after exercise. Secondary outcomes were selected to link function with membrane-associated injury (CK and LDH), energy state (ATP, total creatine, lactate and glycogen), mitochondrial enzyme function (citrate synthase and respiratory-chain Complex I and II), redox status, inflammatory mediators and mitochondrial quality-control/biogenesis transcripts. Histology, microscopy, immunohistochemistry, western blotting and other publication-image-producing biological methods were intentionally excluded so that all biological conclusions were based on numerical functional, biochemical, enzymatic, immunological and transcript data.

Materials and Methods

Study design and ethical approval

The study used a randomized, assessor-masked, controlled six-group parallel design and was reported according to ARRIVE 2.0[23][24]. RT-qPCR procedures were documented according to MIQE 2.0[25]. The primary outcome, exclusion criteria, randomization sequence, group codes, outcome hierarchy and statistical analysis map were fixed before group allocation. Animal procedures were reviewed by an institutional animal research ethics committee (educational record EDU-SIM-2026-014; 12 January 2026)

and were conducted in accordance with institutional standards for animal care and the principles of replacement, reduction and refinement.

Animals and husbandry

Seventy-two healthy Wistar rats (8–10 weeks old; 36 male and 36 female) were obtained from a single accredited laboratory-animal colony. At allocation, mean body mass was 254.5 ± 29.4 g. Animals were acclimated for 7 days and housed two per cage, with males and females housed separately, at 22 ± 2 °C, 50–60% relative humidity and a 12:12-h light–dark cycle (lights on 07:00). AIN-93M purified diet and filtered water were available ad libitum. Cages contained nesting material and a shelter tube. Cage position was rotated weekly. Animals were observed at least twice daily; predefined humane endpoints included persistent inability to eat or drink, inability to bear weight, severe respiratory distress, unrelieved pain, open traumatic injury or >15% body-mass loss accompanied by clinical deterioration.

Randomization and experimental groups

After valid baseline grip-force measurements, rats were randomized in sex-stratified blocks using a computer-generated sequence balanced for baseline body mass and normalized grip force. Allocation was retained by a person not involved in testing or laboratory assays. Treatment tubes, samples, assay plates and analysis files carried coded identifiers. The treadmill operator was necessarily aware of exercise status but remained unaware of supplement identity; grip-force testing, biochemical assays, ELISA, RT-qPCR and statistical analysis were performed under coded group labels until the primary analysis was finalized.

Table 1. Experimental groups and interventions.

Group	Activity	Oral gavage	Diet	Analytical role
SED-CON	Sedentary	Vehicle	Control diet	Sedentary reference
SED-UA+Cr	Sedentary	UA 25 mg/kg/day	2% creatine diet	Combined-treatment sedentary control
EX-CON	Downhill exercise	Vehicle	Control diet	Exercise-injury reference
EX-UA	Downhill exercise	UA 25 mg/kg/day	Control diet	UA treatment
EX-Cr	Downhill exercise	Vehicle	2% creatine diet	Creatine treatment
EX-UA+Cr	Downhill exercise	UA 25 mg/kg/day	2% creatine diet	Combined treatment

Sample-size rationale

The primary comparison was EX-UA+Cr versus EX-CON for 48-h recovery of normalized hindlimb grip force. A two-sided calculation using α=0.05, 80% power and a standardized mean difference of 1.20 required approximately 12 animals per group. Twelve rats were therefore allocated to each of six groups (total n=72). The design also retained repeated within-animal measurements, which increased precision but was not used to reduce the planned group size.

Urolithin A and creatine supplementation

Urolithin A (Sigma-Aldrich/Merck, SML1791; CAS 1143-70-0; stated purity ≥97%) was administered once daily at 25 mg/kg by oral gavage for 28 days before exercise and again during the 48-h recovery interval. The daily dose was calculated from that morning's body mass. UA was freshly suspended in 0.5% (w/v) sodium carboxymethylcellulose in purified water and delivered at 5 mL/kg between 09:00 and 10:00. Vehicle groups received the same gavage volume. Suspensions were vortexed for 60 s and maintained under gentle stirring during dosing.

Creatine monohydrate (Sigma-Aldrich/Merck, C3630; CAS 6020-87-7; stated purity ≥98%) was incorporated into AIN-93M diet at 2% (w/w), corresponding to 20 g creatine/kg final diet, for the same treatment interval. Control and creatine diets were prepared from the same basal batch, stored dry at 4 °C and replaced at least every 7 days. Feed offered and residual feed were weighed daily by cage.

Estimated creatine intake was calculated from feed consumption and mean cage body mass. Certificates of analysis, lot numbers, dose-preparation logs and administration records were retained.

Treadmill familiarization and eccentric-biased exercise

On treatment days 22–24, exercise-assigned rats were familiarized with a motor-driven rodent treadmill at 0° for 10 min/day (5 min at 8 m/min followed by 5 min at 10 m/min). Sedentary animals received matched handling in stationary lanes. On day 29, exercise groups completed 90 min of downhill running at 16 m/min and −16°, an eccentric-biased protocol based on established rat injury studies. No electric shock grid was used; gentle tactile prompting with a soft brush was permitted. Ambient temperature, start time, total running time and prompting events were recorded. Sedentary groups remained in stationary lanes for a matched 90-min exposure to the procedure room. All exercise-assigned animals completed the full 90-min bout.

Hindlimb grip-force assessment

Hindlimb grip force was measured with a calibrated digital grip-force meter (BIO-GS3, Bioseb, Vitrolles, France) at baseline and 2, 24 and 48 h after exercise. Rats underwent two familiarization sessions during acclimation. At each formal time point, five valid pulls were obtained with 60 s between trials. The examiner supported the animal so that only the hind paws engaged the bar and pulled the rat horizontally along the meter axis until release. Trials were rejected and repeated once if a forepaw contributed, the rat twisted markedly or the pull was off-axis. The mean of the three highest valid values was normalized to body mass (N/kg), and recovery was also expressed as percentage of the individual baseline value. The 48-h percentage-of-baseline value was the primary functional endpoint.

Blood collection and serum CK/LDH

Blood was collected at baseline, 2 h, 24 h and 48 h. Nonterminal samples (approximately 200–250 μL) were obtained from a lateral tail vein; the 48-h sample was obtained under terminal isoflurane anesthesia. Blood was allowed to clot for 30 min, centrifuged at 2,000×g for 10 min at 4 °C, and serum was aliquoted and frozen at −80 °C within 60 min. CK and LDH were measured in duplicate by kinetic colorimetry at 37 °C using commercial reagent sets on a temperature-controlled microplate reader (SpectraMax iD3, Molecular Devices, USA). Two levels of quality-control material were included in each run. Duplicate CV ≤10% was the target acceptance criterion. AUC0–48h was calculated by the trapezoidal rule from the four serial measurements.

