Biogerontology (2023) 24:563–580
https://doi.org/10.1007/s10522-023-10032-3

RESEARCH ARTICLE

Age-related differences in response to plasma exchange in male rat liver tissues: insights from histopathological and machine-learning assisted spectrochemical analyses

Hikmet Taner Teker · Taha Ceylani · Seda Keskin · Gizem Samgane · Sina Mansuroglu · Burcu Baba · Huseyin Allahverdi · Eda Acıkgoz · Rafig Gurbanov

Received: 4 March 2023 / Accepted: 27 March 2023 / Published online: 5 April 2023

Abstract This study aimed to examine the biological effects of blood plasma exchange in liver tissues of aged and young rats using machine learning methods and spectrochemical and histopathological approaches. Linear Discriminant Analysis (LDA) and Support Vector Machine (SVM) were the machine learning algorithms employed. Young plasma was given to old male rats (24 months), while old plasma was given to young male rats (5 weeks) for thirty days. LDA (95.83–100%) and SVM (87.5–91.67%) detected significant qualitative changes in liver biomolecules. In old rats, young plasma infusion increased the length of fatty acids, triglyceride, lipid carbonyl, and glycogen levels. Nucleic acid concentration, phosphorylation, and carbonylation rates of proteins were also increased, whereas a decrease in protein concentration was measured. Aged plasma decreased protein carbonylation, triglyceride, and lipid carbonyl levels. Young plasma infusion improved hepatic fibrosis and cellular degeneration and reduced hepatic microvesicular steatosis in aged rats. Otherwise, old plasma infusion in young rats caused disrupted cellular organization, steatosis, and increased fibrosis. Young plasma administration increased liver glycogen accumulation and serum albumin levels. Aged plasma infusion raised serum ALT levels while diminished ALP concentrations in young rats, suggesting possible liver dysfunction. Young plasma increased serum albumin levels in old rats. The study concluded that young plasma infusion might be associated with declined liver damage

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10522-023-10032-3.

H. T. Teker
Department of Medical Biology and Genetics, Ankara Medipol University, Ankara, Turkey

T. Ceylani · H. Allahverdi
Department of Molecular Biology and Genetics, Muş Alparslan University, Muş, Turkey

T. Ceylani
Department of Food Quality Control and Analysis, Muş Alparslan University, Muş, Turkey

S. Keskin · E. Acıkgoz
Department of Histology and Embryology, Van Yuzuncu Yil University, Van, Turkey

G. Samgane · S. Mansuroglu · R. Gurbanov (✉)
Department of Bioengineering, Bilecik Şeyh Edebali University, Bilecik, Turkey
e-mail: rafiq.qurbanov@gmail.com

B. Baba
Department of Medical Biochemistry, Yüksek İhtisas University, Ankara, Turkey

R. Gurbanov
Central Research Laboratory, Bilecik Şeyh Edebali University, Bilecik, Turkey

and fibrosis in aged rats, while aged plasma infusion negatively impacted liver health in young rats. These results imply that young blood plasma holds potential as a rejuvenation therapy for liver health and function.

Keywords Aging · FTIR spectroscopy · Machine learning prediction · Liver · LDA · SVM

Introduction

Aging and age-related disorders can significantly impact the quality of life due to organ and cellular dysfunctions. With the clinical need to address these conditions, various approaches are emerging to improve health, slow the aging process, and increase longevity (Kennedy et al. 2014). One promising approach involves leveraging the healing properties of young blood plasma to limit or reverse aspects of aging in various organs. Early studies investigating the effects of systemic factors in blood on aging and lifespan demonstrated positive effects of young blood on lifespan in older animals (Tripathi et al. 2021). The rejuvenation potential of young blood has been demonstrated in a tibia fracture repair model (Castellano 2019). Studies on other tissues have shown that aged mice transfused with blood from young individuals exhibit reduced cardiac hypertrophy and ventricular myocyte size (Loffredo et al. 2013). In another study, reductions in pancreatic β-cell proliferation and improvements in the parameters of kidney aging were observed in older individuals sharing younger blood (Huang et al. 2018). The young blood, through largely unknown mechanisms, ameliorated age-related brain dysfunction, increased adult neurogenesis, and enhanced hippocampal synaptic plasticity and curative effects on cognitive levels in the brain (Villeda et al. 2014). Impaired autophagy-dependent liver damage has also been associated with aging. However, the administration of young plasma moderately reduced the hepatic damage by restoring the non-functioning autophagy process in old rats. Therefore, it was proposed that juvenile plasma is beneficial in caring for aging-related organ decline (Liu et al. 2018). Recent research by our group has also found that young plasma transfer can recover decreased sperm counts and restore epigenetics in aged testes (Erdogan et al. 2023).

Infrared (IR) spectroscopy, which can detect molecular vibrations and create molecular spectral bands in the mid-infrared regions, is a method used to obtain broad-spectrum data in a fast, simple, and non-invasive way in biochemical analyses with its multiprocessing capability (Severcan and Haris 2012; Baker et al. 2014). There is solid proof that aging has an unfavorable impact on stem cells and/or their niche capacities, which implies expanded senescence cell numbers and expanded disintegration of the self-renewal, multiplication, and differentiation capacities of stem cells (Aksoy and Severcan 2019). IR spectroscopy can be utilized as a novel and non-destructive strategy in stem cell studies, empowering real-time and label-free chemical checking. Collecting high-quality information with less exploratory complexity can be used to recognize novel biomolecular markers (Aksoy and Severcan 2012). The attenuated total reflection (ATR) mode of IR spectroscopy is a capable technique for investigating biological specimens. The preparative procedure is streamlined since samples can be instantly put on a crystal plate before measurement (Kazarian and Chan 2006; Dogan et al. 2021).

This study used LDA and SVM learning methods to analyze IR spectral data of liver tissues from aged and young rats after plasma infusion. The levels of different liver biomolecules were also quantified. The glycogen content was evaluated using spectrochemical analysis and PAS staining, and liver enzyme levels were measured in blood serum samples. Our results showed that plasma administration induced specific and diffuse metabolic responses in the liver, leading to liver regeneration. This study provides valuable insights into the effects of plasma exchange on liver tissues and contributes to anti-aging research.

Material method

Animal studies

The study used the Male Sprague Dawley rat species as a model organism. The aged rats (24 months n = 6) were treated with pooled plasma (0.5 ml per day for 30 days, intravenously into the tail vein) collected from young (5 weeks, n = 51) rats. The young rats (5 weeks, n = 6) were treated with pooled plasma (0.25 ml per day for 30 days, intravenously into the

tail vein) collected from aged (24 months, n = 16) rats. The number of animals sacrificed for plasma supply was determined according to the amount of plasma available from the animal. A 24-month-old rat has an average of 10 ml of blood. In 10 ml of a blood sample, an average of 5 ml of blood plasma is known to be obtained. Similarly, a 5-week-old rat has an average of 5 ml of blood, and this amount has 2.5 ml of blood plasma. The transferred blood plasma was determined according to 1/10 of the blood plasma amount of the animal (Villeda et al. 2014). The young and old rats used in the study came from a center for producing and analyzing experimental animals. Rats in each group were kept in separate cages, and co-housed rats remained in the same group. They were housed in clear Plexiglas cages (6 rats/cage) with free access to food and water under a 12-h light/dark cycle at a constant 21 ℃ temperature. There is no indication that allergies or rejection were observed during the plasma exchange in the rats. No animals were lost during the experiment. All animals were ether-stunned and sacrificed the day after. Liver tissues were collected, immediately shocked on dry ice, and stored at − 80 °C until analysis. The study was carried out with the approval of the Ethics Committee (approval number: 2021/03) from the Saki Yenilli Experimental Animal Production and Practice Laboratory (Ceylani and Teker 2022).

Plasma collection

Pooled rat plasma was collected by terminal cardiac puncture during euthanasia. Plasma was prepared from blood collected with EDTA, followed by centrifugation at 1000 g. For plasma denaturation, plasma was heated for 2–3 min at 95 °C, followed by a short spin at 1000 g. All plasma aliquots were stored at − 80 °C until use. Before administration, plasma was dialyzed using 3.5-kDa D-tube dialyzers (EMD Millipore) in PBS to remove EDTA (Villeda et al. 2014).

Analysis of samples by attenuated total reflectance fourier transform infrared (ATR-FTIR) spectroscopy

Liver samples of all animals were compressed on the Zn/Se crystal of the ATR unit (PerkinElmer) without any pretreatment and examined twice with an ATR-FTIR spectrometer (PerkinElmer) at a resolution of 4 cm^{-1} and a scan number of 32. The spectra were obtained with the Spectrum One (PerkinElmer) software in the wavelength range of 4000–650 cm^{-1} (Gurbanov and Yıldız 2017; Gurbanov et al. 2021).

The quantification of infrared spectral bands

The band quantifications were performed according to previous studies (Ceylani et al. 2022). Spectral data analysis was performed using OPUS 5.5 (Bruker) software. The average spectra obtained from the two replicate spectra of each sample were baseline corrected using the Rubberband correction method with 64 baseline points before the band quantification analyses.

