

## Effect of moderate exercises and curcumin on hepatic transcriptional factors associated with lipid metabolism and steatosis in elderly male rat

Minoo Shirpoor<sup>1</sup>, Asghar Tofighi<sup>1,\*</sup>, Alireza Shirpoor<sup>2,3</sup>, Masoumeh Pourjabali<sup>4</sup>, and Leila Chodari<sup>2</sup>

<sup>1</sup>Department of Exercise Physiology and Corrective Movements, Faculty of Sport Sciences, Urmia University, Urmia, I.R. Iran.

<sup>2</sup>Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, I.R. Iran.

<sup>3</sup>Nephrology and Kidney Transplant Research Center, Clinical Research Institute, Urmia University of Medical Science, Urmia, I.R. Iran.

<sup>4</sup>Department of Pathology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, I.R. Iran.

### Abstract

**Background and purpose:** The specific molecular mediators involved in dyslipidemia in older people are not yet clearly understood. The current study was, thus, an attempt to investigate whether moderate aerobic exercises and curcumin administration alleviates the abnormalities caused by aging in the rats' liver.

**Experimental approach:** Thirty-two eight-year-old young rats were classified into five groups, namely, young control, aged control, aged-curcumin, aged-exercise, and aged-curcumin-exercise co-treatment. The rats in the exercise groups were trained on an animal treadmill for 60 min/day five times per week for eight weeks.

**Findings/Results:** The results revealed a significant increase in FAT/CD36, PTP1B, significantly decreased HNF4 $\alpha$  genes expression, increase in LDL and cholesterol in the aged group compared to the young control. Compared to those in the young control group, no significant changes in HDL and TG amounts in the aged control were observed. Moreover, compared to the young control, the aged group showed liver histological changes such as fibrosis and mild or grade 1 steatohepatitis. Moderate aerobic exercise and curcumin alone or in combination completely masked this effect.

**Conclusion and implications:** The findings revealed dyslipidemia and liver steatosis related to aging might be partly associated with changes in hepatic transcriptional factors which can be mitigated *via* moderate aerobic exercise and curcumin.

**Keywords:** Aerobic exercise; Curcumin; FAT/CD36; PTP1B; Rat; Steatosis.

### INTRODUCTION

Aging is closely associated with hyperlipidemia and various liver diseases including nonalcoholic fatty liver disease (NAFLD), low liver blood flow, and a decreased liver volume (1). At the cellular level, in wild old aging individuals, numerous changes in liver cells, such as polyploidy, accumulation of dense bodies inside liver cells, a decreased area of smooth endoplasmic reticulum, and decline in the number and dysfunction of mitochondria has been reported by previous studies (2). Prevalence and severity

of NAFLD in the general population have been reported to increase with age and, in a population greater than 70 years, over 40% suffer from NAFLD (3). The pathology of NAFLD has been described in a wide range from simple steatosis as the mildest form of NAFLD, characterized by hepatic fat accumulation to necro-inflammatory change, to the perisinusoidal pattern of liver fibrosis disorder called steatohepatitis.

#### Access this article online



Website: <http://rps.mui.ac.ir>

DOI: 10.4103/1735-5362.314828

\*Corresponding author: A. Tofighi

Tel: +98-9144467079, Fax: +98-4412780801

Email: a.tofighi@urmia.ac.ir

The latter is associated with the development of cirrhosis, which frequently happens in the seventh to ninth decades of life (4). Given the deleterious effect of age-associated dyslipidemia, especially its hazardous effects on the cardiovascular system, it is necessary to prevent and manage the mounting risks of aging dyslipidemia such as cardiovascular disease, which are the leading causes of death worldwide (5). Several studies have demonstrated that different types of physical exercises such as resistance training, moderate aerobic exercises, dancing, and Qigong exercises have positive effects on lipid profiles, inflammatory and cardiovascular risk in middle-aged, adults, and elderly individuals (1). Similar to exercise, natural compounds derived from fruit and plant such as polyphenol with antioxidant and anti-inflammatory properties can also improve health and retard the effects of aging on lipid profile alteration and cardiovascular abnormalities (6,7). Over the past 40 years, a large number of studies have focused on different aspects of aging-related liver disease and dyslipidemia and revealed its various effects on cardiovascular abnormalities among the elderly population. Although different aspects of the deleterious effects of aging on liver structure and function have been reported previously, the detailed mechanism of aging on the specific molecular mediators involved in dyslipidemia in old people has been rarely investigated.

