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Research Article

PROTECTIVE EFFECTS OF INTERMITTENT FASTING AND PROBIOTICS USAGE ON OXIDATIVE STRESS AND MOLECULAR ALTERATIONS IN AGING LUNG

Burcu BABA [1] *, Dilek YONAR [2], Hikmet Taner TEKER [3], Taha CEYLANİ [4]

[1]Department of Medical Biochemistry, Faculty of Medicine, Yuksek Ihtisas University, Ankara, Türkiye
[2]Department of Biophysics, Faculty of Medicine, Yuksek Ihtisas University, Ankara, Türkiye
[3]Department of Medical Biology and Genetics, Ankara Medipol University Ankara, Türkiye
[4]Department of Molecular Biology and Genetics, Muş, Alparslan University Muş, Türkiye
*Correspondence: burcubaba@yiu.edu.tr

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ABSTRACT

Objective: Aging is associated with increased oxidative stress and diminished cellular repair mechanisms, particularly in the lungs. This study investigates the protective effects of intermittent fasting (IF), SCD probiotics, and their combination on oxidative stress and molecular alterations in the lungs of aging rats.

Materials and Methods: Sprague-Dawley rats (24 months old) were divided into four groups: control, intermittent fasting, probiotics, and a combination of both treatments. Malondialdehyde (MDA), advanced oxidation protein products (AOPP), and myeloperoxidase (MPO) activity were measured as markers of oxidative stress. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy was employed to detect molecular changes in lung tissues.

Results: Our findings demonstrated that both IF and probiotics, individually and in combination, reduced the MDA and AOPP levels, as well as MPO activity, compared to the control group, indicating a reduction in oxidative stress. Spectral analyses revealed molecular changes in lipid composition, protein conformation and oxidation, as well as phosphodiester groups of nucleic acids. The highest classification accuracy (93.18%) was obtained in the 1300-800 cm^{-1} region by LDA analysis.

Conclusion: Intermittent fasting and probiotics may ameliorate age-related oxidative damage in the lungs and offer promising therapeutic potential for maintaining lung health in aging populations.

Keywords: Aging, intermittent fasting, FTIR spectroscopy, lung, oxidative stress, probiotics.

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INTRODUCTION

Human lungs, which have the largest surface area of any organ in the body, serve as a unique interface with the external environment. They consist of various cell types and are continuously exposed to a range of stresses, including chemical, mechanical, biological, immunological, and xenobiotic factors, throughout a lifetime (1). Age-related alterations in the intrinsic mechanisms responsible for cell regeneration and repair, including the depletion of adult stem cell reserves, mitochondrial dysfunction, and increased oxidative stress, lead to a diminished capacity of lung cells to sustain normal homeostasis (2). Oxidative stress results from the imbalance between the reactive oxygen and nitrogen species (RONS) generation and the capacity of antioxidant defenses. Reactive species are produced by all aerobic cells and are crucial in the aging process and age-related diseases. While RONS production is involved in energy extraction from organic molecules, immune defense, and cellular signaling, it can also have harmful effects (3). Excessive production of oxidants can cause lipid peroxidation and protein oxidation, leading to damage in cells and tissues. Myeloperoxidase (MPO) is an essential enzyme found in neutrophils that generates powerful oxidants, including hypochlorous acid (HOCl), which serves as a defense mechanism against pathogens (4).
The reactive species oxidize amino acid side chains, leading to a conformational alteration, partial unfolding, and fragmentation of the protein backbone. Due to the accumulation of oxidized proteins, cross-linking processes such as the formation of dityrosine, disulphide, and other types of intermolecular bonds occur, generating protein aggregates that polymerize into non-degradable structures, evading proteolysis (5). Aromatic amino acid residues are primary targets for various reactive species, resulting in the generation of cross-links containing dityrosine, which are known as advanced oxidation protein products (AOPPs) (6). Free radicals, including ROS and RNS, also attack the double bonds in lipids by removing hydrogen atoms and adding oxygen, resulting in lipid peroxidation. This process results in primary products like lipid peroxyl radicals and hydroperoxides, along with secondary products including malondialdehyde (MDA) and 4-hydroxynonenal (7).
Intermittent fasting (IF) is associated with reduced oxidative damage and diminished inflammatory responses (8). Intermittent fasting is a dietary pattern that involves a period of food restriction and normal nutrition. It has recently become popular because of its potential health benefits, such as anti-aging and rejuvenation effects (9, 10). IF improves health and combats disease processes through cellular and molecular mechanisms that activate adaptive stress response pathways. These pathways support mitochondrial function, DNA repair, and autophagy (11).

