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Effect of Preoperative Plant-based Versus Animal-based Diets on Myocardial Protection in a Rat Model of Cardiac Surgery

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Abstract

Objectives: Myocardial ischemia-reperfusion injury remains a major source of morbidity in cardiac surgery, and novel strategies for cardioprotection are needed. Preoperative dietary modulation has been proposed as a feasible approach to enhance myocardial resilience. This study investigated the effects of preoperative plant-based versus animal-based diets (ABD) on myocardial protection in a rat model of cardioplegic arrest, with emphasis on apoptosis, oxidative stress, and stress response markers.

Materials and Methods: Sixteen male Wistar albino rats were initially randomized to receive either a plant-based diet (PBD) (soy protein, palm oil) or an ABD (casein, milk fat) for 12 weeks. Due to peri-experimental losses, final analyses were performed on 6 rats in the PBD group and on 7 rats in the ABD group. At the end of the feeding period, rats underwent a standardized cardioplegic arrest induced by St. Thomas II crystalloid solution, resulting in 10 minutes of ischemia; blood cardioplegia was then administered prior to tissue harvesting. Left ventricular tissues were harvested for biochemical analysis. Bcl-2 and Bax, glutathione (GSH), protein carbonyls and malondialdehyde (MDA), and heat

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shock protein 70 (Hsp-70) were measured as markers of apoptosis, redox defense, oxidative damage, and stress response, respectively.

Results: Bcl-2 levels were significantly higher in the PBD group compared to the ABD group (11.28±1.22 vs. 9.50±1.25, p=0.025), indicating enhanced antiapoptotic signaling. Among other markers, Bax, MDA, protein carbonyls, and GSH showed trends favoring the PBD group that were not statistically significant, whereas Hsp-70 levels were numerically higher in the ABD group but not statistically significant.

Conclusion: In this experimental rat model of cardioplegic arrest, preoperative plant-based nutrition enhanced antiapoptotic signaling through significant upregulation of Bcl-2, while other oxidative and apoptotic markers showed favorable but not statistically significant trends. These findings suggest that diet composition can influence myocardial resilience to surgical stress, supporting the potential role of plant-based preoperative regimens as an adjunct to established cardioprotective strategies. Further validation in larger studies is warranted.

Keywords: Plant-based diet, animal-based diet, myocardial protection, ischemia-reperfusion injury, apoptosis, cardiac surgery

Introduction

Cardiovascular diseases remain the leading cause of global mortality despite advances in medical and surgical therapy. Dietary habits are pivotal, modifiable determinants of cardiovascular risk[1]. A growing body of evidence shows that diets emphasizing plant-derived proteins and lipids improve lipid metabolism, reduce oxidative stress and systemic inflammation, and are associated with lower cardiovascular risk and mortality[2-4]. By contrast, patterns rich in animal-derived proteins and fats have been linked to greater atherosclerotic burden and adverse coronary outcomes[5].

In the perioperative setting of cardiac surgery, myocardial ischemia-reperfusion (I/R) injury remains a major driver of morbidity. Cardioplegic arrest and subsequent reperfusion trigger the generation of reactive oxygen species, calcium overload, mitochondrial dysfunction, and apoptosis in cardiomyocytes. However, translation of many experimental cardioprotective strategies to clinical benefit has been challenging[6].

Given this background, preoperative dietary modulation represents a biologically plausible and clinically feasible approach to influence myocardial susceptibility to I/R. However, whether preoperative intake of plant-versus animal-based protein and lipid sources alters myocardial protection during cardiac surgery remains unclear. Recent experimental reports continue to explore adjuncts for I/R mitigation in rat models, underscoring the relevance of this question[7].

Accordingly, we compared the effects of preoperative plant-based and animal-based diet (ABD) on myocardial protection in a rat model of cardiac surgery with cardioplegic arrest. To elucidate these effects, we focused on biologically well-established markers. These included Bcl-2/Bax for apoptosis, glutathione (GSH) for redox defense, protein carbonyls as hallmarks of oxidative protein damage, and heat shock protein 70 (Hsp-70) as a key indicator of the cellular stress response[8-11].