Normal lipid metabolism and turnover, as well as metabolic homeostasis, are regulated by a network of transcriptional factors such as fatty acid translocase/cluster of differentiation 36 (FAT/CD36), protein phosphatase 1B (PTP1B), and hepatocyte nuclear factor 4 alpha (HNF4  $\alpha$ ). Deregulation of these factors contributes to dyslipidemia, liver steatosis, and related disorders (8). Briefly stated, these factors contribute to lipid metabolism through different ways such as an increasing lipogenic gene expression in liver tissue (9), transferring fatty acids into mitochondria for  $\beta$ -oxidation (10), and facilitating the removal of fatty acids from the liver (10). Taken together, in this study, we first investigated the effect that aging may exert upon lipid profile alteration and hepatic structural changes with a particular

focus on transcription factors associated with lipid metabolism and turnover in elderly male rats. It, further, attempted to find out that moderate aerobic exercises and curcumin alleviated the liver structural changes and lipid profile alteration abnormalities and its corresponding molecular mediators (PTP1B, HNF4 $\alpha$ , and FAT/CD36) caused by aging in rat liver. Curcumin as an antioxidant and anti-inflammatory ingredient from *Curcuma longa* L, reveals several biological activities such as anticancer, anti-atherosclerotic, antimutagenic, and immunomodulatory effects (11).

## MATERIALS AND METHODS

### *Experimental design*

Animal care and experimental procedures were conducted according to the Principles of Laboratory Animal Care; in addition, all protocols were approved by the Animal Ethics Committee of the Urmia University of Medical Sciences (Ethics No. IR.umsu.rec.1396.162). Thirty-two male Wistar rats, aged 24 months (550-600 g) and 8 young male rats, aged 4 months (200-250 g) were divided into five groups (8 each): young control, aged control, aged-curcumin (50 mg/kg body weight; Merck KGaA, Darmstadt, Germany) saluted in 5% dimethyl sulfoxide (DMSO) (intragastrically by gavage), aged-exercise, and aged-curcumin-exercise.

### *Exercise protocol*

The rats in the exercise groups were trained on an animal treadmill for 60 min/day during their light phase, with a zero-degree gradient, five times per week for eight weeks. The speed was 18 m/min that is equal to 45-60%  $VO_2$ max.  $VO_2$ , also known as oxygen uptake, is the measurement of the amount of oxygen a person can utilize during exercise. At first, the aged rats in the exercise group were adapted to treadmill running over a one-week period. Based on the training program, the first two weeks encompassed rats' running on a treadmill at a speed of 18 m/min, without inclination for 10 min. The program involved progressive increases every week (+10 min), reaching 60 min in the 7<sup>th</sup> week, which was maintained for the next week. Sedentary rats with no exercise

intervention were placed on the treadmill without running for 10 min each day. We considered the speed of 5-10 m/min for 5 min in both warm-up cool-down stages in each session (12). The experiments were carried out at 9-12 AM.

### Sample preparation

After eight weeks of treatment, the rats were anesthetized by ketamine (10%, 80 mg/kg BW, IP) and xylazine (2%, 10 mg/kg BW, IP). Then, the abdominal cavity was opened and blood samples were taken directly from the portal vein, collected in EDTA tubes and centrifuged at  $4000 \times g$  for 20 min within 30 min of collection. Additionally, the harvested plasma was kept at  $-80^{\circ}\text{C}$  until biochemical parameters were measured. Finally, the liver was excised through the abdomen, cleared away from outer tissues, fat, and blood clots, and washed in ice-cold physiological saline. For isolating total RNA, 100 mg of liver tissue was submerged in 1 mL RiboEX (total RNA isolation solution; GeneAll, Seoul, Korea) and restored at  $-80^{\circ}\text{C}$  until total RNA isolation. For histopathological analysis, a part of the liver was immediately stabilized in 10% buffered formalin, embedded in paraffin, and sectioned at 5  $\mu\text{m}$ .

### Biochemistry analysis

Plasma total cholesterol (TC) and triglycerides (TGs) levels were measured by colorimetric and enzymatic methods. Moreover, plasma low- and high-density lipoprotein (LDL and HDL) amounts were determined by the direct method using kits (Biosystem, Barcelona, Spain).

### Isolation of total RNA, amplification primers, and real-time polymerase chain reaction

Total RNA, according to kit instructions, was taken from 100 mg of liver tissue by means of a kit (Gene All, South Korea, Cat no

305-101). RNA concentration was verified and determined by spectrophotometric measurement of the absorbance at 260-280 nm and TAE-agarose gel electrophoresis, respectively. Reverse transcriptase was performed using Hyperscript™ Reverse Transcriptase (Gene All, South Korea). Real-time polymerase chain reaction (RT-PCR) was carried out using an amplification reagent kit (Ampliqon, Denmark) by the XP-Cycler instrument (TCXPD, Bioer, USA) with PTP1B, HNF4 $\alpha$ , FAT/CD36, and the rats' glyceraldehydes-3-phosphate dehydrogenase (GAPDH) primers. The analyses of 5' and 3' primer sequences (forward and reverse) of the PTP1B, HNF4 $\alpha$ , and FAT/CD36 designed via the Gene Bank revealed that the primers were gene-specific. The forward and reverse primers verified by Gene Runner software were then synthesized to reinforce the cDNA encoding GAPDH as a housekeeping gene. Primers sequences for target genes and housekeeping gene are shown in Table 1:

### Real-time quantification

RT-PCR Master Mix Green kit (Ampliqon, Denmark) with a volume of 25  $\mu\text{L}$  was used for real-time quantification of these three genes according to the instructions provided by the manufacturer. Additionally, we analyzed gene expressions using an iQ5 RT-PCR detection system (Bio-Rad, CA, USA). Afterward, we prepared the reactions at  $95^{\circ}\text{C}$  in a 96-well optimal plate followed by 40 cycles of 20 sat  $59^{\circ}\text{C}$  for 10 min. To confirm the specificity of the reinforcement reactions were recorded a melting curve. The threshold cycle (Ct) value was as much as that of the mean value. The relative expression of each mRNA was calculated by  $2^{-\Delta\Delta\text{Ct}}$  method, with Ct being the threshold cycle. The calculated values were, then, normalized to GAPDH.

**Table 1.** Sequences of the primers used in the real-time polymerase chain reaction.

Genes	Forward sequences (5'-3')	Revers sequences (5'-3')
PTP1B	TTCAAAGTCCGAAGTCAGG	CGGGTCCTTTCCTCTTGTC
HNF4 $\alpha$	TGCGACTCTCTAAAACCCCTC	CTTCAGATGGGGATGTGTCA
FAT/CD36	GACTTGTA CTCTCTCCTCGG	AGTAATGAGCCACAGTTCC
GAPDH	AGACAGCCGCATCTTCTTGT	CTTGCCGTGGGTAGAGTCAT

PTP1B, Protein phosphatase 1B; HNF4 $\alpha$ , hepatocyte nuclear factor 4 alpha; FAT/CD36, fatty acid translocase/cluster of differentiation 36; GAPDH, glyceraldehydes-3-phosphate dehydrogenase.

### **Histopathology**

Hematoxylin and eosin (H&E) staining were applied to assay steatosis and histological changes. The semi-quantitative method suggested by Brunt *et al.* was used to estimate the severity of hepatocyte steatosis (13). Brunt *et al.* described the grading type and scoring for assessment of the hepatocyte steatosis severity as (1) mild or grade 1: steatosis addresses the minimal standards for diagnosing steatohepatitis with mainly macrovesicular, rare intra-acinar inflammation, occasional zone III ballooning, and rare polymorphonuclear collection; (2) moderate or grade 2: hepatocytes ballooning (mainly zone, portal, intra-acinar polymorphonuclear, and intra-acinar chronic inflammation; and (3) severe or grade 3: ballooning and disarray obvious mainly in zone III, augmented lobular inflammation in comparison with grade 2; portal inflammation is as above for grade 2 (13).

To evaluate the liver tissue fibrosis, we stained sections using Masson trichrome while following the manufacturer's instructions (Asiapajohesh, Amol, Iran). The intensity of tissue fibrosis was scored using the semi-quantitative method described by Ashcroft *et al.* and our published protocol (7,14). The criteria employed for scoring liver fibrosis included the following grades: 0, normal liver; 1, thickening of the liver/minimal fibrosis tissue; 2 and 3, moderate thickening of liver tissue with no clear injury to the liver tissue structure; 4 and 5, incremented fibrosis with damage to the liver architecture and development of small fibrosis masses or fibrosis bands; 6 and 7, intense distortion of structure and wide fibrosis areas; and 8, total fibrotic obliteration. The slides were analyzed with a light microscope (Olympus, BH2, Japan), equipped with Sony onboard camera (Zeiss, Cyber-shot, Japan). The captured figures were compiled using Adobe photoshop CS10 (Adobe System, Mountain View, CA, USA). The software analyses for pixel-based intensity were assessed by image pro-insight software version 8.

### **Statistical analysis**

The normal distribution of the data within each group was verified through the Kolmogorov-Smirnov test. In addition, the statistical differences across the groups were tested through one-way ANOVA followed up by Tukey's pair-wise comparisons. The data obtained from each test are presented as the mean  $\pm$  SEM and the significance level was set at  $P < 0.05$ .