Various factors including diet, age, illnesses, stress, and lifestyle can affect the composition of the gut microbiota. The microbes in our gut are more numerous than the total number of body cells by over tenfold. They have important roles in maintaining health, such as aiding digestion, regulating the immune system, and even combating diseases (12). Given the crucial role of diet in shaping the gut microbiome, there has been considerable interest in using prebiotics and probiotics as nutritional strategies to enhance microbiome diversity and promote health (13). Due to the deterioration of physiological and biological systems in the elderly, probiotics present a promising option for mitigating the associated increased disease susceptibility (14).

There is a crosstalk between the respiratory tract and the gastrointestinal tract, defined as the gut-lung axis. Alterations in components of the gut microbiome by diet, disease, or medical interventions are associated with changes in immune responses and the maintenance of homeostasis in the respiratory tract (15, 16). With the rapid increase in the aging population, it is crucial to investigate how physiological and cellular alterations in aging lungs influence the onset and progression of respiratory diseases (2). It is hypothesized that the treatment with IF and SCD probiotics may alleviate increased oxidative damage in aged lung tissue and contribute to the restoration of lung tissue. Additionally, it is suggested that interventions such as IF could further improve lung health by complementing probiotic treatment. Therefore, we aimed to investigate the effects of IF and SCD probiotics, both individually and in combination, on aging lungs by evaluating oxidative stress indicators, including myeloperoxidase, AOPP, and MDA. Moreover, ATR-FTIR spectroscopy combined with multivariate analysis was used to specify the molecular differences in the lung tissue after intermittent fasting and probiotics intake.

MATERIALS AND METHODS

Animal studies

In this study, male Sprague-Dawley rats (24 months old) were used. The animals were divided into four groups: the control group (CNT), the intermittent fasting group (IF), the SCD Probiotics group (PRB), and the group receiving

SCD Probiotics supplementation during intermittent fasting (IFPRB), with 7 rats in each group. The rats in the IF groups were subjected to food restriction for 18 hours a day, with a 6-hour window (between 9 a.m. and 3 p.m.) for food intake. Water was available to them at all times. The animals were fed a typical rodent diet ad libitum (11), and their body weight was recorded throughout the study. The probiotic supplement was given orally at a daily dose of 3 mL (1 x 10^8 CFU) (17), using the product provided by SCD probiotics company (Essential Probiotics XI - 500 ml H.S. Code: 2206.00.7000), which contains 11 different probiotics, including Bacillus subtilis, Bifidobacterium longum, Bifidobacterium bifidum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus fermentum, Lactobacillus casei, Lactobacillus plantarum, Lactococcus lactis, Saccharomyces cerevisiae, and Streptococcus thermophilus species during 30 days (18). At the end of the treatment period, rats in all groups were sacrificed, and then lung tissues were obtained. The lung tissues were immediately shocked on dry ice and stored in a -80°C deep freezer until analysis. The study was approved by the Ethics Committee of the Saki Yenilli Experimental Animal Production and Practice Laboratory (approval number: 2022/03) and conducted according to the standard animal care protocols.

Tissue Preparation

Lung tissues were homogenized to disrupt the tissue structure, leading to the release of cells and intracellular components for further analysis. The lung tissues were homogenized in 10 mM cold phosphate buffered saline (PBS; 1:5 w/v; pH 7.4) with protease inhibitor (PIC002, BioShop, Canada) and 0.5% Triton X-100 to determine the oxidative stress markers. The homogenate was centrifuged at 5,000 x g for 10 minutes at 4°C, and the supernatant was collected. The obtained supernatant was stored in aliquots at -80°C until analysis. Protein concentrations in the samples were measured using the method described by Bradford (19) with bovine serum albumin used as a standard.

Malondialdehyde Measurement

Malondialdehyde, a lipid peroxidation marker, was assessed by measuring the formation of thiobarbituric acid reactive substances. Briefly, the samples were mixed with 20% trichloroacetic acid and 0.67% thiobarbituric acid, heated in a water bath at 100°C, and then cooled on ice. Absorbance was measured at 532 nm, and MDA levels were calculated as nanomoles per milligram of protein.

Advanced Oxidation Protein Products Measurement

AOPPs, which serve as indicators of oxidative protein damage, were assessed by measuring dityrosine-containing and cross-linked protein products. AOPP levels were quantified following the procedure established by Witko-Sarsat et al. (20). In this procedure, samples were first diluted to a concentration of 20 mM with PBS (pH 7.4), and then potassium iodide and acetic acid were added. Absorbance was then measured at 340 nm, and the values were presented as nanomoles per milligram of protein.

