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ORIGINAL ARTICLE

OTHER AREAS

The effects of high-intensity interval training on cardiac fibrosis biomarkers in rats with methamphetamine-induced cardiac fibrosis

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ABSTRACT

BACKGROUND: Methamphetamine (METH) use is associated with significant cardiac damage, including the development of cardiac fibrosis. While high-intensity interval training (HIIT) has demonstrated cardio-protective effects in various cardiovascular conditions, its potential role in mitigating METH-induced cardiac fibrosis remains unclear. This study aimed to investigate the effects of HIIT on the mRNA expression of cardiac fibrosis biomarkers in METH-dependent rats.

METHODS: In this experimental study, the expression of collagen 1a1 (*COL1A1*), collagen 3a1 (*COL3A1*), periostin (*POSTN*), and alpha smooth muscle actin (α-SMA) genes was measured using real-time PCR across four groups: Sham, METH, METH-control (METH-CON), and METH-HIIT. Rats in the METH-HIIT group underwent an 8-week HIIT protocol during the withdrawal period following METH administration.

RESULTS: Twenty-one days of METH administration significantly increased the mRNA expression of all measured fibrosis-related ($P<0.05$). However, the subsequent HIIT intervention did not significantly attenuate the elevated mRNA expression of these markers ($P>0.05$).

CONCLUSIONS: These findings suggest that HIIT does not reverse METH-induced upregulation of cardiac fibrosis markers. Further studies are warranted to explore alternative or complementary interventions in the context of METH-associated cardiac fibrosis.

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KEY WORDS: High-intensity interval training; Fibrosis; Methamphetamine.

Methamphetamine (METH) use is a growing global phenomenon that leads to adverse consequences such as cardiovascular disease.[1] Cardiovascular disease is the second leading cause of death among users of this substance.[2] Cardiac fibrosis arises from aberrant proliferation of cardiac fibroblasts and excessive deposition of extracellular matrix (ECM).[3] Under physiological conditions there is a fine network of collagen fibers around cardio-

myocytes to support and maintain the framework of the cells.[4]

Collagen 1 and collagen 3 are essential for cardiac function and form the major structural components of the myocardial ECM.[5] The synthesis of collagen 1 and collagen 3 differs under conditions of cardiac injury. In experimental models of hypertensive cardiac fibrosis and myocardial infarction, collagen 1 exhibits a more pronounced and sustained increase compared with collagen 3; however, in patients with ischemic cardiomyopathy, the collagen 1/collagen 3 ratio is decreased.[6] Periostin is an ECM protein that contributes to tissue structural integrity and is implicated in fibrosis. In addition, periostin binds to integrin receptors to mediate diverse signaling pathways in multiple cell types, including fibroblasts.[7] Periostin regulates fibroblast adhesion to cardiomyocytes, intercellular fibrosis, and ventricular remodeling following myocardial infarction and pressure overload.[8] Alpha smooth muscle actin (α-SMA) is expressed in smooth muscle cells but not in normal cardiac fibroblasts. α-SMA is significantly expressed only in transdifferentiated myofibroblasts within infarcted and remodeling myocardium. Consequently, α-SMA serves as a reliable marker for myofibroblasts at sites of injury.[9]

Chronic METH administration in mice has been associated with a significant increase in fibrosis, collagen content, periostin, and α-SMA.[10] Chronic METH use causes a higher presence of collagen deposits and collagen 1 and an increase in collagen content, which is dose-dependent, so that its amount increases with the increase of the dose.[11]

Although discontinuation of METH has been shown to attenuate or even reverse myocardial pathologic symptoms,[12] the role of novel therapies in reversing cardiac fibrosis cannot be ignored.[13] Sports science researchers are looking to replace non-drug treatments to reduce treatment costs[14] and drug-related complications.[15] High-intensity interval training (HIIT) is one of the training methods that is characterized by repeated short bursts of intense activity.[16] Although the safety of HIIT has been debated among health professionals, recent studies have shown that the risk of a cardiovascular event is low after HIIT despite the high pressure on the heart.[17] A reduction in cardiac fibrosis as a result of HIIT was observed in patients with heart failure[18] and myocardial infarction.[19] Wang *et al*.[20] showed that HIIT for 4 and 8 weeks in rats with acute myocardial infarct causes a decrease in the percentage of α-SMA area and collagen volume fraction. Despite the studies mentioned above, the interfering role of HIIT on markers involved in cardiac fibrosis, including collagen 1a1, collagen 3a1, α-SMA and periostin in METH users is still unknown. Therefore, the aim of this study was to investigate the effects of eight weeks of HIIT on cardiac fibrosis-related biomarkers in rats with METH-induced cardiac fibrosis.