## **RESULTS**

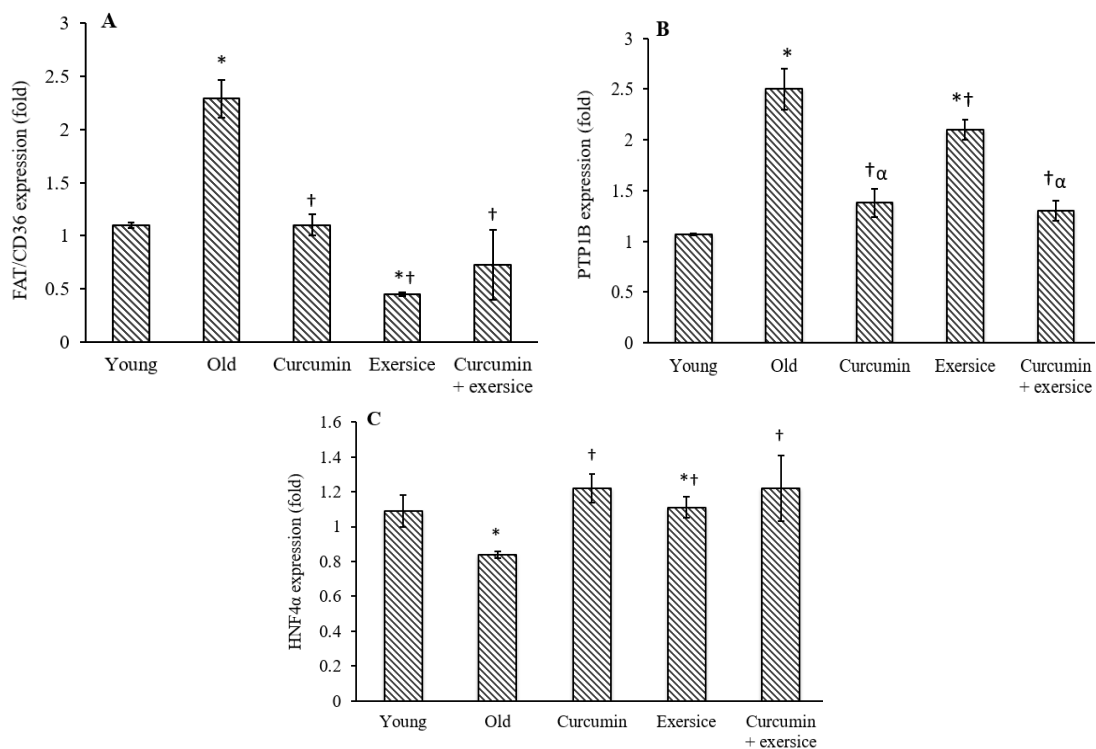
### **Lipid profile**

Table 2 shows lipid profile alteration in different groups. The plasma TG levels in elderly animals were similar to those in young rats. Plasma TG levels were markedly reduced in curcumin, exercises, and curcumin-exercise animals compared to the young and aged groups ( $P < 0.05$ ). Moreover, plasma cholesterol level in the old group was significantly higher than that in the young group. Cholesterol level in the curcumin group was close to the young control ( $P < 0.2$ ), but curcumin treatment significantly reduced cholesterol level compared to the old group ( $P < 0.05$ ). Exercise treatment significantly reduced cholesterol levels compared to the old group ( $P < 0.05$ ), though it was significantly higher than that of the young group. The cholesterol alteration in the curcumin-exercise group was similar to that in the exercise group. Plasma LDL content was notably elevated in the old group compared to the young one. It reduced significantly in curcumin, exercises, and co-treatment groups compared to the old group ( $P < 0.05$ ). Nevertheless, plasma LDL level was still significantly higher in the treatment groups compared to that of the young group ( $P < 0.05$ ). No change was observed in the plasma levels of HDL in the old and curcumin groups compared to the young group. Plasma HDL in the exercises and exercise-curcumin groups significantly increased compared to the young, old, and curcumin groups ( $P < 0.05$ ). No significant differences between exercise and exercise-curcumin groups were obtained.

**Table 2.** Effect of aging and treatment with exercise, curcumin, and their combination on changes of lipid profile after 8-week treatment. Values are mean ± SEM, n = 8.

Lipid type	Groups				
	Young	Old	Curcumin	Exercise	Curcumin + exercise
Triglyceride (mg/dL)	84.1 ± 2.9	85.74 ± 3.02	48.75 ± 4.1*†	57.54 ± 3.1*†	46.57 ± 3.39*† <sup>a</sup>
Cholesterol (mg/dL)	48.17 ± 0.75	69.97 ± 1.5*	50.9 ± 1.64†	58 ± 4*†	60.3 ± 1.27*†
LDL(mg/dL)	9.98 ± 0.75	19.07 ± 0.75*	16.08 ± 1.5*†	17.78 ± 0.85*†	17.44 ± 0.92*†
HDL (mg/dL)	24.85 ± 1.2	27 ± 1.29	23.2 ± 2.1	28.1 ± 3.2* <sup>±</sup>	28.14 ± 0.93* <sup>±</sup>

\**P* < 0.05 Indicates significant difference compared with the young group, †*P* < 0.05 versus the old group, <sup>a</sup>*P* < 0.05 against the exercise group, and <sup>±</sup>*P* < 0.05 in comparison with the curcumin group. LDL, Low-density lipoprotein; HDL, high-density lipoprotein.