Terminal anesthesia and tissue sampling

At 48 h, rats were deeply anesthetized with isoflurane in oxygen (approximately 5% induction, 2–3% maintenance) until loss of pedal withdrawal. Terminal blood was collected by cardiac puncture, followed by exsanguination and bilateral thoracotomy. Soleus, plantaris and the red portion of gastrocnemius were excised, blotted and weighed. Tissue collection was completed within 4 min after confirmation of death. Separate aliquots were allocated to energy metabolites, mitochondrial enzyme activity, redox assays, cytokines and RNA. Samples were snap-frozen in liquid nitrogen and stored at −80 °C. No muscle was fixed or processed for histology.

Homogenization and protein normalization

Analytical domains used separate tissue aliquots. Redox assays used 1:10 (w/v) homogenates in ice-cold 50 mM potassium phosphate buffer, pH 7.4, with 1 mM EDTA. Cytokine assays used 1:10 homogenates in PBS, pH 7.4, containing 0.05% Tween-20 and a broad-spectrum protease-inhibitor cocktail. Mitochondrial enzyme assays used 1:10 homogenates in 225 mM mannitol, 75 mM sucrose, 10 mM Tris-HCl and 0.1 mM EDTA, pH 7.4. Homogenates were clarified at 800×g for 10 min at 4 °C unless otherwise specified. Protein concentration was measured by bicinchoninic acid assay (Pierce BCA, Thermo Fisher Scientific, 23225) against bovine serum albumin standards[26].

Muscle ATP, lactate, glycogen and total creatine

ATP was measured in rapidly frozen soleus using the colorimetric ATP Assay Kit (Abcam, ab83355). Tissue was pulverized over dry ice and immediately deproteinized with perchloric acid, neutralized with KOH, clarified by centrifugation and read at 570 nm against same-plate ATP standards. L-lactate was quantified after deproteinization using Abcam ab65331 at 450 nm. Glycogen was measured using Abcam ab65620 after enzymatic hydrolysis, with a matched glucose-background well for each sample, and read at 570 nm. Total free creatine was quantified using Abcam ab65339 at 570 nm. ATP was

expressed as nmol/mg wet tissue, lactate as μmol/g wet tissue, glycogen as mg/g wet tissue and total creatine as μmol/g wet tissue. All samples were analyzed in duplicate within validated standard ranges.

Mitochondrial enzyme activities

Respiratory-chain enzyme activities were measured spectrophotometrically at 30 °C using established principles[27]. Complex I activity was derived from rotenone-sensitive NADH oxidation at 340 nm. Complex II activity was derived from succinate-dependent reduction of 2,6-dichlorophenolindophenol at 600 nm with a malonate-inhibited blank. Citrate synthase was measured from the rate of 5,5′-dithiobis-(2-nitrobenzoic acid) reduction at 412 nm after addition of oxaloacetate and acetyl-CoA. Activities were calculated from the linear portion of each reaction and expressed as nmol/min/mg protein. Duplicate slopes had to agree within 10%; a pooled skeletal-muscle homogenate was included on every plate to monitor inter-plate drift.

Redox measurements

Malondialdehyde (MDA) equivalents were measured by thiobarbituric-acid reaction with 1,1,3,3-tetramethoxypropane standards and absorbance at 532 nm. Protein carbonyls were quantified after 2,4-dinitrophenylhydrazine derivatization at 370 nm. Reduced and oxidized glutathione were quantified by enzymatic recycling using separate aliquots for total glutathione and GSSG; the GSH/GSSG ratio was calculated. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were quantified by validated kinetic spectrophotometric assays and normalized to total protein. Samples from all groups and both sexes were balanced across plates.

Muscle cytokines

TNF-α, IL-1β, IL-6 and IL-10 were quantified in gastrocnemius homogenate using rat Quantikine ELISA kits (R&D Systems/Bio-Techne: TNF-α RTA00, IL-1β RLB00, IL-6 R6000B, IL-10 R1000). Standard curves, blanks and pooled-homogenate controls were included on every plate. Samples were measured in duplicate and diluted to remain within the validated calibration range. Duplicate CV >15% triggered one repeat if sufficient sample remained. Concentrations were normalized to total protein and expressed as pg/mg protein.

RNA extraction, cDNA synthesis and RT-qPCR

Total RNA was extracted from 20–30 mg frozen soleus in TRIzol reagent (Thermo Fisher Scientific, 15596018), followed by chloroform phase separation, isopropanol precipitation and 75% ethanol washing. Residual genomic DNA was removed with TURBO DNase (Thermo Fisher Scientific, AM2238). RNA concentration and A260/A280 and A260/A230 ratios were recorded; all samples used for expression analysis had A260/A280 values of 1.90–2.10 and passed the laboratory RNA-integrity acceptance criterion. Exactly 1.0 μg RNA was reverse transcribed in 20 μL using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, 4368814) with random primers. cDNA was diluted 1:5 before qPCR.

SYBR Green reactions (10 μL) contained 5.0 μL PowerUp SYBR Green Master Mix (Applied Biosystems, A25742), 0.30 μM each primer, 2.0 μL diluted cDNA and nuclease-free water. Amplification was performed on a QuantStudio 5 Real-Time PCR System (Applied Biosystems) at 95 °C for 2 min followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s, followed by a 65–95 °C melt curve. Each biological sample was analyzed in technical triplicate. Every plate included no-template and no-RT controls and a five-point 1:5 pooled-cDNA dilution series. Assays were accepted at 90–110% efficiency, $R^2 \geq 0.99$ and a single melt peak. Hprt1 and Actb were stable across treatment, exercise and sex (geNorm M<0.5) and their geometric mean was used for normalization. Relative expression was calculated by efficiency-checked 2^−ΔΔCq [28][29].

Table 7. RT-qPCR primers used in the study.

Gene	Accession	Forward primer (5′→3′)	Reverse primer (5′→3′)	Amplicon (bp)
Pink1	NM_001106694	CATGGCTTTGGATGGAGAGT	TGGGAGTTTGCTCTTCAAGG	~120

Gene	Accession	Forward primer (5′→3′)	Reverse primer (5′→3′)	Amplicon (bp)
Park2	NM_020093	CTGGCAGTCATTCTGGACAC	CTCTCCACTCATCCGGTTTG	~120
Ppargc1a	NM_031347.1	CAGTCCTTCCTCCATGCCTG	CTGTGGGTGTGGTTTGCATG	~130
Tfam	NM_031326	ACACCCAGATGCAAAAGTTTCA	TGTACACCTTCCACTCAGCT	~120
Nrf1	NM_001100708	TGCTTCAGAACTGCCAACCA	GGTCATTTCACCGCCCTGTA	~120
Hprt1	NM_012583	CTCATGGACTGATTATGGACAGGAC	GCAGGTCAGCAAAGAACTTATAGCC	123
Actb	NM_031144	CCGCGAGTACAACCTTCTTG	GCAGCGATATCGTCATCCAT	81

Prespecified exclusions and assay quality control

No observation was removed solely because it appeared extreme. Post-randomization exclusion was restricted to welfare withdrawal, administration of the wrong treatment, repeated dosing failure (>2 daily doses), failure to complete ≥60 min of the exercise challenge for a non-medical reason, irrecoverable specimen loss or assay failure that could not be repeated. Grossly hemolyzed serum samples were flagged before group unmasking. Technical replicates were averaged only after prespecified CV criteria were met. No rat met a post-randomization exclusion criterion, and all 72 animals contributed to the primary analysis.