Prediction studies with machine learning algorithms based on big spectral data

The prediction analyses were performed using previous studies (Ceylani et al. 2022). Linear Discriminant Analysis (LDA), a machine learning approach, was applied to differentiate the experimental groups. Spectral data were used in pattern recognition analysis. To make the analyzes as independent as possible from the FTIR spectrometers, each sample spectrum (but not an average spectrum) was preprocessed on The Unscrambler® X 10.3 (CAMO Software AS, Norway) software with a baseline offset transformation in the 4000–650 cm^{-1} region. Spectra processed 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. Lipid (3000–2700 cm^{-1}), protein (1700–1500 cm^{-1}), whole biomolecular (4000–650 cm^{-1}), and nucleic acid and polysaccharide (1200–650 cm^{-1}) spectral windows were examined by Singular Value Decomposition (SVD) 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. The linear method using the projections of the 7 PCA components was used for the prediction (Dogan et al. 2021; Ardahanlı et al. 2022).

The same spectral processings were also applied in SVM modeling, 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 the SVM type using a linear method as the Kernel type. Nu value was set to 0.5, weights as all 1.00. The nine segments of cross-validation were used to calculate training and cross-validation accuracies. Finally, the generated training dataset was applied to all sample datasets to obtain an SVM classification model.

Histopathological analysis

The liver tissue samples were collected and fixed in buffered 10% formaldehyde for about 72 h for histological analysis. After tissue procession, the tissue biopsies were embedded in paraffin blocks, and the blocks were cut into 5 µm thicknesses. The tissue sections were stained with H&E for histological evaluation. However, liver sections were used for Periodic Acid Schiff (PAS) staining according to the commercial kit using a 04-130802A kit (Bio-Optica, Milan, Italy) following the manufacturer's instructions to detect cells containing glycogen for evaluating young and plasma exchange administrations in aged-related liver injury. According to the commercial kit, the tissue sections (n = 6) were deparaffinized and rehydrated for PAS staining. All these steps were conducted at room temperature. To quantify the stained PAS-positive area, the images were changed to a gray scale for binarization. The threshold was set at the minimal level to detect magenta to purple/red stained areas using Image J (National Institutes of Health, Bethesda, Maryland, USA). The stained areas in each condition under the same threshold were measured using ImageJ. Signal intensities from five images in each section for each animal in the group were shown graphically.

An average of 10–15 areas were evaluated by random sampling for each group animal for histopathological evaluation. The findings were semi-quantitatively assessed according to the number of lesions observed in examined regions. Thus, it was performed as normal: (−) negative reaction, (+) weak positive reaction, (++) moderate positive reaction, (+++) strong positive reaction, and (++++) intense positive reaction (Keskin et al. 2022). In all microscopic analyses, the histopathological changes of the study were carried out blindly by two observers. Hepatic morphology in all slides was examined under a light microscope (Olympus BX53, Japan) using a camera attachment (Olympus DP27, Japan) with imaging systems (Olympus cellSens Entry, Japan).

Biochemical analysis

Blood samples were collected under euthanasia and centrifuged at 3500 rpm for 15 min, and serum was separated at four °C by a refrigerated centrifuge. The serum concentrations (IU/mL and g/L) of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and albumin were measured by enzymatic assays using an automated biochemical analyzer (Hitachi C502, Roche, Germany) and commercial kits (Roche Diagnostics). All assays were performed following the procedures described by the manufacturer (Roche Diagnostics).

Statistics

Statistical evaluations and graph plots of the results were made using GraphPad Prism 8.01 (GraphPad, USA). SPSS software (version 20) was used to analyze the histological and biochemical experimental data. The data were analyzed using an unpaired t-test. The significance levels between COLI (control old rat) and POLI (young plasma recipient old rats) and CYLI (young control rat livers) and PYLI (old plasma recipient young rats) groups were stated as $p \leq 0.05$ *, $p \leq 0.01$ **, $p \leq 0.001$ ***, and $p \leq 0.0001$ ****. Results are presented as mean ± SEM (standard error of the mean).

Results

Effect of young and old plasma on liver biomolecules

Plasma exchange between young and aged rats triggered unique changes in liver samples with high discrimination accuracies that are 95.83% for lipids, 97.92% for nucleic acids and polysaccharides, and 100% for proteins and whole biomolecules (Figs. 1–4, Supplementary Tables S1–S8). As shown in the discrimination plot in Fig. 1, the POLI samples (young

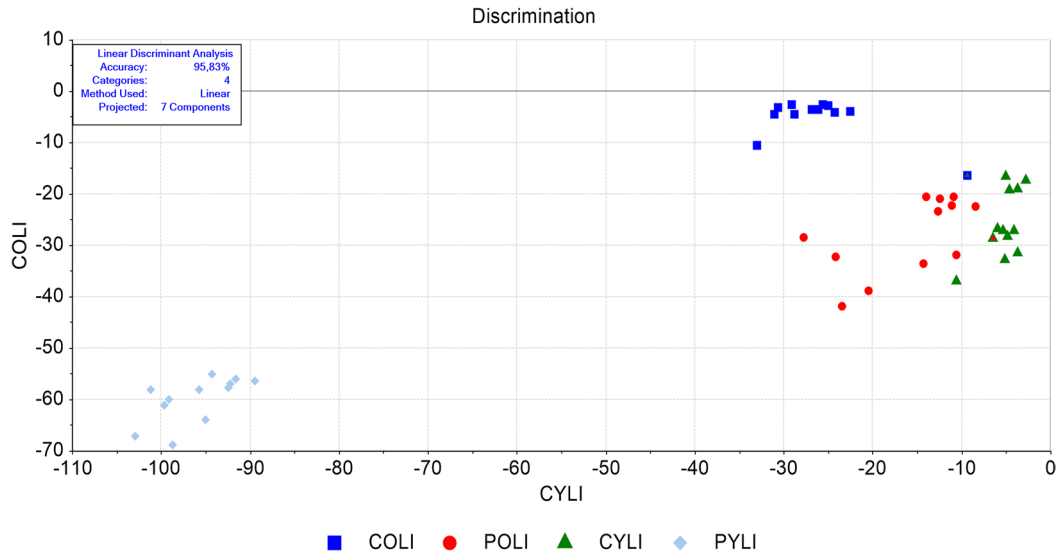

Fig. 1 LDA discrimination plot for liver samples in lipid (3000–2700 cm^{-1}) spectral region. COLI (old control rat livers), CYLI (young control rat livers), POLI (young plasma recipient old rat livers), PYLI (old plasma recipient young rat livers)

plasma recipient old rat livers) were clustered next to the CYLI samples (young control rat livers) and far enough from their control samples (COLI /old control rat livers). On the other hand, PYLI samples (old plasma recipient young rat livers) were located far away from the corresponding control group (CYLI / young control rat livers). The results imply that infusion of young plasma restores the liver lipids of aged rats, whereas the liver lipids of young rats receiving plasma of old animals worsen. In the case of liver proteins, a similar restoring effect was revealed not only for POLI samples but also for PYLI samples (Fig. 2). The detailed confusion and prediction matrices of LDA classes for lipids and proteins revealed almost no confusion between the class members and correct prediction of the samples, respectively (Supplementary Tables S1–S4). Probably due to many other overlapping metabolites, water, etc., masking

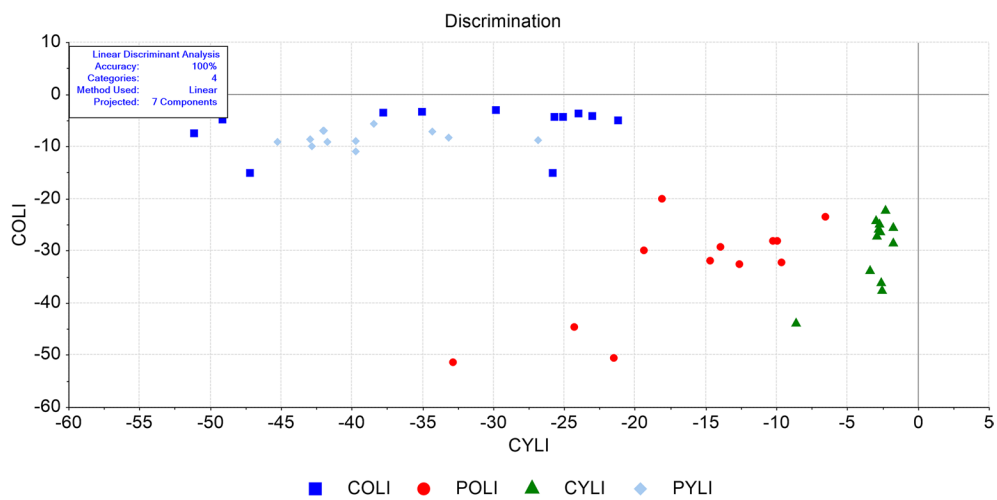

Fig. 2 LDA discrimination plot for liver samples in protein (1700–1500 cm^{-1}) spectral region. COLI (old control rat livers), CYLI (young control rat livers), POLI (young plasma recipient old rat livers), PYLI (old plasma recipient young rat livers)

the overall impact of plasma infusion in liver tissues, not any restoring (for POLI samples), but rather worsening effects (for PYLI samples) were depicted for whole biomolecules in the liver (Fig. 3). Therefore, a separate examination of each biomolecular region is suggested for this kind of research. For nucleic acids and polysaccharides of the liver, the worsening effect was uniquely accepted for POLI samples, while the restoring result was typical for the PYLI group (Fig. 4). Supplementary Tables S5-S8 demonstrate the correct predictions, mostly without confusion, for whole biomolecules, nucleic acids, and polysaccharides. A comparable classification was obtained with the SVM method in which the 91.67% training

