Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2022;47(1):405-414
DOI: 10.17826/cumj.1023909

ARAŞTIRMA / RESEARCH

Aging and gender-related effects of tauroursodeoxycholic acid treatment on liver functions, plasma lipid profile, and oxidative stress

Tauroursodeoksikolik asit tedavisinin karaciğer fonksiyonları, plazma lipit profili ve oksidatif stres üzerindeki etkilerinin yaşlanmaya ve cinsiyete bağlı değişimi

Sevtap Han[1]

[1]Gazi Üniversitesi, Eczacılık Fakültesi, Farmakoloji Anabilim Dalı, Ankara, Turkey

Cukurova Medical Journal 2022;47(1):405-414.

Abstract

Purpose: Aging is related to multiple and systemic dysfunctions in the body, accompanied by metabolic disorders and oxidative stress. Although studies are revealing the role of endoplasmic reticulum (ER) stress in aging-related pathologies, this relationship has not been fully elucidated. In this study, it was aimed to reveal changes in liver function, plasma lipids, and oxidative stress markers due to aging and gender, and to investigate how these parameters change with ER stress inhibitor tauro-ursodeoxycholic acid (TUDCA) treatment.

Materials and Methods: Young (4 months old) and old (24 months old) Wistar albino male and female rats were used in the experiments. The administration of ER stress inhibitor TUDCA was performed for 4 weeks (150 mg/kg/day, ip). Liver function markers (AST and ALT), plasma lipids (LDL, HDL, TG and total cholesterol), and oxidative stress biomarkers (malondialdehyde, (MDA) and myeloperoxidase (MPO)) levels were measured in plasma samples.

Results: ER stress inhibition with TUDCA decreased AST levels, increased HDL value, decreased TG value, and decreased MDA and MPO levels in the elderly. The effects on some parameters varied depending on gender.

Conclusion: Considering the role of oxidative stress and metabolic disorders in the pathogenesis of many age-related diseases, it is thought that these results will contribute to the development of treatment approaches targeting ER stress inhibition in aging.

Keywords:. Aging, endoplasmic reticulum stress, oxidative stress, dyslipidemia

Öz

Amaç: Yaşlanma vücutta çoklu ve sistemik işlev bozuklukları ile ilişkilidir, bu durumlara metabolizma bozuklukları ve oksidatif stres eşlik etmektedir. Endoplazmik retikulum (ER) stresinin yaşlanmaya bağlı patolojilerdeki rolünü gösteren çalışmalar olmakla birlikte bu ilişki tam olarak aydınlatılamamıştır. Bu çalışmada, karaciğer fonksiyonu, plazma lipitleri ve oksidatif stres belirteçlerinin yaşlanmaya ve cinsiyete bağlı değişimlerinin ortaya konması ayrıca bu parametrelerin ER stres inhibitörü tauro-ursodeoksikolik asit (TUDCA) tedavisiyle nasıl değiştiğinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Deneylerde genç (4 aylık) ve yaşlı (24 aylık) Wistar albino erkek ve dişi sıçanlar kullanılmıştır. ER stres inhibitörü olarak TUDCA uygulaması 4 hafta süresince yapılmıştır (150 mg/kg/gün, ip). Plazma örneklerinde karaciğer fonksiyon belirteçleri (AST ve ALT), plazma lipitleri (LDL, HDL, TG ve total kolesterol) ve oksidatif stres biyobelirteçlerinin (malondialdehit, (MDA) ve miyeloperoksidaz (MPO)) seviyeleri ölçülmüştür..

Bulgular: Bu çalışmada TUDCA ile ER stres inhibisyonunun yaşlılarda AST seviyelerini azattığı, HDL değerini yükseltirken TG değerini düşürdüğü, MDA ve MPO düzeylerini azalttığı gösterilmiştir. Bazı parametreler üzerindeki etkileri cinsiyete bağlı olarak değişim göstermiştir.