Materials and methods

Study design and animals

We analyzed data collected from 45 male Wistar rats (180-220 g) obtained from the Animal Facility of Kerman University of Medical Sciences, Iran. Ethical approval for the study was obtained from the Ethics Committee of Hakim Sabzevari University, Sabzevar, Iran (IR.HSU.REC.1400.005). All procedures were conducted in accordance with the ethical principles for the care and use of laboratory animals. All rats were kept under control in environmental conditions (21±2 °C; 40-60% humidity) with a 12-hour light/dark cycle and ad libitum access to standard chow and water. The animals were randomly assigned (simple randomization using a lottery method) into four groups: sham, METH, METH-control (METH-CON), and METH-HIIT (Figure 1). During the study, five rats were excluded due to failure to adapt to the exercise protocol, and eight rats died before completing the training protocol.

METH administration

Animals in the METH, METH-CON, and METH-HIIT groups received intraperitoneal injections of METH (5 mg/kg/day) dissolved in physiological saline (0.9% sodium chloride) for 21 consecutive days.[15] Rats in the sham group received an equivalent volume (0.5 mL) of physiological saline during the same period.

HIIT protocol

Before initiation of the training intervention, rats in the METH-HIIT group underwent a one-week familiarization period, during which they ran on a treadmill for 10 min per session at a speed of 10-20 m/min.[21] An electric shock grid (0.5 mA) was employed to motivate treadmill running. Forty-eight hours after the last familiarization session, rats performed an incremental treadmill test to exhaustion, starting at 10 m/min and increasing 3 m/min every 3 min.[22] The average maximum running speed was calculated to design the exercise program. The maximum running speed was reassessed every two weeks, and the training intensity was adjusted accordingly. The HIIT protocol lasted 8 weeks, with 5 sessions per week (Table I).