Materials and Methods

This experimental study was approved by the Dokuz Eylül University Multidisciplinary Laboratory Animal Experiments Local Ethics Committee (protocol no: 07/2019, date: 30.01.2019) and was conducted in compliance with the Guide for the Care and Use of Laboratory Animals between June 2019 and March 2020.

A total of 16 male Wistar albino rats, weaned at 21 days of age, were randomly assigned to two equal groups:

the plant-based diet (PBD) and ABD (n=8 each). Rats were housed at a controlled temperature and on a 12-hour light/dark cycle, with free access to food and water. The PBD consisted of soy protein as the protein source and palm oil as the lipid source. The ABD contained casein as the protein source and milk fat as the lipid source. Both diets shared the same carbohydrate source and the same vitamin and mineral premixes, and their macronutrient composition was designed to be compatible with standard purified rat chow (based on the D12450J formulation). Rats were fed ad libitum for 12 weeks. During the feeding period, one rat per group died, and an additional rat the PBD group died under deep anesthesia, leaving 7 rats in the ABD group and 6 rats in the PBD group for final analyses.

Surgical Procedure

At the end of week 12, rats were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). A midline laparotomy followed by a thoracotomy extending into the left hemithorax was performed to expose the heart. After visualization, a blood sample was obtained and transferred into a heparinized syringe in preparation for blood cardioplegia. Systemic anticoagulation was provided with heparin (300 IU/kg) administered directly into the left ventricle. Following systemic heparinization, the ascending aorta was cross-clamped, and St. Thomas II crystalloid cardioplegia was delivered via the aortic root to induce cardiac arrest. A 10-minute period of global ischemia was maintained. At the end of this ischemic interval, cardioplegia prepared with the rat's own blood was administered to initiate reperfusion. Immediately thereafter, the hearts were excised under direct vision. The left ventricle was separated, rinsed in phosphate-buffered saline (PBS), wrapped in Parafilm to prevent drying, and stored at -80 °C until biochemical analysis.

Tissue Homogenization and ELISA Procedure

Left ventricular myocardium was homogenized in PBS (100 µL per 0.01 g of tissue) using a bead tissue lyser. Supernatants were obtained after vortexing and centrifugation. Protein quantification was performed using the bicinchoninic acid kit. Blood samples were centrifuged at 2000 rpm for 10 min to separate serum. The following markers were measured using ELISA kits (Bioassay Technology Laboratory, Shanghai, China), according to the manufacturer's instructions, and optical densities were read at 450 nm: Bcl-2, Bax, GSH, protein carbonyls, malondialdehyde (MDA), Hsp-70.

Statistical Analysis

Statistical analyses were performed using SAS software (version 9.4). The significance level for statistical analyses was set at 0.05. Descriptive statistics for each score were presented by study group: n, mean, median, standard deviation, minimum, maximum, 95% lower confidence limit, and 95% upper confidence limit.

Whether there was a statistically significant difference between the group means (arithmetic mean or median) was determined based on the distribution of the scores, using either the two-sample Student's t-test or the Wilcoxon (Mann-Whitney U). For scores that were normally distributed, the Student's t-test was used to compare group means. For scores not conforming to a normal distribution, the Wilcoxon rank-sum test (Mann-Whitney U test) was used to compare group medians. Normal distribution was evaluated by Shapiro-Wilk test prior to choosing parametric or non-parametric analyses.

Results

Analysis of myocardial tissue markers revealed a significant difference between the two dietary groups. Bcl-2 expression was significantly higher in the PBD group compared with the ABD group (11.28±1.22 vs. 9.50±1.25 ng/mg protein, p=0.025), indicating stronger antiapoptotic activity in rats fed a PBD (Table 1).

In contrast, Bax levels, representing pro-apoptotic signaling, were lower in the PBD group than in the ABD group [median 18.03 (14.07-24.62) vs. 22.18 (17.85-49.69)], but this difference was not statistically significant (p=0.100).