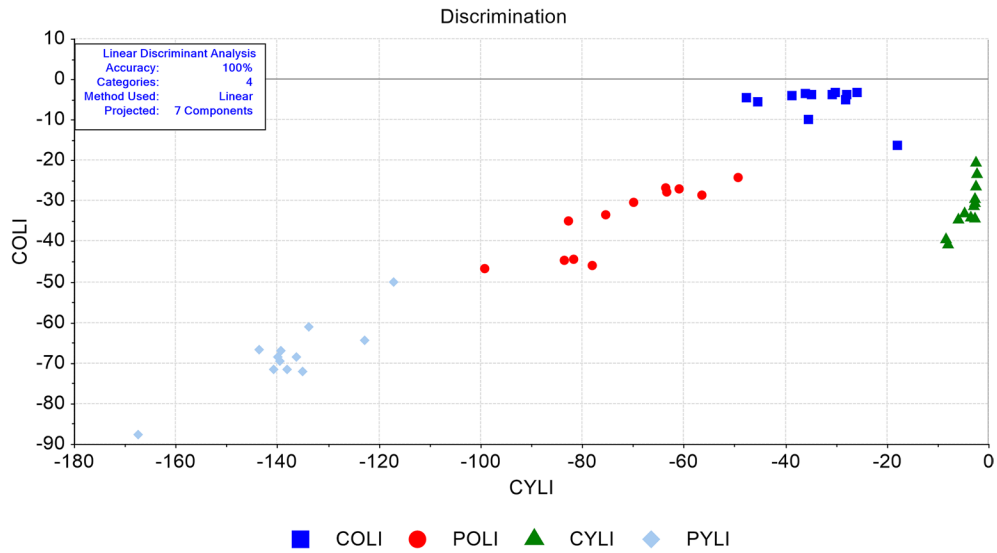

Fig. 3 LDA discrimination plot for liver samples in full (4000–650 cm^{-1}) spectral region. COLI (old control rat livers), CYLI (young control rat livers), POLI (young plasma recipient old rat livers), PYLI (old plasma recipient young rat livers)

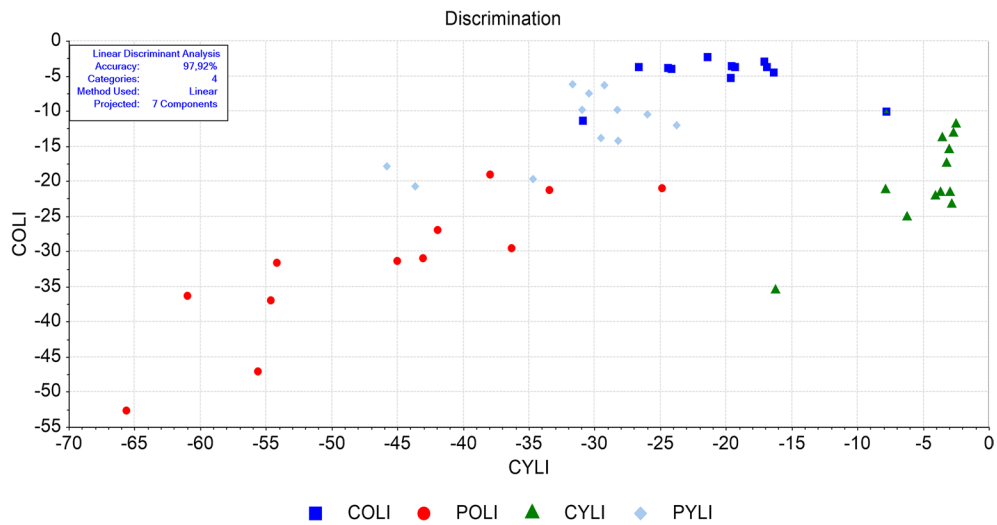

Fig. 4 LDA discrimination plot for liver samples in nucleic acid and polysaccharide (1200–650 cm^{-1}) spectral region. COLI (old control rat livers), CYLI (young control rat livers), POLI (young plasma recipient old rat livers), PYLI (old plasma recipient young rat livers)

and 87.50% cross-validation accuracies were calculated for liver lipids. However, the accuracy of LDA was superior (2 samples with the wrong prediction) to SVM since 5 samples out of 48 were wrongly classified in SVM for liver lipids (Table 1). Similar SVM results were also obtained for liver proteins (data not shown).

To determine the quantitative changes in spectrochemical indices related to different functional groups in liver biomolecules, the band areas and area ratios were measured. Accordingly, the significant changes in indices for an acyl chain length of fatty acids (A_{2928}/A_{2971}), triglycerides ($A_{1741}/A_{2928+2852}$), lipid carbonyl (A_{1741}), glycogen ($A_{1160+1053+1031}$), nucleic acid (A_{1085}), protein concentration ($A_{1644}/A_{1644+1538}$), protein carbonylation (A_{1741}/A_{1538}), and protein phosphorylation (A_{1242}/A_{2971}) were measured (Figs. 5 and 6). A significant increase (36%) in fatty acid acyl chain index was realized for the POLI group (young plasma recipient old rat livers) compared to the COLI group (old control rat livers), indicating longer acyl chains of fatty acids in the liver of aged rats as a result of young plasma infusion. Despite the slightly decreasing trend, there were no significant differences between CYLI (young control rat livers) and PYLI groups (old plasma recipient young rat livers) in terms of fatty acid acyl chains (Fig. 5a). Plasma exchange between aged and young rats led to a significant increase (156%) in liver triglyceride levels for the POLI group. However, a considerable decrease of 47% in triglycerides was realized in the PYLI group (Fig. 5b). Lipid carbonyl level was increased by 180% in the POLI group, while there was a 53% decline in the PYLI group (Fig. 5c). Liver glycogen content was significantly increased by 155% in the POLI group due to plasma exchange; however, there were no significant changes between CYLI and PYLI groups (Fig. 5d). The concentration of nucleic acids was elevated by 26% in the POLI group, and there was no difference between CYLI and PYLI groups (Fig. 5e). The liver protein concentration was 4% lower in the POLI group than in the COLI group, whereas no significant change was depicted in the PYLI group (Fig. 6a). The analysis of marker spectrochemical parameters also assessed post-translation modifications (PTM) in proteins. As an indicator of protein oxidation, higher protein carbonyl levels by 158% were calculated for the POLI group, while reduced by 52% in the PYLI group compared to their

Table 1 SVM classification for liver samples in lipid (3000–2700 cm^{-1}) spectral region

Accuracy (%)			
Training	91.67	Validation	87.50
Classification			
Samples		Class	
COLI	**1**	**1**	***POLI**
COLI	2	2	COLI
COLI	3	3	COLI
COLI	4	4	COLI
COLI	5	5	COLI
COLI	6	6	COLI
COLI	7	7	COLI
COLI	8	8	COLI
COLI	9	9	COLI
COLI	10	10	COLI
COLI	11	11	COLI
COLI	12	12	COLI
POLI	13	13	POLI
POLI	14	14	POLI
POLI	15	15	POLI
POLI	16	16	POLI
POLI	17	17	POLI
POLI	**18**	**18**	***CYLI**
POLI	19	19	POLI
POLI	20	20	POLI
POLI	21	21	POLI
POLI	22	22	POLI
POLI	23	23	POLI
POLI	24	24	POLI
CYLI	25	25	CYLI
CYLI	26	26	CYLI
CYLI	27	27	CYLI
CYLI	28	28	CYLI
CYLI	29	29	CYLI
CYLI	30	30	CYLI
CYLI	31	31	CYLI
CYLI	32	32	CYLI
CYLI	33	33	CYLI
CYLI	34	34	CYLI
CYLI	35	35	CYLI
CYLI	36	36	CYLI
PYLI	37	37	PYLI
PYLI	**38**	**38**	***COLI**
PYLI	**39**	**39**	***COLI**
PYLI	40	40	PYLI
PYLI	41	41	PYLI

Table 1 (continued)

Accuracy (%)			
Training	91.67	Validation	87.50
Classification			
Samples		Class	
PYLI	42	42	PYLI
PYLI	43	43	PYLI
PYLI	44	44	PYLI
PYLI	**45**	**45**	***COLI**
PYLI	46	46	PYLI
PYLI	47	47	PYLI
PYLI	48	48	PYLI

COLI (old control rats), CYLI (young control rats), POLI (young plasma recipient old rats), PYLI (old plasma recipient young rats). SVM type: Classification (nu-SVC). Method: Linear

*Highlights the wrong classification

corresponding control groups (Fig. 6b). The protein phosphorylation index was enhanced by 74% in the POLI group compared to COLI, hence did not change in the PYLI group when compared with the CYLI group (Fig. 6c).

