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Infrared spectrochemical findings on intermittent fasting-associated gross molecular modifications in rat myocardium

İsa Ardahanlı [a], Halil İbrahim Özkan [b], Faik Özel [c], Rafig Gurbanov [d,e], Hikmet Taner Teker [f], Taha Ceylani [g,*]

[a] Department of Cardiology, Faculty of Medicine, Bilecik Şeyh Edebali University Bilecik, Turkey
[b] Department of Biochemistry, Faculty of Medicine, Atatürk University Erzurum, Turkey
[c] Department of Internal Medicine, Faculty of Medicine, Bilecik Şeyh Edebali University Bilecik, Turkey
[d] Department of Bioengineering, Faculty of Engineering, Bilecik Şeyh Edebali University Bilecik, Turkey
[e] Central Research Laboratory, Bilecik Şeyh Edebali University Bilecik, Turkey
[f] Department of Medical Biology, Ankara Medipol University Ankara, Turkey
[g] Department of Food Quality Control and Analysis, Muş Alparslan University Muş, Turkey

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ABSTRACT

Cardiovascular diseases are among the primary life-threatening conditions affecting human society. Intermittent fasting is shown to be functional in the prevention of cardiovascular diseases, however, the information on fasting-associated modifications in myocardial biomolecules is limited. This study aimed to determine the impact of 18-h intermittent fasting administered for five weeks on 12 months-old rats using supervised linear discriminant analysis and support vector machine algorithms constructed on spectrochemical data obtained from myocardial tissues. These algorithms revealed gross biomolecular modifications, while quantitative analyses demonstrated higher amounts of saturated lipids (19%), triglycerides (11%), and lipids (56%), in addition to enhancement in membrane dynamics (18%). The concentrations of nucleic acids and glucose are increased by 52%, while the glycogen content is diminished by 61%. The protein carbonylation/oxidation is reduced by 38%, whereas a 35% increase in protein content was measured. Phosphorylated proteins have been calculated to be at higher concentrations in the 13–62% range. The study findings demonstrated significant molecular changes in the myocardium of rats subjected to intermittent fasting.

1. Introduction

The cardiovascular system (CVS) is one of the critical systems of the human body, responsible for the delivery of oxygen and nutrients to the tissues and organs, and the transport of carbon dioxide and wastes to the outside [1]. CVS diseases are among the leading causes of mortality and morbidity worldwide [2]. Lifestyle, diet, and genetic factors are some of the conditions that can cause cardiovascular diseases (CVD). The frequency of CVDs increases exponentially, especially in high-calorie and irregular nutrition [3].

Previous studies have shown that dietary restrictions, reduced energy intake, or an intermittent fasting diet (IF) increase life expectancy and decrease the incidence of age-related diseases [4,5]. It is known that long-term calorie restriction reduces arterial blood pressure and serum lipid concentrations, which are known metabolic risk factors for cardiovascular disease [6,7]. However, the beneficial effects of IF on the heart at the molecular level are still not fully understood. It has been reported that dietary restriction has antioxidant and anti-inflammatory effects [8]. In this way, it has been reported to reduce endothelial dysfunction. There may be an improvement in coronary artery blood flow and a low incidence of atherosclerotic heart diseases due to decreased endothelial dysfunction [9]. IF is also known to have a cardioprotective effect, providing resistance to ischemic injury in experimental animals. This effect is thought to be possibly related to increases in adiponectin levels [10].

Oxidative stress is characterized by the overproduction of reactive oxygen species (ROS), including free radicals, that cause damage to the cardiovascular system and contribute to the development of cardiovascular disease and aging [8]. The damage caused by free oxygen radicals in the CVS can be in the conduction system of the heart, its contractile

* Corresponding author.
E-mail address: t.ceylani@alparslan.edu.tr (T. Ceylani).

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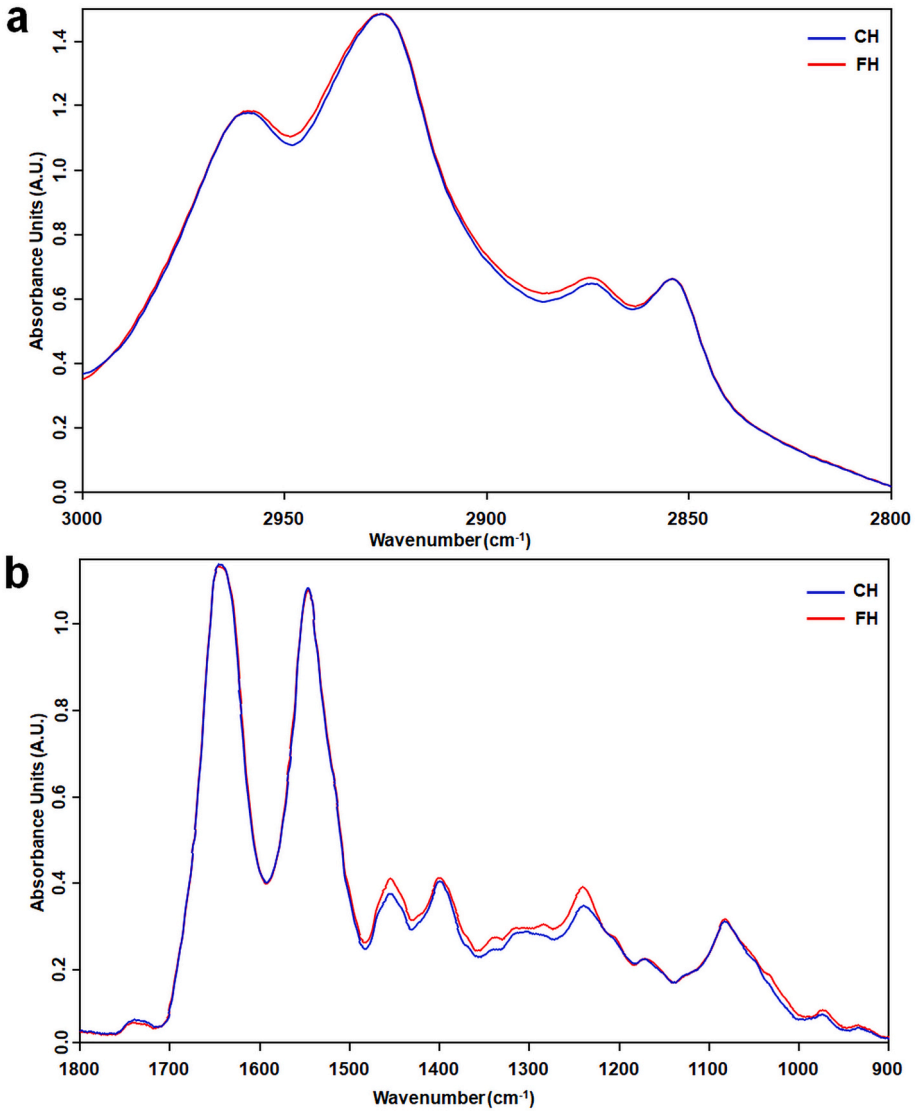

