Indian Journal of Experimental Biology
Vol. 63, April 2025, pp. 291-300
DOI: 10.56042/ijeb.v63i04.11870

Subsarcolemmal mitochondrial dysfunction aggravates ischemia-reperfusion injury in diabetic rat hearts on fructose and cholesterol diets

Mahalakshmi Ansari[1,2], Priyanka N Prem[1,2], Eren Rose Gino[2] & Gino A Kurian[1,2]*

[1]Vascular Biology Lab, [2]School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur, INDIA

Received 31 July 2024; revised 04 February 2025

The rising prevalence of diabetes mellitus and metabolic syndrome, driven by diets high in fat and fructose, has significantly increased the risk of cardiovascular complications, including ischemia-reperfusion (IR) injury. Understanding the mechanisms linking dietary stressors to mitochondrial dysfunction in diabetes is crucial to addressing this health burden. Mitochondrial dysfunction, particularly in the subsarcolemmal fraction, is a hallmark feature of diabetic cardiomyopathy and IR injury. This study assessed the impact of a high-fat and fructose diet on IR injury in diabetic rat hearts. Forty-eight Wistar rats were assigned to four groups: normal, diabetes mellitus (DM), DM with a cholesterol diet (CD), and DM with a fructose diet (FD). Isolated hearts subjected to IR injury via Langendorff perfusion demonstrated cardiac hypertrophy, increased oxidative stress, and mitochondrial dysfunction in CD and FD groups compared to normal rats. FD rats exhibited significantly higher serum glucose, insulin resistance, brain natriuretic peptide expression, and subsarcolemmal mitochondrial damage, resulting in worse cardiac recovery and greater myocardial injury post-IR than CD rats. The findings underscore the detrimental effect of a fructose-rich diet on cardiac and mitochondrial health in diabetic conditions. This study highlights the need for dietary interventions targeting mitochondrial function to reduce cardiovascular risk in diabetic populations.

Keywords: High-fructose diet, Cholesterol diet, Insulin resistance, Langendorff perfusion, Oxidative stress, Diabetic cardiomyopathy

Type 2 diabetes mellitus (T2DM) represents a critical global health concern due to its increasing prevalence and wide-ranging complications[1]. Patients with diabetes have two times greater risk to get the heart failure[2]. Among its ramifications diabetic cardiomyopathy (DCM), a cardiac condition characterized by specific morphological, functional, and metabolic alterations arising as a major complication of T2DM[3]. Sustained hyperglycemia and dyslipidemia contribute to oxidative stress, impaired calcium homeostasis, disrupted mitochondrial function, inflammation, and fibrosis, collectively driving DCM progression[4].

Many documents in the literature recognize the effects of diet on the risk of cardiovascular diseases (CVD) and related lifestyle diseases[5-8]. In fact, dietary interventions can attenuate[9] or aggravate[10] the myocardial pathological alterations, thus need to customize the dietary inclusion according to the requirement of individuals with or without cardiac complications. Diets rich in fructose, a hallmark of modern fast-food culture, increase the risk of obesity, metabolic syndrome, T2DM, and cardiovascular diseases (CVDs)[11]. Excess fructose induces lipogenic effects[12], mitochondrial oxidative damage, impaired DNA repair, and cardiac hypertrophy[13]. Similarly, hypercholesterolemia-triggered by high cholesterol diets worsens oxidative stress and cardiac dysfunction[14,15].

Patients with CVD often undergo revascularization procedures such as percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass graft (CABG), which exacerbate mitochondrial dysfunction through calcium overload and oxidative stress, triggering mitochondrial permeability transition pore (mPTP) opening and subsequent IR injury[16]. Mitochondria play a pivotal role in cellular function by regulating bioenergetics, nutrient metabolism, intracellular signaling, immune regulation, and maintaining stem cell funcionality[17]. Decline in mitochondrial function is a hallmark of diabetic complications, marked by reduced energy production and redox imbalance. In response to stress or damage, mitochondria activate adaptive mechanisms to restore structural integrity and functionality. Mild or transient mitochondrial stress induces mitohormesis, a protective phenomenon that enhances resilience to future

*Correspondence:
Phone: +91 90479 65425 (Mob.)
E-mail: ginokurian@hotmail.com; kurian@scbt.sastra.edu

stressors, offering therapeutic potential for diseases involving mitochondrial dysfunction[18]. Evidence suggests that interventions promoting mitohormesis, such as dietary modification or exercise, may ameliorate metabolic disorders[19].

Diabetes significantly increases the risk of ischemia-reperfusion (IR) injury, yet the role of subsarcolemmal mitochondria (SSM) in this process remains under-explored. Understanding how SSM dysfunction contributes to IR injury in diabetic hearts is essential for developing targeted therapies. High-fructose and high-cholesterol diets induce metabolic stress and mitochondrial dysfunction, and investigating their effects on SSM in the context of IR injury can provide valuable insights into dietary contributions to diabetic cardiac complications. While general mitochondrial dysfunction in diabetic hearts is well-documented, the specific role of SSM—critical for ATP supply to ion channels and membrane integrity—requires further clarification. Moreover, understanding how SSM impairment exacerbates oxidative stress, calcium overload, and metabolic inflexibility during IR injury could identify potential intervention points for cardioprotection. By exploring how diet-induced mitochondrial dysfunction worsens IR injury in diabetes, this study may help the development of mitochondrial-targeted therapies, dietary modifications, or pharmacological interventions to reduce cardiac damage. While diabetes and obesity amplify IR injury vulnerability, the influence of specific diets on diabetic hearts during IR injury remains inadequately explored. This study evaluates and compares the effects of high-cholesterol and fructose-enriched diets on the hearts of diabetic rats subjected to IR injury, addressing a critical gap in understanding diet-mediated cardiac vulnerabilities.

Materials and Methods

Animals

Male Wistar rats (8 weeks old) used in this study were housed in the animal facility in polycarbonate cages exposed to a daily 12 h light/dark cycle with free access to chow and drinking water. All procedures during the investigations followed the guidelines approved by the Committee for Control and Supervision of Experiments on Animals (CPCSEA Approval No.279/SASTRA/IAEC/RPP), India.