Statistical analysis

Analyses were performed in R 4.6-compatible workflows and independently checked in Python 3.13. Two-sided tests were used. Continuous outcomes are presented as mean ± SD. Serial grip force, CK and LDH were analyzed using linear mixed-effects regression with fixed effects for group, time, sex and group×time and a random intercept for rat; CK and LDH were log-transformed for model diagnostics. The confirmatory primary contrast was EX-UA+Cr versus EX-CON at 48 h. Holm adjustment was applied to secondary pairwise contrasts among exercised groups. AUC0–48h was calculated by the trapezoidal method.

Terminal outcomes from the four exercised groups were analyzed in a 2×2 factorial regression containing UA (yes/no), creatine (yes/no), sex and UA×creatine. The interaction term tested non-additivity. Secondary endpoints were organized into bioenergetic, mitochondrial-enzyme, redox, inflammatory and transcript families, with Benjamini–Hochberg false-discovery control at $q<0.05$ within each family[30]. Spearman correlations were used for prespecified cross-domain associations. Effect estimates and 95% confidence intervals accompanied P values. Exploratory sex×treatment terms were examined but were not used for definitive sex-specific claims because the study was not powered for subgroup efficacy.

Results

Study flow, treatment exposure and baseline comparability

All 72 randomized animals completed the intervention and contributed to the primary analysis. No mortality, humane-endpoint withdrawal, major dosing error or irrecoverable specimen loss occurred. All 48 exercise-assigned rats completed the full 90-min downhill-running bout. Mean gavage compliance across groups was 99.6%, and the three creatine-fed groups consumed an estimated 1.67 ± 0.12 g creatine/kg/day. Body mass increased by approximately 5–6% during the intervention with no evidence of a group-specific effect. Baseline normalized grip force, CK and LDH were comparable across groups.

Table 2. Baseline body mass and treatment exposure.

Group	Baseline body mass (g)	Final body mass (g)	Feed intake (g/day)	Creatine intake (g/kg/day)	Gavage compliance (%)
SED-CON	255.7 ± 31.4	270.6 ± 31.8	20.3 ± 1.6	—	99.6
SED-UA+Cr	250.3 ± 29.4	265.6 ± 30.5	21.1 ± 2.0	1.69 ± 0.08	99.6
EX-CON	258.6 ± 29.8	272.9 ± 32.0	20.6 ± 2.1	—	99.7
EX-UA	255.4 ± 32.4	269.3 ± 33.2	20.3 ± 1.7	—	99.8
EX-Cr	253.6 ± 27.3	270.1 ± 28.5	20.9 ± 2.1	1.65 ± 0.11	99.5
EX-UA+Cr	253.7 ± 29.2	269.3 ± 31.2	21.0 ± 2.4	1.66 ± 0.15	99.5

Combined UA and creatine accelerated functional recovery

Downhill running produced an immediate and sustained reduction in hindlimb grip force. EX-CON animals recovered from 60.2 ± 5.6% of baseline at 2 h to 77.0 ± 3.8% at 48 h. Recovery was greater in EX-UA (88.5 ± 4.7%), EX-Cr (90.9 ± 3.7%) and EX-UA+Cr (96.1 ± 6.2%). The prespecified 48-h difference between EX-UA+Cr and EX-CON was 19.1 (14.7 to 23.6) percentage points (P<0.0001). The combination also exceeded EX-UA by 7.6 percentage points (P=0.0027) and EX-Cr by 5.2 percentage points (P=0.021). The group×time term was significant in the repeated-measures model (P<0.0001). No treatment×sex interaction survived multiplicity correction.

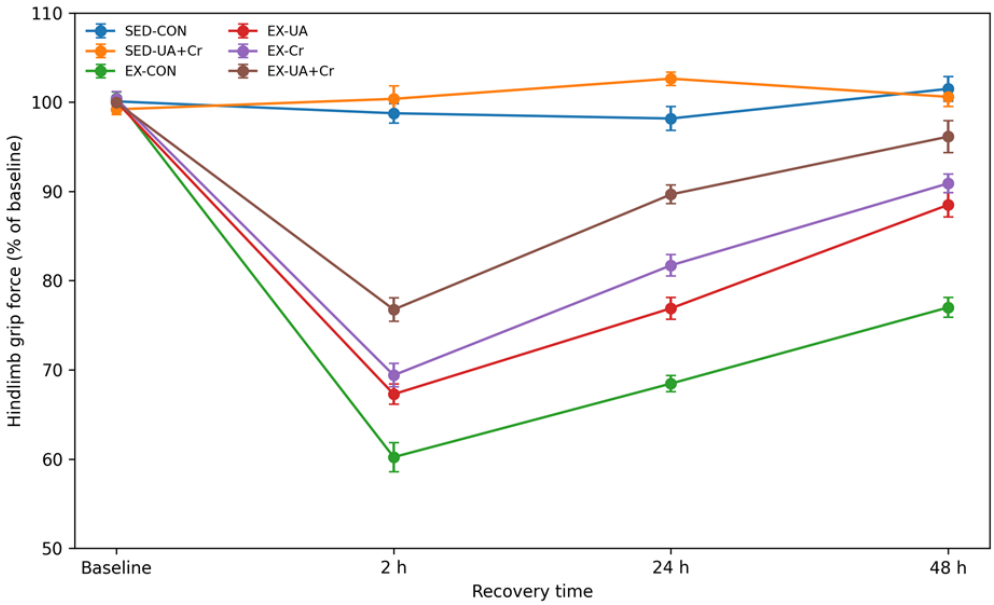


Figure 2. Hindlimb grip-force recovery. Values are mean ± SEM; n=12/group. Exercise control animals showed marked force loss after downhill running, whereas UA and creatine each accelerated recovery and the combined intervention produced the highest 48-h recovery.