Fig. 1. Intermittent fasting modifies the gross biomolecules in rat myocardium. Average baseline corrected and normalized infrared spectra in **a)** C–H (3000–2800 cm^{-1}) and **b)** fingerprint (1800–900 cm^{-1}) regions. CH (control rats), FH (rats on intermittent fasting). Baseline correction was done by the Rubberband correction method with 64 baseline points. Normalization was done by using the vector normalization method. The spectra were pre-processed in OPUS 5.5 (Bruker) software.

structure, and the microvascular circulation that provides perfusion. IF lowers oxidative modifications of proteins and DNA and exerts a cardioprotective effect by reducing lipid peroxidation levels in the heart [11,12]. At the same time, IF inhibits inflammatory processes likely contributing to atherosclerosis, as indicated by low levels of leukocytes, circulating tumor necrosis factor, and other inflammatory cytokines. It can reduce the risk of cardiovascular disease and stroke by suppressing atherosclerosis [13].

Spectroscopy is an orthodox analytical tool. However, it is only in the last few decades that Fourier-transformed infrared (FTIR) spectroscopy has begun to be used in biology and medicine with the invention of modern spectrometers and chemometrics or machine learning tools [14–18]. It is a straightforward, economical, tag-free, and non-destructive approach that only a small quantity of material is enough to provide extremely sensitive and repeatable findings [19]. It also enables a biological sample to be examined in a matter of minutes and a specific spectral signature to be identified. However, sample selection and preparation before spectral capture are critical steps in producing high-quality and repeatable findings. Over the last two decades, it was used in a wide range of scientific investigations providing valuable examinations of different biomolecules [14]. Because of the intricacy of spectrochemical data; chemometric methods should be used to extract relevant information from the material being studied [20,21]. Linear Discriminant Analysis (LDA) is a supervised machine learning method being applied for data mining of big spectrochemical datasets [22,23]. Support Vector Machine (SVM) is a well-known supervised machine learning technique based on statistical learning theory. SVM has been actively used as a classification algorithm in biological systems such as functional genomic prediction, protein homolog identification, and disease assessment [24].

This study aimed to determine the impact of 18-h IF on rat myocardium administered for five weeks. For this purpose, LDA and SVM machine learning methods were applied to large spectrochemical fingerprint data obtained from myocardial tissues in rats. The quantitative measurements of spectrochemical band parameters specific to lipids (3000–2700 cm^{-1}), proteins (1700–1500 cm^{-1}), and nucleic acids with carbohydrates (1300–800 cm^{-1}) were also carried out to explain modified metabolic processes happening at the biomolecular level. Both spectrochemical band analyses and machine learning approaches were implemented for rapid and accurate identification of the IF-induced gross and specific biomolecular modifications in myocardial tissues of rats.

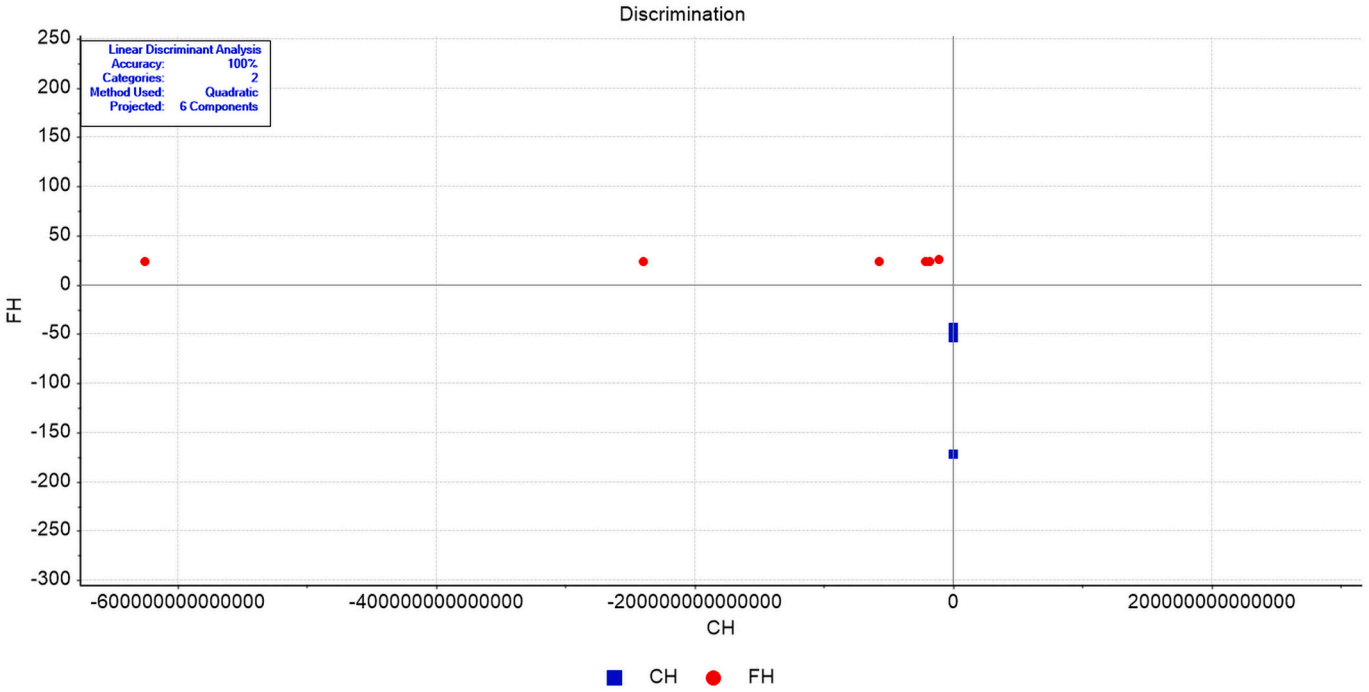

Fig. 2. Intermittent fasting modifies the gross biomolecules in rat myocardium. The discrimination plot of Linear Discriminant Analysis in 4000–650 cm^{-1} infrared spectral region. CH (control rats), FH (rats on intermittent fasting).

Table 1
Intermittent fasting modifies the gross biomolecules in rat myocardium. The confusion and prediction matrices of Linear Discriminant Analysis in full (4000–650 cm^{-1}) infrared spectral region. CH (control rats), FH (rats on intermittent fasting).