Experimental groups

48 young animals (6 week old) with average body weight 200g were used. Rats were divided into four experimental groups (n=12 per each group) namely Normal (N), Diabetes mellitus (DM), Fructose diet fed (FD) and Cholesterol diet fed (CD). The FD and CD groups were treated with fructose (60%/kg b.wt. administered *via* oral route) and cholesterol in coconut oil (2%/kg b.wt. administered *via* oral route) for 8 weeks where the other 2 groups were maintained in normal diet. At the end of 8th week, a single dose of streptozotocin (65 mg/kg, ip) was injected to the fructose and cholesterol-fed animals along with 12 animals from the DM group and maintained for another 4 weeks, resulting in type 2 diabetes, confirmed *via* insulin tolerance test and glucose tolerance tests. Rats were checked for blood glucose level, 72 h after STZ injection using AP Plus glucometer and were monitored for any changes in body weight, food and water intake at regular intervals. In addition, we evaluated insulin resistance by calculating HOMO-IR. Lipid profile and total cholesterol were estimated in the plasma using kit method (Agappe, India) according to the manufacturer's instructions. Hypertrophy in the rat hearts were assessed by measuring heart weight/body weight ratio, and mRNA expression of BNP (Forward primer-3' GACTCCGGCTTCTGACTCTG 5', Reverse primer- 3' ACTGTGGCAAGTTTGTGCTG 5').

Morphology analysis – H&E staining

After the experiment, the heart from each group was fixed in 4% paraformaldehyde followed by embedding. Paraffin-embedded myocardium was sliced and stained with Haematoxylin-Eosin (H&E) and examined under an Olympus BX51 microscope to observe the changes in the myofibril architecture.

Isolated perfused rat heart preparation

At the end of the treatments, the animals were anesthetized by intraperitoneal injection of sodium pentobarbital (80 mg/kg b.wt.) 30 min after heparin administration (1000 IU/kg b.wt.) to prevent coagulation. The heart was excised, mounted on a Langendorff apparatus (AD instruments, Australia) and perfused retrogradely through the aorta with KH buffer (*p*H - 7.4; temperature: 37□) consisting of NaCl (118.5 mM), KCl (5.8 mM), $NaHCO_3$ (25 mM), KH_2PO_4 (1.2 mM), $MgSO_4$ (1.2 mM), glucose (11 mM) and $CaCl_2$ (2.5 mM), bubbled with 5% CO_2 and 95% O_2, at a constant flow rate of 8 mL/min as described elsewhere[20]. Each heart was allowed to stabilize for 20 min before any experiment protocol commenced. The following hemodynamic parameters were recorded and analysed using LabChart Pro

software. (PowerLab; AD Instruments, Australia) -Left ventricular end-diastolic pressure (LVEDP) in mmHg, left ventricular systolic pressure (LVSP) in mmHg, left ventricular developed pressure (LVDP = LVSP-LVEDP) in mmHg, heart rate (HR) in beats per minute (bpm), rate pressure product (RPP = HR * LVSP) in mmHg * bpm. Each experimental group was further divided into 2 subgroups of six animals-Normal perfusion and ischemia-reperfusion group. In the normal perfusion group, after stabilization with KH buffer, hearts were perfused for 90 min. In the ischemia-reperfusion group, hearts were subjected to global ischemia for 30 min after 20 min of stabilization and then reperfused for 60 min. Once the perfusion protocol was over, hearts were stored immediately at -80□ for further biochemical analysis.

Estimation of cardiac injury markers

Lactate dehydrogenase (LDH) and creatine kinase (CK) enzymatic activity were measured spectrophotometrically in cardiac tissue using previously described methods to assess the tissue injury[21]. Protein content was estimated spectrophotometrically at 595 nm using Bio-Rad reagent.

Isolation of mitochondrial subpopulation

The differential centrifugation technique was used to isolate rat heart mitochondrial subpopulations, namely interfibrillar mitochondria (IFM) and subsarcolemmal mitochondria (SSM), essentially according to the method described elsewhere[22]. IFM and SSM were purified using a 60% percoll gradient and western blot analysis of HSP 60, the marker protein (data not included) against β-actin was used to determine the purity.

Mitochondrial electron transport chain complex activities

The activities of electron transport chain (ETC) enzymes in interfibrillar mitochondria (IFM) and subsarcolemmal mitochondria (SSM) were quantified spectrophotometrically using specific donor-acceptor oxidoreductase activities in 0.1 M phosphate buffer. Complex-I activity was assessed using rotenone-sensitive NADH-oxidoreductase. Complex-II activity was measured via succinate decylubiquinone DCPIP reductase. Complex-III activity was evaluated using ubiquinol cytochrome-c reductase, while complex-IV activity (cytochrome c oxidase) was determined following a previously described protocol[22].

Oxidative stress assessment

The antioxidant levels in isolated mitochondria such as reduced glutathione (GSH) and superoxide dismutase (SOD) were estimated by the previously reported methods with slight modifications[21]. Catalase activity was determined by measuring the decrease in absorbance at 240 nm due to the decomposition of H_2O_2 in a UV recording spectrophotometer[21]. The lipid peroxidation product MDA level was evaluated by measuring thiobarbituric acid-reactive substances level as previously reported[21].

Statistical analysis

Values are presented as mean ± SD. Animal groups were compared using analysis of variance (ANOVA) followed by Tukeys post-hoc analysis. Analysis was performed using GraphPad Prism (version 5.01, La Jolla, CA, USA) software. $P< 0.05$ was considered statistically significant.

Results

Basal changes in the rat fed with fructose and cholesterol rich diet

Rats fed with fructose and cholesterol diets exhibited significantly increased body weight at the end of 12-week study, when compared with the normal group. However, no significant change in the body weight between FD and CD group animals were observed (Fig. 1A). Unlike kidney, liver and heart showed increased organ/body weight ratio compared with normal. Both FD and CD fed rats exhibited significant increases in liver weight compared to normal. Heart weight to body weight ratio increased in both FD and CD rat but only FD rat showed significant increase, indicating a presence of hypertrophy (Fig. 1B). This was confirmed by the increased cardiomyocyte cross sectional area in the FD group (Fig. 1F) and elevated mRNA expression of BNP which was prominent in fructose fed animals (Table 1). Diabetic control group did not show any sign of hypertrophy and had low body weight. The collagen content of the myocardium was insignificantly changed in FD and CD from normal (Fig. 1C). Similar to DM group glucotoxicity was high in both FD and CD groups, measured via blood glucose level, plasma insulin level, HbA1C and HOMO-IR index (Table 1) when compared with the normal and diabetic control group. Similarly increased cholesterol was evaluated by measuring total cholesterol, triglycerides, HDL and LDL cholesterol in the blood and found to be high in FD and CD groups compared to normal control (Table 1).