UA and creatine reduced circulating markers of muscle injury

CK and LDH increased after exercise and peaked at 24 h in EX-CON. CK AUC0–48h was 85149 ± 4901 U·h/L in EX-CON, 61427 ± 4271 in EX-UA, 57545 ± 4178 in EX-Cr and 40027 ± 2776 in EX-UA+Cr. The combined intervention lowered CK AUC by 45,122 U·h/L versus EX-CON (P<0.0001). LDH AUC showed the same ordering. At 24 h, serum CK was 2589 ± 189 U/L in EX-CON compared with 1181 ± 104 U/L in EX-UA+Cr.

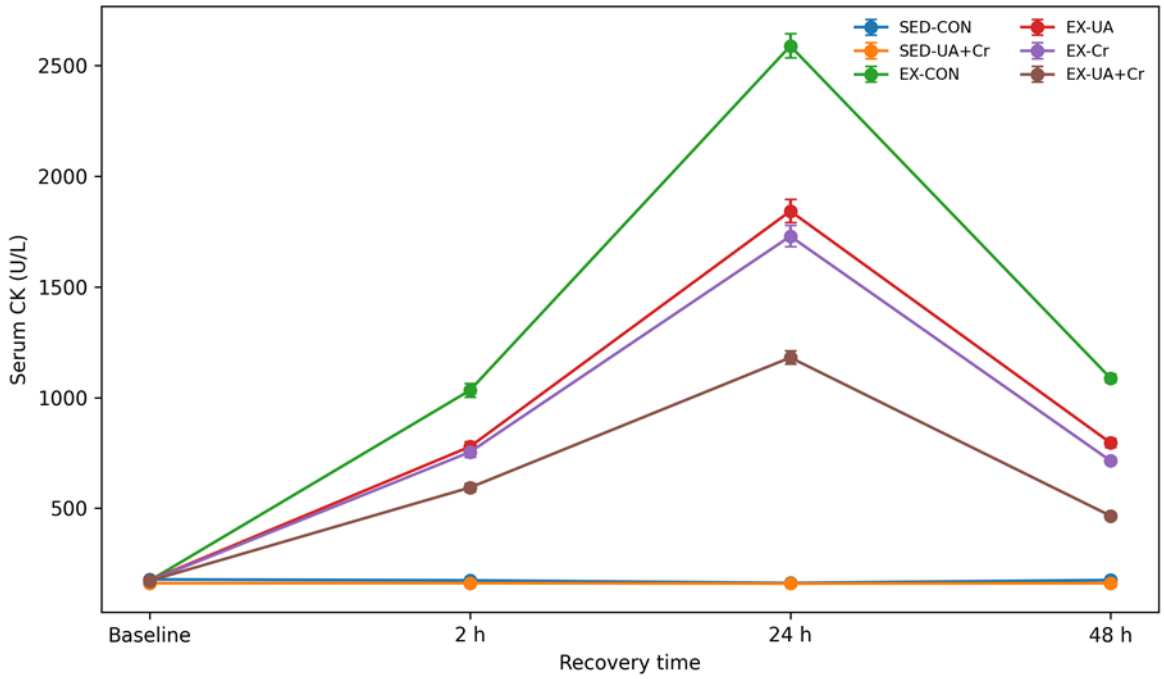


Figure 3. Serum creatine kinase during recovery. Values are mean ± SEM; n=12/group. CK peaked at 24 h after downhill running and was attenuated by both interventions, with the lowest exposure in EX-UA+Cr.

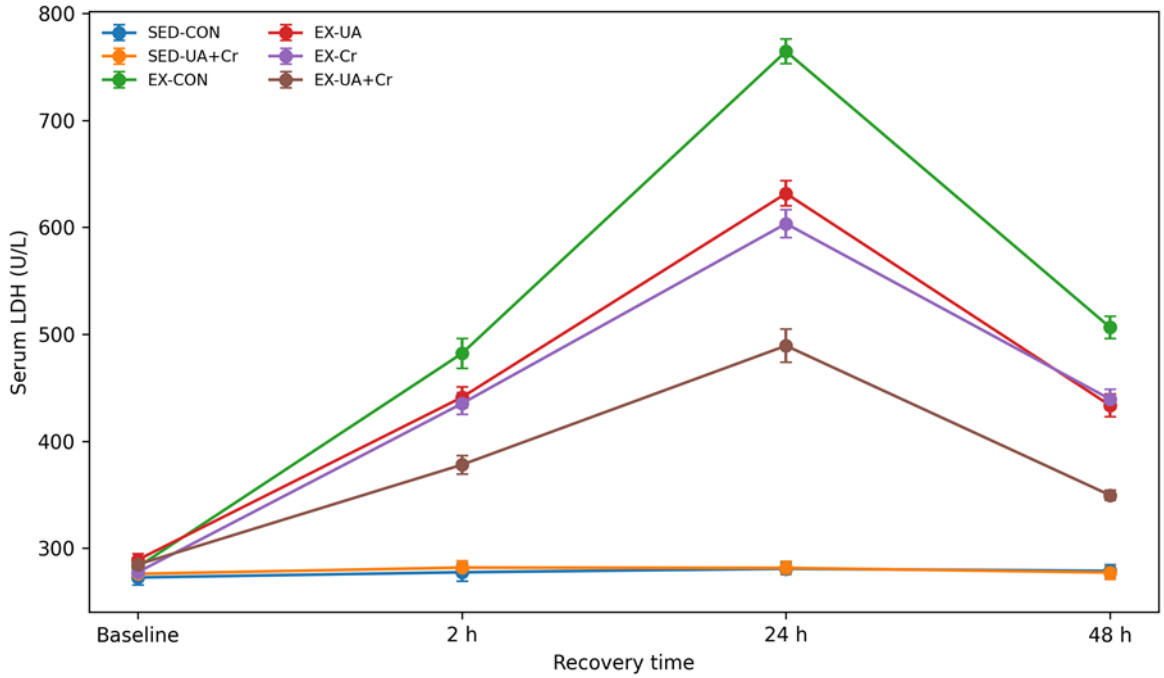


Figure 4. Serum lactate dehydrogenase during recovery. Values are mean ± SEM; n=12/group. The combined intervention produced the smallest post-exercise LDH excursion.

Table 3. Functional recovery and circulating muscle-injury outcomes.