Confusion Matrix			
Predicted	Actual	CH	FH
		1	2
CH	**1**	6	0
FH	**2**	0	8
Prediction Matrix			
Actual	**CH**	**FH**	**Predicted**
AV_CH1.0	35,48,563	−8061,054	CH
AV_CH2.0	41,99,369	−172,3678	CH
AV_CH3.0	42,13,272	−53,4799	CH
AV_CH4.0	40,17,763	−43,25,936	CH
AV_CH5.0	42,06262	−45,02848	CH
AV_CH6.0	42,09597	−46,94,022	CH
AV_FH1.0	−6,25E+14	23,59,776	FH
AV_FH2.0	−1,25E+16	23,56,545	FH
AV_FH3.0	−2,10E+13	23,51,887	FH
AV_FH4.0	−1,83E+13	23,65,122	FH
AV_FH5.0	−5,68E+13	23,96,945	FH
AV_FH6.0	−2,40E+14	24,05723	FH
AV_FH7.0	−1,05E+13	25,4853	FH
AV_FH8.0	−1,57E+15	23,58,929	FH

Table 2
Intermittent fasting modifies the gross biomolecules in rat myocardium. Support Vector Machine classification for heart myocardium samples in full (4000–650 cm^{-1}) infrared spectral region. CH (control rats), FH (rats on intermittent fasting). Support Vector Machine type: Classification (nu-SVC). Method: Linear.

Accuracy (%)			
Training	64.28	Validation	64.28
Classification			
Samples		Class	
AV_CH1.0	1	1	CH
AV_CH2.0	2	2	CH
AV_CH3.0	3	3	CH
AV_CH4.0	4	4	CH
AV_CH5.0	5	5	CH
AV_CH6.0	6	6	CH
AV_FH1.0	7	7	FH
AV_FH2.0	8	8	FH
AV_FH3.0	9	9	FH
AV_FH4.0	10	10	FH
AV_FH5.0	11	11	FH
AV_FH6.0	12	12	FH
AV_FH7.0	13	13	FH
AV_FH8.0	14	14	FH

2. Materials and methods

2.1. Animal studies

Male Wistar rat (12 months, $n = 14$) species was used as a model organism in the study. The control group ($n = 6$) was allowed free access to water and food for 24 h. IF was applied to the rats ($n = 8$) for 35 days. While the rats in the IF group were always able to drink water, their access to food was restricted for 18 h and they were only allowed to feed for 6 h. No food or water restriction was applied to rats in the control group. Animals were fed ad libitum with a standard rodent diet. One day after the end of the application, the animals in the control and IF groups were sacrificed and the cardiac tissues were shocked on dry ice and left in the -80 °C deep freezer until the time to be studied. All animals were housed under standard animal care conditions. The study was carried out with the approval of the Ethics Committee (approval number: 2021/05) from the Saki Yenilli Experimental Animal Production and Practice Laboratory.

2.2. Big data acquisition in the Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy

Myocardial samples (dissected cardiac muscle fibers) of all animals

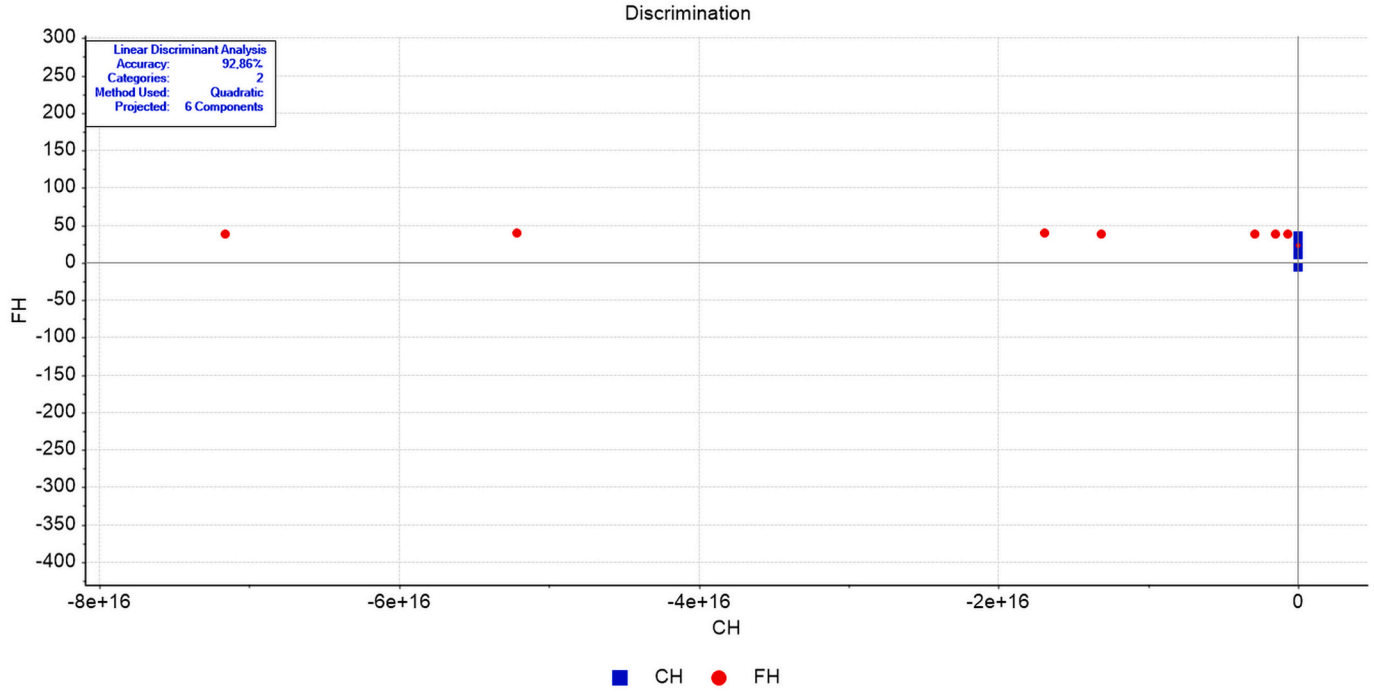

Fig. 3. Intermittent fasting modifies the gross lipids in rat myocardium. The discrimination plot of Linear Discriminant Analysis in 3000–2700 cm^{-1} infrared spectral region. CH (control rats), FH (rats on intermittent fasting).

($n = 14$) were compressed on the Zn/Se crystal of the ATR unit (PerkinElmer) without any pretreatment and each myocardial tissue was examined twice (2) from the vicinal sides with an ATR-FTIR spectrometer (PerkinElmer) at a resolution of 4 cm^{-1} and a scan number of 32. Thus, 28 sample spectra were obtained from 14 animal tissues in total. However, the average spectrum of 2 replicates from each sample was used for all further prediction/classification and quantification analyses. The spectra were obtained with the Spectrum One (PerkinElmer) software in the wavelength range of 4000–650 cm^{-1} [25].