**Fig. 1.** Variations in the (A) FAT/CD36, (B) PTP1B, and (C) HNF4α and genes' expression in the liver of rats in the experimental groups of the study. The relative mRNA values were evaluated by quantitative real-time polymerase chain reaction analysis. The values are set as mean ± SEM, n = 8. \**P* < 0.05 Indicates significant difference compared with the young group, †*P* < 0.05 versus the old group, and <sup>α</sup>*P* < 0.05 against the exercise group. FAT/CD36, Fatty acid translocase/cluster of differentiation 36; PTP1B, protein phosphatase 1B, HNF4A, hepatocyte nuclear factor 4 alpha.

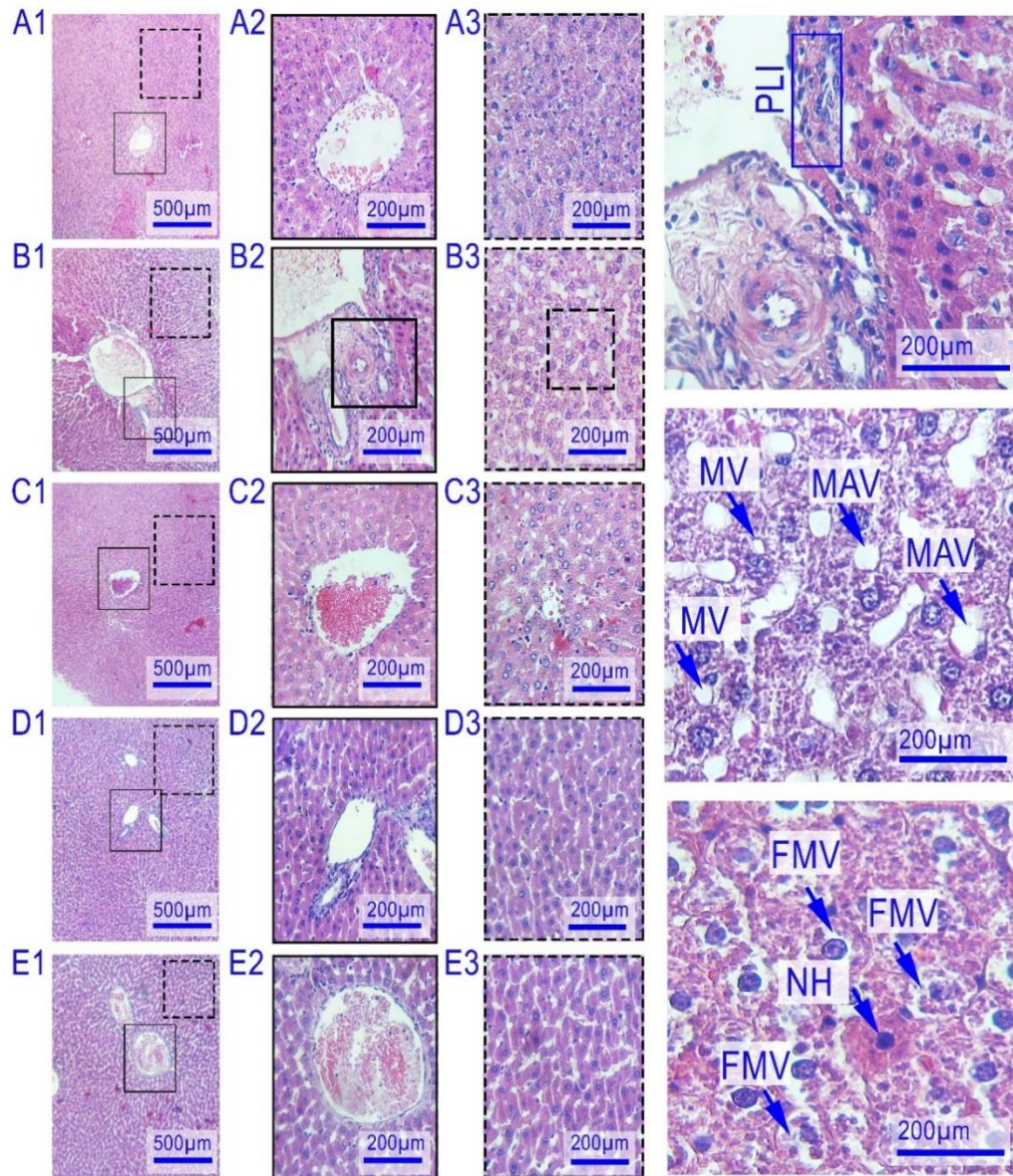
**Gene expression**

Gene expression alteration of the hepatic transcriptional factors is shown in Fig. 1. RT-PCR results indicated that aging significantly increased FAT/CD36 gene expression (*P* < 0.05) in the old group compared to the young control group. FAT/CD36 gene expression was significantly (*P* < 0.05) lower in the curcumin, exercises, and exercise-curcumin groups compared to that of the old control group. No significant difference was noticed between exercises and curcumin-exercise