Effect of fructose and cholesterol rich diet on the hemodynamics of heart subjected to ischemia-reperfusion

Cardiac physiological recovery was measured *via* hemodynamic parameters in diabetic animals fed with

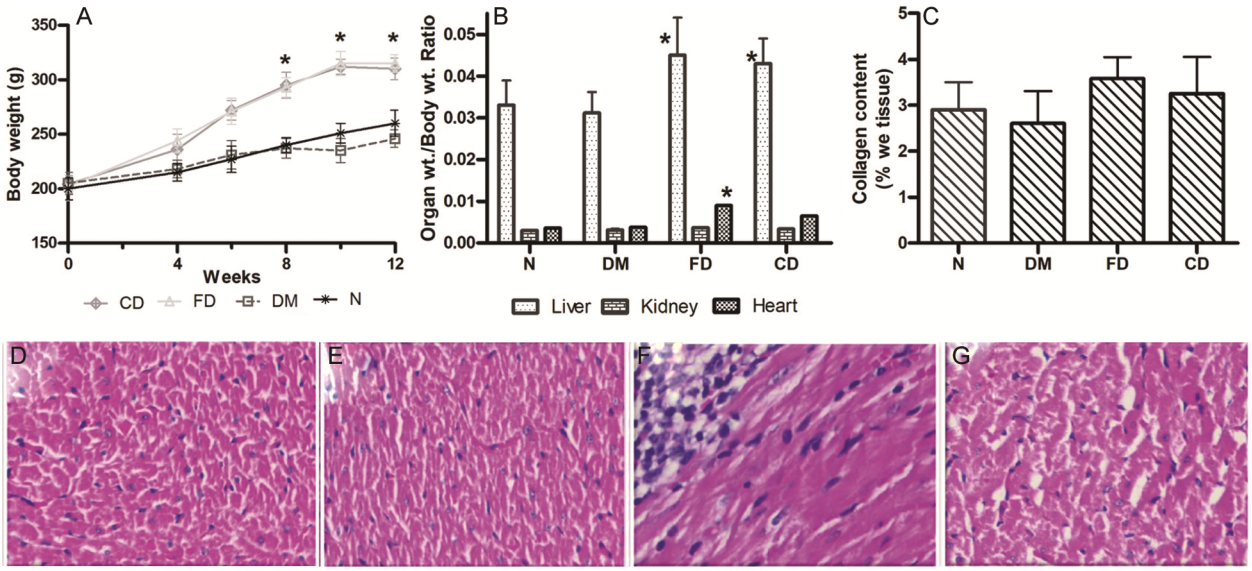

Fig. 1 — Body weight (A), Organ weight /body weight ratio (B), Collagen content (C), H&E stained image of N(D), DM (E), FD (F) and CD (G) myocardium *P<0.05 *vs* Normal

Table 1 — Biochemical and molecular parameters

Parameters	Normal Control	DM Control	Fructose diet	Cholesterol diet
mRNA level of BNP	1.00±0.02	0.04±0.002*	2.44±0.03*	2.10±0.01*
Blood glucose (mmol/L)	4.50±0.6	13.00±3*	16.00±4*	14.00±6*
Plasma Insulin (µIU/mL)	15.27±0.6	8.79±0.44*	11.35±0.41	12.57±0.27
HbA1C (mg/gHb)	0.30±0.01	0.50±0.01	0.61±0.01	0.58 ±0.02*#
HOMO-IR index	3.11 ±0.03	7.89 ± 0.03*	8.24 ± 0.03*	7.99 ± 0.06*
Total Cholesterol (mg/dL)	111±13	137±9	168.7±11*	189.14±14*
Triglycerides	80±11	114±10	145±17*	161±11*
HDL	50±7	38±4	62±6	78±4*
LDL	46±2	48.6±5	50.2±6	53.8±6

[Data are represented as mean ± SD. BNP was normalized to β-actin gene (expressed as arbitrary units). BNP: Brain natriuretic peptide. *P<0.05 *vs* Normal]

Table 2 — Hemodynamic assessment

	N	IR	DM	DM-IR	FD-C	FD-IR	CD	CD-IR
LVDP (mmHg)	98 ± 4	42 ± 3*	87± 2	43 ± 4*	90± 6	33± 4*	93± 2	38± 3*
LVEDP (mmHg)	6 ± 2	45 ± 3*	11± 1	40 ± 2*	8± 3	51± 1*	7± 2	48± 2*
RPP (mmHg*beats/min*10)	35 ± 2	12 ± 2*	24± 2	14 ± 2*	33± 1	9± 3*	32± 2	10± 1*

[Data were represented as mean ± SD; n = 6/group. *P<0.05 *vs* Normal. LVDP: Left ventricular developed pressure; LVEDP: Left ventricular end-diastolic pressure; RPP: Rate pressure product (Heart Rate*LVSP)]

a fructose/cholesterol diet (Table 2). No significant difference in hemodynamic indices were observed between the groups upon normal perfusion. When challenged to IR, FD and CD rat hearts exhibited declined LVDP (FD-66% and CD- 61%) and RPP (FD-74% and CD- 71%) when compared with normal control rat hearts. From DM rat heart subjects IR, CD rat showed 11%, and 28% decline in LVDP and RPP respectively whereas the FD rat showed 23%, and 35% decline. Comparatively FD rat hearts exhibited higher physiological deterioration than CD fed rat hearts (Table 2).