Determination of Myeloperoxidase Activity

The activity of myeloperoxidase was measured using a spectrophotometric method. The samples were mixed with 50 mM PBS (pH 6.0) containing o-dianisidine (0.167 mg/ml) and hydrogen peroxide (0.0005%). The change in absorbance was measured at 460 nm for 5 min. The activity of MPO was presented as units per milligram of protein, following the method as described by Bradley et al. (21).

Sampling for ATR-FTIR spectroscopy and data analysis

The ATR-FTIR spectra of lung tissues were obtained using Perkin Elmer Frontier FTIR spectrometer (Perkin Elmer Inc., USA) with a Quest single reflection ATR accessory (Specac Ltd., UK). The background spectrum was obtained by recording the spectrum of air and automatically subtracted using the Spectrum 10 software program. The spectra of lung tissues compressed on the diamond crystal of the ATR unit were collected at room temperature within the range of 4000-450 cm^{-1} region with 64 scans at 4 cm^{-1} resolution. Under the same conditions, each tissue was scanned from three randomly selected fractions, all of which produced the same spectra. Spectral analyses were performed using the average spectra of these three replicates. The spectra acquisition and data manipulation were obtained by the same software program (Spectrum 10).

Machine learning method in prediction studies

Linear Discriminant Analysis (LDA) as a machine learning tool was implemented on spectral data for differentiating the experimental groups from each other. The goal of LDA is to find the linear combination of original variables that maximizes the difference between classes. In the LDA technique, the best fit parameters for the sample classification are determined with a developed model, and then the model can be applied to classify unknown samples. The development of machine learning-based detection models requires independent training and test/validation data sets. In order to create a classification model and categorize samples, principal component analysis (PCA) has been frequently used to train data sets. Additionally, the detection performance of the classification model has been assessed by a validation data set using LDA. The PCA data of the second derivative and

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normalized spectra in the whole spectral range (4000-450 cm^{-1}), lipid (3020-2800 cm^{-1}), protein (1700-1500 cm^{-1}), and nucleic acid (1300-800 cm^{-1}) regions were used as LDA model inputs using The Unscrambler X 10.3 (CAMO Software AS, Norway) software. Initially, the category variable column was added into the data matrix, and then a training set was created by using all the spectra of the different sample classes. The method used was quadratic, utilizing the projections of 7 principal components and the prior probabilities calculated from the training set for the prediction. LDA results are represented by a discrimination plot, the matrices of prediction and confusion. The prediction matrix exhibits the predicted class for each sample and the probability of membership for each class. However, the confusion matrix reports the predicted and actual classifications of samples (22).

Statistical Analysis

Statistical analyses of the data using one-way ANOVA and the graph plots were performed using GraphPad Prism 10 (GraphPad, USA). The values were reported as mean ± standard error of the mean (SEM). The significance level was denoted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

RESULTS

The body weight, water and food consumption of animals

The IF groups showed significant weight loss, whereas the IFPRB group exhibited less weight loss. The lower weight loss observed between the IF and PRB groups in comparison with the control demonstrates the role of the probiotic in stabilizing weight gain. Although the feed consumption of rats in the IF groups increased because of adaptation, no significant differences in water consumption were observed among the groups, as shown in our previous study (18).

Effects of Treatment with Probiotics and Intermittent Fasting on Oxidative Stress Markers

In the present study, MPO activities, MDA, and AOPP levels as indicators of oxidative stress were measured in lung homogenates from 24-month-old rats treated with intermittent fasting and probiotics, either individually or in combination. MDA levels statistically reduced in aged rats treated with probiotics alone and in those treated with both probiotics and IF compared to the control group. Although there was no statistically significant difference in MDA levels between the IF group and the control group, a notable reduction in MDA levels was observed in the IF group (Figure 1a). The levels of AOPP decreased significantly in all treated groups compared to the controls (Figure 1b). MPO activity in the aging lung was found to be significantly reduced with treatment using probiotics and IF, either administered individually or in combination (Figure 1c). When comparing the groups treated with probiotics and IF, either individually or in combination, no significant difference was observed among them in terms of oxidative stress markers.

