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Aortic Regeneration is Promoted by Intermittent Fasting in Aged Rats

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Abstract

Intermittent fasting (IF) plays important role in health. The regeneration that starts at the cellular level is reflected in all tissues and organs. In this study, molecular changes in the aortic tissue of 12-month-old male Wistar rats that underwent intermittent fasting for 18 hours a day for 35 days were determined by spectrochemical analysis and machine learning algoritm. While IF did not significantly affect body weights or blood glucose levels, it led to increased food and water consumption. Spectrochemical analysis revealed significant differences in the forms of DNA, specifically an increase in the A-DNA form in aortic samples. This form of DNA plays an essential role in cellular defense systems and biological processes. There was also an increase in the Amid I band, providing information about hydration status and lipid molecule interactions. Notably, a significant decrease was observed in protein phosphorylation markers, which could impact a wide range of cellular activities. IF also led to reductions in protein carbonylation, a marker of oxidative damage, and changes in the acyl chain length of fatty acids, impacting membrane fluidity. These findings suggest that IF may offer several health benefits, including improved membrane dynamics, reduced oxidative stress, and potential cellular regeneration through autophagy. Further research is needed to confirm these observations and understand their implications for human health.

Keywords: Intermittent fasting, aorta, ATR-FTIR, wistar rat, support vector machine

1. INTRODUCTION

Cardiovascular diseases are one of the leading causes of morbidity and mortality worldwide [1]. The key pathophysiology for cardiovascular diseases is atherosclerosis, which is characterized by lipid and cholesterol metabolic abnormalities and chronic inflammation. Atherosclerotic plaques can form, worsen, and eventually burst due to a number of different reasons. One of the most important of these variables is hypercholesterolemia, and specifically an elevated low-density lipoprotein cholesterol level [2]. Also the aging promote the development of atherosclerotic lesions and calcifications. The aorta is the largest artery in the human body and arguably one of the most important it receives oxygen-rich blood from the left ventricle of the heart and supplies it to the body via the systemic circulation [3]. Aortic atherosclerosis is well recognized as a significant risk factor for atheroembolic

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events, particularly stroke, following cardiac surgery [4].

Intermittent fasting (IF) is an essential non-pharmaceutical technique for overweight and obese patients. Alternate-day fasting, modified fasting regimens, and time-restricted feeding (TRF) are three types of IF approaches that have been used to reduce body weight in people and rodent models [5]. Standard intermittent fasting, often known as TRF (consisting of 16 hours of fasting and 8 hours of eating), is beneficial for both the maintenance of physical fitness and the amelioration of metabolic disorders [6]. Recently, it was observed that intermittent fasting for 18 hours a day for 35 days increased the species diversity in the gut microbiota, changed the presence of these species towards healthy microbiota criteria, and played a role in the recovery of the dysbiotic structure [7]. When the molecular profiles of the colon, ileum and liver tissues of the rats belonging to the same application were evaluated, it was seen that the intermittent fasting program provided significant rejuvenation in these tissues [8]. Significant molecular improvements were also determined in the study with heart tissue [9].

With its ability to detect molecular vibrations and generate molecular spectral bands in the mid-infrared region, Fourier Transform Infrared (FTIR) Spectroscopy is a useful tool in biological investigations because it can collect broad-spectrum data rapidly, easily, and without causing any damage to the sample [10]. The FTIR spectroscopic mode of attenuated total reflection (ATR) is effective for examining biological substances [11]. Chemometrics is a discipline of chemistry that deals with computer-assisted chemical data processing as well as statistics and mathematics. Chemometric techniques (machine learning approaches) are used to analyze data from a wide range of analytical processes in fields like analytical chemistry, clinical and forensic medicine, biology, and archaeology [12]. In this study, significant changes in the aortic tissue at the molecular level after intermittent fasting for 18 hours a day for 35 days were determined by ATR-FTIR. Using the FTIR data collected, it was also evaluated with the machine learning-based Support Vector Machine learning algorithm.