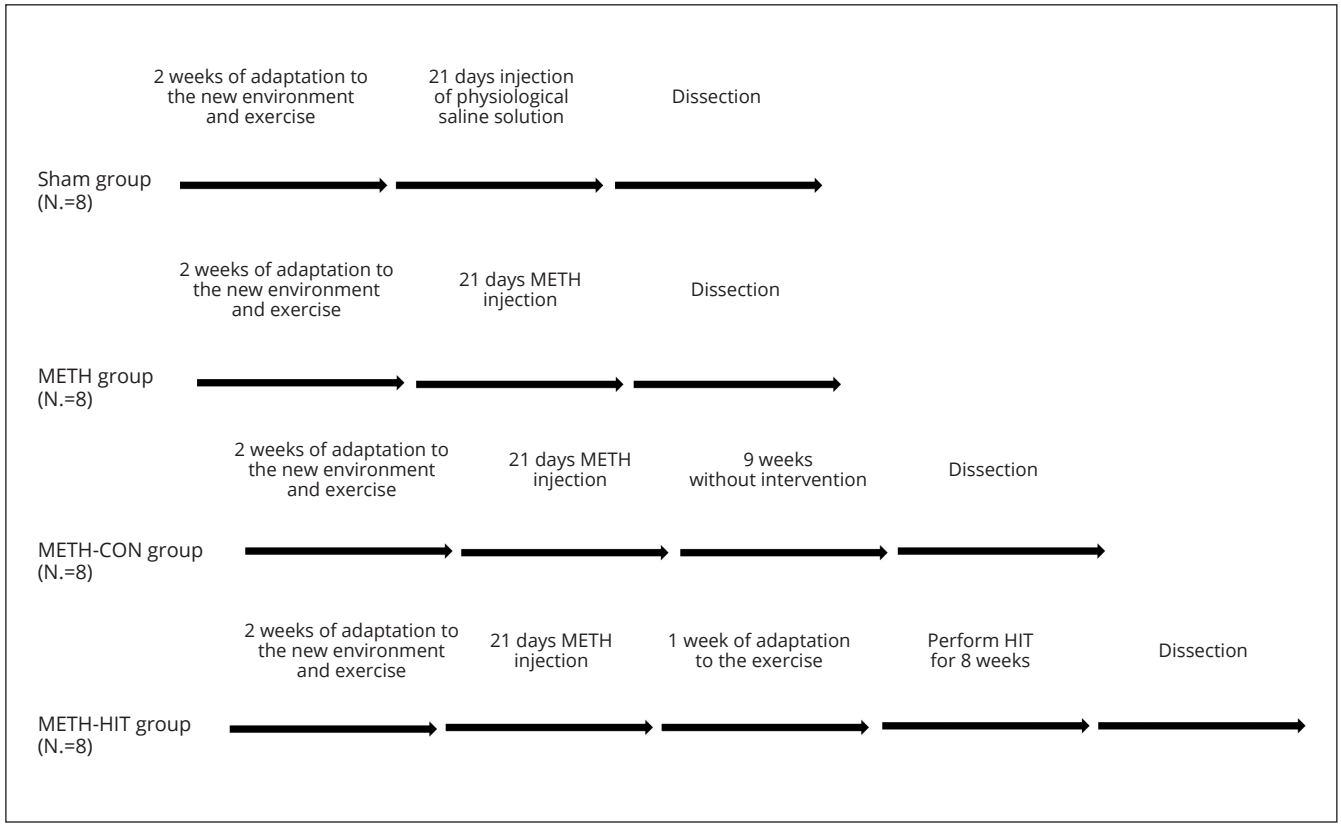

Figure 1.—Schematic overview of the experimental protocol.
METH: methamphetamine; HIIT: high-intensity interval training; CON: control.

TABLE I.—*The HIIT protocol employed in this study.*

Exercise procedures	Weeks	1	2	3	4	5	6	7	8
Intense interval (85% of maximum speed)	Number of intervals	4	4	4	4	4	4	4	4
	Duration of each interval (min)	2	2	2	2	2	2	2	2
	Running speed during each interval (m/min)	22	24	26	28	30	32	34	36
Active recovery interval (40% of maximum speed)	Number of intervals	4	4	4	4	4	4	4	4
	Duration of each interval (min)	2	2	3	3	4	4	4	4
	Running speed during each interval (m/min)	10	10	11	11	12	12	13	13
	Mean maximum speed (m/min)	26	29	32	35	38	40	41	42

HIIT: high-intensity interval training.

Each training session comprised a 6-min warm-up and a 6-min cool-down at 8 m/min. The main exercise bout was conducted at 85% of each rat's maximum speed, with no treadmill incline. To control environmental factors, rats in the METH-CON group were placed on a stationary treadmill (turned off) for the same duration.

Tissue collection and primer design and synthesis

Tissue collection was performed after 21 days of METH or saline administration in the sham and METH groups, and 24 hours after the final training session in the METH-CON and METH-HIIT groups. Rats were anesthetized with CO_2 gas and euthanized by decapitation. The hearts were excised immediately; the left ventricles were separated, weighed, and frozen in liquid nitrogen before storage at -70 °C. Primers for collagen 1a1 (*COL1A1*), collagen 3a1 (*COL3A1*), periostin (*POSTN*), α-smooth muscle actin (α-SMA), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were designed using Primer-BLAST (NCBI) and synthesized by Sina-

TABLE II.—*Primer sequences used for Real-Time PCR analysis.*

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
COL1A1	GTACATCAGCCCAAACCCCA	CAGGATCGGAACCTTCGCTT
COL3A1	AGGGCAGGGAACAACTGATG	GGTCCCACATTGCACAAGC
POSTN	AAAGAGACCCGGGAAGAACG	TTGCAGGTGTGTCTTTTTGC
α-SMA	TTGTCCACCGCAAATGCTTC	TGAAGGCGCTGATCCACAAA
GAPDH	CAACTCCCTCAAGATTGTCAGCAA	GGCATGGACTGTGGTCATGA

COL1A1: collagen 1a1; *COL3A1*: collagen 3a1; *POSTN*: periostin; α-SMA: α-smooth muscle actin; *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase.

clon Co. (Tehran, Iran). Primer sequences are reported in Table II.

RNA extraction, cDNA synthesis and quantitative real-time PCR

Total RNA was extracted from 50 mg of left ventricular tissue using TRIzol reagent (Yekta Tajhiz Azma, Iran) according to the manufacturer's protocol. Chloroform and isopropanol were used for RNA phase separation and precipitation, respectively. RNA concentration and purity were assessed using a Picodrop spectrophotometer (Picodrop limited, Hinxton, England). First-strand cDNA was synthesized from 1 μg of total RNA using a commercial cDNA synthesis kit (cat N.: YT4500; Yekta Tajhiz Azma, Tehran, Iran). Gene expression was analyzed by real-time PCR using Rotor Gene Q (Qiagen, Germany) and Real Q Plus 2× Master Mix Green-high Rox™ (Ampliqon, Odense, Denmark). PCR conditions were: initial denaturation at 95 °C for 15 minutes, followed by 40 cycles of 95 °C for 10 seconds, 60 °C for 20 seconds, and 72 °C for 20 seconds. Gene expression levels were normalized to GAPDH, and the relative expression was calculated using the 2^-ΔΔCt method.

