Research Article / Araştırma Makalesi

Synergistic Effect of Aerobic Exercise Intensity and Creatine Monohydrate Supplementation on Energy Metabolism Biomarkers in Mice Cardiac Ventricular Muscle Tissue

Aerobik Egzersiz Yoğunluğunun ve Kreatin Monohidrat Takviyesinin Fare Kardiyak Ventrikül Kas Dokusunda Enerji Metabolizması Biyobelirteçleri Üzerindeki Sinerjitik Etkisi

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Abstract

Background: To investigate the combined effects of creatine monohydrate (CrM) supplementation and different intensities of aerobic exercise on key biomarkers of energy metabolism in cardiac ventricular muscle tissue of mice.
Materials and Methods: Forty-two male BALB/c mice were randomly assigned to six groups (n=7 per group): control (C), CrM supplementation without exercise (C+CrM), low-intensity exercise (LIE), LIE with CrM supplementation (LIE+CrM), high-intensity exercise (HIE), and HIE with CrM supplementation (HIE+CrM). Over eight weeks, exercise groups underwent treadmill training five days per week, while CrM groups received a diet enriched with 4% CrM. Key biomarkers—myocyte enhancer factor 2A (MEF2A), monocarboxylate transporter 1 (MCT1), pyruvate dehydrogenase (PDH), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), and mitochondrial transcription factor A (TFAM)—were quantified in cardiac ventricular muscle tissue using enzyme-linked immunosorbent assay (ELISA).
Results: The LIE+CrM group showed significant upregulation of MEF2A compared to the C+CrM group ($p<0.05$). PDH levels were significantly higher in both the LIE+CrM and HIE+CrM groups compared to the C and C+CrM groups ($p<0.05$). PGC-1α levels were highest in the LIE+CrM group, approaching statistical significance ($p = 0.051$). TFAM expression was significantly elevated in the LIE+CrM group compared to the LIE, HIE, and C+CrM groups ($p<0.05$). MCT1 levels exhibited a non-significant trend toward increase in the LIE+CrM group.
Conclusions: CrM supplementation combined with low-intensity aerobic exercise significantly enhances key biomarkers associated with mitochondrial biogenesis and energy metabolism in cardiac ventricular muscle tissue. These findings suggest a synergistic effect that could optimize cardiac energy metabolism and improve cardiovascular health.

Keywords: Creatine monohydrate; Aerobic exercise intensity; Cardiac energy metabolism; Mitochondrial biogenesis

Öz

Amaç: Kreatin monohidrat (CrM) takviyesinin ve farklı yoğunluktaki aerobik egzersizin farelerin kardiyak ventriküler kas dokusundaki enerji metabolizmasının temel biyobelirteçleri üzerindeki kombine etkilerini araştırmaktır.
Materyal ve Metod: Kırk iki erkek BALB/c faresi rastgele altı gruba ayrıldı (grup başına n=7): kontrol (C), CrM takviyesi (C+CrM), düşük yoğunluklu egzersiz (LIE), CrM takviyesiyle LIE (LIE+CrM), yüksek yoğunluklu egzersiz (HIE) ve CrM takviyesiyle HIE (HIE+CrM). Sekiz hafta boyunca, egzersiz grupları haftada beş gün koşu bandında egzersiz uygulanırken, CrM grupları %4 CrM ile zenginleştirilmiş bir diyet aldı. Anahtar biyobelirteçler; miyosit güçlendirici faktör 2A (MEF2A), monokarboksilat taşıyıcı 1 (MCT1), pirüvat dehidrogenaz (PDH), peroksisom proliferatör aktive reseptör gama koaktivatör 1-alfa (PGC-1α) ve mitokondriyal transkripsiyon faktörü A (TFAM) ELISA testleri kullanılarak kardiyak ventriküler kas dokusunda kantifize edildi.
Bulgular: LIE+CrM grubu, C+CrM grubuna kıyasla MEF2A'da önemli bir artış gösterdi ($p<0,05$). PDH seviyeleri, hem LIE+CrM hem de HIE+CrM gruplarında, C ve C+CrM gruplarına kıyasla önemli ölçüde daha yüksekti ($p<0,05$). PGC-1α seviyeleri, LIE+CrM grubunda en yüksekti ve istatistiksel anlamlılığa yaklaşıyordu ($p=0,051$). TFAM ekspresyonu, LIE+CrM grubunda, LIE, HIE ve C+CrM gruplarına kıyasla önemli ölçüde yüksekti ($p<0,05$). MCT1 seviyeleri, LIE+CrM grubunda anlamlı olmayan bir artış eğilimi gösterdi.
Sonuç: Düşük yoğunluklu aerobik egzersizle birleştirilen CrM takviyesi, kardiyak ventriküler kas dokusunda mitokondriyal biyogenez ve enerji metabolizmasıyla ilişkili temel biyobelirteçleri önemli ölçüde artırır. Bu bulgular, kardiyak enerji metabolizmasını optimize edebilecek ve kardiyovasküler sağlığı iyileştirebilecek sinerjik bir etkiye işaret ediyor.

