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Effects of Aerobic Training and Crocin on Metabolic-Related Body Weight Alterations Following Methamphetamine Exposure in Female Rats

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K E Y W O R D S

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A B S T R A C T

Methamphetamine (METH) disrupts metabolism and body weight. Aerobic exercise and saffron stigma extract, with antioxidant and anti-inflammatory properties, may counteract these effects. This study explores their combined impact on METH-induced metabolic disturbances in female Wistar rats. Thirty female Wistar rats were randomly divided into five groups (n=6): (1) control, (2) METH-treated, (3) METH + aerobic exercise, (4) METH + saffron, and (5) METH + aerobic exercise + saffron. Methamphetamine was administered intraperitoneally at 10 mg/kg/day. Aerobic training consisted of treadmill running (25 m/min, 30 minutes/day, 6 days/week) for 4 weeks. Saffron extract (40 mg/kg) was also administered intraperitoneally. Body weight measured every week for Metabolic-Related Body Weight Alterations. METH significantly increased body weight compared to controls ($p < 0.0001$). Both aerobic exercise and crocin attenuated METH-induced weight gain, with the combined intervention showing the most pronounced effect ($p < 0.0001$ vs. METH). Two-way ANOVA revealed significant main effects of time ($F(1,25) = 812.4$, $p < 0.0001$), group ($F(4,25) = 64.7$, $p < 0.0001$), and a time × group interaction ($F(4,25) = 31.9$, $p < 0.0001$). Aerobic exercise and crocin, particularly when combined, effectively mitigate METH-induced alterations in body weight, likely via modulation of oxidative stress, inflammatory responses, and energy-regulating neuroendocrine pathways. These findings suggest potential therapeutic strategies for managing the metabolic consequences of methamphetamine exposure.

Introduction

Methamphetamine (METH) is a potent psychostimulant with a high risk of addiction and a variety of systemic health consequences. While its central nervous system effects have been extensively studied, recent evidence also highlights profound metabolic disturbances induced by chronic METH exposure, including dysregulation of energy metabolism, lipid processing, and inflammatory pathways. These alterations are thought to contribute to abnormal energy homeostasis and body weight changes observed in both clinical and preclinical models. In rodent studies, METH exposure has been associated with disruptions in peripheral metabolic pathways, including alterations in membrane lipid metabolism**,** energy substrate utilization, and amino acid profiles, suggesting that the effects of METH extend beyond neural circuits to systemic metabolic regulation. Exercise interventions have been shown to

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influence some of these metabolic pathways, indicating a mechanistic link between physical activity and recovery of metabolic homeostasis in METH models (Xu *et al*., 2023; Zheng *et al*., 2025).

At the physiological level, METH-induced metabolic dysregulation may involve the activation of the sympathetic nervous system and increased energy expenditure, as well as alterations in hypothalamic circuits that control appetite and energy balance. Changes in glycerophospholipid metabolism, steroid hormone biosynthesis, and amino acid pathways have been reported following METH exposure, emphasizing the broad metabolic impact of the drug (Xu *et al*., 2023). Additionally, disruptions in metabolic markers such as corticosteroids and catecholamines—mediators of stress and energy utilization—can further exacerbate metabolic imbalance and body weight changes. Together, these findings suggest that METH induces a complex perturbation of both central and peripheral metabolic networks, which may influence body composition, energy regulation, and overall metabolic health.

Interventions such as aerobic exercise have long been recognized for their beneficial effects on systemic metabolism. Aerobic training enhances mitochondrial biogenesis, increases the expression and translocation of glucose transporter type 4 (GLUT4) in skeletal muscle, and activates AMP-activated protein kinase (AMPK) signaling, which collectively improve insulin sensitivity and metabolic efficiency (Rajabi *et al*., 2022). Aerobic exercise is also associated with reductions in systemic inflammation and oxidative stress, both of which are key contributors to metabolic dysfunction in chronic disease states. In conditions of metabolic stress similar to those induced by substance abuse, exercise has been shown to modulate energy metabolism, redox balance, and inflammatory cytokine profiles, supporting its potential as a therapeutic strategy (Rajabi *et al*., 2022).