Statistical analysis

Data normality was assessed using Shapiro-Wilk Test, and homogeneity of variance was evaluated by Brown-Forsythe Test. If residuals were not normally distributed, group comparisons were conducted using the Kruskal-Wallis Test followed by Dunn's Multiple Comparisons Test. When residuals were normally distributed and variances were homogeneous, group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Sidak's Multiple Comparisons Test. In cases of heterogeneous variances, Welch's ANOVA followed by Dunnett's T3 *post-hoc* multiple comparisons test was applied. All statistical analyses were performed using GraphPad Prism software (version 8; GraphPad Inc., La Jolla, CA, USA), with statistical significance defined as $P<0.05$.

Results

The general characteristics of the rats are presented in Figure 2. After eight weeks of training, the average body weight was significantly higher in both the METH-CON ($P=0.0096$) and METH-HIIT ($P=0.0159$) groups compared with the METH group. Moreover, the heart-to-body weight ratio ($P=0.0268$) and left ventricular-to-body weight ratio ($P=0.0076$) were significantly lower in the METH-CON group compared with the METH group. No significant differences were observed in the other parameters assessed ($P>0.005$).

The statistical results for cardiac fibrosis markers are presented in Figure 3. A significant overall difference between groups was observed in the expression of *COL1A1* ($P=0.0106$), *COL3A1* ($P=0.0002$), *POSTN* ($P=0.0003$), and α-SMA ($P=0.0011$) genes. However, no significant difference was observed in the *COL1A1*/*COL3A1*ratio ($P=0.3392$).

Pairwise comparisons revealed that *COL1A1* gene expression was significantly elevated in the METH group compared with the sham group ($P=0.0143$). *COL3A1* gene expression was significantly elevated in the METH ($P=0.0058$) and METH-CON ($P=0.0175$) groups compared with the sham group. Similarly, *POSTN* gene expression was significantly elevated in the METH ($P=0.0087$) and METH-CON ($P=0.0002$) groups compared with the sham group. α-SMA gene expression was also significantly elevated in the METH ($P=0.0018$) and METH-CON ($P=0.0054$) groups compared with the sham group. No significant differences in the *COL1A1*/*COL3A1* ratio were observed among the groups.

Discussion

The present study aimed to investigate the changes of cardiac fibrosis markers in METH-dependent rats following a period of HIIT. Twenty-one days of METH administration increased the expression of *COL1A1*, *COL3A1*, *POSTN*, and α-SMA genes in rats. The expression of *COL3A1*, *POSTN*, and α-SMA genes remained elevated in

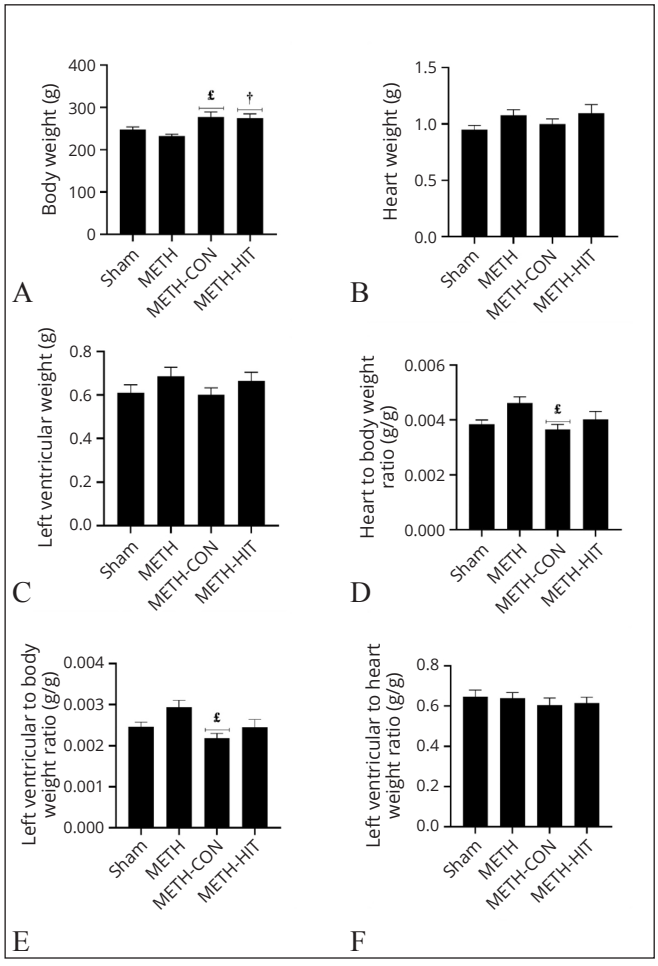

Figure 2.—General characteristics of rats in the sham and METH groups at the end of METH administration, and in the METH-CON and METH-HIIT groups at the end of the training program: A) body weight; B) heart weight; C) left ventricular weight; D) heart to body weight ratio; E) left ventricular to body weight ratio; and F) left ventricular to heart weight ratio.
Data are presented as mean±SEM.
METH: methamphetamine, HIIT: high-intensity interval training, CON: control; SEM: standard error of measurement.
†Significant differences between METH-HIIT and METH groups, £significant differences between METH-CON and METH groups.