Lipid, protein and nucleic acid profiles of lung tissues altered by intermittent fasting and probiotics supplementation

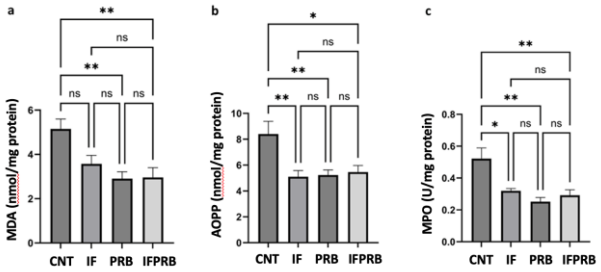

Figure 1 Effects of probiotics and intermittent fasting on MDA (a), AOPP (b) and MPO (c) activity of the lung tissue of aged rats. CNT (control), IF (intermittent fasting), PRB (SCD Probiotics) and IFPRB applications (in which IF and PRB were applied together). The significance levels were denoted as * $p < 0.05$, ** $p < 0.01$.

FTIR spectroscopy has arisen as a potent technique for simultaneously analyzing the structure, conformation, and function of all molecules in a biological system (23). ATR-FTIR spectroscopy was performed to elucidate the altered biomolecular changes in rat lung tissues after intermittent fasting (IF), probiotics supplementation (PRB), and in combination (IFPRB). For this purpose, quantitative measurements of spectral parameters such as shifts in the peak positions, alterations in bandwidths, band areas of spectral bands or the area ratios and machine learning approaches were carried out. Figure 2a–b exhibits the averaged IR spectra of all groups (CNT, IF, PRB and IFPRB) in the 3025–2800 and 1800–800 cm^{-1} regions where the absorption bands originate from the functional groups of lipids, proteins, nucleic acids, and carbohydrates. The below panels in Figure 2a–b show difference spectra obtained by subtracting the average spectrum of each group from the average spectrum of the control to explore the differences between the groups more plainly. The difference spectra showed notable changes in the functional groups of specific biomolecules between the control group and different treatment groups (Figure 2a-b). In order to determine the quantitative differences between the groups, spectral band areas and area ratios for individual bands were calculated. According to Beer-Lambert's law, the concentration of the functional groups in the relevant molecule is exactly proportional to the signal intensity/area under the spectral bands. To exclude potential artifacts from experimental conditions, the

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integrated area ratios of a few selected bands were assessed for relative quantitative analysis (22, 23).
Some prominent bands for lipids such as olefinic C=CH at 3011 cm^{-1}, CH_3 asymmetric stretching at 2960 cm^{-1}, CH_2 asymmetric stretching at 2923 cm^{-1}, CH_2 symmetric stretching at 2852 cm^{-1} and C=O stretching at 1740 cm^{-1}

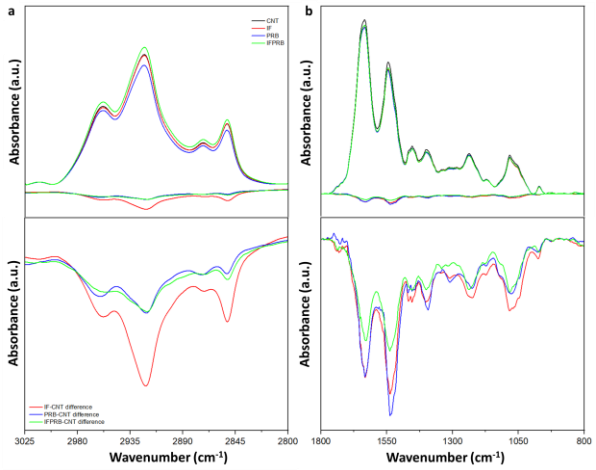

Figure 2. The normalized average IR spectra of the groups in the a) 3025–2800 and b) 1800–800 cm^{-1} regions. The normalization was performed according to the amide A band located at 3280 cm^{-1}. Difference IR spectra were acquired via subtracting the control spectrum from the spectra of the groups treated by intermittent fasting (IF), probiotics supplementation (PRB) and the combined administration of both (IFPRB) in the below panels for both regions.