Effect of fructose and cholesterol rich diet on the cardiac injury of heart subjected to ischemia-reperfusion

Cardiac injury associated with ischemia-reperfusion injury was assessed by TTC heart sections and measured corresponding level of cardiac markers (Fig. 2). Representative images of stained TTC (Fig. 2A-H) showed significantly increased infarct size in the IR group from normal, cholesterol and fructose fed rats. In fact, the infarct size was more prominent in fructose fed rats as compared to other experimental groups (Fig. 2I). The TTC results were supported by the elevated levels of cardiac marker

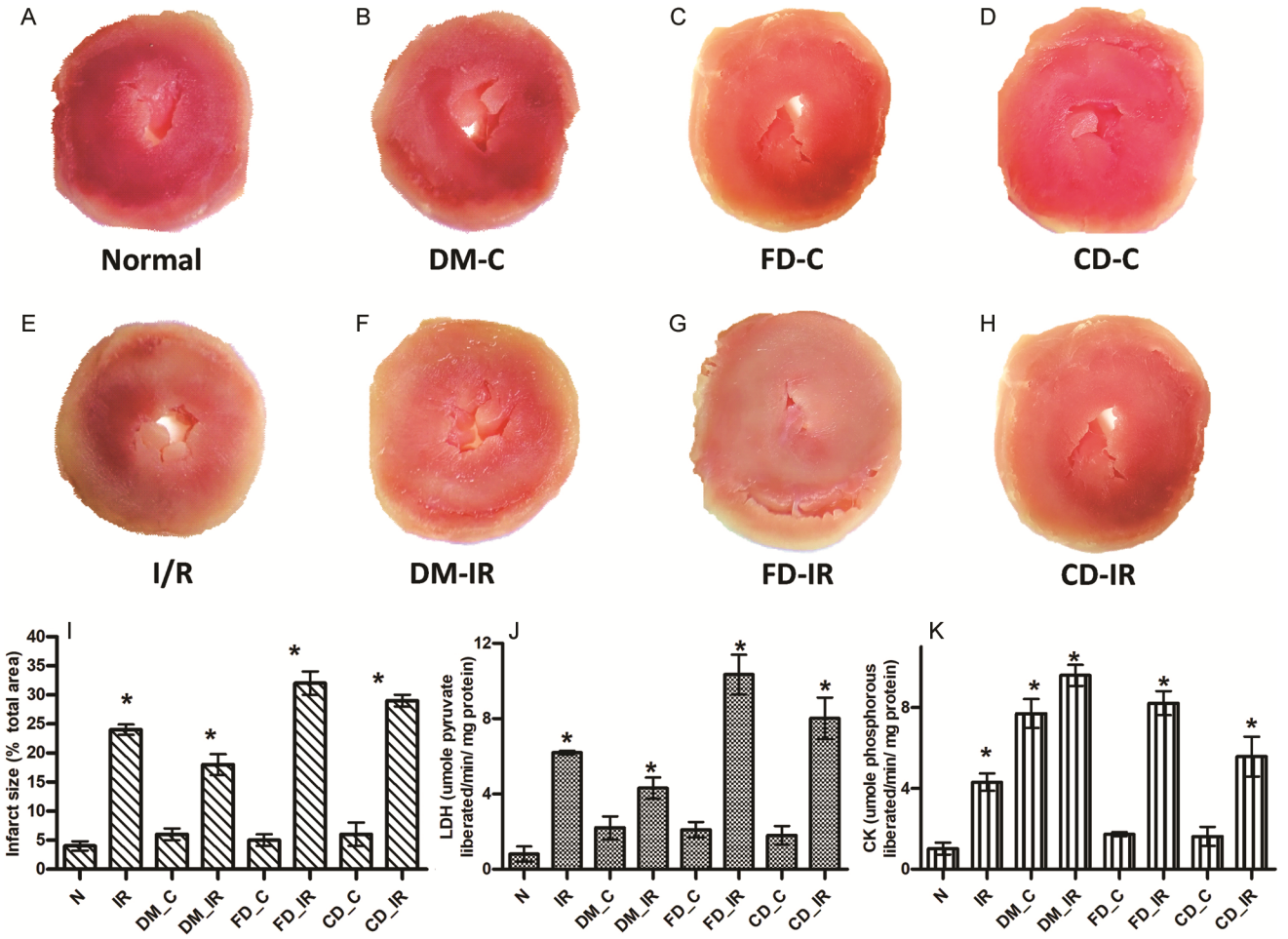

Fig. 2 — Photograph of TTC stained rat hearts subjected to normal perfusion (A-D) and IR (E-H) from N, DM, FD, and CD; Infarct size (% total area) (I), LDH (J) and CK (K) levels in coronary perfusate of normal and IR subjected groups. **P*<0.05 *vs* Normal

enzymes in the coronary perfusate of both FD & CD groups (Fig. 2J & 2K).

Effect of fructose and cholesterol rich diet on the oxidative stress of heart subjected to ischemia-reperfusion

The oxidative stress and mitochondrial dysfunction are the key mediators for IR pathology. Fig. 3 provides the lipid peroxidation and corresponding antioxidant enzyme activities in rat heart underwent different experiment protocols. As shown in Fig. 3, rat hearts challenged to IR from different experimental groups exhibited significantly increased TBARS level (Fig. 3A), decreased GSH (Fig. 3B) and decreased catalase and SOD activity (Fig. 3C & 3D). Fructose treated rat heart experienced prominent oxidative stress as compared with cholesterol fed and standard diet fed normal rat heart. Incidentally, lipid peroxidation level was distinct between the mitochondrial sub populations, where the IFM fraction of mitochondria showed higher levels of lipid peroxide products.

Effect of fructose and cholesterol rich diet on the mitochondrial function of heart subjected to ischemia-reperfusion

Mitochondrial electron transport chain enzyme activities were measured to find the functional efficiency of mitochondrial subpopulations. The baseline activities of proximal ETC enzymes in both IFM and SSM showed decreased activity in fructose fed rats, compared with normal and cholesterol fed rats (Fig. 4A & 4B). However, the distal ETC enzymes declined significantly in IFM and SSM in both CD and FD rat groups without significant difference between the subpopulations (Fig. 4C, 4D). Upon IR treatment, both proximal and distal ETC enzymes were significantly (P<0.05) declined in all experimental groups. In fact, IR induced decline was small cholesterol and fructose fed rats as compared with standard feed fed rats.