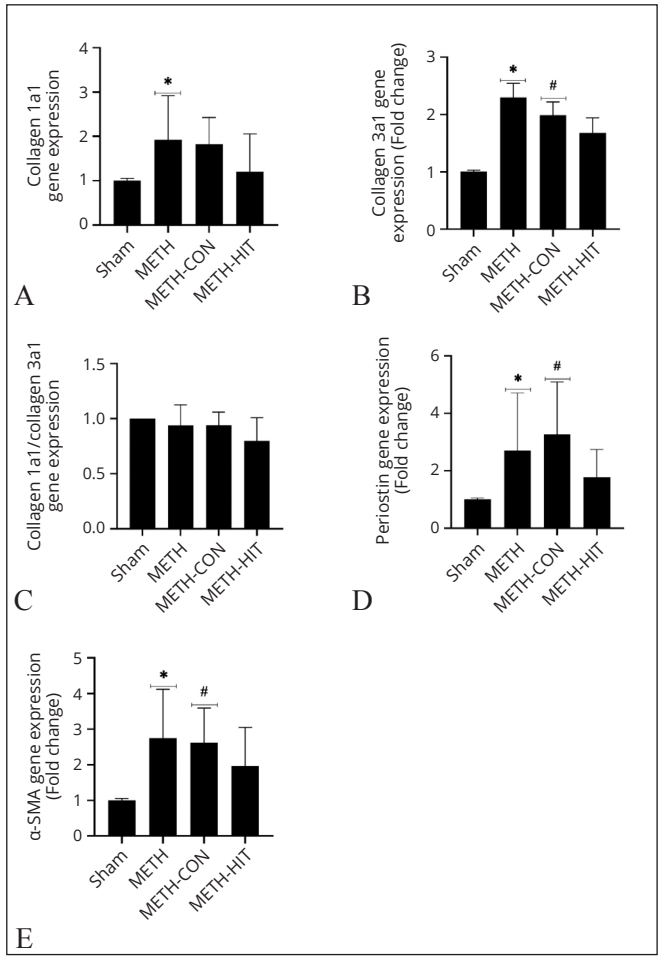

Figure 3.—Effects of METH exposure and HIIT training on left ventricular gene expression: A) *COL1A1* gene expression; B) *COL3A1* gene expression; C) *COL1A1*/*COL3A1* ratio gene expression; D) *POSTN* gene expression; and E) α-SMA gene expression. Values are expressed as mean±SEM for B, and median±IQR for A, C, D, and E.
METH: methamphetamine, HIIT: high-intensity interval training, CON: control, α-SMA: α-Smooth muscle actin; SEM: standard error of measurement; IQR: interquartile range; *COL1A1*: collagen 1a1; *COL3A1*: collagen 3a1; *POSTN*: periostin; α-SMA: α-smooth muscle actin.
*Significant differences between METH and sham groups; #significant differences between METH-CON and sham groups.

the METH-CON group even after the withdrawal period. HIIT did not attenuate the METH-induced upregulation of cardiac fibrosis markers.

Elevated α-SMA area and collagen volume fraction have previously been linked to impaired cardiac function, manifested as a decline in ejection fraction and fractional shortening.[20] Consistent with our findings, Abdullah *et al*.[10] reported that METH administration (0-6 mg/kg, 5 days per week for 4 weeks) resulted in increased cardiac fibrosis, elevated collagen content, and higher expression levels of α-SMA and periostin in mice. Similarly, Kolluru *et al*.[23] reported that chronic METH administration (0-6 mg/kg, 5 days per week for 4 weeks) exacerbated cardiac fibrosis in mice. Zhang *et al*.[11] also observed greater collagen deposition and increased collagen content with chronic METH exposure; however, in their zebrafish model, changes in mRNA expression of *COL1A1* and *COL3A1* were not statistically significant. These discrepancies may be attributed to variations in drug dosage, treatment duration, and animal models. METH did not alter the *COL1A1*/

COL3A1 ratio in our study, suggesting that collagen phenotype remodeling may vary depending on the experimental model.[6]