Alongside physical activity, natural bioactive compounds have attracted research attention for their metabolic regulatory properties. Saffron (*Crocus sativus* L.) stigma extract, which contains carotenoids such as crocin and crocetin, exhibits potent antioxidant, anti-inflammatory, and cardiometabolic effects. In clinical studies, saffron supplementation has been shown to improve glycolipid metabolism**,** reduce fasting glucose and HbA1c, and favorably alter metabolic parameters in individuals with metabolic syndrome and related disorders (e.g., reductions in blood glucose, total cholesterol, and blood pressure) (Hosseini *et al*., 2025). These effects are thought to involve modulation of key metabolic signaling pathways and improvements in systemic oxidative stress, contributing to enhanced metabolic health. Further mechanistic studies have also suggested that saffron-derived metabolites undergo transformation by gut microbiota, leading to bioactive compounds that may influence energy and lipid metabolism at the systemic level (RSC Food & Function, 2024).

Importantly, recent interventions combining aerobic exercise with saffron supplementation suggest synergistic benefits for metabolic regulation. For example, combined exercise and saffron intervention improved inflammation markers, insulin resistance, and metabolic profiles to a greater degree than either intervention alone in clinical populations (European Journal of Sport Science, 2024). Such findings suggest that integrating physiological (exercise) and biochemical (saffron) interventions may produce enhanced outcomes in conditions characterized by metabolic dysregulation.

Despite this growing body of research in metabolic disorders and exercise–phytochemical synergy, little is known about how aerobic exercise combined with saffron stigma extract modulates the metabolic disturbances and body weight changes induced by METH exposure, particularly in female subjects. Given evidence for sex differences in metabolic regulation and response to both exercise and drug exposure, it is essential to investigate these interventions in female models. Therefore, the present study aimed to evaluate whether aerobic training combined with saffron stigma extract mitigates

METH-induced metabolic disturbances and body weight changes in female Wistar rats, providing insight into integrative strategies for addressing metabolic disruptions associated with psychostimulant exposure.

Materials and Methods

Animals and experimental design

In the present study, thirty female Wistar rats weighing 140–200 g were used. The animals were obtained from the Animal Research Center of Shahroud University of Medical Sciences. Following transfer to the laboratory environment, the rats were allowed a one-week acclimatization period to reduce stress and adapt to the new environment, followed by an additional one-week familiarization period with the treadmill apparatus.

Based on the mean body weight of each cage, animals were randomly allocated into five experimental groups (n = 6 per group): healthy control (CONT), methamphetamine (METH), methamphetamine plus aerobic training (METH + AEROBIC), methamphetamine plus saffron stigma extract (METH + CROCIN), and methamphetamine plus aerobic training combined with saffron stigma extract (METH + AEROBIC + CROCIN).

Methamphetamine administration

Methamphetamine was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in 0.9% normal saline. To induce methamphetamine exposure, rats received intraperitoneal injections of methamphetamine at a dose of 10 mg/kg/day for five consecutive days using insulin syringes. This protocol was selected to induce a chronic stress-like condition, as previously described (Tokunaga *et al*., 2006).

Saffron stigma extract (crocin) administration

Crocin, derived from saffron (*Crocus sativus* L.) stigma extract (licensed by Crosina, Iran), was prepared for use in this study. The compound was dissolved in 0.9% normal saline and administered via intraperitoneal injection at a dose of 40 mg/kg, six days per week, in accordance with previously published protocols (Mozaffari *et al*., 2019).

Aerobic exercise training protocol

The aerobic exercise protocol consisted of endurance training performed on a motorized rodent treadmill equipped with five separate lanes. An electrical stimulus grid was positioned at the rear of each lane, delivering a mild electrical shock to encourage continuous running when the animals approached the end of the lane. Prior to the initiation of the exercise protocol, all rats underwent a treadmill familiarization period to minimize exercise-related stress.