Outcome	SED-CON	SED-UA+Cr	EX-CON	EX-UA	EX-Cr	EX-UA+Cr	P, EX-UA+Cr vs EX-CON
48-h grip (% baseline)	101.5 ± 4.8	100.6 ± 3.7	77.0 ± 3.8	88.5 ± 4.7	90.9 ± 3.7	96.1 ± 6.2	<0.0001

Outcome	SED-CON	SED-UA+Cr	EX-CON	EX-UA	EX-Cr	EX-UA+Cr	P, EX-UA+Cr vs EX-CON
CK AUC0–48h (U·h/L)	8098 ± 404	7700 ± 610	85149 ± 4901	61427 ± 4271	57545 ± 4178	40027 ± 2776	<0.0001
LDH AUC0–48h (U·h/L)	13408 ± 635	13464 ± 552	29725 ± 1149	25310 ± 1285	24651 ± 1406	20269 ± 1219	<0.0001

The combination preserved muscle energy status and creatine availability

At 48 h, muscle ATP remained depressed in EX-CON (2.84 ± 0.24 nmol/mg wet tissue) relative to SED-CON (4.64 ± 0.32), whereas ATP was progressively higher in EX-UA (3.60 ± 0.34), EX-Cr (3.71 ± 0.19) and EX-UA+Cr (4.46 ± 0.38). The combination exceeded EX-CON by 1.62 (1.34 to 1.89) nmol/mg (P<0.0001). Total muscle creatine increased strongly in creatine-fed groups: 40.23 ± 3.65 µmol/g in EX-Cr and 42.75 ± 3.18 µmol/g in EX-UA+Cr versus 29.25 ± 2.75 µmol/g in EX-CON (P<0.0001). UA alone did not materially alter total creatine (P=0.51). Muscle lactate was lower and glycogen was higher in each intervention group than in EX-CON, with EX-UA+Cr closest to sedentary values.

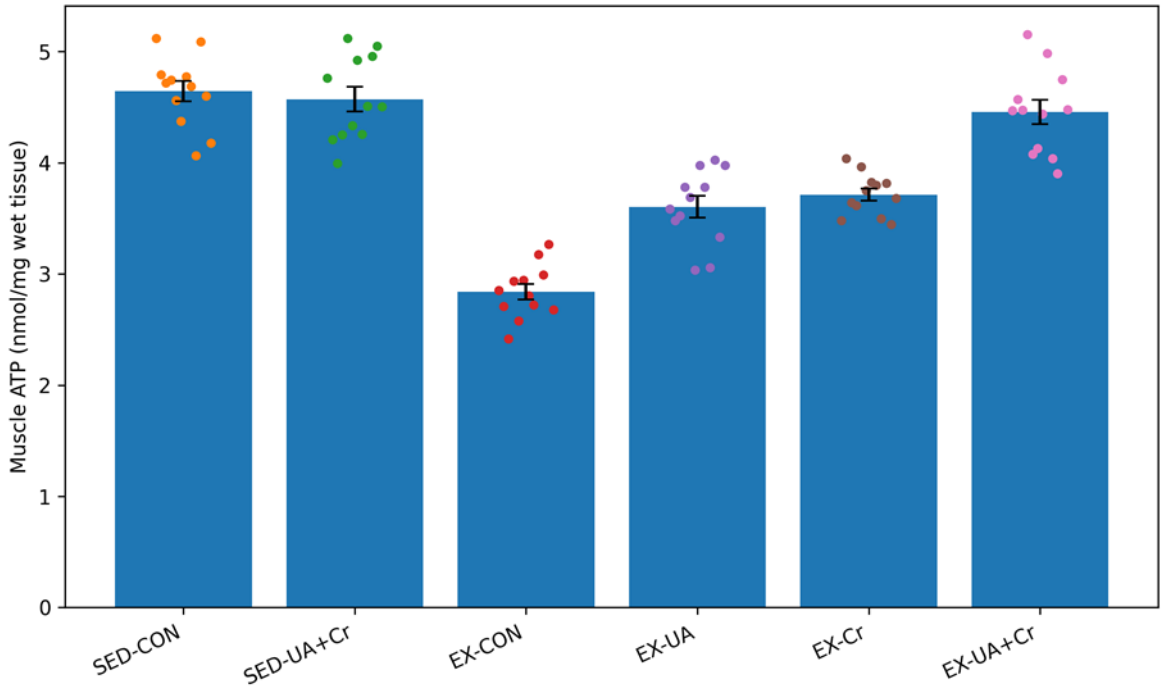


Figure 5. Muscle ATP concentration at 48 h. Bars are mean ± SEM and points are individual rats (n=12/group). ATP depletion after downhill running was attenuated by UA and creatine and nearly normalized by combined treatment.

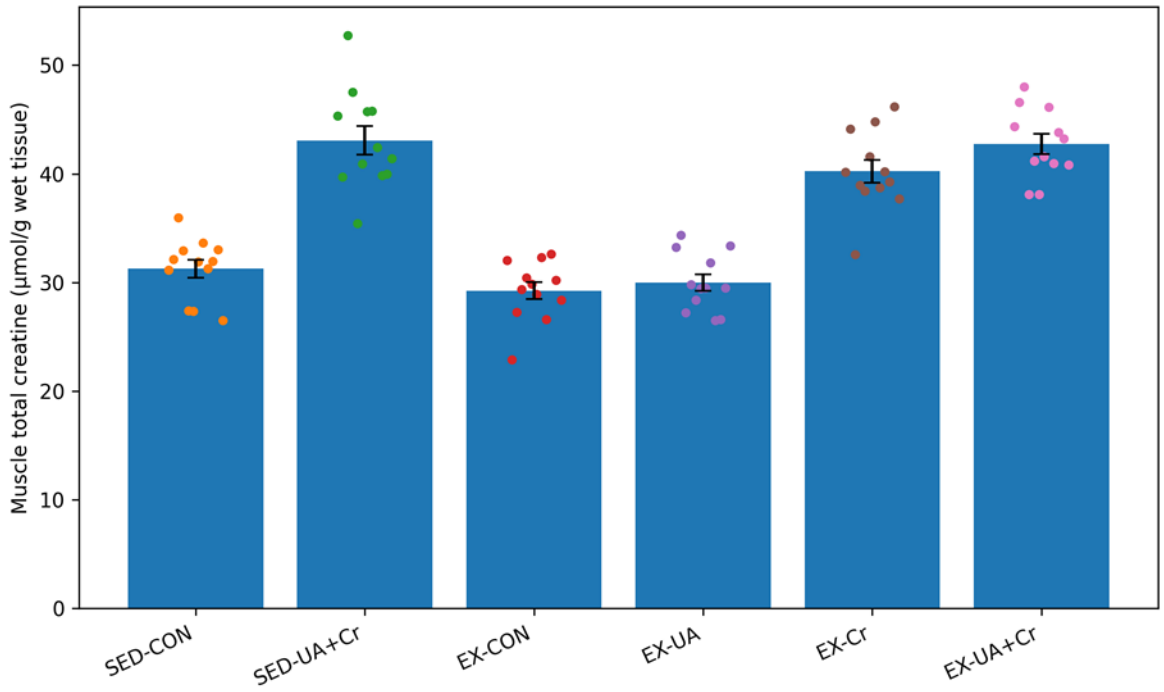


Figure 6. Total skeletal-muscle creatine at 48 h. Bars are mean ± SEM and points are individual rats (n=12/group). Creatine-fed groups showed clear biochemical target engagement.