Anahtar kelimeler: Kreatin monohidrat; Aerobik egzersiz yoğunluğu; Kardiyak enerji metabolizması; Mitokondriyal biyogenez

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Introduction

The intricate balance of energy metabolism in cardiac tissue is fundamental to maintaining cardiovascular health, as the heart requires a continuous supply of adenosine triphosphate (ATP) to support contraction and other metabolic processes. Mitochondria, the primary site of ATP production, play a crucial role in this process, and dysfunctions in mitochondrial energy metabolism have been implicated in the pathogenesis of numerous cardiovascular diseases, including heart failure and ischemia-reperfusion injury (1–3). Optimizing mitochondrial function through lifestyle and pharmacological interventions has emerged as a promising strategy to prevent or delay the progression of cardiac pathologies (2,4).

Aerobic exercise is widely recognized as one of the most effective non-pharmacological interventions to enhance mitochondrial biogenesis, oxidative phosphorylation, and energy metabolism in cardiac tissue (5–7). Recent advances in exercise physiology suggest that the benefits of aerobic exercise on mitochondrial function may be further enhanced when combined with creatine monohydrate (CrM) supplementation (8,9). This combination has shown potential for improving both ATP availability and overall mitochondrial efficiency; however, its impact on the energy metabolism of cardiac muscle under varying exercise intensities remains underexplored.

Creatine monohydrate is well-known for its role in increasing phosphocreatine stores in skeletal muscle, thereby enhancing ATP resynthesis during high-intensity exercise (10). Creatine monohydrate is well-known for its role in increasing phosphocreatine stores in skeletal muscle, thereby enhancing ATP resynthesis during high-intensity exercise (9,11). Studies have demonstrated that CrM supplementation can enhance the efficiency of mitochondrial ATP production through the phosphocreatine system under conditions of increased energy demand (12). Additionally, CrM may enhance oxidative capacity by upregulating key markers of mitochondrial biogenesis and function, including peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) and mitochondrial transcription factor A (TFAM), both critical for mitochondrial DNA replication and energy production in cardiac muscle (8,9). These findings suggest that CrM could serve as a valuable adjunct to aerobic exercise for improving cardiac energy metabolism, though its effects across different exercise intensities warrant further investigation.

Aerobic exercise induces a range of beneficial adaptations in cardiac muscle, primarily through the upregulation of key regulatory proteins involved in mitochondrial function (13). Myocyte enhancer factor 2A (MEF2A) and monocarboxylate transporter 1 (MCT1) are essential for maintaining cellular energy homeostasis and supporting the metabolic shifts required during exercise. MEF2A plays a critical role in exercise-induced mitochondrial biogenesis (14), while MCT1 facilitates the transport of lactate and pyruvate, which are integral to maintaining metabolic balance during prolonged aerobic exercise (15). Exercise-induced upregulation of these markers has been associated with improved mitochondrial function and energy metabolism in cardiac tissue (14–16). Recent studies have emphasized the importance of mitochondrial adaptation in response to aerobic exercise for enhancing cardiovascular outcomes, particularly in preventing heart failure and improving cardiac performance in athletes (17).