2. MATERIAL METHOD

2.1. Animal Studies

In the study, the male Wistar rat (12-month-old) was used. For 35 days, rats (n = 7) in the study's experimental group were subjected to intermittent fasting. While the rats in the experimental group could always drink water, their access to food was limited for 18 hours, and they could only feed for 6 hours. The experimental group's meal access period was determined to be between $9:^{00}$ a.m. and $3:^{00}$ p.m. For 24 hours, the control group (n = 7) had access to water and food. Ad libitum, the mice were fed a conventional rat diet [13]. For 35 days, the animals' body weight, feed, and water consumption were tracked. When the application process was complete, blood glucose levels were also assessed. One day after the end of the 35-day intermittent fasting program, the rats in the experimental and control groups were lightly stunned with ether, then sacrificed, and aortic tissues were removed. The excised aorta tissues were shocked on dry ice and stored in a -80^{o}C deep freezer until further study. Aorta samples from the descending aorta were used. All of the animals were kept in conventional animal care conditions.

2.2. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy for Sample Analysis

To examine the aorta samples, an ATR-FTIR spectrometer (PerkinElmer) was used, with a resolution of 4 cm-1 and a scan number of 32, after compressing all of the samples (2x24=48) on the Zn/Se crystal of the ATR unit. The spectra were obtained from 4000-

650 cm-1 using a Spectrum One (PerkinElmer). [5].

2.3. Studies of Prediction Utilizing a Variety of Machine Learning Techniques, All Driven by Massive Amounts of Spectral Data

Pattern recognition uses spectral data. For the most unbiased results from the FTIR spectrometers, spectra were preprocessed using The Unscrambler® X 10.3 (CAMO Software AS, Norway) by applying a baseline offset transformation in the 4000-650 cm1 region to each spectrum. Spectra were initially submitted to unsupervised Principal Component Analysis (PCA). Standard deviation normalization and leverage or full-cross random validation passed spectra. The spectra were then studied in lipid (3000-2700 cm-1), protein (1700-1500 cm-1), nucleic acid (1200-650 cm1), and complete (4000-650 cm-1) areas [5].

Support Vector Machine (SVM) is a popular machine learning approach. The Unscrambler® X 10.3 (CAMO Software AS, Norway) performed SVM classification. After preprocessing all spectra, distinct sample categories were utilized to create a training set. Nu-SVC was chosen as SVM type with a linear Kernel. Nu=0.5, weights=1.00. Training and cross-validation accuracies employed 9 cross-validation segments. The training dataset was used to all sample datasets to create an SVM classification model. [5].

2.4. FTIR Spectral Band Quantification Studies

OPUS 5.5 (Bruker) program analyzed spectral data. Before band quantification analysis, each sample's average spectrum was baseline adjusted using 128 baseline points. In thorough band studies, the bands with the greatest absorbance values in different spectral areas were picked and their beginning and ending frequencies were calculated. Using OPUS 5.5 (Bruker) software, the integral regions of biomolecule-specific frequency ranges were investigated. A virtual line was created from the band's midway to its top, and its length was measured with a virtual ruler. Bandwidth values were then calculated by drawing a horizontal line down the band at the location where 0.75 times the length of the line corresponded with the line [5].

2.5. Statistics

GraphPad Prism 6.01 was used for all statistical analyses and graphical representation of the findings (GraphPad, USA). Statistical significance was determined using an unpaired t-test, and results were indicated as $P \leq 0.05$ *. The results are shown as a mean standard error of the mean (standard error of the mean).

3. RESULTS AND DISCUSSION

IF didn't affect rats' body weights ($p \leq 0.7950$). However, rats in the control group significantly gained weight ($p \leq 0.001$). Also significant ($p \leq 0.0001$) was the difference between the experimental and control groups. Additionally, there was a significant difference in food consumption ($p \leq 0.0001$) and water ($p \leq 0.0001$). In the days following the application, the rats in the experimental group tended to consume more food and water. There was no significant difference in blood glucose ($p \leq 0.250$). [5].

Changes in various spectrochemical bands, each linked with different functional groups of biomolecules, are clearly apparent in the average spectra (whole infrared range /4000-650 cm-1) of aorta samples from the control and IF groups. A comparable classification was obtained with the SVM method with 100% accuracy, for the whole content of aorta tissues (Table 1). The PO2 antisymmetric band from the absorbance spectrum located in between 1242–1238 cm^{-1} that is assigned to total nucleic acids [14]. The absorbance of the main B and A-forms of DNA are located at 1221 cm^{-1} and 1240 cm^{-1}, respectively [15]. It is seen that intermittent fasting caused a

significant increase in A-DNA form in samples taken from the aorta Figure 1a. Double-stranded DNA may have many forms, including A-, B-, and Z-DNA. Despite the fact that the B-form DNA is the most often seen structure in solution under physiological settings, the A-form DNA serves an important biological function in the context of cellular defense systems under hard conditions [16].