Mitochondrial enzyme activities were preserved by UA and further improved by combined treatment

EX-CON showed lower citrate synthase, Complex I and Complex II activities than sedentary controls. Complex I activity was 66.6 ± 8.3 nmol/min/mg protein in EX-CON and increased to 88.5 ± 10.9 with UA, 78.4 ± 6.6 with creatine and 99.2 ± 8.6 with the combination. EX-UA+Cr differed from EX-CON by 32.6 (25.5 to 39.8) nmol/min/mg (P<0.0001) and also exceeded EX-UA (P=0.014) and EX-Cr (P<0.0001). Citrate synthase and Complex II followed a similar pattern. Factorial analysis showed strong UA main effects on the respiratory-chain endpoints and smaller but significant creatine contributions; the UA×creatine interaction was not significant for ATP or respiratory-chain enzyme activity, indicating predominantly additive rather than supra-additive energetic protection.

Table 4. Skeletal-muscle bioenergetic and mitochondrial-enzyme outcomes at 48 h.

Outcome	SED-CON	SED-UA+Cr	EX-CON	EX-UA	EX-Cr	EX-UA+Cr	P, combo vs EX-CON
ATP (nmol/mg wet tissue)	4.64 ± 0.32	4.57 ± 0.38	2.84 ± 0.24	3.60 ± 0.34	3.71 ± 0.19	4.46 ± 0.38	<0.0001
Total creatine (µmol/g)	31.28 ± 2.83	43.07 ± 4.58	29.25 ± 2.75	29.99 ± 2.68	40.23 ± 3.65	42.75 ± 3.18	<0.0001
Lactate (µmol/g)	1.55 ± 0.15	1.50 ± 0.11	4.01 ± 0.49	3.02 ± 0.54	2.94 ± 0.33	2.18 ± 0.22	<0.0001
Glycogen (mg/g)	13.27 ± 1.36	13.79 ± 1.46	8.61 ± 0.71	10.26 ± 1.33	11.15 ± 0.85	12.03 ± 1.07	<0.0001
Citrate synthase (nmol/min/mg)	131.4 ± 8.9	129.8 ± 12.0	99.8 ± 9.1	117.3 ± 11.0	107.0 ± 13.9	125.3 ± 8.2	<0.0001
Complex I (nmol/min/mg)	105.0 ± 9.6	115.9 ± 15.9	66.6 ± 8.3	88.5 ± 10.9	78.4 ± 6.6	99.2 ± 8.6	<0.0001
Complex II (nmol/min/mg)	76.2 ± 6.9	80.3 ± 7.8	44.9 ± 5.3	61.1 ± 4.2	56.5 ± 6.0	72.0 ± 5.5	<0.0001

Redox homeostasis improved with each intervention and was closest to sedentary values after combined treatment

Exercise increased MDA and protein carbonyls and reduced the GSH/GSSG ratio, SOD, CAT and GPx. MDA was 3.12 ± 0.25 nmol/mg protein in EX-CON compared with 2.34 ± 0.32, 2.43 ± 0.26 and 1.70 ± 0.17 in EX-UA, EX-Cr and EX-UA+Cr, respectively (combo vs EX-CON P<0.0001). The combination restored the GSH/GSSG ratio to 8.20 ± 0.89 versus 4.14 ± 0.50 in EX-CON and produced the highest SOD, CAT and GPx activities among exercised groups. The UA×creatine interaction was not significant for MDA, supporting an additive redox effect.

Muscle inflammatory signaling was attenuated, with the strongest anti-inflammatory profile in EX-UA+Cr

TNF-α, IL-1β and IL-6 were markedly elevated in EX-CON. TNF-α fell from 46.7 ± 5.4 pg/mg protein to 30.5 ± 3.7 with UA, 32.6 ± 4.4 with creatine and 22.6 ± 2.4 with combined treatment. IL-1β and IL-6 showed the same ordering. IL-10 increased from 24.1 ± 2.2 pg/mg in EX-CON to 41.7 ± 4.1 pg/mg in EX-UA+Cr. Factorial analysis identified significant UA and creatine main effects across the inflammatory domain and an outcome-specific interaction for TNF-α (P=0.013), indicating that non-additivity was not uniform across cytokines.

Table 5. Skeletal-muscle redox and cytokine outcomes at 48 h.

Outcome	SED-CON	SED-UA+Cr	EX-CON	EX-UA	EX-Cr	EX-UA+Cr	P, combo vs EX-CON
MDA (nmol/mg protein)	1.38 ± 0.14	1.33 ± 0.14	3.12 ± 0.25	2.34 ± 0.32	2.43 ± 0.26	1.70 ± 0.17	<0.0001
Protein carbonyls (nmol/mg)	0.81 ± 0.09	0.75 ± 0.07	1.99 ± 0.22	1.39 ± 0.17	1.35 ± 0.18	1.04 ± 0.11	<0.0001
GSH/GSSG ratio	8.56 ± 0.59	9.80 ± 1.39	4.14 ± 0.50	6.34 ± 0.67	6.17 ± 0.41	8.20 ± 0.89	<0.0001
SOD (U/mg protein)	19.0 ± 1.5	19.5 ± 1.3	11.4 ± 1.0	15.4 ± 1.3	14.4 ± 1.3	17.8 ± 0.9	<0.0001
CAT (U/mg protein)	46.5 ± 4.4	46.5 ± 3.2	29.1 ± 2.6	36.7 ± 3.4	36.0 ± 3.4	41.2 ± 2.7	<0.0001
GPx (U/mg protein)	32.3 ± 2.1	34.8 ± 3.3	20.3 ± 2.0	28.1 ± 3.1	25.6 ± 1.9	31.0 ± 1.6	<0.0001
TNF-α (pg/mg protein)	14.9 ± 2.2	14.0 ± 2.4	46.7 ± 5.4	30.5 ± 3.7	32.6 ± 4.4	22.6 ± 2.4	<0.0001
IL-1β (pg/mg protein)	18.7 ± 2.1	17.6 ± 1.9	62.6 ± 7.6	39.7 ± 4.5	41.4 ± 4.9	29.4 ± 2.8	<0.0001
IL-6 (pg/mg protein)	20.8 ± 2.4	21.5 ± 2.0	83.6 ± 9.5	52.0 ± 4.3	56.2 ± 3.7	33.9 ± 4.9	<0.0001
IL-10 (pg/mg protein)	19.3 ± 1.6	21.9 ± 2.9	24.1 ± 2.2	30.4 ± 4.7	29.0 ± 3.7	41.7 ± 4.1	<0.0001

UA-associated mitochondrial quality-control and biogenesis transcripts increased most in the combined group

UA-containing groups showed the clearest transcriptional response. Pink1 expression increased from 1.20 ± 0.14-fold in EX-CON to 1.81 ± 0.15-fold in EX-UA and 2.13 ± 0.21-fold in EX-UA+Cr. Park2 showed a parallel pattern. Ppargc1a, Tfam and Nrf1 were depressed or near sedentary levels in EX-CON but increased with UA and were highest with the combined treatment. Creatine alone produced smaller changes in these transcripts. A significant UA×creatine interaction was observed for Pink1 (P=0.023), whereas the interaction was not significant for Ppargc1a, indicating that the combination did not produce uniform transcriptional supra-additivity.