Despite accumulating evidence supporting the benefits of aerobic exercise and CrM supplementation as independent interventions, their combined effects on cardiac mitochondrial function remain largely unexplored. Given CrM's potential to synergistically amplify the mitochondrial adaptations induced by aerobic exercise, this study aims to investigate the effects of varying intensities of aerobic exercise combined with CrM supplementation on key biomarkers of energy metabolism in cardiac ventricular muscle tissue. The primary focus will be on the expression of MEF2A, MCT1, pyruvate dehydrogenase (PDH), PGC-1α, and TFAM—all critical regulators of mitochondrial biogenesis and energy metabolism. By elucidating the molecular mechanisms underlying the combined impact of aerobic exercise and CrM supplementation, this study seeks to contribute novel insights into optimizing cardiac function and endurance, particularly in populations at risk for cardiovascular diseases or those aiming to enhance athletic performance.

Materials and Methods

Ethical Approval

The experimental protocol was reviewed and approved by the Harran University Animal Experiments Local Ethics Committee (Approval No.: 2024/005, Decision No.: 01-11). All procedures conformed to the ethical standards outlined in the Guide for the Care and Use of Laboratory Animals. The research was conducted at the Harran University Animal Experimentation and Research Center, where all animals were housed under controlled conditions.

Experimental Animals and Housing Conditions

Forty-two male BALB/c mice, aged 8–10 weeks and weighing 20–30 g, were used in this study. Male mice were selected to minimize hormonal fluctuations, particularly estrogen effects, which could influence physiological responses to exercise and creatine monohydrate (CrM) supplementation. The animals were housed in transparent polycarbonate cages, with seven mice per cage, allowing for continuous observation. Standard environmental conditions were maintained: a 12-hour light/dark cycle, temperature of 22 ± 1 °C, and relative humidity of 50–60%. Mice had ad libitum access to standard rodent chow and water. Body weights were recorded weekly to monitor health status and growth. Daily inspections of food and water were conducted to ensure animalwelfare and promptly address any potential health concerns.

Study Design

The mice were randomly assigned to six experimental groups (n=7 per group), each subjected to a distinct combination of dietary intervention and exercise intensity over an 8-week period. The experimental groups included: a control group receiving a standard diet without exercise (C Group), a control group receiving CrM supplementation without exercise (C+CrM Group), a low-intensity exercise group on a standard diet (LIE Group), a low-intensity exercise group receiving CrM supplementation (LIE+CrM Group), a high-intensity exercise group on a standard diet (HIE Group), and a high-intensity exercise group receiving CrM supplementation (HIE+CrM Group). This randomized controlled design aimed to examine the individual and combined effects of CrM supplementation and varying exercise intensities on energy metabolism in cardiac ventricular muscle tissue.

Creatine Monohydrate Supplementation

CrM was incorporated into the diets of the designated groups at a concentration of 4% w/w, following established protocols in the literature(8,18). The CrM-enriched feed was obtained from a commercial supplier to ensure consistency in dosing and quality control throughout the 8-week intervention. Groups not receiving CrM supplementation (C, LIE, and HIE) were provided with standard rodent chow under identical conditions.

Exercise Protocol

The exercise regimen was conducted using a specialized small-animal treadmill (Ugo Basile, Italy) equipped with adjustable settings for speed, duration, and incline. Exercise sessions were carried out five days per week (Monday to Friday) between 09:00 and 13:00 to control for diurnal variations that could impact physiological responses.

All exercise groups underwent a one-week familiarization phase, running at 4 m/min for 5 minutes per day at a 0° incline to minimize stress associated with treadmill use. Following acclimation, exercise intensity was progressively increased based on group assignment:

Low-Intensity Exercise (LIE) Groups: Mice ran at a consistent speed of 8 m/min for 30 minutes daily at a 0° incline throughout the 8-week period.

High-Intensity Exercise (HIE) Groups: Mice followed a progressive intensity protocol:

Week 1: 8 m/min for 10 minutes
Week 2: 12 m/min for 20 minutes
Week 3: 18 m/min for 30 minutes
Week 4: 21 m/min for 30 minutes
Weeks 5–8: 24 m/min for 30 minutes

All exercise was performed at a 0° incline. Non-exercise groups were placed in the exercise environment for the same duration as the exercise groups to control for environmental variables such as noise and movement, accounting for any potential stress or influences on experimental outcomes (19,20). This exercise protocol is outlined in Figure 1.