Effect of young and old plasma on liver histopathology

Given that aging induces liver damage and fibrosis, the effect of young plasma on aging-induced liver damage and fibrosis was investigated. As shown in Fig. 7, the healthy histological architecture was observed in the liver of the CYLI (young control rat livers) group. However, the formation of fibrosis in the intravascular and perivascular areas due to aging and tissue damage, including collagenous protein accumulations in the portal and the centrilobular regions, was detected in the COLI (control old rat

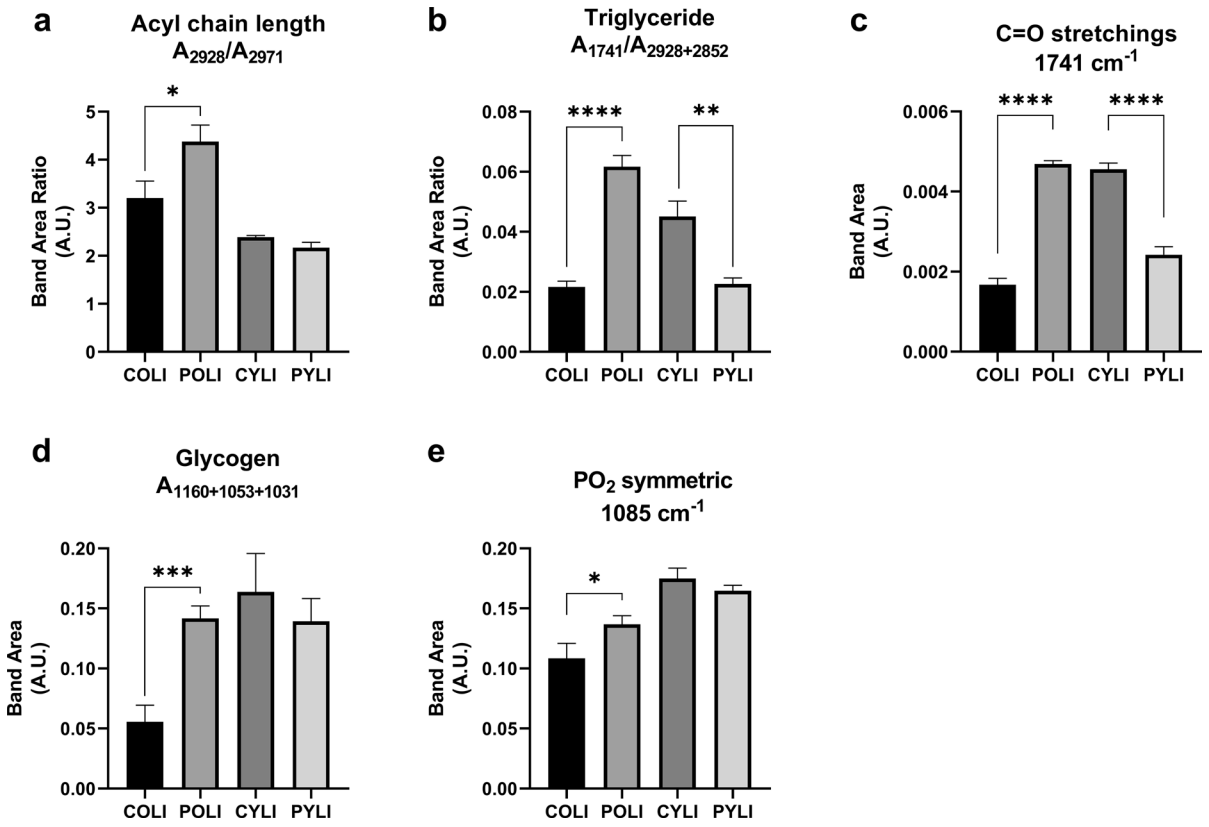

Fig. 5 The quantitative changes in lipid, polysaccharide, and nucleic acid-associated spectrochemical parameters. The indices for **a** acyl chain length of fatty acids (A_{2928}/A_{2971}), **b** triglycerides ($A_{1741}/A_{2928+2852}$), **c** lipid carbonyl (A_{1741}), **d** glycogen ($A_{1160+1053+1031}$), and **e** nucleic acid (A_{1085}). A (Absorbance), COLI (old control rat livers), CYLI (young control rat livers), POLI (young plasma recipient old rat livers), PYLI (old plasma recipient young rat livers)

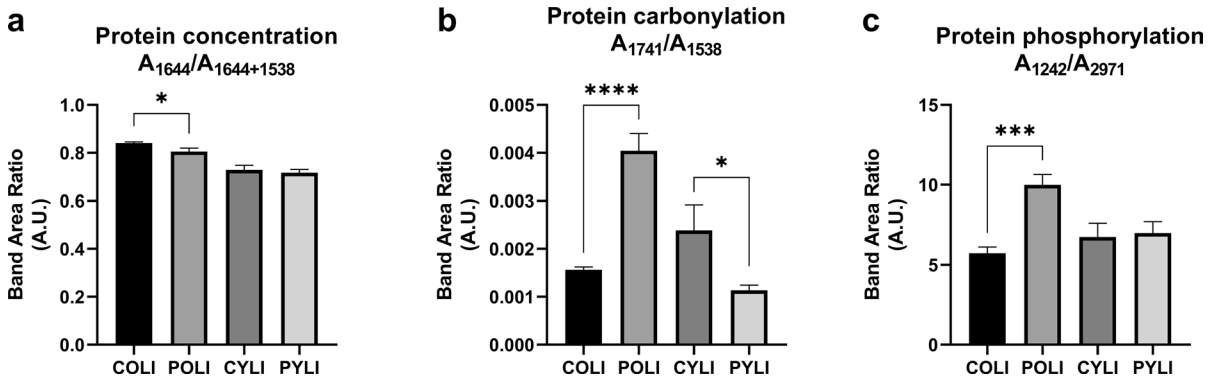

Fig. 6 The quantitative changes in protein-associated spectrochemical parameters. The indices for **a** protein concentration ($A_{1644}/A_{1644+1538}$), **b** protein carbonylation (A_{1741}/A_{1538}), and **c** protein phosphorylation (A_{1242}/A_{2971}). A (Absorbance), COLI (old control rat livers), CYLI (young control rat livers), POLI (young plasma recipient old rat livers), PYLI (old plasma recipient young rat livers)

livers) group. In addition, liver sections of the COLI group exhibited more dense lipid droplets than the POLI (young plasma recipient aged rat livers) group, irregular arrangement in hepatocytes, and an increase in sinusoidal areas compared to the CYLI group. The formation of microvesicular steatosis was due to the deterioration in the histological structure of the liver and increased fat tissue in the hepatocyte lobules.

The young plasma administration improved hepatic fibrosis in the POLI group and cellular degenerations in hepatocytes in other histological findings (Fig. 7). When the effect of plasma exchange on aging-related hepatic microvesicular steatosis was evaluated, a significant reduction in the amount of fatty tissue in hepatocytes was revealed in the POLI group compared to the COLI and PYLI groups. This indicates that young plasma to aged rats may reduce hepatic microvesicular steatosis. In the PYLI group (aged plasma recipient young rat livers), disruption of cellular organization in liver tissues, hepatocytes irregular arrangement, degeneration, and vacuolization were observed. Accordingly, microvesicular steatosis and increased fibrosis were detected in young rat livers receiving aged plasma. At the same time, the increase of Kupffer cells in the portal areas of the liver and intense lymphatic and neutrophil infiltration and acidophilic hepatocytes in the sinusoidal regions were detected in the PYLI group (Fig. 7).

Besides, degenerations in the histological structure, such as swelling of the cell, loss of cell membrane integrity, and disruption of nuclear chromatin structure, which are among the typical morphological features of necroptosis in hepatocytes were observed in the COLI and PYLI groups (Fig. 7). The obtained data indicate that there may be a cell death mechanism like necroptosis in the hepatocytes. Since the rupture of the plasma membrane in necroptotic cells causes the release of cell contents, inflammatory responses may be triggered by exposure to damage-related molecular patterns (Damp-associated molecular patterns, DAMPs) (Gong et al. 2019). The activation and infiltration of many inflammatory cells were observed in tissues where inflammation occurs (Liu et al. 2018). Therefore, as shown in Fig. 8, lymphatic infiltration density was significantly increased in the COLI and PYLI groups compared to the CYLI and POLI groups. Especially in the POLI group, the lymphatic infiltration density was significantly decreased compared to the COLI and PYLI groups. This situation suggests that administering young plasma may reduce necroptosis in the aged liver.

Glycogen histochemistry

The glycogen content of the liver is generally examined in organ damage due to aging and impaired metabolic activity. Therefore, PAS staining was performed to determine the effects of young and old plasma administration on glycogen content in hepatocytes during the aging process. PAS staining indicated that the administration of young plasma significantly increased liver glycogen accumulation in the POLI and CYLI groups on the cytoplasm of