Table 1. Biochemical parameters

Parameter	PBD Mean ± SD/median (min-max)	ABD Mean ± SD/median (min-max)	p-value
Bcl-2	11.28±1.22	9.50±1.25	0.025
Bax	18.03 (14.07-24.62)	22.18 (17.85-49.69)	0.100
MDA	1.41 (1.23-1.72)	1.72 (1.24-3.23)	0.080
Protein carbonyl	57.93±9.76	62.17±13.44	0.536
GSH	116.19±26.01	99.08±25.02	0.253
Hsp-70	3.60±0.47	4.01±1.03	0.381

Data are presented as mean ± SD or median (min-max), depending on the statistical test used. Bold values indicate statistical significance (p<0.05). PBD: Plant-based diet group, ABD: Animal-based diet group, MDA: Malondialdehyde, GSH: Glutathione, SD: Standard deviation, Hsp-70: Heat shock protein 70

Regarding oxidative stress markers, MDA levels were lower in the PBD group [median 1.41 (1.23-1.72)] compared with the ABD group [median 1.72 (1.24-3.23)], representing a borderline trend that did not reach statistical significance (p=0.080). Protein carbonyl content was also lower in the PBD group (57.93±9.76 vs. 62.17±13.44), although the difference was not statistically significant (p=0.536).

GSH levels were higher in the PBD group (116.19±26.01) than in the ABD group (99.08±25.02), but this increase was not statistically significant (p=0.253).

Finally, Hsp-70 levels were slightly lower in the PBD group than in the ABD group (3.60±0.47 vs. 4.01±1.03), but the difference was not statistically significant (p=0.381).

Discussion

In this experimental model of cardioplegic arrest, PBD was associated with significantly higher myocardial Bcl-2 levels compared with ABD, indicating a stronger antiapoptotic profile. Other markers, including Bax, MDA, protein carbonyls, GSH, and Hsp-70, showed no significant differences between groups. Nevertheless, the overall trend favored PBD, with lower Bax expression, reduced oxidative stress indices, and higher GSH levels. These data suggest that preoperative dietary modulation can shift myocardial signaling toward cell survival, even when oxidative stress readouts do not reach statistical significance.

The Bcl-2 family is a critical regulator of apoptosis in cardiomyocytes exposed to I/R. Increased Bcl-2 stabilizes mitochondrial membranes, while Bax promotes permeabilization and release of cytochrome c, triggering apoptosis[8,12,13]. Our finding of significantly higher Bcl-2 levels in the PBD group highlights a potential mechanism for diet-induced cardioprotection. Although Bax reduction was not statistically significant, its directionality suggests attenuation of proapoptotic signaling. The balance between Bcl-2 and Bax is widely regarded as a determinant of myocardial survival after I/R injury.

I/R injury generates reactive oxygen species that induce lipid peroxidation and protein oxidation. In our study, MDA and protein carbonyls were lower in the PBD group, although the differences were not statistically significant. Protein carbonylation is a robust marker of oxidative protein damage and has been linked to adverse cardiovascular outcomes[14,15]. At the same time, higher GSH levels in the PBD group suggest enhanced antioxidant reserves. GSH is crucial for detoxifying ROS, and impaired GSH metabolism exacerbates myocardial I/R injury[16,17]. Although not statistically significant, the favorable GSH trend supports the biological plausibility that plant-based nutrients augment endogenous redox defenses.

Hsp, particularly Hsp-70, are protective mediators during I/R stress. They stabilize proteins, regulate calcium handling, and reduce apoptosis[11,18]. In our study, Hsp-70 levels were numerically higher in the ABD group compared to the PBD group, although the difference was