were evaluated. The area of CH_3 antisymmetric ($p<0.001$), CH_2 antisymmetric and symmetric stretching bands ($p<0.05$) in all treatment groups decreased significantly compared to controls. Total saturated lipid content calculated from the sum of CH_2 symmetric and antisymmetric stretching bands decreased in treated groups compared to the control group (Figure 3a). Moreover, the CH_2/CH_3 antisymmetric stretching area ratio provides acyl chain length information. A higher value indicates the presence of relatively longer chain lipids, while a lower value implies the presence of shorter chain and/or more branched lipids. No remarkable changes in acyl chain length were observed among the groups. The bandwidth of CH_2 symmetric or antisymmetric stretching bands indicates membrane dynamics, as it is connected with the motional rates of the lipid molecule. An increase in membrane dynamics is indicated by the increase in the bandwidths of these bands. A nonsignificant decrease was observed in the bandwidth values of CH_2 antisymmetric stretching bands for treated groups when compared to the control group (Figure 3b). The olefinic band points out the content of double bonds in the lipid structure, which is utilized for monitoring the unsaturated lipid content. There was also a decrease in the area of the olefinic C=CH stretching band compared to the control as seen from Figure 3c. C=O stretching band indicates the carbonyl ester concentration in lipids. Moreover, this band can be used to visualize protein oxidation due to resulting in the production of some additional carbonyls. A nonsignificant decrease in the C=O stretching band was observed in all groups.

The protein associated bands Amide I at 1635 cm^{-1} (mainly from the C=O stretching vibration), Amide II at 1545 cm^{-1} (arises from N–H bending vibration and from C–N stretching vibration) and some major nucleic acids related bands such as PO_2^- antisymmetric stretching at 1238 cm^{-1}, PO_2^- symmetric stretching at 1083 cm^{-1} and $PO_4^=$ stretching at 971 cm^{-1} were also evaluated. Amide I and amide II bands are sensitive to conformational alterations in the proteins. The shifts in the band positions and the alterations in the bandwidth of amide I and amide II as well as the amide I/amide II band area ratio can provide the change in the protein conformation and structure. Our results show that no significant change in these parameters is observed. However, the area of amide I and amide II bands dropped significantly ($p<0.05$) in all treatment groups. Figure 3d reveals an increase in the amide I/amide II ratio in all of the treated groups in comparison with the control. This increase is significant in fasting and SCD probiotics groups, which proposes the higher decrease in the content of N–H bending and C–N stretching relative to the content of C=O stretching in the proteins of IF and PRB treated groups. The broadening of the amide I band and area ratio of C=O stretching band to

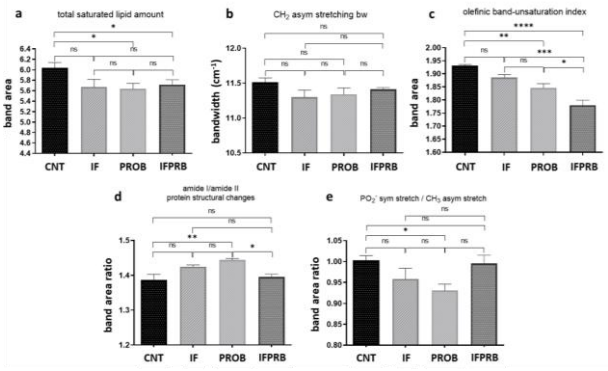

Figure 3. Quantitative changes in spectral parameters including band areas and band area ratios for: (a) total saturated lipid amount, (b) bandwidth of CH_2 antisymmetric stretching band, (c) olefinic band area, (d) protein conformation (A1653/A1545) and (e) protein phosphorylation (A1083/A2955). The significance levels were denoted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