2.3. Prediction studies with machine learning methods based on big spectral data

Linear Discriminant Analysis (LDA), a machine learning approach, was applied to differentiate the experimental groups from each other. Spectral data were used in pattern recognition analysis. To make the analyzes as independent as possible from the FTIR spectrometers, each sample's average spectrum was preprocessed on The Unscrambler® X 10.3 (CAMO Software AS, Norway) software with a baseline offset transformation and unit vector normalization in the 4000–650 cm^{-1} region. Spectra processed in this way were first subjected to Principal Component Analysis (PCA), an unsupervised pattern processing technique. Spectra were passed from standard deviation normalization (mean centering normalization) and full-cross random validation with 14 segments. Subsequently, the spectra were examined in lipid (3000–2700 cm^{-1}), protein (1700–1500 cm^{-1}), nucleic acid (1300–800 cm^{-1}), and full (4000–650 cm^{-1}) regions by the NIPALS (Non-linear Iterative Partial Least Squares) algorithm. PCA data were used as LDA model inputs with The Unscrambler® X 10.3 (CAMO Software AS, Norway) multivariate analysis (MVA) software. The category variable column was included in a data matrix and then all spectra of different sample categories were used to generate a training set. A quadratic method using the projections of the 6 principal components was used for the prediction. Prior probabilities were calculated from the training set. The results are presented as a discrimination plot, as well as prediction and confusion matrices.

Same spectral processings were applied in SVM modeling as well, using The Unscrambler® X 10.3 (CAMO Software AS, Norway) multivariate analysis (MVA) software. All spectra of different sample categories were used to generate a training set. Classification (nu-SVC) was chosen as SVM type using a linear method as Kernel type. Nu value was set to 0.5, weights as all 1.00. The 14 segments of cross-validation were used in the calculation of training and cross-validation accuracies. Finally, the generated training dataset was applied to all sample datasets to obtain an SVM classification model.

2.4. Quantification studies of spectrochemical bands

Spectral data analysis was performed using OPUS 5.5 (Bruker) software. The average spectra of each sample were baseline corrected using the Rubberband correction method with 64 baseline points before the band quantification analyses. In detailed band analyses, the bands with the highest absorbance values in different spectral regions of the spectra were selected and the beginning and ending frequencies of the bands were determined with precision. The integrated areas of bands specific to various biomolecules were analyzed by taking the integral areas of the determined frequency ranges with the OPUS 5.5 (Bruker) software. Furthermore, a virtual line was drawn vertically from the midpoint of the band baseline to the peak of the band, and the length of the line was measured with the help of a virtual ruler. Then, by marking the point where 0.75 times the length of the line coincides with the line, a horizontal line was drawn along the band from this point and bandwidth values were obtained.

2.5. Statistics

Statistical evaluations and graph plots of the results were made using GraphPad Prism 8.01 (GraphPad, USA). The data were analyzed using an unpaired *t*-test, and the significance levels were stated as $P \leq 0.05$ * and $P \leq 0.01$ **. Results are presented as mean ± SEM (standard error of the mean).

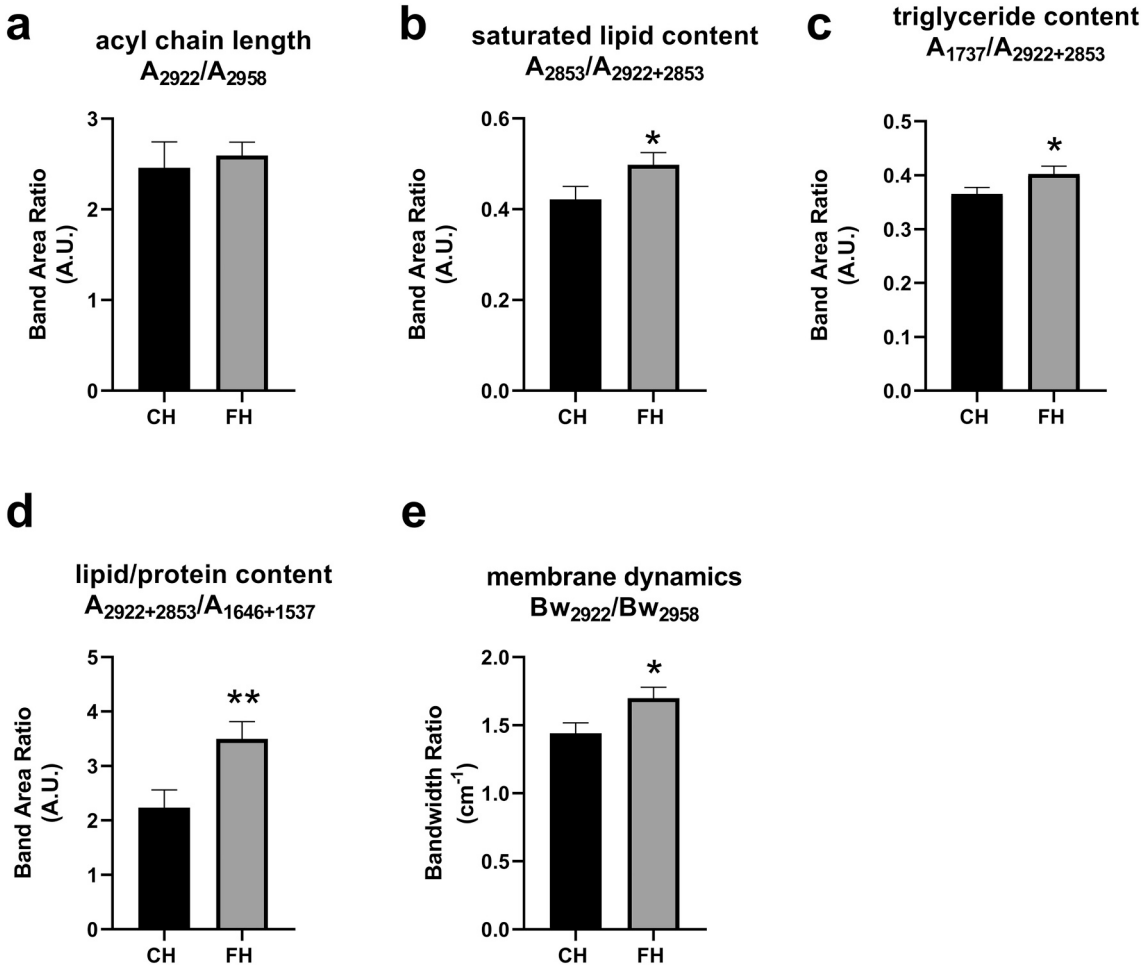

Fig. 4. Intermittent fasting modifies the lipid-associated particular parameters in rat myocardium. The indices for **a)** acyl chain length of fatty acids (A_{2922}/A_{2958}), **b)** saturated lipid content ($A_{2853}/A_{2922\ +\ 2853}$), **c)** triglyceride content ($A_{1737}/A_{2922\ +\ 2853}$), **d)** lipid/protein content ($A_{2922\ +\ 2853}/A_{1646\ +\ 1537}$), and **e)** membrane dynamics (Bw_{2922}/Bw_{2958}). A (Absorbance), Bw (Bandwidth), CH (control rats), FH (rats on intermittent fasting).