Beyond fibrotic markers, METH has been shown to promote a proinflammatory environment by elevating cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β, while suppressing the anti-inflammatory cytokine IL-10.[24] Following the inflammatory phase, cardiac repair mechanisms mediated by IL-10 and transforming growth factor-β (TGF-β) are activated; however, these processes may also lead to maladaptive fibrosis.[9] TGF-β is a key regulator of fibroblast-to-myofibroblast conversion, a process associated with the secretion of ECM proteins and tissue inhibitors of metalloproteinases (TIMPs) production.[25] Cardiac fibrosis is pathologically characterized by excessive deposition of collagens, particularly types I and III, the principal structural proteins of the cardiac ECM and scar tissue, secreted by activated cardiac fibroblasts.[26, 27] In injured myocardium, TGF-β activates the Sma- and MAD-related protein 3 (Smad3) signaling cascade, leading to increased α-SMA expression in fibroblasts,[9] which enhances their contractility and promotes focal adhesion maturation.[28] Additionally, periostin has been implicated in cardiac fibrosis by amplifying TGF-β/Smad signaling[27] and directly interacting with collagen 1 to regulate fibril diameter.[29] Several signaling pathways have been implicated in fibrosis, including phosphatidylinositol 3-kinase (PI3K) / protein kinase B (AKT), Janus kinase (JAK) / signal transducer and activator of transcription (STAT), and wingless-type MMTV integration site family (WNT) / β-catenin.[30] The increase in the METH-induced fibrotic response may involve the G protein coupled receptor (GPCR)/cyclic adenosine monophosphate (cAMP) pathway. Binding of METH to trace amine-associated receptor 1 (TAAR1) increases intracellular cAMP levels in cardiomyocytes, potentially leading to fibrotic dysregulation *via* upregulation of lysyl oxidase (LOX).[11]

Our results showed that METH administration did not significantly alter cardiac weight. This contrasts with the findings of Abdullah *et al*.[10] who reported an increase in heart weight, but aligns with the observations of Chauva *et al*.[31] Increased cardiac weight may reflect hypertrophic changes induced by chronic administration of drugs that influence ventricular inotropism.[32] Following METH withdrawal, we observed a reduction in the heart-to-body weight and left ventricular-to-body weight ratios, suggesting a potential attenuation of hypertrophy.

Following a 9-week withdrawal period, the expression of *COL3A1*, *POSTN*, and α-SMA genes remained elevated, and HIIT was unable to normalize their levels. This suggests that an extended recovery period may be necessary to achieve meaningful improvements in METH-induced cardiac fibrosis. A decrease in the signs of cardiac fibrosis (*i.e.*, percentage of α-SMA area, collagen volume fraction) was observed in the rat myocardial infarct model during HIIT.[20] However, in a diabetic cardiomyopathy model, four weeks of high-intensity exercise at 80% of maximal capacity resulted in only a non-significant reduction in fibrosis.[33] Moreover, a study reported that cardiac recovery following METH withdrawal is delayed, with reductions in cardiac lesions typically observed after four weeks.[12] Although mRNA expression of *COL1A1* and *COL3A1* were slightly decreased during HIIT in our study, these changes did not reach statistical significance. By contrast, significant reductions in collagen 1 and 3 mRNA expression have been observed in ischemia-reperfusion and diabetic cardiomyopathy models, respectively.[34, 35] It is possible that specific adaptations during exercise, such as inhibition of the TGF-β1/Smad2/3 pathway, and consequent decreased the mRNA expression of collagen 1 and 3, mediate reductions in fibrosis.[36] Nonetheless, further research is warranted to elucidate the mechanisms through which exercise may modulate cardiac remodeling following METH exposure.

Limitations of the study

Despite the valuable findings, the present study has several limitations. First, the duration of the HIIT protocol was relatively short, which limits the ability to assess the long-term and sustained effects of this training method in reducing or reversing METH-induced cardiac damage. Second, the small sample size reduces the statistical power and generalizability of the results. Third, we measured only four fibrosis-related genes and did not evaluate additional biomarkers such as suppression of tumorigenicity-2 (ST2), Galectin-3 (Gal3), and others, which are recognized indicators of fibrotic activity. We recommend that future studies include these biomarkers to obtain a more comprehensive understanding of the mechanisms involved in cardiac fibrosis. Additionally, studies with longer intervention periods and larger sample sizes are necessary to better elucidate and potentially reverse METH-induced cardiac damage.

Conclusions

METH administration induces cardiac fibrosis, as evidenced by increased expression of *COL1A1*, *COL3A1*, *POSTN*, and α-SMA genes. Although HIIT is recognized for its beneficial effects on cardiac remodeling, the present

study demonstrated that it did not significantly attenuate METH-induced cardiac fibrosis under the current experimental conditions.