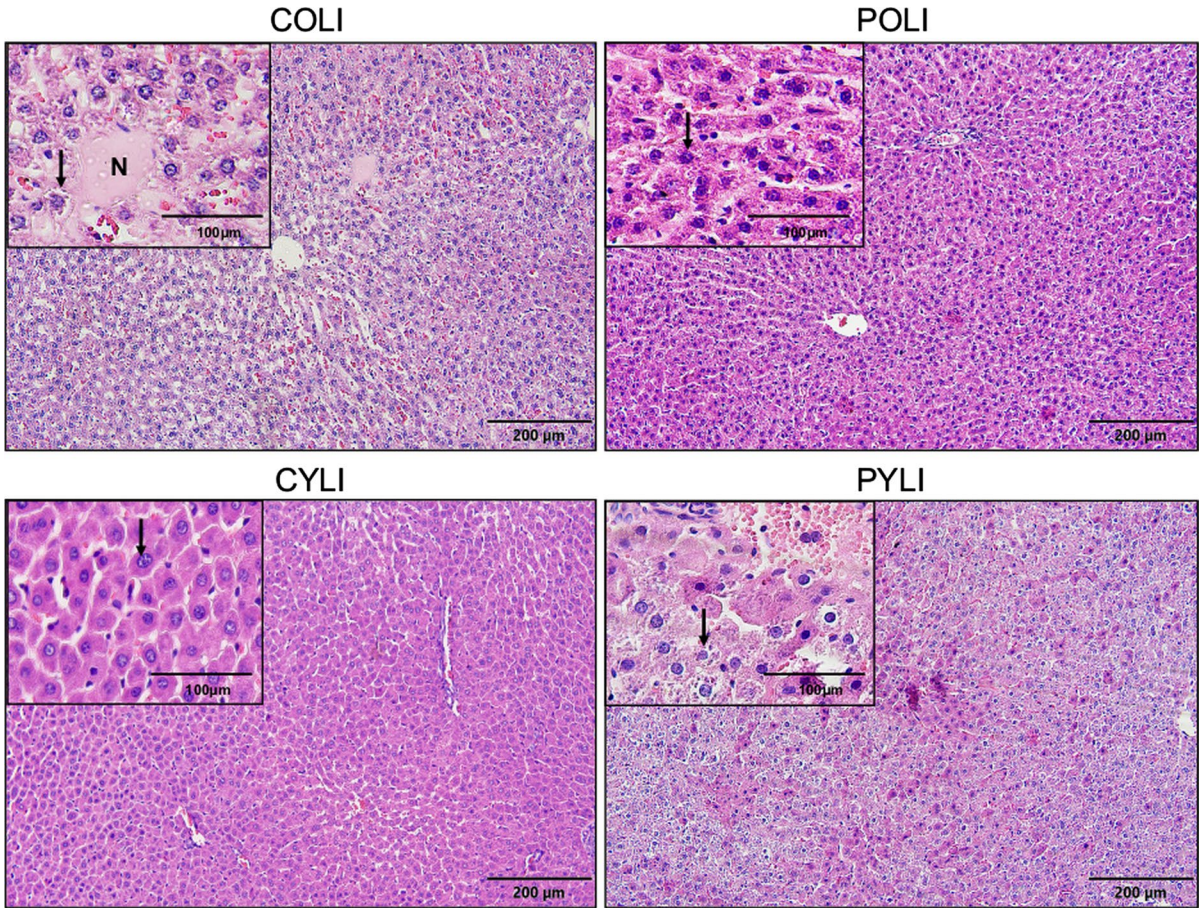

Fig. 7 H&E photomicrograph of the liver sections from all rat groups. Morphological changes in the liver: CYLI group had the normal structure of the young rat liver. In the COLI and PYLI group, the mass granular degeneration and the swelling of hepatic cells around the central vein, vague cell boundaries, liver cell cord derangement, and necrotic areas in the liver portal areas (represented N) were observed. In the POLI group, pathological changes included the slight vacuolar degeneration of hepatic cells with a completed structure and clear boundaries and fibrosis in some rats' portal areas of the liver. Black arrows show necrotic regions, and H represents hepatocytes. Magnification, 200 × and 400x. Scale bars, 100 µm and 200 µm

the hepatocytes (Fig. 9a). However, the administration of young plasma strengthened the PAS reaction. It resulted in many reddish-colored fine granules of different sizes, indicating increased glycogen content in the POLI group shown in Fig. 9b. On the other hand, aged plasma significantly reduced glycogen in most hepatic parenchyma with deposits only at the periphery (Fig. 9b). Therefore, the glycogen content showed the weak intensity of the reaction and became hardly detectable, i.e., a decrease in the hepatic glycogen stores was observed in the COLI and PYLI groups (Fig. 9a).

Activities of liver marker enzymes in blood serum

The aging liver is often associated with decreased liver function or tissue/serum enzymes (Timchenko 2009). During this process, metabolic changes such as elevated ALT and AST levels, lipofuscin deposition, steatosis, fibrosis, and defective liver regeneration occur. Given that aging induces liver injury and fibrosis, we determined the effect of young plasma administrations on aging-induced liver injury and fibrosis. As shown in (Fig. 10a), serum levels of AST were increased in young rats (CYLI group) when compared to those in old controls (COLI group).

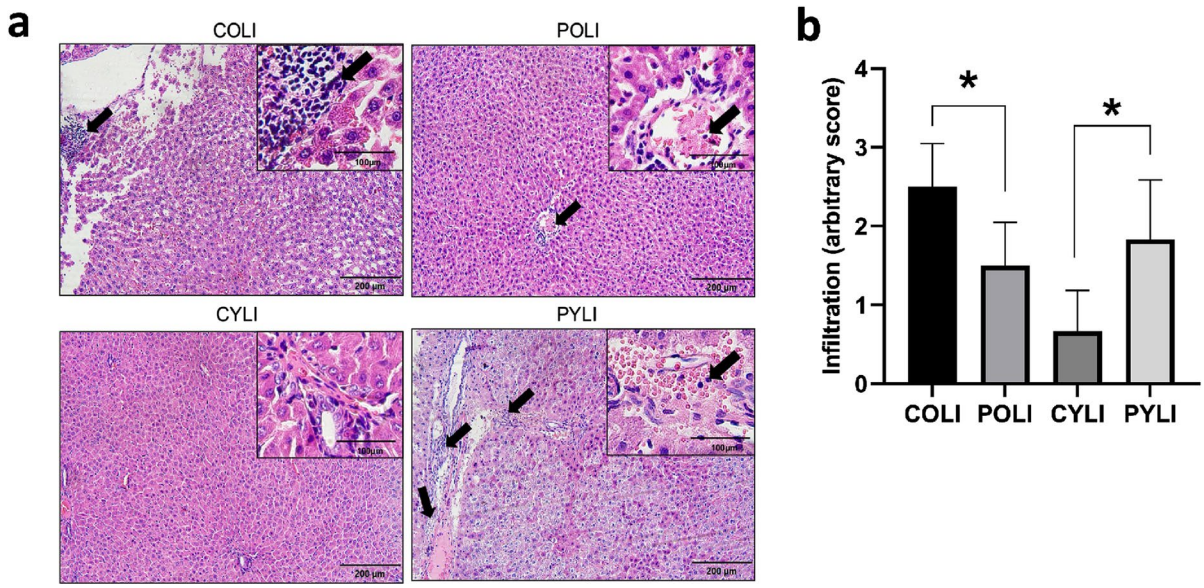

Fig. 8 Effect of young and old plasma administration on liver lymphatic infiltration alterations score in all groups. **a** Representative liver inflammation morphology and H&E-stained liver sections. **b** Hepatic neutrophil infiltration score was graded on a scale of 0 (absent), 1 (rare), 2 (mild), 3 (moderate), and 4 (severe). Black arrows show inflammatory cell infiltration in intravascular and perivascular areas. Magnification, 200 × and 400x. Scale bars, 100 µm and 200 µm

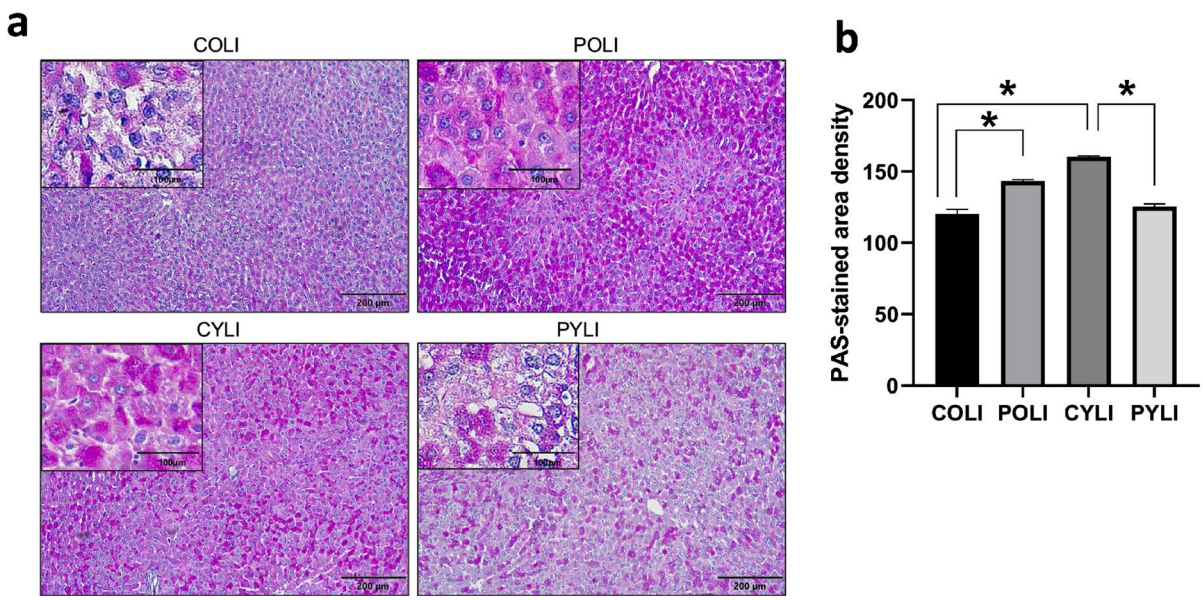

Fig. 9 Photomicrographs of the liver sections from COLI, POLI, CYLI, and PYLI groups for detecting glycogen deposition demonstrated by purple-red PAS staining. **a** Representative liver PAS-stained morphology and PAS-stained liver sections. **b** PAS-stained area densities of blots were measured using the NIH Image J program. COLI and PYLI: Liver glycogen in the old control rats and old plasma recipient young rats: PAS-positive cells were significantly reduced, mainly at the edge of the portal area, with only scattered, sporadic purple liver cells surrounding the central vein. CYLI and POLI: Liver glycogen in the young plasma recipient old rats and young control rats: PAS-positive cells remained mainly in the area around the portal vein, and the number of PAS-positive cells increased around the central vein. Magnification, 200 × and 400x. Scale bars, 100 µm and 200 µm