3. Results and discussion

3.1. Intermittent fasting modifies the gross biomolecules of rat myocardium

The qualitative differences were detected with the inspection of average infrared spectra of control (CH group) rats and rats subjected to IF (FH group) given at two major spectral regions: C—H (3000–2800 cm^{-1}) and fingerprint (1800–900 cm^{-1}) (Fig. 1a–b). The unsupervised PCA method was conducted before supervised LDA and SVM analyses (Fig. S1). The scores plots of PCA are given in the 4000–650 cm^{-1} (Fig. S1a), 3000–2700 cm^{-1} (Fig. S1b), 1300–800 cm^{-1} (Fig. S1c), and 1700–1500 cm^{-1} (Fig. S1d) infrared spectral regions. LDA analysis revealed clear-cut biomolecular modifications in the myocardium of rats subjected to IF, with a 100% accuracy rate in the full infrared region (4000–650 cm^{-1}). As can be seen from the discrimination plot, the data for CH and FH groups are stationed in completely different coordinates (Fig. 2). This indicates the absolute effect of IF on myocardial tissue. According to the confusion matrix, the actual samples of the FH group ($n = 8$) were not confused with the CH samples ($n = 6$). All the actual samples were correctly predicted in the matrix of prediction (Table 1). The SVM method also revealed the correct classification of all samples in their corresponding classes with 64.28% training and cross-validation accuracies, for the gross biomolecules of myocardial tissues (Table 2). According to literature, fasting is associated with improvement of serum lipid profiles, weight loss, body mass index, alterations in gut microbiota, and an increase in serum butyrate. Therefore, it was assumed to be beneficial against several chronic disturbances like hyperlipidemia and CVD [26].

To explain the possible origin of IF-induced gross molecular alterations in the myocardial tissue, LDA modeling was obtained for particular biomolecule groups. Moreover, the quantitative changes in specific spectrochemical bands related to different functional groups in these biomolecules were measured. Accordingly, the spectral parameters such as band area ratios and bandwidths were analyzed for the elucidation of biochemical modifications happening in lipids, proteins, nucleic acids, and carbohydrates of the myocardium. The analyses mainly covered the bands at 2958 cm^{-1} (CH_3 antisymmetric stretching: lipids and proteins), 2922 cm^{-1} (CH_2 antisymmetric stretching: mainly lipids), 2853 cm^{-1} (CH_2 symmetric stretching: mainly lipids), 1737 cm^{-1} (Ester C═O stretching: triglyceride, cholesterol esters), 1646 cm^{-1} (Amide *I*: C═O stretching vibration of amide groups: proteins), 1537 cm^{-1} (Amide II: N—H bending strongly coupled to C—N stretching vibration of amide groups: protein), 1232 cm^{-1} (PO_2 antisymmetric stretching: nucleic acids with a little contribution of phospholipids), 1083 cm^{-1} (PO_2 symmetric stretching: nucleic acids and phospholipids), 1047 cm^{-1} (νC–O + δC–O stretching: glycogen), and 1031 cm^{-1} (C—O stretching: carbohydrates /glycogen, glucose) spectral positions.

3.2. Intermittent fasting modifies the myocardial lipids

A similar observation with high accuracy (92.86%) was obtained in

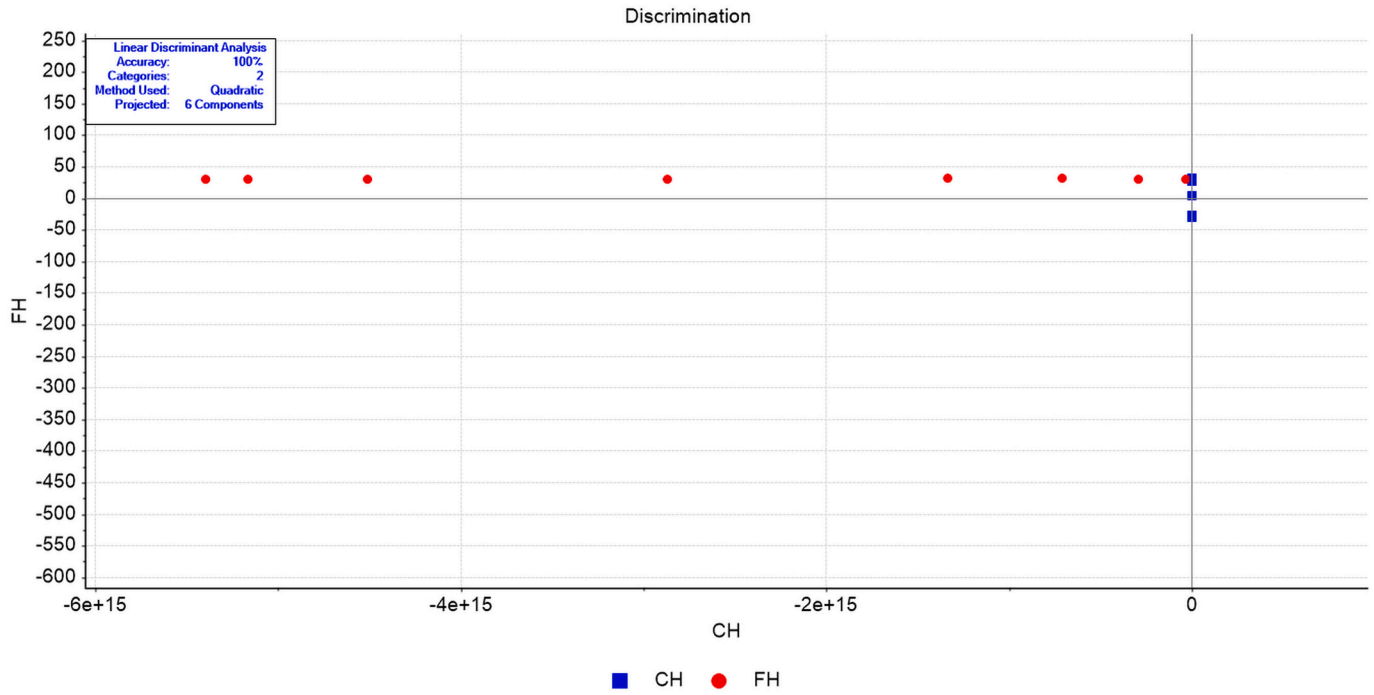

Fig. 5. Intermittent fasting modifies the gross nucleic acids and carbohydrates in rat myocardium. The discrimination plot of Linear Discriminant Analysis in 1300–800 cm^{-1} infrared spectral region. CH (control rats), FH (rats on intermittent fasting).