Discussion

Dietary habits have long been implicated in the etiology of cardiovascular diseases, with excessive

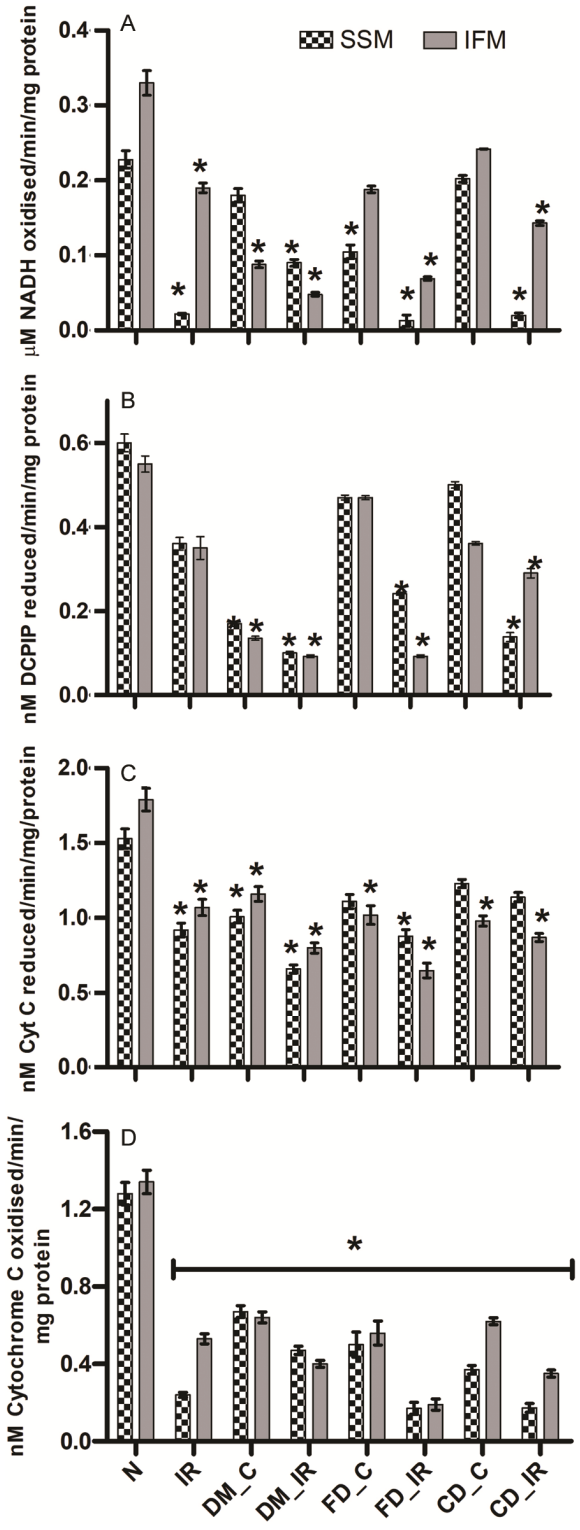

Fig. 3 — Oxidative stress analysis in cardiac mitochondria subpopulation. Image represents the levels of lipid peroxidation (A), reduced glutathione – GSH (B), Catalase (C), and Superoxide dismutase (D) in cardiac mitochondria subpopulation. Data were represented as mean ± SD of 6 individual experiments. **P*<0.05 *vs* Normal

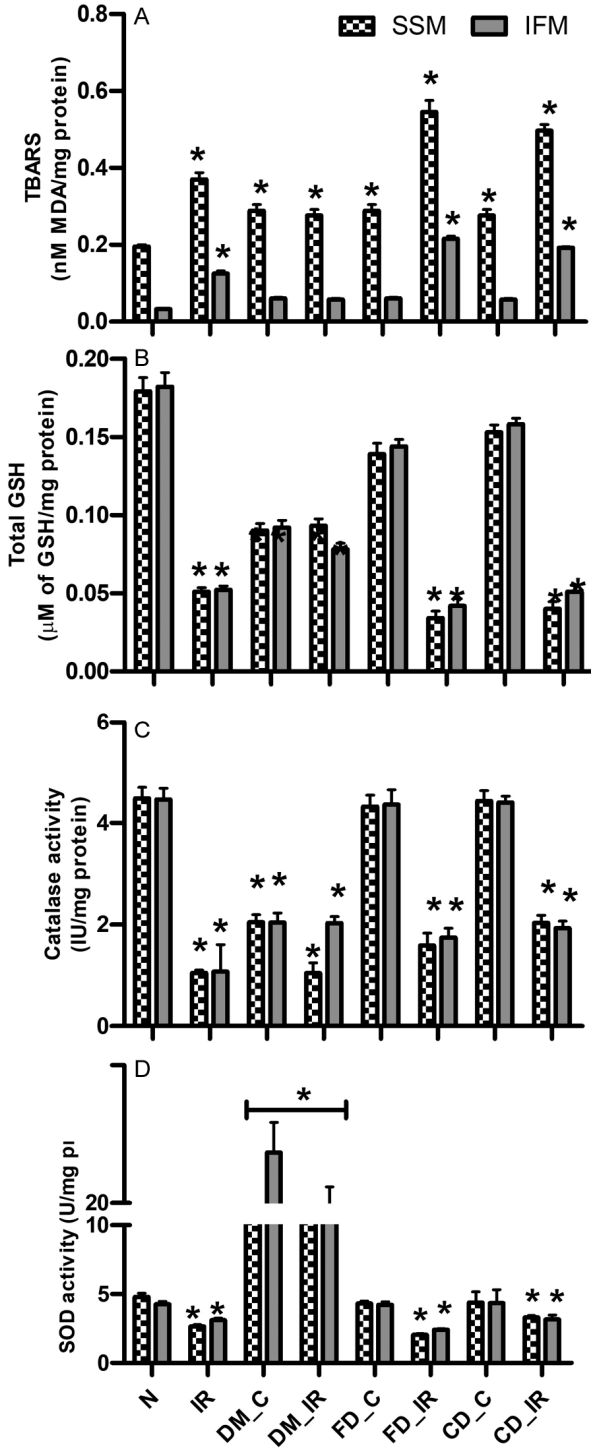

Fig. 4 — Electron transport enzyme activities in cardiac mitochondria subpopulation. NQR (Complex-I) enzymatic activity (A), SQR (complex-II) enzymatic activity (B) QCR (complex-III) enzymatic activity (C), and COX (complex-IV) enzymatic activity (D) of mitochondrial subpopulations from N, DM, FD, and CD rat heart subjected to normal an IR perfusion. Data were represented as mean ± SD of 6 individual experiments. **P*<0.05 *vs* Normal

intake of cholesterol, fructose, and other high-calorie components being significant contributors to premature heart disease[10]. Recent research highlights the role of diet in developing cardiac diseases and modulating the heart's resilience to stress, including surgical interventions such as ischemia-reperfusion (IR) injury management[8]. This unmet clinical challenge underlines the need for a deeper understanding of how specific diets affect cardiac tolerance and recovery during IR challenges.