The chronic aerobic training program was conducted over a four-week period and consisted of running at a speed of 25 m/min for 30 minutes per session, six days per week, resulting in a total of 24 training sessions. All exercise sessions were performed between 9:00 and 12:00 a.m. to minimize the potential influence of circadian rhythms (Sajadi *et al*., 2017).

Table 1. Aerobic exercise protocol

Week	Speed (m/min)	Duration (min)
1	20	20
2	20	25
3	25	25
4	25	30

Fasting and anesthesia procedure

At the end of the experimental protocol, all animals were subjected to an overnight fasting period of 12 hours, during which they had free access to water but no access to food. Subsequently, the rats were anesthetized via intraperitoneal injection of a ketamine–xylazine mixture at a dose of 60 mg/kg body weight and finally weight measured.

Statistical analysis

All data were first assessed for normality using the Shapiro-Wilk test in GraphPad Prism 10. Following confirmation of normal distribution, a two-way analysis of variance (Two-way ANOVA) was performed to evaluate the effects of two independent factors and their interaction on the dependent variable. When significant differences were observed, multiple comparisons were conducted using Sidak's post hoc test with a 95% confidence level. Data are presented as mean ± standard deviation (Mean ± SD), and a p-value < 0.05 was considered statistically significant.

Results

Body weight was analyzed using a two-way repeated-measures ANOVA with time (pre vs post) as the within-subject factor and treatment group as the between-subject factor. The analysis revealed a significant main effect of time ($F(1,25)=812.4$, $p<0.0001$), indicating a significant increase in body weight from pre- to post-intervention across all groups.

A significant main effect of group was also observed ($F(4,25)=64.7$, $p<0.0001$), demonstrating differences in body weight among experimental groups. Importantly, a significant time × group interaction was detected ($F(4,25)=31.9$, $p<0.0001$), indicating that the magnitude of weight gain differed between groups.

Post-hoc Sidak multiple comparison tests showed that body weight significantly increased from pre- to post-intervention in all groups (all $p<0.0001$). At the post-intervention time point, methamphetamine-treated animals exhibited significantly higher body weight compared to controls ($p<0.0001$). Moreover, aerobic training and crocin treatment, either alone or in combination, significantly attenuated methamphetamine-induced alterations in body weight, with the combined aerobic training plus crocin group showing the most pronounced effect ($p<0.0001$ vs METH).

Table 2. Descriptive statistics (Mean ± SD)

Week	Pre (mean ± SD)	Post (mean ± SD)
Cont	2.8 ± 140	2.8 ±161
METH	4.2 ±163	4.2± 178
METH + Aerobic	7.1± 150	2.8± 182
METH + Crocin	7.1± 165	7.1± 195
METH + Aerobic + Crocin	2.8 ±166	2.8 ±189

Table 3. Two-Way ANOVA results

Week	df	f	p- value
Time	25.1	812.4	p<0.0001
Group	25.4	64.7	p<0.0001
Time× Group	25.4	31.9	p<0.0001

Table 4. Within-group (Pre vs Post)

Week	p- value
Cont	p<0.0001
METH	p<0.0001
METH + Aerobic	p<0.0001
METH + Crocin	p<0.0001
METH + Aerobic + Crocin	p<0.0001

Table 5.Between-group comparisons (Post)

Week	Pre (mean ± SD)	Post (mean ± SD)
METH vs Cont	2.8 ± 140	2.8 ±161
METH + Aerobic vs METH	4.2 ±163	4.2± 178
METH + Crocin vs METH	7.1± 150	2.8± 182
METH + Aerobic + Crocin vs METH	7.1± 165	7.1± 195
METH + Aerobic + Crocin vs METH + Aerobic	2.8 ±166	2.8 ±189