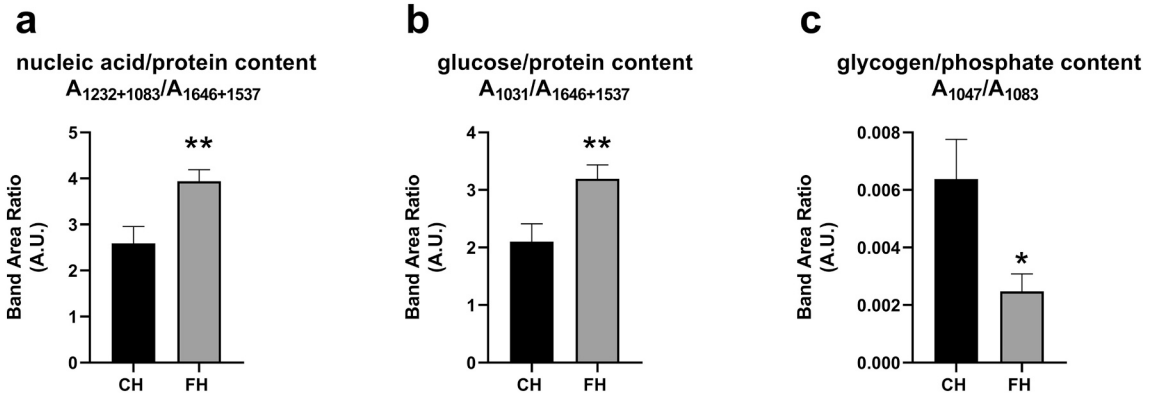

Fig. 6. Intermittent fasting modifies the nucleic acid and carbohydrate-associated particular parameters in rat myocardium. The indices for **a)** nucleic acid/protein content ($A_{1232\ +\ 1083}/A_{1646\ +\ 1537}$), **b)** glucose/protein content ($A_{1031}/A_{1646\ +\ 1537}$), and **c)** glycogen/phosphate content (A_{1047}/A_{1083}). A (Absorbance), CH (control rats), FH (rats on intermittent fasting).

LDA analysis administered for the inspection of lipid profiles of the myocardium. The CH and FH groups are discriminated from each other across the discrimination plot in terms of their lipid profiles (Fig. 3). Moreover, the prediction and confusion matrices revealed that 5 actual CH samples and all actual 8 FH samples were correctly predicted, except for 1 mispredicted CH sample (Data not shown).

On the other hand, the IF diet has an impact on various lipid molecules as revealed by the calculation of band area ratios for an acyl chain length of fatty acids (A_{2922}/A_{2958}), saturated lipid ($A_{2853}/A_{2922\ +\ 2853}$), triglyceride ($A_{1737}/A_{2922\ +\ 2853}$), and lipid/protein ($A_{2922\ +\ 2853}/A_{1646\ +\ 1537}$) contents [23,27] (Fig. 4a–d). These band ratio indices were significantly elevated in myocardial tissues due to IF (saturated lipid content: 19%, triglyceride content: 11%, lipid/protein content: 56%), except for the acyl chain length of fatty acids which was increased only by 6%. Although the metabolic state of the liver condition is lipolytic [28], the elevated levels of free fatty acid (FFA) in plasma, such as those in fasting, increase the delivery of FFA to the liver, which may cause excessive hepatic triglyceride accumulation despite accompanying increases in fatty acid oxidation [29]. Plasma FFA concentration is a major regulator of intramyocardial triglyceride content. Intramyocardial triglyceride synthesis was shown to be increased with elevated plasma FFA concentrations in diabetes, fasting, and starvation [30]. The contribution of beta-adrenergic stimulation to the mobilization of fat during fasting was previously reported [31]. Adrenergic stimulation in isolated cardiac myocytes activates glycerol phosphate acyltransferase enzyme and incorporates palmitate into triglyceride stores (synthesis) while simultaneously increasing triglyceride breakdown (lipolysis), suggesting that adrenergic stress increases the turnover of the intramyocardial triglyceride pool [30]. Perilipin 5 (PLIN5) protects against lipotoxicity and stimulates lipid oxidation. Fasting transiently increased circulating free fatty acids and PLIN5 mRNA levels in human skeletal muscle. This would suggest that lipid droplet formation and storage within the myocyte were transiently augmented in response to fasting [32].

The bandwidth of CH_2 antisymmetric stretching bands gives

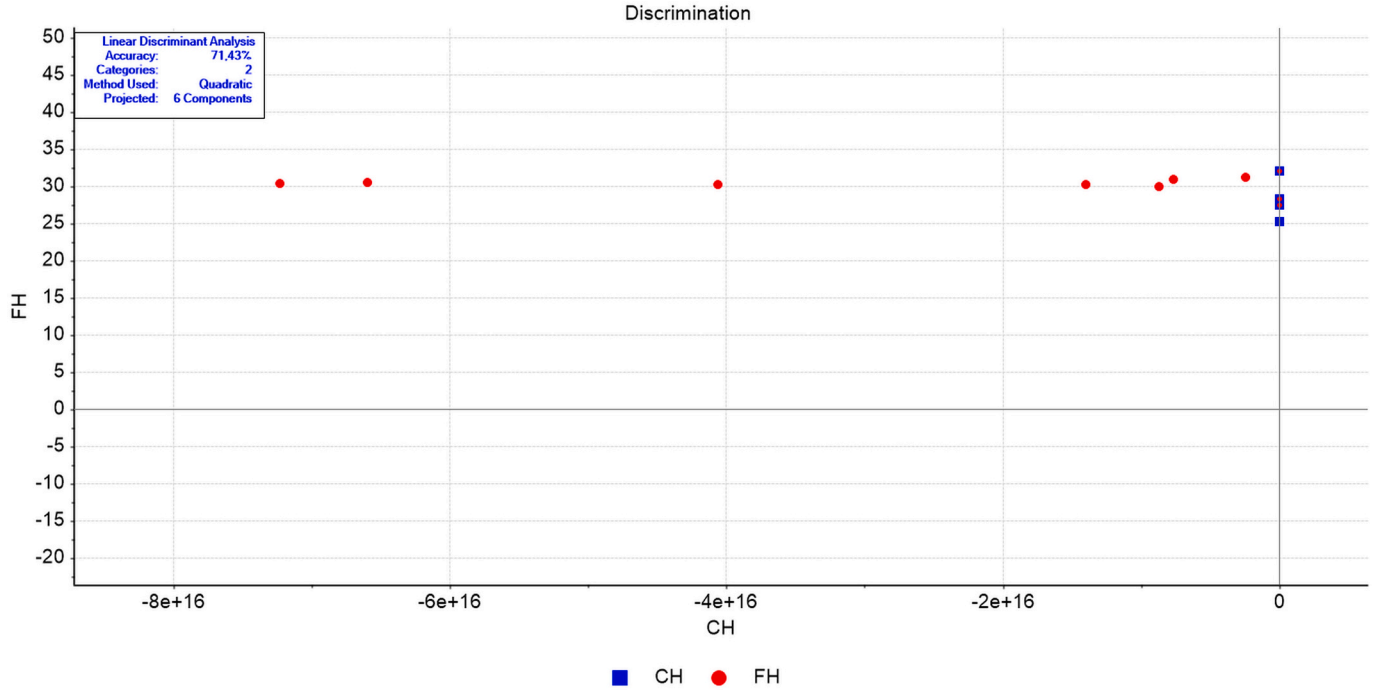

Fig. 7. Intermittent fasting modifies the gross proteins in rat myocardium. The discrimination plot of Linear Discriminant Analysis in 1700–1500 cm^{-1} infrared spectral region. CH (control rats), FH (rats on intermittent fasting).