The present study investigated the differential effects of cholesterol- and fructose-rich diets on diabetic rat hearts exposed to IR injury. The findings reveal that diabetic hearts from fructose-fed rats were more vulnerable to IR injury compared to those from cholesterol-fed rats. This increased vulnerability in fructose-fed rats was accompanied by severe mitochondrial dysfunction, particularly in subsarcolemmal mitochondria (SSM), increased oxidative stress (Fig. 3), and greater myocardial damage, as evidenced by elevated infarct size (Fig. 2) and impaired hemodynamics (Table. 2)

SSM is found close to the cell membrane and is positioned to support membrane-specific energy requirements like ion transport and signaling, where as IFM are situated near the contractile machinery of the heart muscle to supply energy for contraction and relaxation[23]**.** Diet plays a crucial role in influencing the function of subsarcolemmal mitochondria (SSM) and interfibrillar mitochondria (IFM), particularly in the context of their spatial orientation and specialized functions. Diet significantly influences the spatially driven functions of subsarcolemmal mitochondria (SSM) and interfibrillar mitochondria (IFM) in the heart. High-fat diets promote lipid accumulation around SSM, impairing their oxidative function and ATP generation for membrane-specific energy demands, while antioxidant-rich diets reduce oxidative stress near the sarcolemma, maintaining ion channel function and calcium signaling[24,25]. In contrast, ketogenic or low-carb diets can negatively impact IFM by limiting glucose availability, leading to impaired ATP generation from glucose essential for contractile function[26]. Conversely, high-quality protein diets enhance IFM efficiency by supplying amino acids necessary for the repair and maintenance of contractile machinery[27].

The role of mitochondrial dysfunction in diabetic cardiomyopathy (DCM) is well-established, with studies reporting that mitochondrial damage contributes significantly to cardiac remodeling and reduced cardiac resilience[21,28]. Mitochondrial dysfunction is known to exacerbate oxidative stress, inflammation, and calcium dysregulation, key mediators of IR injury. Our findings corroborate prior evidence, demonstrating that subsarcolemmal mitochondria are particularly susceptible to dietary insults, resulting in compromised ATP production and increased oxidative damage[29]. This aligns with studies showing that fructose-rich diets induce mitochondrial DNA damage and alter mitochondrial DNA repair enzymes, exacerbating tissue vulnerability to IR stress[13].

Interestingly, the differential effects of cholesterol and fructose diets on mitochondrial function were evident in our study. Cholesterol-rich diets primarily affect mitochondrial function by altering membrane composition, increasing cholesterol content, and reducing membrane fluidity, which impairs oxidative phosphorylation and ATP production[30]. While, fructose-rich diets promote mitochondrial dysfunction through excessive reactive oxygen species (ROS) generation, mitochondrial DNA damage, and impaired biogenesis, leading to reduced oxidative capacity[31]. Comparative studies reveal that fructose exacerbates mitochondrial damage via lipid peroxidation and oxidative stress, whereas cholesterol primarily disrupts membrane potential and protein function. Mechanistically, cholesterol diets impair mitochondrial dynamics by disrupting membrane integrity, while fructose depletes cardiolipin and promotes inflammation, collectively causing energy deficits[32,33]. While cholesterol-rich diets have been associated with altered mitochondrial membrane composition and cholesterol content, leading to a paradoxical improvement in mitochondrial efficiency in certain contexts[34], fructose-rich diets disrupt cardiolipin composition, impairing mitochondrial bioenergetics and dynamics[35]. These compositional changes may account for the greater decline in electron transport chain (ETC) enzyme activity observed in SSM from fructose-fed rats compared to those fed cholesterol-rich diets.

Previous studies have shown that dietary interventions, such as caloric restriction, can improve cardiac outcomes in IR injury by enhancing mitochondrial resilience and reducing oxidative stress[36]. Conversely, excessive fructose intake has been linked to lipotoxicity and the formation of cytosolic lipid inclusions in cardiac tissue, as noted in diabetic patient atrial samples[34]. This lipotoxicity,

coupled with the observed hypertrophic changes in fructose-fed rats in our study, further underscores the detrimental effects of a fructose-rich diet on cardiac recovery following IR injury.

The observed differences in infarct size and myocardial function between cholesterol-fed and fructose-fed rats also highlight the critical influence of dietary components on signaling pathways that govern cardio-protection. While hypercholesterolemia has been reported to diminish the protective effects of remote ischemic conditioning against IR injury[37], some studies suggest that controlled high-fat diets may enhance oxidative phosphorylation and improve contractile performance in experimental models of heart failure[28]. This dichotomy underscores the complex interplay between diet, mitochondrial function, and cardiac resilience, warranting further investigation into the specific mechanisms underlying these effects.

Our study adds to the growing body of evidence that highlights the distinct roles of mitochondrial subpopulations in cardiac pathophysiology. Interfibrillar mitochondria (IFM), which supply ATP for muscle contraction, and subsarcolemmal mitochondria, responsible for maintaining cellular homeostasis, exhibit differential responses to pathological conditions such as diabetes and IR injury[29]. The pronounced dysfunction in SSM observed in fructose-fed rats suggests an inefficient compensatory adaptation to meet the heightened energy demands during IR stress, contributing to accelerated cardiac myopathy.