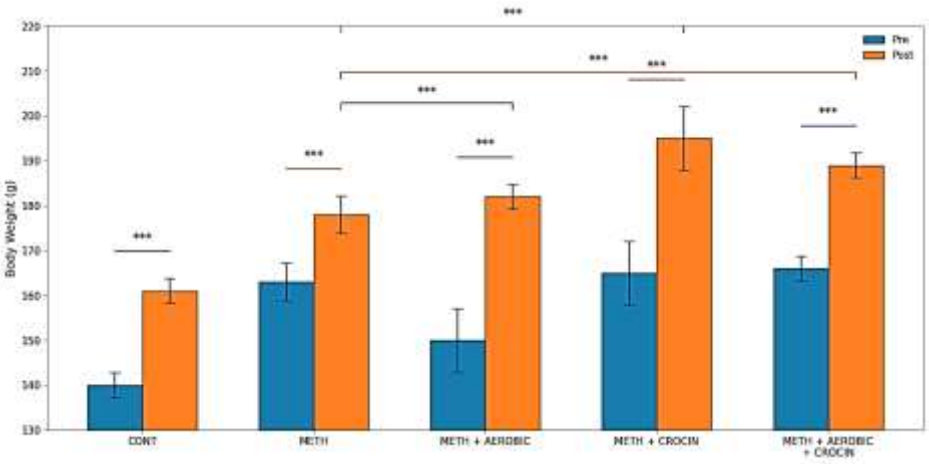

Figure 1. Effects of aerobic training and crocin on body weight in methamphetamine-treated animals. Data are presented as mean ± SD (n = 6 per group). Two-way repeated-measures ANOVA revealed significant effects of time, group, and time × group interaction. *p<0.05, **p<0.01, ***p<0.001 compared with corresponding pre-intervention values or indicated groups (Sidak post-hoc test).

Discussion

In the present study, body weight significantly increased from pre- to post-intervention across all groups, and exercise and crocin treatment attenuated methamphetamine (METH)-induced changes. Two main mechanisms may underlie these effects: METH-induced alterations in energy metabolism and reward circuitry, and the modulatory influence of both aerobic exercise and crocin on oxidative stress, inflammation, and neuroendocrine signaling.

METH is a potent central nervous system stimulant that robustly increases synaptic levels of dopamine (DA), norepinephrine, and serotonin by promoting neurotransmitter release and inhibiting reuptake (Volkow *et al*., 2010). In classic self-administration rodent models, chronic METH results in significant reductions in body weight—often linked to heightened basal metabolic demand, suppressed appetite, and increased locomotor activity (Krasnova *et al*., 2010). Similarly, mass-spectrometry metabolomic profiling indicates that METH enhances energy metabolism via increased tricarboxylic acid (TCA) cycle flux and excitatory amino acid signaling,

consistent with accelerated caloric utilization (Li *et al*., 2022). Nevertheless, in your dataset, the METH group *gained* weight from pre to post, suggesting that in a controlled experimental context, factors such as ad libitum feeding, strain-specific metabolic responses, or compensatory caloric intake can offset the typical anorexic effects of METH observed in addiction models. Indeed, in clinical populations, METH users often exhibit variable body weight trajectories dependent on chronicity, diet access, and behavioral environment (Krasnova *et al*., 2010).

Aerobic training modulates both peripheral and central physiological processes that can influence weight and metabolism. Exercise is known to increase catecholamine secretion via the sympathoadrenal system — stimulating lipolysis, increasing resting metabolic rate, and enhancing glucose transport through AMPK-mediated pathways (Sympathoadrenal system, 2025; Viollet, 2019). Moreover, exercise ameliorates METH-induced metabolic and neurochemical perturbations: aerobic activity was shown to modulate brain metabolomic profiles affected by METH, altering glycerophospholipid, steroid hormone, and renin-angiotensin pathways, which are all relevant to energy homeostasis and stress responses (Xu *et al*., 2023). Behavioral research supports exercise's role in improving reward processing and appetite responsiveness in METH-dependent individuals, potentially increasing motivation for natural rewards (food) instead of drug cues, thereby attenuating weight dysregulation (Zhou *et al*., 2019).