Table 1 Intermittent fasting modifies the gross biomolecules in rat aorta tissues. Support Vector Machine classification for aorta samples in full (4000-650 cm-1) infrared spectral region. CA (control rats), FA (rats on intermittent fasting). Support Vector Machine type: Classification (nu-SVC). Method: Linear.

Accuracy (%)	100	
Classification		
Samples		**Class**
CA1	1	CA
CA2	2	CA
CA3	3	CA
CA4	4	CA
CA5	5	CA
CA6	6	CA
FA1	7	FA
FA2	8	FA
FA3	9	FA
FA4	10	FA
FA5	11	FA
FA6	12	FA
FA7	13	FA
FA8	14	FA

The A-form of DNA is not only involved in cellular defense systems, but also has a striking presence in many other biological processes. For certain proteins to bind to DNA, the sugar phosphate backbone of the DNA must be unprotected so that direct recognition processes may take place. Local B-DNA to A-DNA transition is triggered by proteins like polymerases, endonucleases, etc., which execute cutting and sealing actions. The B-A transition widens the main groove and narrows the minor groove, exposing previously inaccessible regions of DNA. During transcription, some transcription factors use an indirect readout method by looking for an A-form in the genome to bind to [17]. Keeping A-DNA "hydrated" is another benefit of local B-A transition, which occurs when water molecules establish bridges between the different hydrophilic atoms of DNA bases in the A-form [18].

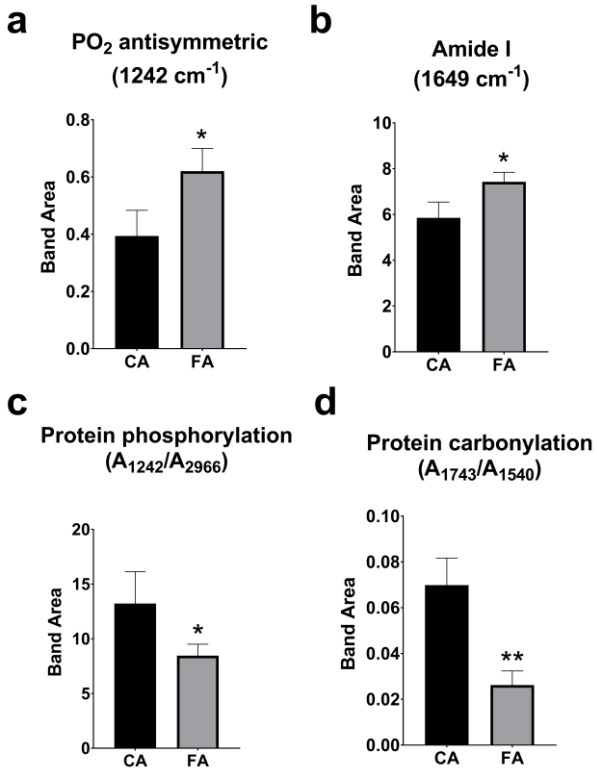

Figure 1 The changes in the FTIR spectral band areas for aorta samples. The area values of a) PO2 antisymmetric (1242 cm-1), b) Amide I (1649 cm-1), c) protein phosphorylation (A1242/A2966), and d) protein carbonylation (A1743/A1540). CA (control rats), FA (rats on intermittent fasting).

There was also a significant increase in the Amid I band in aortic tissue samples after intermittent fasting Figure 1b. Amide I (1649 cm−1) which associated with C=O stretching The SVM method revealed 100% training and 100% cross-validation accuracies for the whole content of aorta tissue vibration [19]. This is a particularly useful band for evaluating environmental changes, hydrogen bonding of lipid molecules, and differentiating ligand interactions [20]. As a result, the location and intensity of the carbonyl band provide useful information about the hydration status of lipid molecules

near the water interface. Water content varies as lipid density changes. As the lipid layer thickens, the headgroup becomes less hydrated, causing the carbonyl bands to shift to higher wavenumbers [21].