Dietary alterations, including high-fat and high-fructose diets, impair subsarcolemmal mitochondrial function in diabetic hearts, increasing ischemia-reperfusion (IR) injury risk. Excess fatty acid load and β-oxidation from a high-fat diet cause oxidative stress, insulin resistance, and mitochondrial proteome changes[38]. Hyperglycemia worsens dysfunction via advanced glycation end-products (AGEs), mitochondrial ROS, electron transport chain disruption, and calcium dysregulation[39]. Impaired AMPK, mTOR, PGC-1α, and SIRT1 signaling further reduce metabolic flexibility[40]. Ischemia depletes ATP, overloads calcium, and primes the mitochondrial permeability transition pore (mPTP), while reperfusion triggers oxidative bursts, mPTP opening, cytochrome c release, and inflammation, accelerating cell death. Diabetic hearts, with impaired ischemic preconditioning and recovery, suffer greater IR damage. Structural changes, including altered membrane composition and cristae, worsen dysfunction. Therapeutic strategies include dietary interventions (caloric restriction, Mediterranean/ketogenic diets, nutrient supplementation) and pharmacological approaches (mitochondrial antioxidants, mPTP inhibitors, metabolic modulators). Combined lifestyle strategies like exercise, stress reduction, and metabolic control may restore mitochondrial function and improve cardiac resilience.

Conclusion

In conclusion, our findings demonstrate that fructose-rich diets exacerbate cardiac vulnerability to IR injury through mechanisms involving severe mitochondrial dysfunction, increased oxidative stress, and impaired bioenergetics. These results highlight the need for dietary interventions and targeted therapeutic strategies to mitigate the adverse effects of high-fructose diets, particularly in diabetic patients at risk of ischemic heart disease. Future studies should explore the molecular underpinnings of these dietary effects, including the role of mitochondrial biogenesis, dynamics, and signaling pathways, to develop effective cardio-protective approaches.

Animal Ethical statement

The experimental procedures involving animals were done following the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA Approval No.279/SASTRA/IAEC/RPP), India

Acknowledgements

Dr. Mahalakshmi Ansari sincerely thanked the Department of Science and Technology, India for supporting this research through the INSPIRE fellowship (DST/INSPIRE Fellowship/2013/326). The authors acknowledge Dr. C. David Raj for his valuable inputs during animal experiments.

Conflict of interest

The authors declare no potential conflict of interest to disclose.

References

1 Hossain MJ, Al-Mamun M & Islam MR, Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. *Health Sci Rep,* 7(3), (2024) e2004.

2 Kenny HC, Abel ED. Heart failure in type 2 diabetes mellitus: impact of glucose-lowering agents, heart failure therapies, and novel therapeutic strategies. *Circ Res*, 124(1), (2019) 121.

3 Zhao X, Liu S, Wang X, Chen Y, Pang P, Yang Q, Lin J, Deng S, Wu S, Fan G &Wang B, Diabetic cardiomyopathy: Clinical phenotype and practice. *Front Endocrinol (Lausanne)*, 13, (2022) 1032268.

4 Ghosh N, Chacko L, Bhattacharya H, Vallamkondu J, Nag S, Dey A, Karmakar T, Reddy PH, Kandimalla R & Dewanjee S, Exploring the Complex Relationship between Diabetes and Cardiovascular Complications: Understanding Diabetic Cardiomyopathy and Promising Therapies. *Biomedicines*, 11(4), (2023) 1126.

5 Mazur M, Przytuła A, Szymańska M & Popiołek-Kalisz J, Dietary strategies for cardiovascular disease risk factors prevention. *Curr Probl Cardiol*, 49(9), (2024) 102746.

6 Shan Z, Li Y, Baden MY, Bhupathiraju SN, Wang DD, Sun QI, Rexrode KM, Rimm EB, Qi LU, Willett WC & Manson JE, Association between healthy eating patterns and risk of cardiovascular disease. *JAMA Intern Med*, 180(8), (2020) 1090.

7 Peila R, Xue X, Qi Q, Dannenberg AJ, Allison MA, Johnson KC, LaMonte MJ, Wild RA, Haring B, Pan K & Tindle HA, Healthy lifestyle index and risk of cardiovascular disease among postmenopausal women with normal body mass index. *J Am Heart Assoc*, 12(12), (2023) e029111.

8 Tada H, Takamura M & Kawashiri MA, The Effect of Diet on Cardiovascular Disease, Heart Disease, and Blood Vessels. *Nutrients*, 14(2), (2022) 246.

9 Lazzarin T, Garcia LR, Martins D, Queiroz DAR, Tonon CR, Balin PDS, Polegato BF, Paiva SAR, Azevedo PS, Minicucci M & Zornoff L, Role of Nutrients and Foods in Attenuation of Cardiac Remodeling through Oxidative Stress Pathways. *Antioxidants (Basel)*, 11(10), (2022) 2064.

10 Xiao YL, Gong Y, Qi YJ, Shao ZM & Jiang YZ, Effects of dietary intervention on human diseases: molecular mechanisms and therapeutic potential. *Sig Transduct Target Ther*, 9(1), (2024) 59.

11 Clemente-Suárez VJ, Beltrán-Velasco AI, Redondo-Flórez L, Martín-Rodríguez A & Tornero-Aguilera JF, Global Impacts of Western Diet and Its Effects on Metabolism and Health: A Narrative Review. *Nutrients*, 15(12), (2023) 2749.

12 Geidl-Flueck B & Gerber PA, Fructose drives de novo lipogenesis affecting metabolic health. *J Endocrinol*, 257(2), (2023) e220270.

13 Ma F, Qin X, Yang J, Zhou X, Zhao J, Cai K, Zhao R, Shi Y & Sun K, High fructose levels inhibit the proliferation of cardiomyocytes via the Notch1 signaling pathway. *Cell Signal*. 127, (2025) 111600.

14 Alhashemi M, Mayo W, Alshaghel MM, Brimo Alsaman MZ & Haj Kassem L, Prevalence of obesity and its association with fast-food consumption and physical activity: A cross-sectional study and review of medical students' obesity rate. *Ann Med Surg (Lond)*, 79 (2022) 104007.

15 Smith RE, Ozben T & Saso L, Modulation of Oxidative Stress: Pharmaceutical and Pharmacological Aspects 2018. *Oxid Med Cell Longev*, (2019) 6380473.