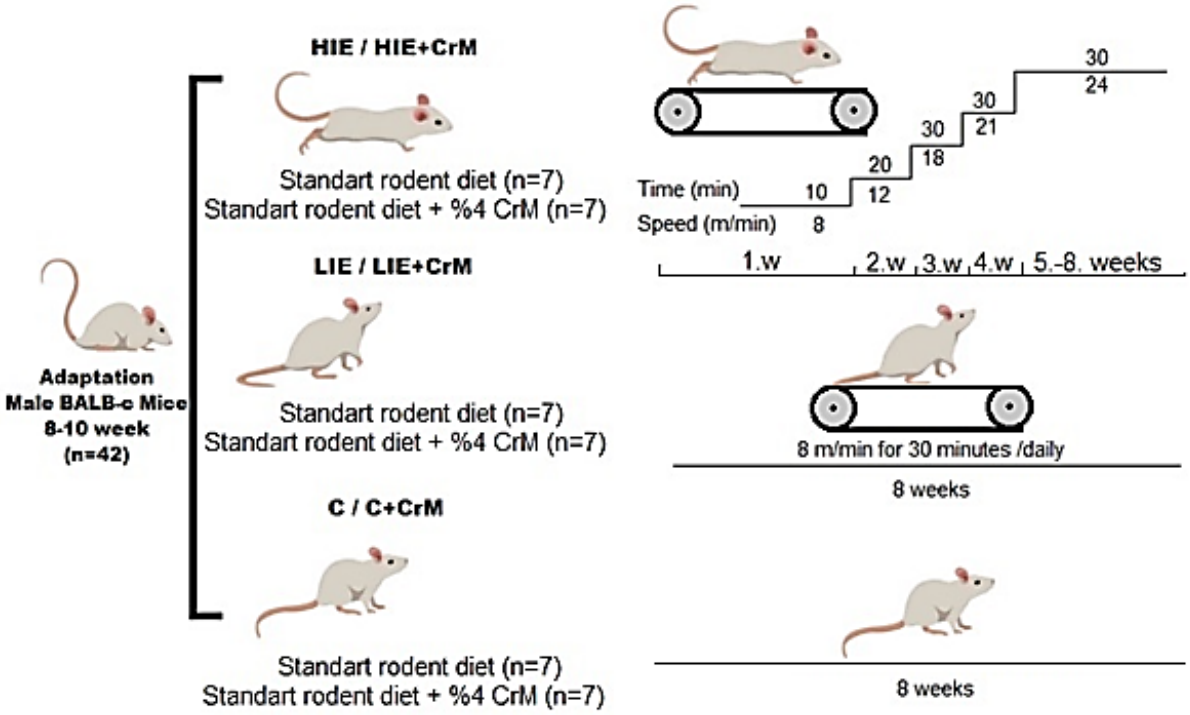

Figure 1. Exercise protocol. **C**: Control; **C+CrM**: Control + Creatine Monohydrate; **LIE**: Low-Intensity Exercise; **LIE+CrM**: Low-Intensity Exercise + Creatine Monohydrate; **HIE**: High-Intensity Exercise; **HIE+CrM**: High-Intensity Exercise + Creatine Monohydrate.

Tissue Collection and Homogenization

Forty-eight hours after the final exercise session, to avoid capturing acute exercise-induced effects, all animals were anesthetized via intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Left ventricular muscle tissue was immediately excised, flash-frozen in liquid nitrogen, and stored at –86 °C until analysis.

On the day of analysis, cardiac tissue samples were homogenized at a concentration of 10% (w/v) in 0.1 M phosphate-buffered saline (PBS, pH 7.4) using a bead homogenizer (Retsch MM 400, Germany). Homogenates were centrifuged at 10,000 rpm for 5 minutes at 4 °C, and the supernatant was collected for biomarker measurements. Protein concentrations in the supernatants were determined using the µDrop Plate method (Varioskan™ LUX, Thermo Fisher Scientific), with results expressed in mg/mL.

M*easurement of Energy Metabolism Biomarkers*

Key biomarkers related to mitochondrial function and energy metabolism were quantified in cardiac ventricular muscle tissue using enzyme-linked immunosorbent assay (ELISA) kits (FineTest, Wuhan Fine Biotech Co., Ltd., China). The biomarkers analyzed included:

Myocyte Enhancer Factor 2A (MEF2A) (Catalog No.: EM7629): A transcription factor regulating muscle differentiation and mitochondrial adaptation to exercise.