References

1. Curran L, Nah G, Marcus GM, Tseng Z, Crawford MH, Parikh NI. Clinical Correlates and Outcomes of Methamphetamine-Associated Cardiovascular Diseases in Hospitalized Patients in California. J Am Heart Assoc 2022;11:e023663.

2. Darke S, Duflou J, Kaye S. Prevalence and nature of cardiovascular disease in methamphetamine-related death: A national study. Drug Alcohol Depend 2017;179:174–9.

3. Lin LC, Liu ZY, Tu B, Song K, Sun H, Zhou Y, *et al.* Epigenetic signatures in cardiac fibrosis: focusing on noncoding RNA regulators as the gatekeepers of cardiac fibroblast identity. Int J Biol Macromol 2024;254:127593.

4. Horn MA, Trafford AW. Aging and the cardiac collagen matrix: novel mediators of fibrotic remodelling. J Mol Cell Cardiol 2016;93:175–85.

5. Singh D, Rai V, Agrawal DK. Regulation of Collagen I and Collagen III in Tissue Injury and Regeneration. Cardiol Cardiovasc Med 2023;7:5–16.

6. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. Cell Mol Life Sci 2014;71:549–74.

7. Iino Y. Eosinophilic otitis media; state-of-the-art diagnosis and treatment. Auris Nasus Larynx 2023;50:479–89.

8. Snider P, Hinton RB, Moreno-Rodriguez RA, Wang J, Rogers R, Lindsley A, *et al.* Periostin is required for maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. Circ Res 2008;102:752–60.

9. Chen W, Frangogiannis NG. Fibroblasts in post-infarction inflammation and cardiac repair. Biochim Biophys Acta 2013;1833:945–53.

10. Abdullah CS, Aishwarya R, Alam S, Morshed M, Remex NS, Nitu S, *et al.* Methamphetamine induces cardiomyopathy by Sigmar1 inhibition-dependent impairment of mitochondrial dynamics and function. Commun Biol 2020;3:682.

11. Zhang J, Nguyen AH, Jilani D, Trigo Torres RS, Schmiess-Heine L, Le T, *et al.* Consecutive treatments of methamphetamine promote the development of cardiac pathological symptoms in zebrafish. PLoS One 2023;18:e0294322.

12. Islam MN, Kuroki H, Hongcheng B, Ogura Y, Kawaguchi N, Onishi S, *et al.* Cardiac lesions and their reversibility after long term administration of methamphetamine. Forensic Sci Int 1995;75:29–43.

13. Raziyeva K, Kim Y, Zharkinbekov Z, Temirkhanova K, Saparov A. Novel Therapies for the Treatment of Cardiac Fibrosis Following Myocardial Infarction. Biomedicines 2022;10:2178.

14. Duijvestijn M, de Wit GA, van Gils PF, Wendel-Vos GC. Impact of physical activity on healthcare costs: a systematic review. BMC Health Serv Res 2023;23:572.

15. Shahrabadi H, Haghighi AH, Askari R, Asadi-Shekaari M, Souza DC, Gentil P. Effect of High-Intensity Interval Training on Cardiac Apoptosis Markers in Methamphetamine-Dependent Rats. Curr Issues Mol Biol 2022;44:3030–8.

16. Atakan MM, Li Y, Koşar ŞN, Turnagöl HH, Yan X. Evidence-Based Effects of High-Intensity Interval Training on Exercise Capacity and Health: A Review with Historical Perspective. Int J Environ Res Public Health 2021;18:7201.

17. Weston KS, Wisløff U, Coombes JS. High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. Br J Sports Med 2014;48:1227–34.

18. Hsu CC, Wang JS, Shyu YC, Fu TC, Juan YH, Yuan SS, *et al.* Hypermethylation of ACADVL is involved in the high-intensity interval training-associated reduction of cardiac fibrosis in heart failure patients. J Transl Med 2023;21:187.

19. Shi X, Chen X, Qiu X, Luo W, Luo X, Liu H, *et al.* Effect of High-Intensity Interval Training, Moderate Continuous Training, or Guideline-Based Physical Activity on Peak Oxygen Uptake and Myocardial Fibrosis in Patients With Myocardial Infarction: Protocol for a Randomized Controlled Trial. Front Cardiovasc Med 2022;9:860071.

20. Wang B, Zhou R, Wang Y, Liu X, Shou X, Yang Y, *et al.* Effect of high-intensity interval training on cardiac structure and function in rats with acute myocardial infarct. Biomed Pharmacother 2020;131:110690.

21. Banaei P, Nazem F, Nazari A, Arjomand A. Preconditioning Effect of High-Intensity Interval Training (HIIT) and Berberine Supplementation on the Gene Expression of Angiogenesis Regulators and Caspase-3 Protein in the Rats with Myocardial Ischemia-Reperfusion (IR) Injury. BioMed Res Int 2020;2020:4104965.