Phosphorylation of proteins is the most significant post-translational modification, with effects on a wide range of cellular activities including gene expression, metabolism, cell cycle control, differentiation, and death [22]. In this study, after intermittent fasting, there was a significant decrease in the bands area ratio A_{1242}/A_{2966} that is markers of protein phosphorylation in the aortic tissue Figure 1c. Proteins serve diverse purposes depending on the specific alteration state they are in, such as after acetylation, methylation, or phosphorylation. A protein's function may respond quite differently to the same change made at two distinct locations. Some protein phosphorylation sites may activate the protein's activities, whereas other phosphorylation sites can inhibit them. So, the functional regulation of a protein can be changed in many ways, such as by adding or removing residues in certain places [23].

Protein oxidation plays a crucial role in the pathophysiology of aging and the regulation of physiological processes, limiting tissue injury. According to the oxidative stress hypothesis of aging, senescence is primarily governed by an advanced accumulation of oxidized molecules that disrupt biotic homeostasis and cause a functional deterioration in cellular physiology. High amounts of protein carbonylation, known as a marker of protein oxidative damage [24]. The protein carbonylation bands area ratio A_{1743}/A_{1540} was also significantly reduced after intermittent fasting Figure 1d. With the application of intermittent fasting, damage to age-related oxidative stress mechanisms may be improved. However, recent proteomic studies revealed opposite findings on the key doctrine of oxidative stress theory were identified in the soluble cell portions from the exceptionally long-living rodent (naked-mole rats) compared to short-living mice [25].

The acyl chain length of fatty acids can be calculated by band area ratio A_{2922}/A_{2966}. In this study, it was observed that there was a significant decrease in the acyl chain length band area ratio after intermittent fasting Figure 2a. The length of the fatty acid tail influences membrane fluidity. This is due to the intermolecular interactions of the phospholipid tails, which provide membrane rigidity. Longer phospholipid tails allow for tail-to-tail interactions while decreasing membrane fluidity. At physiological temperatures, saturated lipid acyl chains tend to produce non-fluid, closely packed gel phases, while unsaturated lipid acyl chains fluidize the bilayer [26]. When the stiffness of the aorta and other blood vessels due to age is taken into account, it is clear that intermittent fasting can help get rid of this problem.
Membranes, which are composed of lipids and proteins, are very complex structures that perform several functions in living organisms. By separating cells and cell compartments from their environment, they serve as an effective barrier against a variety of chemicals. The membranes, on the other hand, must permit contact with the same environment by actively or passively transporting things into and out of the cell. The cell surface receives external signals, and the membranes' role is to transfer them to the cell's internal machinery. Cells may adapt to their constantly changing surroundings by doing so. To execute all of these functions, a biological membrane must maintain dynamic equilibrium between its components and with their surroundings [27]. The band area ratio $A_{2922}/_{2966}$ of membrane dynamics increased significantly after intermittent fasting Figure 2b. One of the most important features of intermittent fasting applications exceeding 16 hours is that they provide regeneration at the cellular level by activating autophagy mechanisms [28]. A significant increase in membrane dynamics may indicate this renewal.

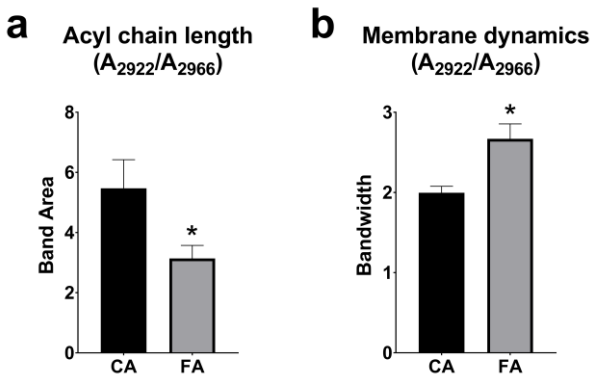

Figure 2 The changes in the FTIR spectral band areas for aorta samples. The area values of **a)** Acyl chain lenght (A2922/A2966), **b)** Membrane dynamics (A2922/A2966). CA (control rats), FA (rats on intermittent fasting).

3. CONCLUSIONS

In this study, the effects of intermittent fasting on aortic tissue were evaluated with ATR-FTIR spectroscopy. The practice of intermittent fasting caused significant molecular changes. When the significant increase in membrane dynamics was evaluated together with the other results obtained, it was determined that the application of intermittent fasting supported the regeneration of the aortic tissue. In this respect, intermittent fasting may be considered an effective therapeutic approach for age-related aortic deformations.

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Authors' Contribution
The authors contributed equally to the study.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the authors.

The Declaration of Ethics Committee Approval
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.

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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