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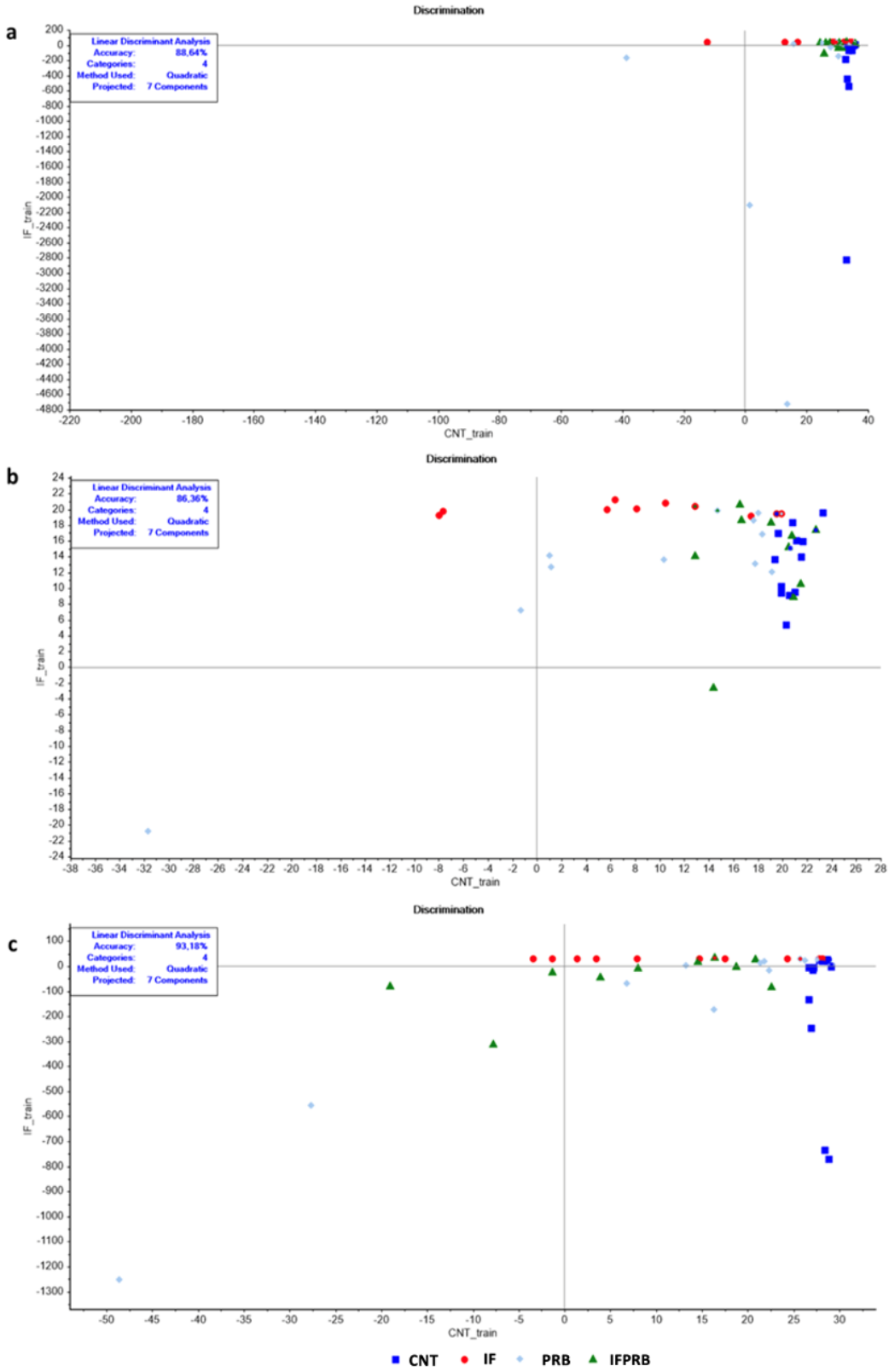

Figure 4. LDA discrimination plot for lung tissue samples in a) the lipid region (3020-2800 cm^{-1}), b) the protein region (1700-1500 cm^{-1}) and c) the nucleic acid region (1300-800 cm^{-1}). CNT (control), IF (intermittent fasting), PRB (SCD Probiotics) and IFPRB applications (in which the IF and PRB were applied together).

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the amide I band (A1740/A1635) were measured to assess the relative level of protein carbonylation in the studied groups. No significant change in the broadening of amide I band was obtained and although there was a decreasing trend in A1740/A1635 ratio, no significant change was achieved.

The PO_2^- antisymmetric, symmetric stretching (antisymmetric phosphate and symmetric phosphate) bands are attributed to the phosphodiester groups of nucleic acids, whereas $PO_4^=$ stretching band is attributed to the phosphate monoester groups of phosphorylated proteins and cellular nucleic acids. All of these bands decreased in all treated groups compared to controls. The band ratios of PO_2^- antisymmetric, symmetric stretching to the CH_3 antisymmetric stretching (A1238/A2955, A1083/A2955) are related to protein phosphorylation. A decrease in protein phosphorylation was observed in all treated groups, but was not significant in groups other than the PROB group (Figure 3e).

LDA analyses were performed in regions where functional groups related to lipids, proteins, and nucleic acids are predominant. LDA analysis indicated a clear distinction in the biomolecular content of lung tissue among the control groups, IF, SCD Probiotics, and the combined treated groups. The discrimination plots obtained as a result of LDA analyses are given in Figure 4. The prediction matrix and the confusion matrix are given in the supplementary document (Table S1-S6). LDA analysis revealed notable differentiation in the regions associated with functional groups related to lipids, proteins, and nucleic acids, achieving accuracy rates of 88.64%, 86.36%, and 93.18%, respectively (Figure 4). While all treatments caused similar differences in lipid (Figure 4a, Table S1, S2) and nucleic acid profiles, the intermittent fasting group had the most distinct protein profile (Figure 4b; Table S3, S4). A high level of accuracy with 93.18% in the nucleic acid region (1300-800 cm^{-1}) was obtained (Figure 4c, Table S5, S6).