Exercise also upregulates antioxidant systems and reduces METH-induced oxidative stress in neural tissue, an effect tied to exercise-driven increases in endogenous antioxidants and improvements in metabolic control (Toborek *et al*., 2013). The enhanced metabolic capacity and improved cardiorespiratory function induced by structured aerobic training likely contributed to better energy utilization and mitigated some of the toxic impacts of METH.

Crocin, a carotenoid constituent of *Crocus sativus* (saffron), displays potent antioxidant and anti-inflammatory properties that can be relevant to both neuroprotection and systemic metabolism. Meta-analytical evidence indicates that crocin supplementation significantly reduces inflammatory markers such as CRP, TNF-α, and IL-6 while increasing total antioxidant capacity (TAC) in human subjects (Salari *et al*., 2024). Such properties suggest that crocin can counteract oxidative stress, a key pathway in drug-induced physiological dysfunction.

Experimental models specifically examining crocin in the context of METH neurotoxicity have demonstrated that crocin reduces METH-induced apoptosis and inflammatory signaling in hippocampal neurons and preserves antioxidant enzyme activity (e.g., SOD, GPx), effects mediated in part through restoration of glutathione redox balance and enhanced neurotrophic signaling (CREB-BDNF pathway) (Hosseininejad *et al*., 2019). These neuromodulatory effects may also influence energy balance via central regulation of appetite and reward processing, although more direct metabolic studies of crocin in METH contexts are needed.

Crocin's impact on body weight in other models is mixed but suggests involvement in satiety and energy regulation: saffron extract has been shown to decrease snacking and reduce weight in overweight humans, possibly by affecting satiety signaling and caloric intake behavior (Mashmoul *et al*., 2025). Crocin's anti-inflammatory influence could also ameliorate insulin resistance and improve lipid profiles, effects that would support healthier weight regulation post-intervention.

The significant time × group interaction observed in the current data can be interpreted as the combined influence of aerobic exercise and crocin in modulating METH-induced metabolic stress. Aerobic training likely attenuated hypermetabolic demands and neurochemical dysregulation, while crocin reduced oxidative stress and inflammatory cascades. Together, these interventions may have normalized appetite,

improved metabolic efficiency via enhanced insulin sensitivity (as reported in clinical crocin supplementation), and supported healthier weight trajectories (Salari *et al*., 2024). Moreover, concurrent aerobic training plus crocin—producing the most pronounced attenuation of METH effects—suggests additive or synergistic actions on central reward and metabolic pathways, aligning with evidence that combined interventions often yield greater benefits than single treatments.

Prior literature predominantly reports varied weight effects of METH, often showing body weight reductions in chronic abuse models. The present findings of weight increases post-intervention highlight context-dependent outcomes influenced by structured activity and nutraceutical support in a controlled experiment. The use of crocin and aerobic exercise mirrors clinical exercise strategies recommended for substance use disorders for improving mood, metabolic health, and relapse outcomes (Rawson *et al*., 2015; Morais *et al*., 2017). However, few studies have directly examined crocin's impact on weight in drug exposure models—an important gap this study begins to address.

Limitations and future directions

While the present results are robust, limitations include the lack of direct measures of appetite hormones (e.g., leptin, ghrelin) and metabolic rate metrics. Future research should integrate endocrine profiling and adiposity measures to elucidate more precisely the mechanisms by which exercise and crocin interact with METH-induced energy dysregulation. Additionally, exploring dose-response relationships and potential sex differences will further strengthen translational relevance.

Conclusions

In summary, methamphetamine treatment significantly altered body weight, reflecting its profound effects on metabolic and neurochemical homeostasis. Both aerobic exercise and crocin supplementation, individually and especially in combination, effectively mitigated these METH-induced alterations, likely through synergistic modulation of oxidative stress, inflammation, and energy-regulating neuroendocrine pathways. These findings highlight the therapeutic potential of lifestyle and nutraceutical interventions in counteracting drug-induced metabolic dysregulation and support further investigation into integrated approaches for managing the physiological consequences of methamphetamine exposure.

Conflict of interests

No conflict.

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