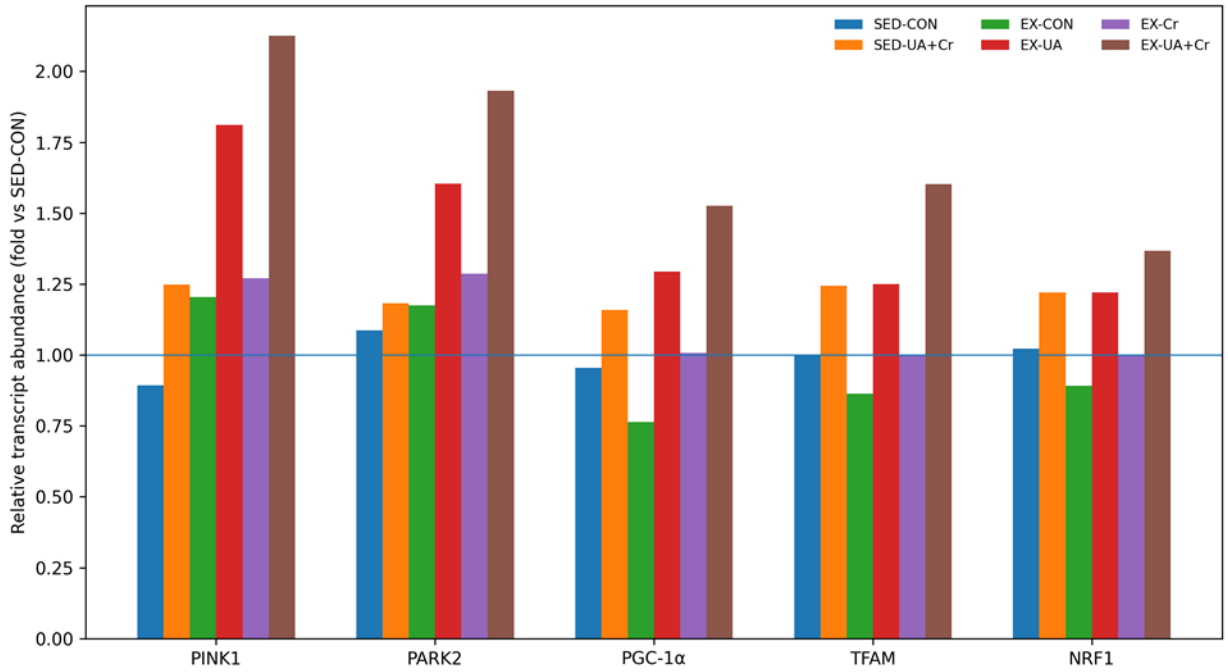


Figure 7. Relative transcript abundance of mitochondrial quality-control and biogenesis genes at 48 h. Values are group means normalized to the sedentary control calibrator. UA-containing groups showed the largest increases in PINK1/Parkin and PGC-1α/TFAM/NRF1-associated transcripts.

Table 6. Relative transcript abundance at 48 h (fold vs SED-CON calibrator).

Gene	SED-CON	SED-UA+Cr	EX-CON	EX-UA	EX-Cr	EX-UA+Cr	P, combo vs EX-CON
Pink1	0.89 ± 0.14	1.25 ± 0.17	1.20 ± 0.14	1.81 ± 0.15	1.27 ± 0.21	2.13 ± 0.21	<0.0001
Park2	1.09 ± 0.19	1.18 ± 0.16	1.18 ± 0.14	1.60 ± 0.17	1.29 ± 0.08	1.93 ± 0.24	<0.0001
Ppargc1a	0.95 ± 0.09	1.16 ± 0.13	0.76 ± 0.10	1.29 ± 0.13	1.01 ± 0.11	1.53 ± 0.14	<0.0001
Tfam	1.00 ± 0.11	1.24 ± 0.13	0.86 ± 0.12	1.25 ± 0.10	1.00 ± 0.10	1.60 ± 0.16	<0.0001
Nrf1	1.02 ± 0.13	1.22 ± 0.14	0.89 ± 0.11	1.22 ± 0.14	1.00 ± 0.16	1.37 ± 0.17	<0.0001

Cross-domain associations linked functional recovery to energetic preservation

Across the 48 exercised animals, 48-h grip-force recovery correlated positively with muscle ATP (Spearman ϱ=0.76, P<0.0001) and Complex I activity (ϱ=0.56, P=0.0000) and inversely with CK AUC (ϱ=-0.78, P<0.0001). ATP itself correlated with Complex I activity (ϱ=0.67, P<0.0001). These associations remained significant after false-discovery correction and supported concordance between functional recovery and the bioenergetic/mitochondrial phenotype.