DISCUSSION

In the present study, we assessed the effects of IF and probiotics, both individually and in combination, on markers related to oxidative damage in aging lungs. According to our findings, treatment with probiotics and IF has a beneficial effect on aging lungs by leading to a reduction in oxidative damage indicators, including AOPP, MDA, and myeloperoxidase. In addition, the efficacy of IF and PRB supplementation on the molecular content of lung tissues was investigated using FTIR. We detected prominent changes in the lipid, protein, and nucleic acid profiles in the lung tissue using spectral analysis and machine learning techniques. According to spectral analyses, we observed decreases in all lipid-related bands, such as olefinic groups of unsaturated lipids, C–H stretching groups indicating total saturated lipids, and C=O stretching bands representing the triglyceride and cholesterol ester amounts by IF and SCD probiotics supplementation. These results support the reduced fat mass in old rats with treatment (24).

Aging is a process associated with a decline in the function and efficiency of tissues and organs. According to the oxidative stress theory of aging, these age-related functional declines are attributed to damage caused by reactive species (25). Luceri et al. observed that during aging, excessive ROS production results in oxidative damage at both the liver and systemic levels, which occurs as early as middle age (26). In a previous study, elevated levels of protein oxidation and lipid peroxidation in the liver tissue of aged female rats were demonstrated through AOPP and MDA measurements, which indicate the extent of oxidative damage to proteins and lipids (27). Higher MDA and AOPP levels were found in older rats compared to their younger counterparts (28–30). In addition, myeloperoxidase, a heme protein found in neutrophils and monocytes, plays a role in multiple stages of inflammation by generating various potent oxidants. MPO activity is significantly elevated with aging (4).

Intermittent fasting may enhance resistance to oxidative stress, reduce inflammation, and support longevity at the cellular level (24). A study in rats reported that both time-restricted feeding and alternate-day fasting improve metabolic profile and redox homeostasis by increasing the antioxidant defense system and decreasing oxidative stress markers such as MDA and AOPP, indicating their potential for aging intervention (31). A previous study demonstrated that alternate-day fasting reduced mitochondrial oxidative stress in aged mice, suggesting it may contribute to diminishing age-related cellular damage (32). According to our findings, a notable reduction in the MDA levels was observed in the IF group, but it was not statistically significant. This finding may be influenced by sample variability due to individual differences among rats. Therefore, further research with larger sample sizes is needed to confirm these findings. However, both AOPP levels and MPO activity were significantly reduced with IF treatment. The reductions in lipid peroxidation and protein oxidation markers as well as MPO activity may result from an increase in the antioxidant defense system, indicating a reduction in inflammation and oxidative stress.

As individuals age, their biological and physiological processes undergo changes, leading to alterations in

gastrointestinal and immune functions (14). The gut microbiota is crucial for various functions, including signaling pathways, homeostasis, nutrient and drug metabolism, intestinal barrier integrity, protection against pathogens, and immune system interaction. There's increasing interest in the link between microbiota dysbiosis and oxidative stress. Disruptions in gut microbiota can lead to excessive ROS production, increasing oxidative stress. It was suggested that the probiotics have antioxidant effects by modulating oxidative stress mechanisms in healthy individuals (33). Lactic acid bacteria are considered a promising source of antioxidants, with potential benefits in mitigating the detrimental effects of oxidative stress, a key factor in aging (34). Research shows that these bacteria produce various antioxidants that neutralize free radicals and alleviate oxidative stress, potentially improving cellular health and promoting healthy aging (35, 36). Administration of Lactobacillus helveticus led to a significant reduction in MDA and AOPP levels in a randomized double-blind study (37). Probiotics, prebiotics, and, when used together, synbiotics are widely recognized as immunomodulators and promoters of gut health. In addition to their proven effects in supporting a healthy microbiota composition and gut health, there is emerging evidence on how these components may improve lung health (15, 16). Our study demonstrated that probiotic treatment resulted in a decrease in MPO activity, lipid peroxidation, and protein oxidation, which suggests a reduction in oxidative stress in the aged lung. A decrease in short-chain lipid peroxidation product, MDA, was observed, while no change in total short-chain fatty acids was found between the groups according to the spectral analysis. It is suggested that this might be due to an increase in short-chain fatty acids (acetate, propionate, and butyrate), which are important bacterial fermentation metabolites that regulate many essential aspects of human physiology (15, 38). Protein carbonylation is commonly used to indicate oxidative stress and serve as a marker of oxidative damage. However, protein carbonyls are not only important as a biomarker for protein oxidation in aging and disease, but also they can disrupt protein structure and function by changing the conformation of the polypeptide chain and leading to partial or complete inactivation of proteins (39). No significant change was observed in the bandwidth of the amide I band and A1740/A1635 area ratio values, which are used to evaluate protein carbonylation, possibly due to sample variability. However, a decreasing trend was observed in the A1740/A1635 ratio of the treatment group compared to the control. This may indicate that treatments such as IF and probiotic supplementation applied in old age contribute to reducing carbonylation. It was suggested that IF and probiotic supplementation might promote the expression of antioxidant genes and boost the activity of antioxidant enzymes in the development of age-related lung diseases, leading to lessening inflammation and oxidative stress. Exposure to oxidative stress triggers a series of lipid peroxidation and phosphorylation reactions in cells. Certain protein phosphorylation events take place during apoptosis and are crucial for controlling programmed cell death (40). Aging is largely caused by increased reactive oxygen species and enhanced apoptosis (41). In our study, decreases in the band ratios related to protein phosphorylation and the band area of phosphate monoester groups of phosphorylated proteins by treatments were observed, suggesting a potential modulation of the apoptotic pathway.