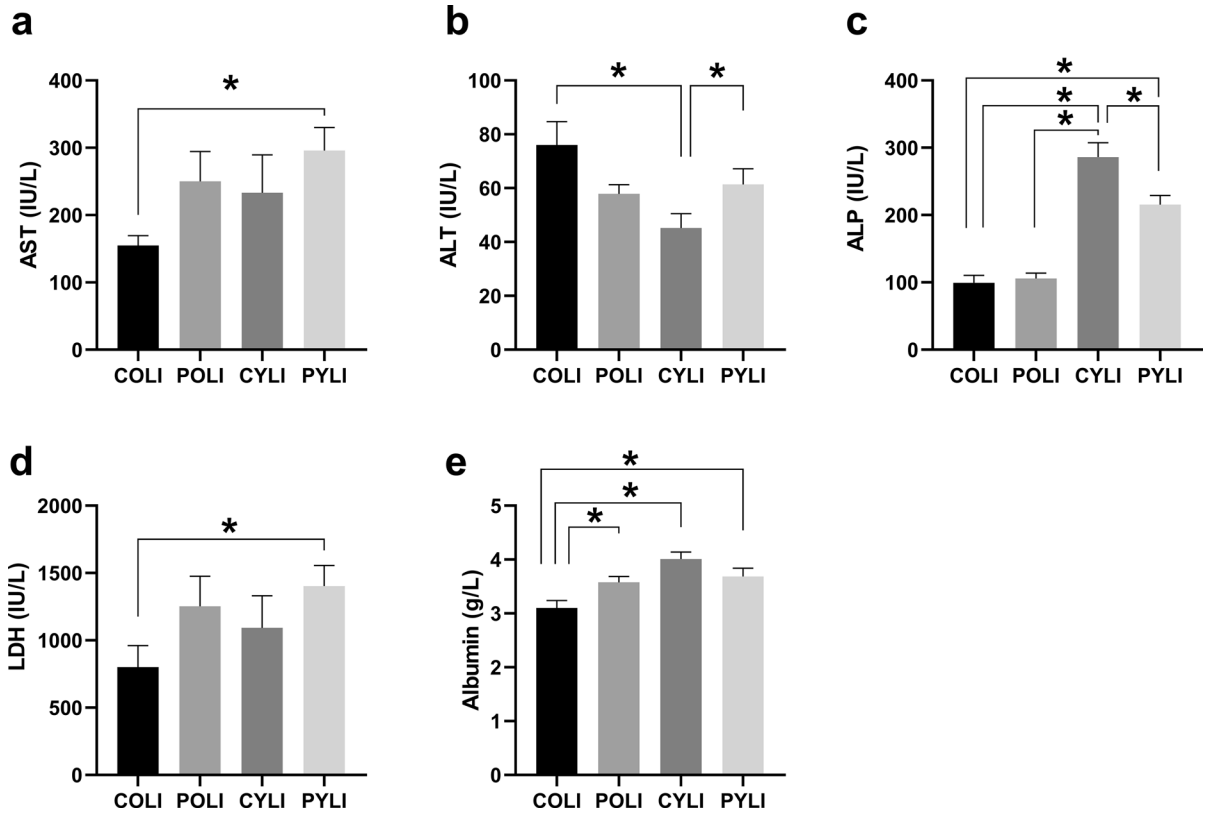

Fig. 10 Effects of young and old plasma on serum **a** AST, **b** ALT, **c** ALP, **d** LDH, and **e** Albumin levels in COLI, POLI, CYLI, and PYLI groups. Values are expressed in Mean ± SE. n = 6, *p < 0.05 compared with COLI = Old Control Rats group, POLI = Young Plasma Recipient Old Rats Group, CYLI = Young Control Rats Group, and PYLI = Old Plasma Recipient Young Rats Group

However, young plasma administrations increased serum AST levels in the POLI group compared to the COLI group. But there was no statistical significance between the POLI and COLI groups. Old plasma administrations significantly increased serum AST levels in the PYLI group compared to the COLI and CYLI groups. AST is not the only liver-specific enzyme but is also abundantly found in muscle tissue. Young individuals have a high muscle ratio, whereas the muscle ratio decreases in elderly individuals due to cellular degeneration during aging. The AST levels may therefore be low in aged rats. The serum levels of ALT were significantly increased in old rats (COLI group) when compared to those in young controls (CYLI group) (Fig. 10b). Although the difference between the groups was not statistically significant, young plasma administrations in the POLI group decreased serum ALT levels in old rats (COLI group). Old plasma administrations significantly increased serum ALT levels in young rats (PYLI group) compared to young controls (CYLI group).

Intestinal barrier dysfunction and gut-derived chronic inflammation have crucial roles in human aging. The intestinal brush border enzyme ALP is an essential positive regulator of intestinal barrier function and microbial homeostasis by inhibiting inflammatory mediators. This enzyme also plays a critical role in regulating the aging process. Changes in the levels of ALP, an enzyme associated with liver, kidney, and bone tissue, in systemic and portal circulation in elderly individuals may affect the aging process. The serum levels of ALP were significantly increased in the CYLI group compared to the COLI, POLI, and PYLI groups (Fig. 10c). Young plasma administrations slightly increased serum ALP levels in the POLI group compared to the COLI group. But there was no statistical significance between POLI and COLI groups. In addition, old plasma

administration significantly increased serum ALP levels in the PYLI group compared to the COLI and the POLI groups.

LDH is an essential enzyme responsible for pyruvate formation in anaerobic glycolysis and converting NADH to NAD^+ during glycolysis under hypoxia conditions. Although this enzyme is found in all cells, it is mainly found in muscle, the liver, and the kidney. A wide range of diseases has been associated with altered LDH levels. Measurement of activities of marker enzymes (notably LDH activity) in serum is a diagnostic parameter for ascertaining inherent, biological, and xenobiotic-induced systemic toxicity. Elevated serum LDH activity is a clinical indicator of organ injuries, especially those affected by heart, liver, and muscle aging. The serum levels of LDH were increased in young rats (CYLI group) when compared to those in old controls (COLI group) (Fig. 10d). But there was no statistical significance between POLI and COLI groups. However, young plasma administration increased serum LDH levels in the POLI group compared to the COLI group. Although the difference between the groups was not statistically significant, old plasma administrations significantly increased serum LDH levels in the PYLI group compared to the COLI group.

The serum levels of albumin were significantly increased in the CYLI group compared to those in the COLI group (Fig. 10e). However, young plasma administration significantly increased serum albumin levels in the POLI group compared to the COLI group. Old plasma administration decreased serum albumin levels in the PYLI group compared to the CYLI group. But there was no statistical significance between PYLI and CYLI groups.

Discussion

This study aimed to investigate the effects of blood plasma exchange on the liver tissues of aged and young rats using machine learning methods and spectrochemical analyses. Histological examinations were also conducted to determine the effects of plasma on cellular and tissue architecture. The study utilized two machine learning methods, LDA and SVM, and plasma was exchanged between young and old rats for 30 days. The results showed significant changes in liver biomolecules. Interestingly, the study found a substantial increase in the acyl chain length of liver lipids in the POLI group (young plasma recipient old rats), which may have implications for membrane fluidity (Ballweg et al. 2020). Specifically, longer phospholipid tails can reduce membrane fluidity by permitting tail-to-tail interactions and forming non-fluid, tightly packed gel phases. This finding is in line with previous studies that have shown a link between changes in membrane fluidity and cancer progression (Bompard et al. 2020).

Moreover, the study found an increase in liver triglyceride concentrations in the POLI group, which negatively correlated with membrane fluidity (Ballweg et al. 2020). This suggests that young plasma may enhance liver health by maintaining membrane fluidity and reducing the risk of chronic liver diseases and tumors. The study also found that the 1741 cm^{-1} spectral band (C=O stretching) for lipid carbonyl groups was increased in the POLI group while decreasing in the PYLI group (old plasma recipient young rats) (Dreier et al. 2019). This band is functional for characterizing environmental changes, hydrogen bonding of lipid molecules, and distinguishing interactions with ligands. Thus, the position and intensity of the carbonyl band deliver practical facts about the hydration state of lipid molecules at the water interface. Water content changes with alter in lipid density. As the lipid layer becomes denser, the headgroup becomes less hydrated, which in turn causes relocation of the carbonyl bands to higher wavenumbers (Dicko et al. 1998; Ohto et al. 2015). The findings of this study provide novel insights into the mechanisms underlying the beneficial effects of young plasma on liver health. However, further research is needed to confirm these findings and elucidate the underlying molecular mechanisms.

The spectrochemical data from this study showed an increase in protein carbonyl groups in the liver of the POLI group. In contrast, a decrease in protein carbonylation was observed in the PYLI group. Protein oxidation plays a crucial role in the pathophysiology of aging and is intricately involved in the management of physiological events and minimizing tissue injury (Paraboni et al. 2022). The oxidative stress theory of aging suggests that senescence is predominantly controlled by the accumulation of oxidized molecules that adversely affect biotic homeostasis and induce a functional decline in cellular physiology. Contrary to this theory, a

proteomic study found that the soluble cell portions of long-living naked mole rats had high levels of protein carbonylation in comparison to short-living mice (Finch and Crimmins 2016; Krisko and Radman 2019). Another study found higher protein carbonylation in long-living naked-mole rodents than in mice (Andziak et al. 2006). It should be noted that, besides being an indicator of oxidative stress, protein carbonylation also appears as a biomarker of misfolded proteins or an index to check protein folding quality (Krisko and Radman 2019). In addition, our spectrochemical data revealed an increase in phosphorylated proteins in the POLI group, indicating that the phosphorylation machinery may play a role in healthy aging. Changes in protein phosphorylation have been associated with many operational modifications that occur with aging, such as learning and memory deficits and changes in energy metabolism (Krisko and Radman 2019).