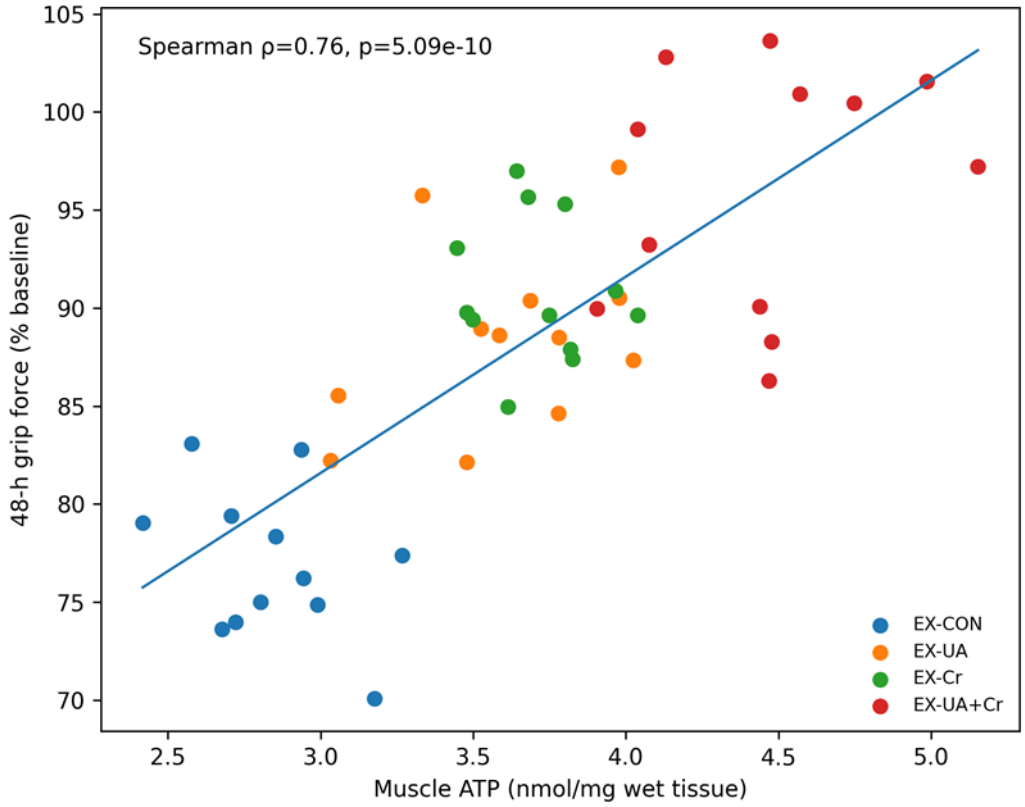


Figure 8. Relationship between 48-h muscle ATP and grip-force recovery among exercised rats (n=48). Points represent individual animals; the line is a simple least-squares visual guide. Inference used Spearman correlation.

Discussion

The principal finding was that UA and creatine, given before a standardized eccentric-biased exercise challenge, accelerated functional recovery and preserved multiple dimensions of skeletal-muscle energy homeostasis. The combined intervention produced the strongest 48-h grip-force recovery, the lowest CK/LDH exposure, the greatest preservation of ATP and respiratory-chain enzyme activity, the most favorable redox and inflammatory profile and the highest expression of several mitochondrial quality-control/biogenesis transcripts. The cross-domain correlations are particularly important because they connect functional recovery to ATP status, Complex I activity and the magnitude of circulating muscle-injury enzyme exposure rather than relying on any single biomarker.

The downhill-running response was consistent with the established characteristics of eccentric-biased injury in rats. Armstrong and colleagues showed that prolonged downhill running at a 16° decline produces delayed biochemical and structural manifestations of muscle injury, including late enzyme elevations [1,2]. In the present study, force loss was greatest soon after exercise, whereas CK and LDH peaked later, emphasizing the different biological information carried by functional and circulating markers. This distinction is consistent with contemporary understanding that exercise-induced muscle damage is a multi-component process involving mechanical strain, disturbed calcium handling, membrane permeability, redox signaling, inflammation and mitochondrial stress [3,4].

The UA response is mechanistically consistent with prior observations. UA was originally reported as a mitophagy-inducing metabolite that enhanced rodent muscle function and metabolism [5]. human trials have reported improvements in endurance and/or strength and associated changes in mitochondrial health [6-10]. One trial involving endurance athletes reported that UA improved and protected muscle function, decreased creatine kinase levels, and altered muscle pathways associated with inflammation and mitochondria. However, changes in muscle function and race performances were not significantly altered [9]. The present studies extended these observations by providing

additional insights regarding muscle function, ATP, the respiratory chain and the PINK1/Parkin pathway.

Creatine also altered muscle metabolism. Increased muscle creatine, reduced atp breakdown and enhanced muscle recovery were observed in both the ex-cr and ex-ua + cr groups. Increased glycogen levels and reduced blood lactate levels were observed in the ex-ua + cr group. Increased strength and faster recovery of muscle function were observed in athletes following the ingestion of creatine. The studies reporting the effects of creatine were consistent and positively influenced the observed outcomes. However, studies have reported no effects of creatine supplementation. The variation in findings was primarily influenced by the creatine loading period, duration of the exercise and the health status of participants at baseline.

The cautious interpretation of the combined ua and creatine study was more appropriate than a simple "synergy" observation.

The ua + creatine interaction was numerically greater than either single treatment for each outcome measured but was not statistically significant. Given that ua and creatine altered different pathways (quality control pathway and atp regeneration pathway, respectively), these results may suggest that ua and creatine act additively and/or synergistically to improve a range of mitochondrial function related outcomes (e.g. tnf-α and pink1). That said, it is important to note that the ua + creatine interventions may have acted synergistically to alter multiple systems in a way that improved the overall health of the muscle and, in turn, improved the overall recovery of the subject. Thus, even in the absence of statistical significance, ua + creatine interventions warrant further consideration.

In line with clinical findings of declining inflammatory markers during ua supplementation, ua-containing groups demonstrated the greatest decrease in the levels of inflammatory cytokines (tnf-α, il-1β, and il-6) of all study groups. Similar to ua, creatine also lowered inflammatory and oxidative stress markers. However, changes in these variables were smaller, compared to the effect of creatine on total creatine and atp levels, indicating that the primary role of creatine is to augment energy level, rather than influence mitochondrial quality control.

Overall, the study has several major strengths. The statistical design included the random assignment of participants to the treatment groups, stratified by sex. There was masking of the study personnel and study participants for the functional and laboratory assessments, respectively. The statistical plan accounted for main effects and interaction effects. There was termin primarily evaluated paired functional and serologic assessments, with biochemical and immunologic assessments of study specimens. Numerous outcomes, including enzymatic, immunologic, and transcript assessment, also facilitated interrogation of numerous mechanisms. Imaging of study specimens was not performed, and therefore, considerable depth was not required for imaging analyses.

Important limitations should be addressed. The 48-hour time point reflects only early recovery, with long-term muscle remodeling occurring over days to weeks. The level of a given transcript does not necessarily reflect the amount of the corresponding protein, and several post-translational modifying events may occur. Further, assessments of histology and ultrastructure were lacking to fully determine fiber type and level of remodeling. The design of the study balanced the number of males and females, but the number of animals in each group may have limited the study from fully assessing possible sex-related differences. The mitochondrial targeted-up take two (ua) and creatine supplementation reduced skeletal muscle recovery time in rats following exhaustive eccentric exercise.

Declarations Ethical

Statement

The authors declare that the conducted studies fully comply with generally accepted norms of humane treatment of animals and the principles of bioethics. Ethical approval was officiall y granted by the Bioethics Committee of the Department of Internal and Preventive Medicine at the College of Veterinary Medicine, University of Wasit, Iraq (Protocol No. 874 dated 03.09.2026).

To minimize animal stress and discomfort, all handling, restriction, and blood sampling proce dures were performed in strict accordance with standard veterinary guidelines and current international principles of animal welfare.

Conclusion

Creatine supplementation increased the muscle creatine store and helped to reduce ATP depletion. UA further augmented the mitochondrial quality control and biogenesis. The combination of the two supplements improved muscle recovery the most. This study demonstrated that some strategies to mitigate the loss of bioenergetics in skeletal muscle may reduce the impact of dysfunction.

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