information about lipid bilayer membrane dynamics since it is related to the motion rates of the lipid molecule [33]. Previously, reduced membrane dynamics have been linked with obesity, diabetes, and diabetes-associated cardiovascular disease in human subjects [34,35]. Animal studies have also shown similarly reduced membrane fluidity in diabetic rat tissues [36]. Interestingly, IF enhanced the membrane lipid dynamics/fluidity by 18% as assessed through the measurement of bandwidth ratio for lipid dynamics parameter that is Bw_{2922}/Bw_{2958} ratio index (Fig. 4e). According to these results, it is apparent that IF modulates lipid mobilization and synthesis processes in myocardial tissues of rats.

3.3. Intermittent fasting modifies the myocardial nucleic acids and carbohydrates

The significant biochemical modifications caused by IF were similarly realized for nucleic acids and carbohydrates with the highest accuracy (100%) in LDA analysis. It is seen that the samples are located at completely different coordinates in the 1300–800 cm^{-1} spectral region (Fig. 5). The results presented in prediction and confusion matrices were comparable (no confusion and/or incorrect prediction) with that of gross biomolecules (Data not shown).

Moreover, the IF diet produced significant changes in nucleic acid and carbohydrate concentrations as measured through the calculation of band area ratios for nucleic acid/protein ($A_{1232\ +\ 1083}/A_{1646\ +\ 1537}$), glucose/protein ($A_{1031}/A_{1646\ +\ 1537}$), and glycogen/phosphate (A_{1047}/A_{1083}) contents (Fig. 6a–c). The band ratio indices for both nucleic acid/protein and glucose/protein contents are increased by 52%, whereas the index for glycogen/phosphate content is diminished by 61%.

The major metabolic substrate for the heart is fatty acids. However, up to 30% of myocardial ATP is generated by glucose and lactate [37]. Glucose is a universal substrate for energy production for cells, and therefore, the modifications of glucose metabolism are crucial for cellular homeostasis. The trafficking and translocation of glucose transporter (GLUT4) in cardiac muscle are mostly similar to that observed in skeletal muscle [37]. Excess glucose is stored as glycogen via the glycogenesis pathway in the liver as well as skeletal and cardiac muscles. The results of this study demonstrated elevated glucose and diminished glycogen levels in myocardial tissues of animals subjected to an IF diet. Fasting was reported to be associated with altered glycogen contents in different tissues of rats dependent on increased plasma levels of non-esterified fatty acids [38]. Considering that fasting metabolism is unique and different from fed-state physiology, the adaptive mutual actions of fat and glucose metabolism during fasting/starvation are also crucial [39]. Therefore, the change in metabolic preference of myocardium due to modulated lipid and nucleic acid metabolism during the IF diet might be involved in the modulation of metabolic events such as enhanced glycogen breakdown and glucose transport to meet the energy expenditure of cell.

3.4. Intermittent fasting modifies the myocardial proteins

In the case of proteins; the LDA discrimination accuracy was obtained as 71.43%, and most of the samples are located in different coordinates. However, this discrimination is lower compared to gross biomolecules (100%), lipids (92.86%), as well as nucleic acids, and carbohydrates (100%) (Fig. 7). The 4 actual members of the CH group were wrongly predicted as FH group members in the prediction matrix, and confused in the confusion matrix. The actual samples of the FH group were precisely predicted without any confusion (Data not shown).

The calculation of protein-associated band area ratios demonstrated IF-induced significant modifications in proteins (Fig. 8). While the index for protein conformation (A_{1646}/A_{1537}) did not change (Fig. 8a), the ratio index for protein concentration ($A_{1646}/A_{1646\ +\ 1537}$) demonstrated a significant increment by 35% (Fig. 8b), indicating IF-induced enhancement of protein synthesis or reduced breakdown of proteins in myocardial tissues. The band area ratios A_{1232}/A_{2958} and A_{1083}/A_{1537} are specified as protein phosphorylation indices [23,40]. The phosphorylation indices A_{1232}/A_{2958} and A_{1083}/A_{1537} were respectively increased by 62% and 13% due to IF, indicating high concentrations of phosphorylated proteins (Fig. 8c–d). Protein phosphorylation is a regulatory mechanism that is crucial in most cellular processes. Many biomolecules involved in protein synthesis, cell proliferation, cell signaling, morphogenesis, progression, and aging are regulated by