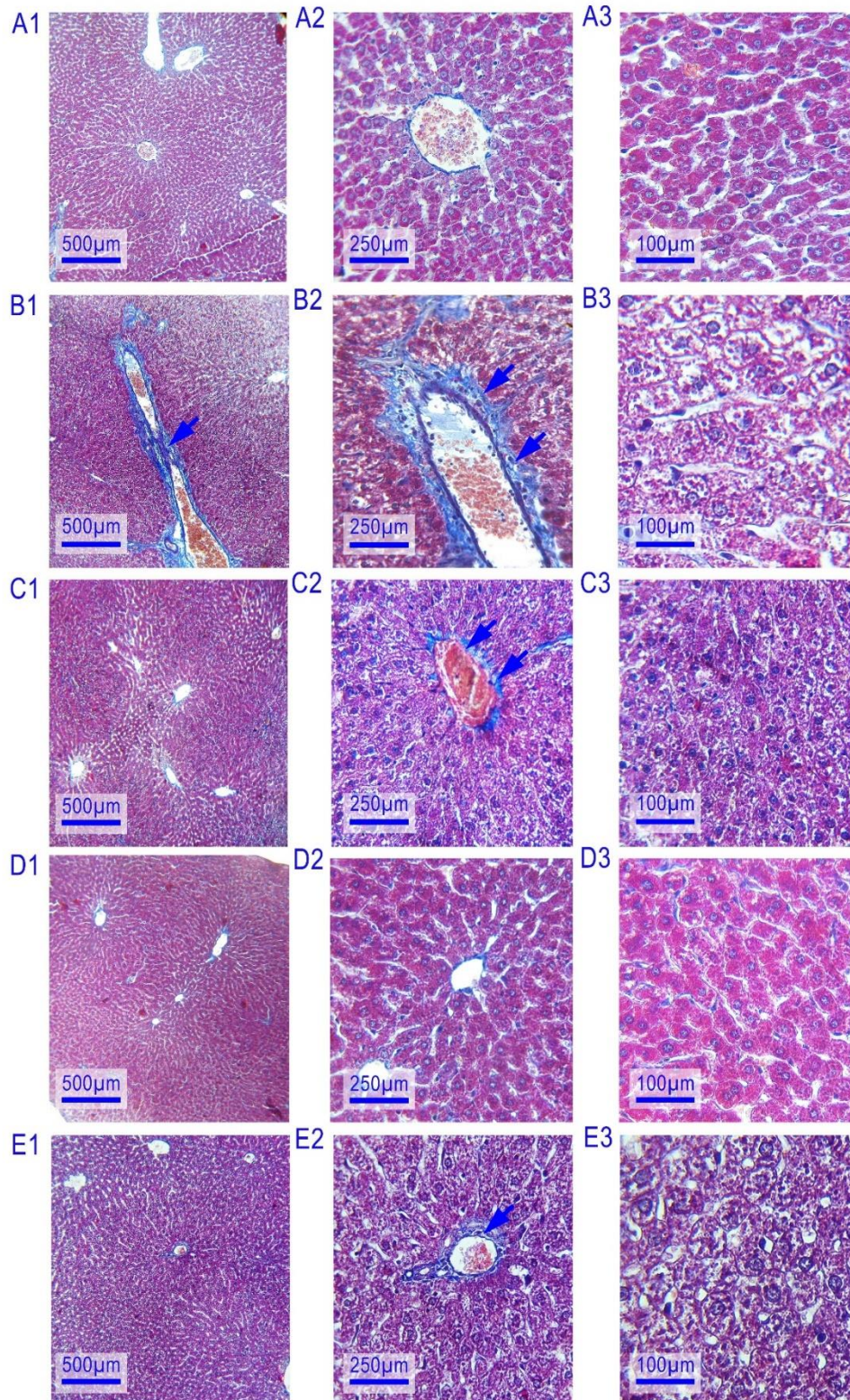
groups. The PTP1B mRNA level was significantly elevated in the old group compared to the young control group. PTP1B gene expression reduced significantly in the curcumin administration group as compared to the old group. In addition, no significant difference was found between the old group and the exercise groups, on the other hand, in the exercises-curcumin groups, the PTP1B mRNA level revealed a significantly decreasing pattern compared to the old and exercise groups (*P* < 0.05). Gene expression of HNF4α in the

old group, compared to the young group, was notably depleted. HNF4 $\alpha$  mRNA levels in exercise, curcumin, and curcumin-exercise groups were significantly higher than that of the old group ( $P < 0.05$ ). There was no significant alternation in terms of HNF4 $\alpha$  mRNA level among curcumin, exercise, and curcumin-exercise groups. Histological examination of the liver tissue is shown in Figs. 2 and 3. As depicted in Fig. 2B, aging resulted in a feature of nonalcoholic steatohepatitis (mild or grade 1) including mild portal lymphocyte infiltration, microvesicular steatosis, rare macrovesicular steatosis, focal microvesicular, and necrotic hepatocytes. Curcumin co-treatment and