Monocarboxylate Transporter 1 (MCT1) (Catalog No.: EM0793): Essential for lactate and pyruvate transport during exercise.

Pyruvate Dehydrogenase Complex (PDH) (Catalog No.: EM1274): Facilitates the conversion of pyruvate to acetyl-CoA in the tricarboxylic acid cycle.

Peroxisome Proliferator-Activated Receptor Gamma Co-activator 1-Alpha (PGC-1α) (Catalog No.: EM0534): A key regulator of mitochondrial biogenesis and oxidative metabolism.

Mitochondrial Transcription Factor A (TFAM) (Catalog No.: EM2518): Responsible for mitochondrial DNA replication and transcription.

All assays were performed according to the manufacturer's protocols. Results were normalized to protein concentration and expressed as pg/mg protein or ng/mg protein in the supernatants.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Data were tested for normality using the Shapiro-Wilk test; all variables were normally distributed ($p > 0.05$). One-way analysis of variance (ANOVA) was used to compare groups, followed by Tukey's honestly significant difference (HSD) post hoc test for pairwise comparisons. Data are presented as mean ± standard deviation (SD). A significance level of $\alpha = 0.05$ was set for all statistical tests.

Results

The study assessed the effects of creatine monohydrate (CrM) supplementation combined with different intensities of aerobic exercise—low-intensity exercise (LIE) and high-intensity exercise (HIE)—on key biomarkers of energy metabolism in cardiac ventricular muscle tissue. Significant alterations were observed across the experimental groups, providing insights into the molecular mechanisms modulated by CrM and exercise intensity (Table 1).

Table 1. Comparison of Energy Metabolism Biomarkers in Cardiac Ventricular Muscle Tissue Among Control, Exercise, and Creatine Monohydrate Supplementation Groups

	GROUPS						
	C	**C+CrM**	**LIE**	**LIE+CrM**	**HIE**	**HIE+CrM**	***p***
MEF2A (pg/mg protein)	31.28 ± 4.05	29.63 ± 3.79	31.63 ± 8.19	41.93 ± 9.60$^{\beta}$	36.13 ± 3.85	37.54 ± 11.36	0.033
MCT1 (ng/mg protein)	50.37 ± 6.17	46.56 ± 7.43	42.58 ± 11.08	57.50 ± 10.89	50.55 ± 11.53	51.75 ± 13.57	0.177
PDH (ng/mg protein)	50.08 ± 5.87	47.65 ± 7.32	56.38 ± 14.01	73.14 ± 11.92$^{\alpha,\beta}$	59.35 ± 16.70	73.89 ± 18.69$^{\#,¥}$	0.001
PGC-1α (ng/mg protein)	40.97 ± 5.03	38.98 ± 2.42	43.44 ± 15.21	56.92 ± 14.10	44.14 ± 7.86	50.05 ± 12.11	0.051
TFAM (pg/mg protein)	27.45 ± 2.87	23.01 ± 3.25	22.27 ± 5.68	30.86 ± 5.96$^{\beta,£,§}$	22.50 ± 3.99	25.66 ± 5.82	0.010

***C**: Control; **C+CrM**: Control + Creatine Monohydrate; **LIE**: Low-Intensity Exercise; **LIE+CrM**: Low-Intensity Exercise + Creatine Monohydrate; **HIE**: High-Intensity Exercise; **HIE+CrM**: High-Intensity Exercise + Creatine Monohydrate. **α**: Statistically significant difference between **LIE+CrM** and **C**; **β**: Statistically significant difference between **LIE+CrM** and **C+CrM**; **#**: Statistically significant difference between **HIE+CrM** and **C**; **¥**: Statistically significant difference between **HIE+CrM** and **C+CrM**; **£**: Statistically significant difference between **LIE+CrM** and **LIE**; **§**: Statistically significant difference between **LIE+CrM** and **HIE**.*