Oxidative stress is a key factor in the development of age-related lung diseases including chronic obstructive pulmonary disease (42), pulmonary hypertension (43, 44), and fibrotic lung disease (45). In many respiratory disorders, the gut-lung axis plays an essential role and provides the bidirectional communication between the respiratory system and the gut microbiota. The gut microbiota is affected by age, diet, and lifestyle, which may have an effect on lung health and the emergence of both acute and chronic lung diseases. Altering the gut microbiota using strategies such as probiotics and dietary interventions could serve as potential therapeutic approaches for lung diseases through microbial balance restoration and the growth of beneficial strains in the gut (15, 16). Probiotic supplementation during IF has been demonstrated to be more effective in restoring colon and ileum tissues in aged rats (18). Moreover, both IF and SCD probiotics supplementation may help mitigate age-associated liver alterations (46). The findings of our study suggest that lipid and protein oxidative damage in the aging lung was reduced with IF and probiotic supplementation, whether administered individually or in combination. Additionally, a reduction in MPO activity was observed, which may indicate a decrease in inflammation and oxidative stress. The reductions in MPO activity, lipid peroxidation, and protein oxidation indicators might result from an enhancement of the antioxidant defense system, supporting the notion that IF and probiotic supplementation exert antioxidant effects. All the results proposed that both IF and probiotic treatments, individually or combined, are promising strategies for reducing age-related oxidative damage in the lungs. However, little is known about the gut-lung axis, and the mechanisms by which the gut microbiota influences lung homeostasis and diseases are not yet fully understood and require further investigation (15, 16).

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CONCLUSION

This study demonstrates that IF and probiotics, both individually and in combination, have significant protective effects against age-related oxidative damage and molecular alterations in the lungs of aged rats. The reduction in oxidative stress markers such as MDA, AOPP, and MPO activity suggests that these interventions can mitigate lipid and protein oxidation, key contributors to lung aging and dysfunction. Additionally, the application of ATR-FTIR spectroscopy, combined with machine learning, revealed distinct biomolecular changes further supporting the beneficial impact of these treatments on aging lung tissues. The findings emphasize the potential of intermittent fasting and probiotics as non-invasive, dietary-based strategies to enhance cellular resilience and reduce oxidative damage in the aging lung. However, further research is required to explore the long-term efficacy, underlying mechanisms, and potential clinical applications in aging-related lung diseases. These insights offer promising avenues for future interventions targeting age-related oxidative stress and respiratory decline. Besides, we acknowledge the challenges of applying IF regimens to elderly individuals. Therefore, a better understanding of the complex interactions between the gut microbiota, dietary interventions such as IF, and the host is essential for developing personalized medical treatments for a range of health conditions.

Acknowledgments

None to declare.

Authorship contributions

Concept and design: T.C., H.T.T.; Data Collection and Processing: B.B., D.Y.; Analysis or Interpretation: B.B., D.Y., H.T.T., T.C.; Literature Search: and Writing: B.B., D.Y., H.T.T., T.C.

Data availibity statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declared no conflict of interest.

Ethics

The study was approved by the Ethics Committee of the Saki Yenilli Experimental Animal Production and Practice Laboratory (approval number: 2022/03)

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