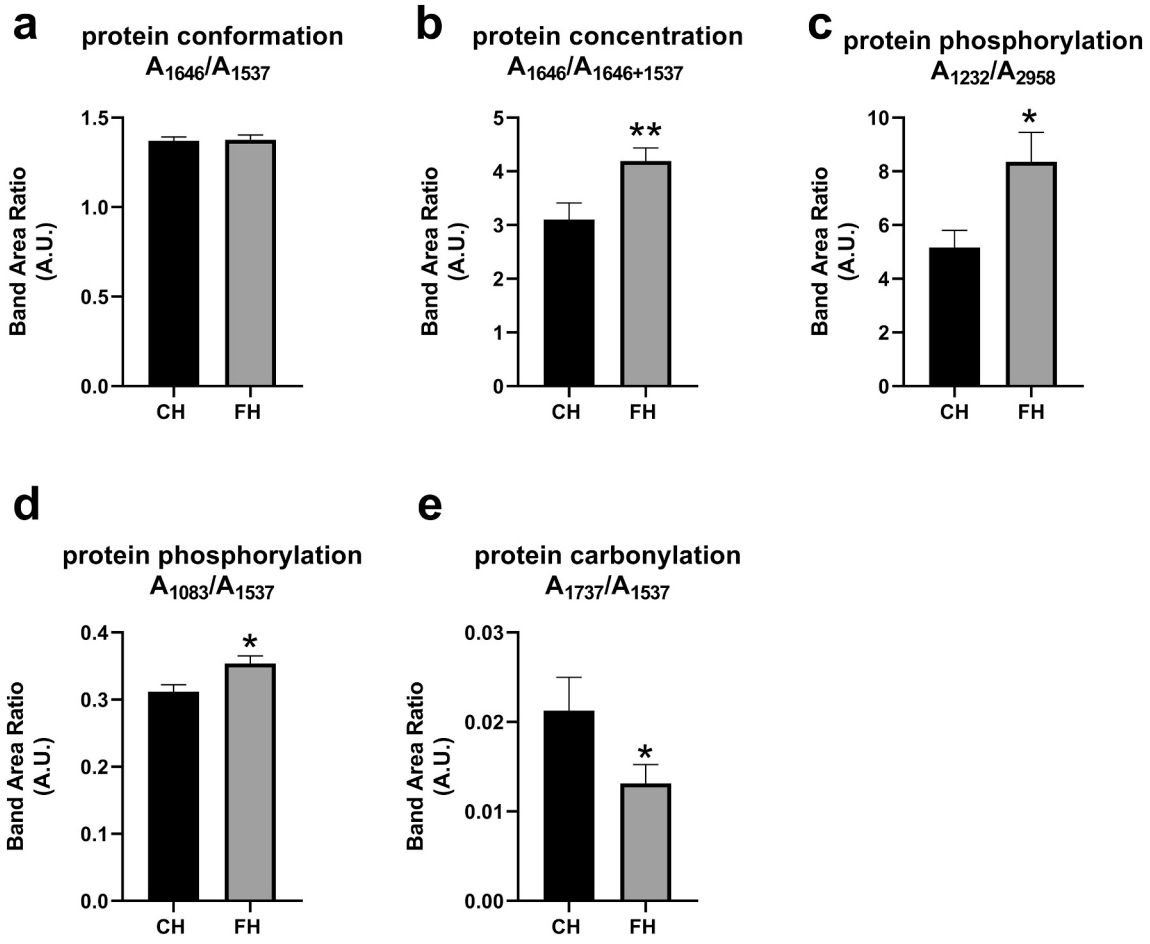

Fig. 8. Intermittent fasting modifies the protein-associated particular parameters in rat myocardium. The indices for **a)** protein conformation (A_{1646}/A_{1537}), **b)** protein concentration ($A_{1646}/A_{1646+1537}$), **c–d)** protein phosphorylation (A_{1232}/A_{2958} and A_{1083}/A_{1537}), and **e)** protein carbonylation (A_{1737}/A_{1537}). A (Absorbance), CH (control rats), FH (rats on intermittent fasting).

phosphorylation-dephosphorylation cascades caused by various kinases and phosphatases [41,42]. In reality, the human genome contains 568 protein kinases and 156 protein phosphatases that control phosphorylation processes. As a result, they play a vital role in the regulation of biological processes including proliferation, differentiation, and apoptosis [42]. Like a keto diet plan, fasting causes AMPK (5′ adenosine monophosphate-activated protein kinase), a key player in mitochondrial biogenesis and function, to be phosphorylated. The fed status, on the other hand, activates the mTOR (the mechanistic target of rapamycin /serine/threonine protein kinase) pathway, which favors biosynthetic reactions under extra energy supply and may interact with the AMPK pathway. This connection bolsters the close linkage between fed/fasting states and biochemical pathways [43].

On the other hand, the band area ratio A_{1737}/A_{1537} can be used for the measurement of carbonyl levels in proteins [40]. IF diet lowered the protein carbonylation by 38%, therefore, protein oxidation in myocardial tissues (Fig. 8e). Protein carbonylation, one of the most harmful irreversible oxidative protein modifications, is considered a major hallmark of oxidative stress-related disorders [44]. It is a type of protein oxidation that can be promoted by ROS [45]. IF may be associated with a reduction in ROS production in human skeletal muscle, meaning that lower levels of antioxidant enzymes were required to maintain systemic redox homeostasis, as assessed by serum protein carbonyls [32]. Previously, decreased carbonylation in heart proteins was reported for rats subjected to the IF diet [46]. In rats, IF can block and restore all features of diabetes and obesity. As a result, excess fat tissue, inflammatory processes, and hypertension are lowered, insulin sensitivity is improved, and the neurological, neuromuscular, and cardiovascular systems' functional capabilities are enhanced. In myocardial infarction models, the heart is also safeguarded from ischemic damage by IF [47]. Autophagy is important not just during cardiac embryonic development but also for optimal circulatory function in the heart and blood vessels. Many proteins and hormones implicated in cardiovascular biology have been hypothesized to be controlled by autophagy, hence deregulation of autophagy may be related to high blood pressure, metabolic syndrome, and terminal organ impairment [48,49]. Fasting induces autophagy; hence these two determinants may be associated with the documented cardioprotective activity [43].

4. Conclusions

According to the findings of this study; the IF diet initiates serious biochemical modifications in the myocardial tissues of rats. IF was effective on both broad and particular spectrochemical parameters, therefore, leading to the comprehensive remodeling of metabolism in myocardial tissues. Indicatively, LDA and SVM algorithms revealed gross biomolecular modifications in rats subjected to the IF diet. The discrimination/prediction accuracies of LDA were calculated as 100% for gross biomolecules, nucleic acids, and carbohydrates. In the case of lipids and proteins, the LDA accuracies were 92.86% and 71.43%, respectively. On the other hand, the training and cross-validation accuracies of SVM were calculated as 64.28%. Therefore, the discrimination capacity of the LDA algorithm is superior to that of SVM in detecting IF-induced biomolecular changes in myocardial tissues. On the other

hand, the quantitative analyses of particular biomolecules revealed higher amounts of saturated lipids (19%), triglycerides (11%), and lipids (56%), in addition to the enhanced membrane lipid dynamics/fluidity (18%). Moreover, the concentrations of nucleic acids and glucose are increased by 52%, while the glycogen content is diminished by 61%. The protein carbonylation/oxidation is reduced by 38%, whereas a 35% increase in protein content was measured. Furthermore, the phosphorylated proteins have been calculated to be at higher concentrations in the range of 13–62%. Diet is a modifiable risk factor for CVD, and IF seems to be effective at molecular level modifications of disease-associated bio-spectral markers.

Statements and declarations

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No financial support was requested from any institution or organization for this study.

Ethics approval

This study was carried out with the approval of the Ethics Committee (approval number: 2021/05) from the Saki Yenilli Experimental Animal Production and Practice Laboratory.

Consent to participate

For this type of study, consent is not required.

Consent for publication

Consent for publication is not required in this study.

Availability of data and material

All data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

There is not any custom computer code or algorithm used to generate the results reported in the manuscript.

CRediT authorship contribution statement

İsa Ardahanlı: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Writing - original draft. **Halil İbrahim Özkan:** Data curation, Formal analysis, Funding acquisition, Investigation, Resources, Visualization, Writing - review & editing. **Faik Özel:** Investigation, Methodology, Resources, Software, Visualization, Conceptualization, Writing - review & editing. **Rafig Gurbanov:** Investigation, Methodology, Software, Supervision, Validation, Conceptualization, Data curation, Formal analysis, Project administration, Writing - review & editing. **Hikmet Taner Teker:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources. **Taha Ceylani:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpc.2022.106873.

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