moderate exercise alone and curcumin-exercise co-treatment resulted in a marked attenuation of histological alterations in the liver tissue compared to the old group. Figure 3 represents microscopic fibrosis scores in the liver tissue obtained from different study groups. The 2 and 3 microscopic lesion scores in the liver tissue of the old group were an indication of a moderate thickening of liver tissue without obvious damage to its structure. In the curcumin co-treatment and moderate exercise alone and their combination, the fibrosis score was similar to that in the young control (grade 1 indicates minimal fibrosis thickening in the perivascular due to collagen fibers in adventitia).



**Fig. 2.** Photomicrographs of H&D stained sections of liver tissues. (A) Young control, (B) old control, (C) curcumin, (D) exercise, (E) exercise + curcumin groups. PLI, mild portal lymphocyte infiltration; MV, microvesicular steatosis; MAV, macrovesicular steatosis; FMV, focal microvesicular; HN, necrotic hepatocyte.



**Fig. 3.** Photomicrographs of liver tissue stained by Masson Trichrome illustrate the score of the microscopic lesion in the liver tissues 2 and 3, a symptom of a moderate thickening of liver tissue with no clear damage to the liver tissue structure. (A) Young control, (B) old control, (C) curcumin, (D) exercise, (E) exercise + curcumin groups. In the curcumin co-treatment and moderate exercise alone or in combination form fibrosis score was similar to that in young control (grade 1 = minimal fibrosis thickening in the perivascular due to collagen fibers in adventitia).

## DISCUSSION

The positive effect of aerobic exercises on dyslipidemia is reported by several studies, the findings in the literature on the topic are still contradictory. For example, consistent with our results, de Piano *et al.* and Johanson and colleagues reported that aerobic physical activity reduced plasma cholesterol, but it was not a significant reduction (15,16). Regular aerobic exercise alone or combined with dietary intervention, in contrast, has been reported to lead to increased HDL-C levels in most individuals (17). Our findings are in accord with experiments that revealed a significant decrease in plasma cholesterol and LDL levels following aerobic exercises (18,19). Similarly, Bae *et al.* indicated that fixed aerobic exercises among the individuals with NAFLD resulted in a decrease in plasma TG and total cholesterol, and an increase in plasma HDL (20). The present study manifested that histological alteration of the liver was observed by mild or grade 1 nonalcoholic steatohepatitis and fibrosis in the livers of the elderly groups in comparison with the young control group. This may have been induced by inflammatory reactions and oxidative stress, although the mechanism *via* which the elderly developed steatohepatitis and fibrosis is not perceived completely. Day and James' 'two-hit' hypothesis can be proposed to explain the pathogenesis of steatohepatitis and fibrosis (21). Based on this hypothesis, the first hit arises from TG accumulation in hepatocytes due to metabolic imbalance, which, in turn, leads to steatosis. Then, through the 'second hit process', the lipid accumulation predisposes the liver vulnerable to damaging processes such as oxidative stress and inflammation (21). The Achilles heel of this older concept as a predictor of steatohepatitis and fibrosis is that, in this concept, TG acculturation in liver cells is introduced as a trigger of consequent processes such as steatohepatitis and fibrosis. Recently, it has been established that TG accumulation in tissues such as in the liver is nontoxic safe storage of lipid (22). To acknowledge Day and James' hypothesis, it is thought that instead of TG storage, accumulation of molecules including free fatty acids (FFAs), ceramide, diacylglycerides, cholesterol, oxysterol

metabolites, in the liver is responsible molecules that cause an imbalance in hepatic lipid turnover (23). Then, exceeded elimination of FFA from the lipid-laden liver *via* oxidation and re-esterification pathway could result in steatosis and fibrosis in liver tissue (23). In agreement with the 'second hit' of Day and James' hypothesis, several studies have demonstrated that the aging-associated changes such as fibrosis and nonalcoholic steatohepatitis in the liver of the elderly population are accompanied by increased oxidative stress and inflammatory response in liver tissue (24). Our findings showed that treatment of elderly rats with moderate exercise and curcumin improved liver steatohepatitis and fibrosis; these manifest oxidative stress and inflammatory reaction in the liver tissues of aged rats. In a similar vein, it has been previously reported that moderate exercise exerts its benefits through improving redox balance, plasma lipids profile, and restoring immunosenescence induced by aging (25). In the current study, curcumin administration turned out to induce a similar improving effect on unfavorable lipid profile and liver structural alteration among the aged rats. Even though curcumin is a very well-known antioxidant and anti-inflammatory agent, previous experiments have reported the paradoxical effect of curcumin supplementation on lipid profile levels. According to Sahebkar's meta-analysis as well as other experimental studies, curcumin supplementation showed no effect on plasma cholesterol, LDL, triacylglycerols, TG, and HDL in animal and human models (27,28). Meanwhile, other researchers have reported that curcumin supplementation could improve lipid profile and liver damage *via* counteracting oxidative stress and inflammation (29,30). As mentioned earlier, another important finding of this study was the significant alteration in gene expression of hepatic transcription factors involved in all aspects of hepatic lipid metabolism processes (*e.g.* synthesis, transport, and catabolism) in the aged rats compared to those in the young control rats. Compared to the young control rats, in the liver of aged rats, we observed increased mRNA expression of FAT/CD36 gene, which, as a transmembrane glycoprotein receptor, plays an important role in lipid metabolism in the liver. FAT/CD36