Furthermore, the chemical environment of pH plays a pivotal role in post-translational events such as phosphorylation/dephosphorylation during aging, as previously reported in the dephosphorylation of acidic proteins in the microsome and hyperphosphorylation of essential proteins in the nuclear fractions of hepatocytes (Heydari et al. 1989; Liu et al. 2022). Additionally, intravenous administration of phospho-peptides of small nuclear ribonuclear protein (snRNP) has been shown to stimulate immune tolerance, decline disease severity, and extend the survival of lupus-prone mice. At the same time, parental non-phosphorylated peptides were ineffective (Cloos and Christgau 2004).

Age-related diseases are closely linked to immune dysregulation and inflammation. Previous studies have demonstrated increased expression of inflammation-related genes in the aged liver (Singh et al. 2011) and higher levels of inflammatory cytokines, chemokines, and genes in immune-related changes in the aged liver of animals. In our study, increased immune cells in the livers of aged mice were observed in clusters or foci, especially near perivascular regions, consistent with previous research. Additionally, the livers of aged rats exhibited marked inflammation and increased immune cell infiltration, a similar phenomenon observed in the liver tissues of young rats receiving aged plasma. Our findings suggest that young plasma applications can treat the side effects of inflammatory processes in the tissue microenvironment associated with aging and other metabolic diseases.

Defective autophagy has been implicated in aging and aging-related organ injuries. Liu et al. (2018) investigated the effects of young plasma on liver damage in aging rats. They pooled plasma from rats and administered it (1 ml, i.v) three times a week for 4 weeks. The present study found that young plasma injections significantly reduced high ALT and AST levels, lipofuscin deposition, steatosis, fibrosis, and defective liver regeneration. Young plasma also restored impaired autophagy activity caused by aging in elderly individuals. The beneficial effect of young plasma on aging-associated liver damage was inhibited by 3-methyladenine or wortmannin, indicating that the restoration of autophagic activity by young plasma is necessary for its healing effects (Liu et al. 2018). In this study, we administered 0.5 ml (i.v) of young plasma to 24-month-old rats daily for 30 days, while aged plasma was administered to young rats at 5 weeks of age by 0.25 ml (i.v) daily during the same period. Our results showed that young plasma improved age-related liver damage, steatosis, and lymphatic infiltration. The healing role of young plasma was more effective in aging-induced cellular liver damage, considering the plasma content, application time, and amount applied.

It is known that serum ALT levels provide insight into hepatocyte damage in liver tissue during the aging process (Morsiani et al. 2019). The significantly higher serum ALT levels in aged rats than young rats are consistent with the histopathological findings on liver damage due to aging. In elderly subjects, it has been reported that ALT levels decrease with age, independent of adiposity signal biomarkers and other commonly used liver function tests. The decrease in ALT levels during aging may be associated with changes in liver volume, function, and immune response (Goh et al. 2015). Our findings demonstrated the curative effects of young plasma during metabolic processes in age-associated liver injury, which supports the previous observations suggesting that the liver is more prone to aging (Mitchell and Hilmer 2010). The biochemical enzyme levels (AST, ALP, LDH, and albumin) and histopathological changes detected in the present study for COLI and CYLI groups are consistent with the study mentioned above. Furthermore, our spectrochemical analyses revealed triglyceride, protein carbonylation, and

protein phosphorylation results in the elderly groups that correlate with the histopathological data.

The accumulation of glycogen in the liver is a well-known phenomenon associated with aging and has been attributed to cellular degeneration or wear and tear. GSK3β, a ubiquitous multifunctional serine/threonine protein kinase (Wang et al. 2022), was initially identified as a regulator of glycogen synthesis. Previous studies have mainly focused on GSK3β's ability to phosphorylate, inactivate, or degrade specific target substrates (Doble and Woodgett 2003). However, research has shown that the downregulation of GSK3β in young mice inhibits liver proliferation after partial hepatectomy via the cyclin D3-C/EBPa pathway, while the elevation of GSK3β in aged mice accelerates liver proliferation (Jin et al. 2009). Thus, GSK3β has been identified as a crucial regulator of liver proliferation, and the decrease of GSK3β with age may cause a loss of regenerative capacities in the liver. In the present study, we found that the regenerative capacity of liver tissue was significantly reduced in aged rats compared to young rats, which may be associated with a decrease in GSK3β and glycogen accumulation. Experiments with parabiotic animals have demonstrated that certain systemic factors in the circulatory systems of young mice can stimulate progenitor cells in the skeletal muscle and liver of aged mice, restoring the proliferation of these tissues (Jin et al. 2009). Therefore, the significant increase in glycogen density in the POLI group may have been due to the activation of GSK3β by factors present in young plasma. Our findings are interesting as the molecules enhancing liver regeneration in young plasma increased age-related glycogen accumulation in hepatocytes. Previous research has reported a significant decrease in hepatic glycogen storage in aged mice and decreased phosphorylation of the hepatocellular insulin receptor Akt (Mohamad et al. 2016). The overall findings of biochemical, histopathological, and spectrochemical analyses in old and young rats are consistent with the literature on age-related changes in glycogen metabolism.

Furthermore, commensal bacterial populations in the gut microbiota may also play a critical role in the biomolecule profile of the liver tissue of aged rats receiving young plasma. Our research group recently completed a study that demonstrated a considerable increase in gut microbiota species diversity following the transfer of young plasma into 12-month-old Wistar rats. Additionally, we observed a significant normalization in the Firmicutes to Bacteroidetes (F/B) ratio, an established marker for healthy gut microbiota. Species from these phyla are recognized for producing short-chain fatty acids (SCFAs) such as butyrate, acetate, and propionate (Ceylani and Teker 2022). These compounds serve as an energy source for intestinal epithelial cells and are vital in maintaining gut and overall health. SCFAs have been shown to possess anti-inflammatory properties, promote gut barrier integrity, and modulate immune responses. Maintaining healthy gut microbiota and SCFA levels can improve liver function and overall health during aging (Ticinesi et al. 2019). Additionally, an analysis of the gut microbiota profiles in rats whose livers were used for this study showed that transferring young plasma to aged rats significantly increased alpha diversity indices and considerably decreased the F/B ratio. In contrast, transferring old plasma to young rats led to a marked reduction in species diversity. Our findings reveal that young plasma promotes a shift in the aged gut microbiota composition towards a more health-supporting state, while old plasma transfer induces dysbiosis within the gut microbiota of young rats (Ceylani et al. 2023). Our recent investigations also indicated that young plasma transfer not only augments sperm concentrations but also restores epigenetic patterns within the testes of aged rats (Erdogan et al. 2023).

This study offers valuable insights into the effects of plasma exchange on liver health in both aged and young rats; however, several limitations should be considered when interpreting the findings. A larger sample size could yield more reliable results and potentially uncover additional significant findings. Further research should explore the impact of plasma exchange on a broader range of organs and tissues to gain a more comprehensive understanding of this treatment's systemic effects. Another limitation is that only male animals were used in this study. Including female animals and conducting analyses by sex will contribute to a more comprehensive understanding of plasma transfusion in both sexes. Although the study sheds light on some possible mechanisms, additional research is needed to pinpoint the specific factors in young plasma responsible for these effects and to elucidate their underlying molecular pathways. Long-term studies are essential for determining the enduring impact of plasma exchange on liver health

and the aging process. To better comprehend the potential clinical applications of this treatment, future studies should investigate plasma exchange's effects on human subjects or more relevant animal models. Lastly, further research should examine the more extensive implications of plasma exchange on overall health and well-being and any potential risks and side effects associated with the treatment.

Conclusion

The results of this study indicate that plasma exchange between aged and young animals significantly impacts liver biomolecules and tissue architecture. The machine learning algorithms and spectrochemical band analyses revealed specific changes in the levels of biomolecules in the liver, including lipids, glycogen, nucleic acids, and proteins. Moreover, young plasma treatment improved cellular architecture, reduced oxidative stress and inflammation and altered the metabolic activities of hepatocytes. These findings highlight the rejuvenation potential of plasma infusion and emphasize the need for further research in this area. This study contributes to the growing body of literature investigating the anti-aging effects of plasma exchange. It also provides essential insights into the biological consequences of this approach in liver tissues.

Acknowledgements The authors are grateful to the Department of Chemistry at Bilecik Şeyh Edebali University for providing an FTIR spectrometer and to Adem Kurtcuoğlu for his contributions.

Author contributions HTT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing. TC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing. SK: Histopathological analysis, Biochemical and Histopathological interpretation, Visualization, Writing–original draft, Writing—review & editing. GS: Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization. SM: Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization. BB: Resources, Funding acquisition, Supervision, Visualization. HA: Resources, Funding acquisition, Supervision, Visualization. EA: Histopathological and Biochemical interpretation, Visualization, Writing—original draft, Writing—review & editing. Rafig Gurbanov: Investigation, Methodology, Resources, Software, Supervision, Validation, Writing—review & editing, Visualization.

Funding This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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

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

Declarations

Competing interests The authors have declared that no competing interests exist.