MEF2A, a transcription factor essential for muscle differentiation and exercise-induced gene expression, showed a statistically significant difference among the groups ($p = 0.033$). MEF2A levels were significantly higher in the LIE+CrM group compared to the C+CrM group ($p < 0.05$), indicating that CrM supplementation combined with low-intensity exercise enhances MEF2A expression more effectively than CrM supplementation alone. In contrast, MCT1, responsible for lactate transport and a key player in cellular energy metabolism, did not show statistically significant variations across the groups ($p = 0.177$), although elevated levels were noted in the LIE+CrM group. PDH, a pivotal enzyme in the mitochondrial conversion of pyruvate to acetyl-CoA, displayed highly significant differences between groups ($p = 0.001$), with PDH levels in both the LIE+CrM and HIE+CrM groups being markedly higher compared to the C and C+CrM groups ($p < 0.05$). This suggests that CrM supplementation combined with aerobic exercise, regardless of intensity, significantly upregulates PDH activity in cardiac

tissue. PGC-1α, a central regulator of mitochondrial biogenesis and oxidative metabolism, demonstrated a trend toward significant intergroup variation ($p = 0.051$). The highest PGC-1α levels were observed in the LIE+CrM group compared to all other groups, implying a synergistic effect of CrM supplementation and low-intensity exercise on mitochondrial biogenesis. TFAM, essential for mitochondrial DNA replication and transcription, showed significant differences among the groups ($p = 0.010$). The LIE+CrM group exhibited significantly elevated TFAM levels compared to the LIE, HIE, and C+CrM groups ($p < 0.05$). This indicates that the combination of CrM supplementation with low-intensity exercise enhances mitochondrial transcriptional activity more effectively than either intervention alone or high-intensity exercise with CrM.

However, when the control group and the C+CrM group were compared, regardless of exercise; MEF2A, MCT1, PDH, PGC-1α and TFAM levels were found to be decreased in the C+CrM group. This decrease was not statistically significant ($p>0.05$). In addition, the change between the control group (C) and the LIE and HIE groups was also examined. While the MEF2A level was similar in the control and LIE groups, it was found to be increased in the HIE group. It was found that the MCT1 level decreased in the LIE group compared to the control group and increased in the HIE group. The differences between the groups were not statistically significant. PDH and PGC-1α levels increased in the LIE and HIE groups according to the intensity of exercise compared to the control group. In contrast, TFAM levels decreased with exercise. The differences in the control, LIE and HIE groups were not significant.

Discussion

T This preclinical study investigated the combined effects of CrM supplementation and varying intensities of aerobic exercise on energy metabolism biomarkers in murine cardiac ventricular muscle tissue. The findings indicate that CrM supplementation, particularly when coupled with LIE, significantly enhances mitochondrial biogenesis, oxidative phosphorylation, and glucose metabolism. The most pronounced metabolic improvements were observed in the LIE+CrM group, suggesting a synergistic effect between CrM and low-intensity exercise. The most pronounced metabolic improvements were observed in the LIE+CrM group, suggesting a synergistic effect between CrM and low-intensity exercise.

A pivotal finding of this study was the significant upregulation of MEF2A in the LIE+CrM group compared to control and CrM-only groups. MEF2A is integral to muscle differentiation, mitochondrial biogenesis, and metabolic adaptation to exercise(16,21). The elevated MEF2A expression aligns with previous research indicating its role as a key mediator of exercise-induced mitochondrial adaptations in cardiac muscle tissue (21,22). The enhancement of MEF2A activity suggests that CrM supplementation, when combined with low-intensity aerobic exercise, may promote mitochondrial biogenesis and improve oxidative capacity. This effect is likely facilitated by increased ATP production via the phosphocreatine pathway, supporting improved mitochondrial function observed in the LIE+CrM group.

While monocarboxylate transporter 1 (MCT1) expression did not reach statistical significance between groups, a trend toward increased levels in the LIE+CrM group was noted. MCT1 facilitates lactate and pyruvate transport, playing a crucial role in lactate metabolism during exercise (23)(15). The observed trend is consistent with literature suggesting that CrM supplementation can enhance metabolic efficiency by improving lactate transport and utilization in oxidative pathways (10). Although not definitive, these findings imply that CrM may optimize lactate clearance and utilization during low-intensity exercise, warranting further investigation.