plays a basic role in hepatic fatty acids transportation (31). Moreover, it facilitates the uptake of long-chain fatty acids from the plasma pool and oxidized LDL in the liver (32). Consequently, the elevated levels of FFAs and their metabolites in hepatocytes could promote the oxidative facility of metabolism, leading to the production of reactive oxygen species and oxidative stress, and can cause lipotoxicity (8). The exiting evidence from the previous studies reveals that FFAs uptake by the liver from plasma pool and TG storage is magnified by overexpression of CD36 in human and animal livers (33). It is also maintained that overexpression of FAT/CD36 contributes to high-fat-induced steatohepatitis and dyslipidemia associated with diet-induced obesity (34). The expression of hepatocyte HNF4 $\alpha$  in the aged group in the current experiment was reduced compared to the young control group. Much interest in the hepatic transcription factors has been drawn to NF4 $\alpha$ . This molecule regulates the transcription of a great number of genes involved in gluconeogenesis, bile acid synthesis, lipid metabolism. It also directs transportation of substances such as Apo-A, Apo-B, and microsomal triglyceride transfer protein, through direct binding to their promoters (8). Besides, HNF4 $\alpha$  facilitates the transportation of fatty acids into mitochondria and augments their beta-oxidation in the mitochondria (8). Due to its pivotal role in lipid metabolism and its transcriptional properties, HNF4 $\alpha$  is accepted as the master manager of hepatocyte lipid metabolism. Previous studies have demonstrated that knockout of HNF4 $\alpha$  gene in mice, accompanied by massive lipid accumulation in the hepatocyte, increased ketogenesis and serum  $\beta$ -hydroxybutyrate levels (8). Another important result of the present study was the overexpression of liver PTP1B, which has emerged as an important key regulator, in the aged group as compared with the control young group. As documented in biochemical, genetic, and inhibitor studies, PTP1B plays an important role in the regulation and improvement of metabolic parameters including insulin signaling, lower serum and hepatic TG and cholesterol levels (8). Moreover, high-fat diet-induced endoplasmic reticulum stress is

rescued by PTP1B (35). Overexpression of liver PTP1B results in increased insulin resistance and promotes the development and progression of steatosis (8). Knockout of PTP1B expression in mice leads to a decrease in liver fat store and TG levels; it brings about increased systemic insulin sensitivity, reduced serum and hepatic TG and cholesterol levels, and enhanced glucose tolerance compared with normal control (36). Interestingly, in the current study, the alteration of transcriptional factor gene expression was parallel with dyslipidemia and liver structural changes including fibrosis and mild steatohepatitis. Moderate exercise or curcumin administration masked liver structural changes, plasma dyslipidemia, and gene expression alteration induced by aging in aged rats. Similar to the mere exercise, curcumin and exercise combined with curcumin supplementation improved unfavorable lipid profile, liver structural changes, and hepatic transcriptional factor expression; no synergistic effect, however, was detected with exercise and curcumin co-treatment.

## CONCLUSION

In conclusion, current study findings showed that dyslipidemia and liver steatosis related to aging might be partly associated with changes in hepatic transcriptional factors; and these effects can be mitigated *via* moderate aerobic exercise and curcumin. In line with the previous literature, we suppose that specific prevention of gene expression alteration of hepatic-transcription factors such as HNF4 $\alpha$ , FAT/CD36, and PTP1B may prove to be a novel strategy that normalizes hepatic lipid metabolism in the elderly population and thus may be useful in down-aging and lowering the incidence of age-associated diseases.

## Acknowledgments

This work is extracted from a Ph.D. thesis in the Exercise Physiology Department of Urmia University, Urmia, Iran.

## Conflict of interest statement

The authors declared no conflict of interest in this study.

**Authors' contribution**

M. Shirpoor and A. Tofighi contributed to the conception, design, data analyses, and drafting of the manuscript. A. Shirpoor analyzed the data and drafted the manuscript. M. Pourjabali and L. Chodari conceived and designed the experiments.

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