16 Heusch G, Myocardial ischaemia–reperfusion injury and cardioprotection in perspective. *Nat Rev Cardiol*, 17(12), (2020) 773.

17 Chen W, Zhao H & Li Y, Mitochondrial dynamics in health and disease: mechanisms and potential targets. *Signal Transduct Target Ther*, 8, (2023) 333.

18 Cheng YW, Liu J & Finkel T, Mitohormesis. *Cell Metab*, 35, (2023) 1872.

19 Musci RV, Hamilton KL & Linden MA, Exercise-Induced Mitohormesis for the Maintenance of Skeletal Muscle and Healthspan Extension. *Sports (Basel)*, 7, (2019) 170.

20 Kurian GA, Ansari M & Prem PN, Diabetic cardiomyopathy attenuated the protective effect of ischaemic post-conditioning against ischaemia-reperfusion injury in the isolated rat heart model. *Arch Physiol Biochem*, 129(3), 2023 711.

21 Mahalakshmi A & Kurian GA, Evaluating the impact of diabetes and diabetic cardiomyopathy rat heart on the outcome of ischemia-reperfusion associated oxidative stress. *Free Radic Biol Med*, 118 (2018) 35.

22 Ravindran S & Kurian GA, Preconditioning the rat heart with sodium thiosulfate preserved the mitochondria in response to ischemia-reperfusion injury. *J Bioenerg Biomembr*, 51 (2019) 189.

23 Nakamura E, Aoki T, Endo Y, Kazmi J, Hagiwara J, Kuschner CE, Yin T, Kim J, Becker LB & Hayashida K, Organ-Specific Mitochondrial Alterations Following Ischemia-Reperfusion Injury in Post-Cardiac Arrest Syndrome: A Comprehensive Review. *Life (Basel)*, 14(4), (2024) 477.

24 Gualtieri P, Marchetti M, Frank G, Smeriglio A, Trombetta D, Colica C, Cianci R, De Lorenzo A & Di Renzo L, Antioxidant-Enriched Diet on Oxidative Stress and Inflammation Gene Expression: A Randomized Controlled Trial. *Genes (Basel)*, 14(1), (2023) 206.

25 Tan BL & Norhaizan ME, Effect of High-Fat Diets on Oxidative Stress, Cellular Inflammatory Response and Cognitive Function. *Nutrients*, 11(11), (2019) 2579.

26 Zhu H, Bi D, Zhang Y, Kong C, Du J, Wu X, Wei Q & Qin H, Ketogenic diet for human diseases: the underlying mechanisms and potential for clinical implementations. *Sig Transduct Target Ther*, 7(1), (2022) 11.

27 Lesnefsky EJ, Chen Q & Hoppel CL, Mitochondrial metabolism in aging heart. *Circ Res*, 118(10), (2016) 1593.

28 Heyne E, Schrepper A, Doenst T, Schenkl C, Kreuzer K & Schwarzer M. High-fat diet affects skeletal muscle mitochondria comparable to pressure overload-induced heart failure. *J Cell Mol Med*, 24 (2020) 6741.

29 Kurian GA, Berenshtein E, Kakhlon O & Chevion M, Energy status determines the distinct biochemical and physiological behavior of interfibrillar and sub-sarcolemmal mitochondria. *Biochem Biophys Res Commun*, 428, (2012) 376.

30 Schell M, Chudoba C, Leboucher A, Alfine E, Flore T, Ritter K, Weiper K, Wernitz A, Henkel J & Kleinridders A, Interplay of Dietary Fatty Acids and Cholesterol Impacts Brain Mitochondria and Insulin Action. *Nutrients*, 12(5), (2020) 1518.

31 Diniz MS, Tocantins C, Grilo LF & Pereira SP, The Bitter Side of Sugar Consumption: A Mitochondrial Perspective on Diabetes Development. *Diabetol*, 3(4), (2022) 583.

32 Cheng H, Zhou J, Sun Y, Zhan Q & Zhang D, High fructose diet: A risk factor for immune system dysregulation. *Human Immunol*, 83(6), (2022) 538.

33 Goicoechea L, Conde de la Rosa L, Torres S, García-Ruiz C & Fernández-Checa JC, Mitochondrial cholesterol: Metabolism and impact on redox biology and disease. *Redox Biol*, 61, (2023) 102643.

34 Astorga J, Gasaly N, Dubois-Camacho K, De la Fuente M, Landskron G, Faber KN, Urra FA & Hermoso MA, The role of cholesterol and mitochondrial bioenergetics in activation of the inflammasome in IBD. *Front Immunol*, 13, (2022) 1028953.

35 Wu KL, Wu CW, Chen LW, Chang HH, Cheng CL, Wu CY, Lee YC, Chen IC, Hung CY & Liu WC, Dysregulation of mitochondrial dynamics mediated aortic perivascular adipose tissue-associated vascular reactivity impairment under excessive fructose intake. *Nutr Metabol*, 21(1), (2024) 4.

36 Guo Z, Wang M, Ying X, Yuan J, Wang C, Zhang W, Tian S & Yan X, Caloric restriction increases the resistance of aged heart to myocardial ischemia/reperfusion injury via modulating AMPK-SIRT1-PGC1a energy metabolism pathway. *Sci Rep*., 13(1), (2023) 2045.

37 Kövamees O, Mahdi A, Wodaje T, Verouhis D, Brinck J & Pernow J, The protective effect of remote ischemic conditioning is lost in patients with hypercholesterolemia. *Am J Physiol Heart Circ Physiol*, 323(5), (2022) H1004.

38 Čolak E & Pap D, The role of oxidative stress in the development of obesity and obesity-related metabolic disorders. *J Med Biochem*, 40(1), (2021) 1.

39 González P, Lozano P, Ros G & Solano F, Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections. *Int J Mol Sci*, 24(11), (2023) 9352.

40 Yang L, Liu D, Jiang S, Li H, Chen L, Wu Y, Essien AE, Opoku M, Naranmandakh S, Liu S & Ru Q, SIRT1 signaling pathways in sarcopenia: Novel mechanisms and potential therapeutic targets. *Biomed Pharmacother*, 177, (2024) 116917.