not statistically significant. This pattern may suggest that animal-based feeding induced a modest stress response, potentially reflecting an early or compensatory activation of Hsp-70. In contrast, the lower, though not significantly different, values in the PBD group could indicate a reduced cellular stress burden, consistent with the favorable trends observed in apoptotic and oxidative markers. Nonetheless, given the short ischemic duration and immediate tissue sampling, Hsp-70 expression may not have fully manifested, and these findings should be interpreted with caution. Future studies with longer reperfusion times are required to clarify whether diet type meaningfully modulates Hsp-70 dynamics. Epidemiological and clinical studies consistently associate PBDs with reduced cardiovascular morbidity[19,20]. Beyond systemic benefits, our findings indicate potential direct myocardial effects during surgical I/R. The significant increase in Bcl-2 suggests that antiapoptotic signaling may be a robust contributor to perioperative cardioprotection. By contrast, oxidative stress and antioxidant markers did not differ significantly; nevertheless, their consistent trends in favor of PBD suggest additional biological contributions that may require larger or differently timed studies to confirm. Importantly, these observations indicate that the protective impact of plant-based nutrition is unlikely to be mediated by a single pathway. Rather, it may reflect the convergence of multiple mechanisms, including modulation of apoptosis, redox homeostasis, inflammatory responses, and mitochondrial function. Recognizing this multifactorial nature is critical for translating nutritional interventions into perioperative strategies and long-term cardiovascular prevention.

Beyond apoptosis and oxidative stress, ischemia-reperfusion injury is strongly mediated by inflammatory cascades. PBDs are rich in bioactive compounds such as polyphenols, which have been shown to modulate inflammatory pathways and reduce the production of proinflammatory cytokines in cardiovascular and inflammatory models[21]. Although we did not directly assess inflammatory mediators in this study, the favorable trends in oxidative and apoptotic indices within the PBD group may indirectly reflect lower inflammatory activation. Future studies should incorporate inflammatory biomarkers to clarify whether dietary modulation exerts additive protective effects through suppression of inflammatory signaling pathways.

From a translational standpoint, these findings may have particular relevance for patients undergoing heart surgery, especially those at high risk of perioperative myocardial injury. Short-term preoperative nutritional optimization with plant-based regimens could augment myocardial protection when combined with conventional strategies such as cardioplegia and pharmacologic conditioning. While current evidence is preliminary, it suggests that perioperative dietary interventions may represent a simple, low-cost, and non-invasive adjunct for improving surgical outcomes and merit further evaluation in clinical trials.

Study Limitations

This study has several limitations that should be acknowledged. First, the relatively small sample size reduces statistical power and increases the likelihood of a type II error, particularly for oxidative stress parameters. Second, the ischemic protocol consisted of only a 10-minute cardioplegic arrest followed by immediate tissue sampling, which may have led to underestimation of the full extent of reperfusion-related oxidative injury and stress protein induction. In addition, the absence of a sham or standard chow control group limits the ability to distinguish the effects of dietary composition from baseline physiology. Furthermore, histopathological assessment was not performed, which could have provided additional validation of the biochemical findings. Finally, although the 12-week dietary period was sufficient to induce measurable tissue changes, it may not fully reflect the long-term effects of plant- and ABD. These limitations should be considered when interpreting our results, as they highlight the need for larger, more comprehensive studies.

Conclusion

Our findings demonstrate that preoperative plant-based nutrition can modulate myocardial responses to surgical I/R, most notably by enhancing antiapoptotic signaling through significant upregulation of Bcl-2. While other biochemical markers showed only favorable but non-significant trends, the overall profile supports the concept that diet composition may influence myocardial tolerance to perioperative stress. These results provide a biological rationale for exploring plant-based strategies as adjuncts to established cardioprotective approaches in cardiac surgery. Validation in larger experimental models and clinical trials will be essential to determine whether such dietary interventions can translate into meaningful perioperative and long-term benefits.

Ethics

Ethics Committee Approval: This experimental study was approved by the Dokuz Eylül University Multidisciplinary Laboratory Animal Experiments Local Ethics Committee (protocol no: 07/2019, date: 30.01.2019) and was conducted in compliance with the Guide for the Care and Use of Laboratory Animals.

Informed Consent: Experimental research.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Kemahlı MB., Gençpınar T., Concept: Koçtürk S., Metin K., Design: Koçtürk S., Metin K., Data Collection and/or Processing: Kemahlı MB., Erkmen T., Sert Serdar B., Analysis and/or Interpretation: Erkmen T., Sert Serdar B., Yüksel K., Literature Search: Kemahlı MB., Writing: Kemahlı MB.

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