22. Fashi M, Agha-Alinejad H, Mahabadi HA, Rezaei B, Pakrad BB, Rezaei S. The effects of aerobic exercise on NF-κB and TNF-α in lung tissue of male rat. Nov Biomed 2015;3:131–4.

23. Kolluru GK, Glawe JD, Pardue S, Kasabali A, Alam S, Rajendran S, *et al.* Methamphetamine causes cardiovascular dysfunction via cystathionine gamma lyase and hydrogen sulfide depletion. Redox Biol 2022;57:102480.

24. Li X, Wu F, Xue L, Wang B, Li J, Chen Y, *et al.* Methamphetamine causes neurotoxicity by promoting polarization of macrophages and inflammatory response. Hum Exp Toxicol 2018;37:486–95.

25. Frangogiannis N. Transforming growth factor-β in tissue fibrosis. J Exp Med 2020;217:e20190103.

26. Hinderer S, Schenke-Layland K. Cardiac fibrosis - A short review of causes and therapeutic strategies. Adv Drug Deliv Rev 2019;146:77–82.

27. Ma Y, Iyer RP, de Castro Brás LE, Toba H, Yabluchanskiy A, Deleon-Pennell KY, *et al.* Cross talk between inflammation and extracellular matrix following myocardial infarction. In: Blankesteijn WM, Altara R, editors. Inflammation in heart failure. Cambridge, MA: Academic Press; 2015. p.67–79.

28. Shinde AV, Humeres C, Frangogiannis NG. The role of α-smooth muscle actin in fibroblast-mediated matrix contraction and remodeling. Biochim Biophys Acta Mol Basis Dis 2017;1863:298–309.

29. Snider P, Standley KN, Wang J, Azhar M, Doetschman T, Conway SJ. Origin of cardiac fibroblasts and the role of periostin. Circ Res 2009;105:934–47.

30. Zhao M, Wang L, Wang M, Zhou S, Lu Y, Cui H, *et al.* Targeting fibrosis, mechanisms and cilinical trials. Signal Transduct Target Ther 2022;7:206.

31. Chavva H, Brazeau DA, Denvir J, Primerano DA, Fan J, Seeley SL, *et al.* Methamphetamine-induced changes in myocardial gene transcription are sex-dependent. BMC Genomics 2021;22:259.

32. Richig JW, Sleeper MM. Electrocardiography of laboratory animals. Cambridge, MA: Academic Press; 2018.

33. Novoa U, Arauna D, Moran M, Nuñez M, Zagmutt S, Saldivia S, *et al.* High-Intensity Exercise Reduces Cardiac Fibrosis and Hypertrophy but Does Not Restore the Nitroso-Redox Imbalance in Diabetic Cardiomyopathy. Oxid Med Cell Longev 2017;2017:7921363.

34. Golbashi R, Gaeini A, Kordi MR, Aboutaleb N, Ghardashi Afousi A. Effect of one period of high-intensity interval training on myocardial collagen-1 and TGF-Î^21 and cardiac function in post ischemia-reperfusion rats. Daneshvar Med 2018;26:65–74.

35. Akbari N, Azarbayjani MA, Delfan M. Effect of high intensity interval training (HIIT) on the gene expression of MMP-2, COL-III and myocardial function in type 2 diabetic rats; 2020 [Internet]. Available from: https://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=17355311&AN=144308176&h=uk2X7qKBGb548R9Nm2LxNH2cAu0DbQ9vOzR0WM1NBH2%2BPotEF33TDjcBv370B%2FHgIwniiw04M0L%2F60Qq8LvdNw%3D%3D&crl=c [cited 2026, Jan 8]

36. Cui X, Wang K, Zhang J, Cao ZB. Aerobic exercise ameliorates myocardial fibrosis via affecting vitamin D receptor and transforming growth factor-β1 signaling in vitamin D-deficient mice. Nutrients 2023;15:741.

Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions
Amir H. Haghighi, Roya Askari and Majid Asadi-Shekaari have given substantial contributions to the study conception and design; Hadi Shahrabadi contributed to the data collection; Ahad Shafiei, Alfredo Caturano, Vincenzo Russo, Giuseppe Caminiti, Ferdinando Iellamo, and Pasquale Farsetti contributed to the data analysis and interpretation; Rosario Barone, Maurizio Volterrani and Attilio Parisi contributed to the manuscript draft, Roberto Bei and Marco Alfonso Perrone contributed to the manuscript editing and revised it critically. All authors read and approved the final version of the manuscript.

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