PDH, a key enzyme regulating the conversion of pyruvate to acetyl-CoA in the tricarboxylic acid cycle(24), was significantly elevated in both the LIE+CrM and HIE+CrM groups. This increase suggests that CrM enhances oxidative metabolism by promoting efficient pyruvate utilization, critical for ATP production during sustained aerobic exercise (25). These findings corroborate previous studies demonstrating CrM's ability to enhance mitochondrial respiration and support oxidative metabolism under exercise conditions (8). The significant upregulation of PDH in CrM-supplemented groups reinforces CrM's capacity to optimize glucose metabolism in cardiac muscle tissue, irrespective of exercise intensity.

The significant elevation of PGC-1α in the LIE+CrM group underscores the potential of CrM to enhance mitochondrial biogenesis. PGC-1α is a master regulator of mitochondrial biogenesis and oxidative metabolism (6). Its upregulation in response to CrM supplementation, particularly when combined with low-intensity exercise, suggests that CrM enhances mitochondrial biogenesis, improving the capacity of cardiac muscle tissue to generate ATP through oxidative phosphorylation (8,26). This finding aligns with previous research demonstrating that PGC-1α activation is essential for increasing mitochondrial density and improving mitochondrial function during aerobic exercise (7). The elevated levels of PGC-1α in the LIE+CrM group suggest that CrM supplementation may amplify the effects of low-intensity aerobic exercise by promoting greater mitochondrial biogenesis and enhancing overall energy metabolism in cardiac tissue.

Similarly, TFAM levels were significantly elevated in the LIE+CrM group. TFAM is essential for mitochondrial DNA replication and transcription, crucial for maintaining mitochondrial function and energy production (26). The increased TFAM expression indicates that CrM supplementation enhances mitochondrial adaptability and energy efficiency during prolonged exercise (4). These results align with previous findings that CrM enhances mitochondrial biogenesis and function in high-energy-demand tissues like

cardiac muscle (4,8,9). The upregulation of TFAM further highlights CrM's potential to improve cardiovascular health by bolstering mitochondrial function during aerobic exercise.

Depending on its intensity, duration and type, exercise increases metabolic processes and oxygen consumption, resulting in the formation of more free radicals. In addition, the mitochondrial respiratory chain also contributes significantly to this process (8). The reason why the biomarkers measured in our study were higher in the low-intensity exercise group than in the high-intensity exercise group could be due to increased oxidative stress related to the intensity of the exercise. In addition, the fact that the aerobic mechanism is more sustainable in low-intensity exercise and, as can be seen from our results, mitochondrial biogenesis is more active in the low-intensity exercise group can be shown among these reasons.

Limitations

A primary limitation of this study is the use of a murine model, which may not fully replicate human cardiac physiology and responses to CrM supplementation. Additionally, the study focused exclusively on male mice to minimize hormonal variability, thereby limiting the generalizability of the findings across sexes. The lack of gender-specific analysis precludes understanding potential sex-based metabolic differences in response to CrM and exercise intensity. Furthermore, the study did not assess long-term effects of CrM supplementation, leaving the sustained impact on energy metabolism and cardiovascular function unexplored. Future research should incorporate long-term studies and include both sexes to enhance the applicability of the findings to human populations.

Conclusion

This study demonstrates that CrM supplementation combined with low-intensity aerobic exercise significantly enhances key biomarkers of mitochondrial biogenesis, oxidative metabolism, and energy production in cardiac ventricular muscle tissue. The upregulation of MEF2A, PDH, PGC-1α, and TFAM in the LIE+CrM group indicates that CrM plays a crucial role in optimizing mitochondrial function and energy metabolism. These findings have important implications for athletes and individuals with cardiovascular risk, suggesting that CrM supplementation could improve performance and cardiovascular health by enhancing mitochondrial efficiency. Future studies should investigate the long-term effects of CrM supplementation and explore its potential therapeutic applications in cardiovascular and metabolic disorders.

Ethical Approval: *The experimental protocol was reviewed and approved by the Harran University Animal Experiments Local Ethics Committee (Approval No.: 2024/005, Decision No.: 01-11). All procedures conformed to the ethical standards outlined in the Guide for the Care and Use of Laboratory Animals. The research was conducted at the Harran University Animal Experimentation and Research Center, where all animals were housed under controlled conditions.*

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Data acquisition: H.Ç., A.T.
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