Ethical approval This study was carried out with the approval of the Ethics Committee (approval number: 2021/03) 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.

References

Aksoy C, Severcan F (2012) Role of vibrational spectroscopy in stem cell research. Spectroscopy 27:167–184. https://doi.org/10.1155/2012/513286

Aksoy C, Severcan F (2019) Infrared spectroscopy and imaging in stem cells and aging research. Methods Mol Biol 2045:201–215. https://doi.org/10.1007/7651_2018_119

Andziak B, O'Connor TP, Qi W et al (2006) High oxidative damage levels in the longest-living rodent, the naked mole-rat. Aging Cell 5:463–471. https://doi.org/10.1111/j.1474-9726.2006.00237.x

Ardahanlı İ, Özkan Hİ, Özel F et al (2022) Infrared spectrochemical findings on intermittent fasting-associated gross molecular modifications in rat myocardium. Biophys Chem 289:106873. https://doi.org/10.1016/j.bpc.2022.106873

Baker MJ, Trevisan J, Bassan P et al (2014) Using fourier transform IR spectroscopy to analyze biological materials. Nat Protoc 9:1771–1791. https://doi.org/10.1038/nprot.2014.110

Ballweg S, Sezgin E, Doktorova M et al (2020) Regulation of lipid saturation without sensing membrane fluidity. Nat Commun 11:756. https://doi.org/10.1038/s41467-020-14528-1

Bompard J, Rosso A, Brizuela L et al (2020) Membrane fluidity as a new means to selectively target cancer cells

with fusogenic lipid carriers. Langmuir 36:5134–5144. https://doi.org/10.1021/acs.langmuir.0c00262
Castellano JM (2019) Blood-based therapies to combat aging. Gerontology 65:84–89. https://doi.org/10.1159/000492573
Ceylani T, Teker HT (2022) The effect of young blood plasma administration on gut microbiota in middle-aged rats. Arch Microbiol 204:541. https://doi.org/10.1007/s00203-022-03154-8
Ceylani T, Taner H, Samgane G, Gurbanov R (2022) Intermittent fasting-induced biomolecular modifications in rat tissues detected by ATR-FTIR spectroscopy and machine learning algorithms. Anal Biochem 654:114825. https://doi.org/10.1016/j.ab.2022.114825
Ceylani T, Allahverdi H, Teker HT (2023) Role of age-related plasma in the diversity of gut bacteria. Arch Gerontol Geriatr 111:105003. https://doi.org/10.1016/j.archger.2023.105003
Cloos PAC, Christgau S (2004) Post-translational modifications of proteins: implications for aging, antigen recognition, and autoimmunity. Biogerontology 5:139–158. https://doi.org/10.1023/B:BGEN.0000031152.31352.8b
Dicko A, Bourque H, Pézolet M (1998) Study by infrared spectroscopy of the conformation of dipalmitoylphosphatidylglycerol monolayers at the air-water interface and transferred on solid substrates. Chem Phys Lipids 96:125–139. https://doi.org/10.1016/S0009-3084(98)00084-X
Doble BW, Woodgett JR (2003) GSK-3: tricks of the trade for a multi-tasking kinase. J Cell Sci 116:1175–1186. https://doi.org/10.1242/jcs.00384
Dogan A, Gurbanov R, Severcan M, Severcan F (2021) CoronaVac (Sinovac) COVID-19 vaccine-induced molecular changes in healthy human serum by infrared spectroscopy coupled with chemometrics. Turk J Biol 45:549–558. https://doi.org/10.3906/biy-2105-65
Dreier LB, Bonn M, Backus EHG (2019) Hydration and orientation of carbonyl groups in oppositely charged lipid monolayers on water. J Phys Chem B 123:1085–1089. https://doi.org/10.1021/acs.jpcb.8b12297
Erdogan K, Ceylani T, Teker HT et al (2023) Young plasma transfer recovers decreased sperm counts and restores epigenetics in aged testis. Exp Gerontol 172:112042. https://doi.org/10.1016/j.exger.2022.112042
Finch CE, Crimmins EM (2016) Constant molecular aging rates vs. the exponential acceleration of mortality. Proc Natl Acad Sci USA 113:1121–1123
Goh GB-B, Pagadala MR, Dasarathy J et al (2015) Age impacts ability of aspartate-alanine aminotransferase ratio to predict advanced fibrosis in nonalcoholic fatty liver disease. Dig Dis Sci 60:1825–1831. https://doi.org/10.1007/s10620-015-3529-8
Gong Y, Fan Z, Luo G et al (2019) The role of necroptosis in cancer biology and therapy. Mol Cancer 18:100. https://doi.org/10.1186/s12943-019-1029-8
Gurbanov R, Yıldız F (2017) Molecular profile of oral probiotic bacteria to be used with functional foods. J Food Health Sci 12:117–131. https://doi.org/10.3153/jfhs17015
Gurbanov R, Karadağ H, Karaçam S, Samgane G (2021) Tapioca starch modulates cellular events in oral probiotic streptococcus salivarius strains. Probiotics Antimicrob Proteins 13:195–207. https://doi.org/10.1007/s12602-020-09678-z
Heydari AR, Butler JA, Waggoner SM, Richardson A (1989) Age-related changes in protein phosphorylation by rat hepatocytes. Mech Ageing Dev 50:227–248. https://doi.org/10.1016/0047-6374(89)90102-4
Huang Q, Ning Y, Liu D et al (2018) A Young blood environment decreases aging of senile mice kidneys. J Gerontol A Biol Sci Med Sci 73:421–428. https://doi.org/10.1093/gerona/glx183
Jin J, Wang G-L, Shi X et al (2009) The age-associated decline of glycogen synthase kinase 3beta plays a critical role in the inhibition of liver regeneration. Mol Cell Biol 29:3867–3880. https://doi.org/10.1128/MCB.00456-09
Kazarian SG, Chan KLA (2006) Applications of ATR-FTIR spectroscopic imaging to biomedical samples. Biochimica et Biophysica Acta (BBA)—Biomembr. https://doi.org/10.1016/j.bbamem.2006.02.011
Kennedy BK, Berger SL, Brunet A et al (2014) Geroscience: linking aging to chronic disease. Cell 159:709–713
Keskin S, Acikgoz E, Ertürk FY, Ragbetli MC (2022) Histopathological changes in liver and heart tissue associated with experimental ultraviolet radiation a and b exposure on wistar albino rats. Photochem Photobiol. https://doi.org/10.1111/php.13664
Krisko A, Radman M (2019) Protein damage, ageing and age-related diseases. Open Biol. https://doi.org/10.1098/rsob.180249
Liu A, Guo E, Yang J et al (2018) Young plasma reverses age-dependent alterations in hepatic function through the restoration of autophagy. Aging Cell. https://doi.org/10.1111/acel.12708
Liu J-F, Wu Y, Yang Y-H et al (2022) Phosphoproteome profiling of mouse liver during normal aging. Proteome Sci 20:12. https://doi.org/10.1186/s12953-022-00194-2
Loffredo FS, Steinhauser ML, Jay SM et al (2013) Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell 153:828–839. https://doi.org/10.1016/j.cell.2013.04.015
Mohamad M, Mitchell SJ, Wu LE et al (2016) Ultrastructure of the liver microcirculation influences hepatic and systemic insulin activity and provides a mechanism for age-related insulin resistance. Aging Cell 15:706–715. https://doi.org/10.1111/acel.12481
Morsiani C, Bacalini MG, Santoro A et al (2019) The peculiar aging of human liver: a geroscience perspective within transplant context. Ageing Res Rev 51:24–34. https://doi.org/10.1016/j.arr.2019.02.002
Ohto T, Backus EHG, Hsieh C-S et al (2015) Lipid carbonyl groups terminate the hydrogen bond network of membrane-bound water. J Phys Chem Lett 6:4499–4503. https://doi.org/10.1021/acs.jpclett.5b02141
Paraboni MLR, Kalinoski J, Braciak BG et al (2022) Protein carbonyl products, malondialdehyde, glutathione and vitamins C/E of breast cancer patients subjected to chemotherapy. Braz J Oncol 18:1–8. https://doi.org/10.5935/2526-8732.20220302
Severcan F, Haris PI (2012) Vibrational spectroscopy in diagnosis and screening. IOS Press, Amsterdam

Singh P, Goode T, Dean A et al (2011) Elevated interferon gamma signaling contributes to impaired regeneration in the aged liver. J Gerontol A Biol Sci Med Sci 66:944–956. https://doi.org/10.1093/gerona/glr094

Ticinesi A, Nouvenne A, Cerundolo N et al (2019) Gut microbiota, muscle mass and function in aging: a focus on physical frailty and sarcopenia. Nutrients. https://doi.org/10.3390/nu11071633

Timchenko NA (2009) Aging and liver regeneration. Trends Endocrinol Metab 20:171–176. https://doi.org/10.1016/j.tem.2009.01.005

Tripathi SS, Kumar R, Arya JK, Rizvi SI (2021) Plasma from young rats injected into old rats induce anti-aging effects. Rejuvenation Res 24:206–212. https://doi.org/10.1089/rej.2020.2354

Villeda SA, Plambeck KE, Middeldorp J et al (2014) Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med 20:659–663. https://doi.org/10.1038/nm.3569

Wang L, Li J, Dijun L (2022) Glycogen synthesis and beyond, a comprehensive review of GSK3 as a key regulator of metabolic pathways and a therapeutic target for treating metabolic diseases. Med Res Rev 42:946–982