Sonuç: Oksidatif stres ve metabolik bozuklukların yaşa bağlı pek çok hastalığın patogenezindeki rolü göz önüne alındığında; bu sonuçların yaşlanmada ER stres inhibisyonunu hedef alan tedavi yaklaşımlarının geliştirilmesine katkı sunacağı düşünülmektedir.

Anahtar kelimeler: Yaşlanma, endoplazmik retikulum stresi, oksidatif stres, dislipidemi

Yazışma Adresi/Address for Correspondence: Dr. Sevtap Han, Gazi Üniversitesi, Eczacılık Fakültesi, Farmakoloji Anabilim Dalı, Ankara, Turkey E-mail: sevtap.han@gazi.edu.tr
Geliş tarihi/Received: 15.11.2021 Kabul tarihi/Accepted: 04.03.2022

INTRODUCTION

The elderly population is increasing worldwide, and many chronic diseases such as cardiovascular diseases (CVD), diabetes mellitus, liver diseases, and chronic kidney disease are common in the aging population. Aging is one of the most important risk factors associated with CVD. It is predicted that by 2030, approximately one-fifth of the world population will be 65 years or older and the prevalence of CVD will increase exponentially[1].

Aging induced dysfunctions in the body are accompanied by lipid metabolism disturbance[2]. Dyslipidemia is defined as high total cholesterol or low-density lipoprotein (LDL) cholesterol concentrations or low levels of high-density lipoprotein (HDL) cholesterol or apolipoprotein A-1[3]. In 25% of men and 42% of women older than 65 years have dyslipidemia, and available data suggest a positive association between dyslipidemia and increased CVD risk in the aged population[4]. It is estimated that dyslipidemia is the cause of more than half of coronary artery disease cases worldwide. Several studies found a strong association between serum cholesterol levels and the risk of developing coronary artery disease [2]. On the other hand, it has been reported that aging increases oxidative stress markers under dyslipidemic conditions[5].

Oxidative stress is a component of many diseases, from CVD to Alzheimer's disease and cancer. With aging, the balance between reactive oxygen and nitrogen species (RONS) and antioxidant defenses that neutralize their negative effects is disrupted; as a result, the amount of oxidative stress increases[6,7]. According to the oxidative stress theory of aging, age-induced dysfunctions result from the accumulation of RONS-induced damage[6]. Due to the important role of oxidative stress in the pathogenesis of aging, it is thought that antioxidant treatments may positively affect the natural course of many aging-related diseases.

The endoplasmic reticulum (ER) has many functions including lipid biosynthesis, protein folding, calcium storage and release. Disruptions in ER homeostasis lead to protein misfolding or unfolding and eventually ER stress. Thus, it causes the activation of a signaling pathway called the ER stress response or unfolded protein response (UPR). The UPR is characterized by increased chaperones, degradation of misfolded proteins, and decreased protein translation. ER stress may increase with aging, and age-related declines occur in these regulation mechanisms[8,9]. The altered ER stress response in the elderly plays a role in the pathogenesis of many diseases ranging from CVD to neurodegenerative, metabolic, and inflammatory diseases[10-12]. The roles of ER stress in the onset and progression of many age-related diseases are quite complex and have not yet been fully elucidated.

While the aging process is characterized by tissue and function reductions and the onset of age-related diseases, it is not permanent and can be modulated by a variety of genetic and environmental pathways. One of these interventions may be the modulation of cellular stress responses, including the UPR, in the ER. Understanding how the UPR alters with age and how it influences disease progress may open new therapeutic avenues for the treatment of many age-related diseases[8].

The role of ER stress in many aging-induced diseases has been demonstrated. However, the effect of ER stress inhibition in the elderly has not been studied yet. On the other hand, metabolic disorders and oxidative stress are involved in the pathogenesis of many age-related diseases and ER stress is associated with these parameters. Therefore, it was hypothesized that inhibiting ER stress might have a beneficial effect on age-induced disorders. The gender-related variation of the effects of ER stress has also not been revealed yet; therefore, it was considered to investigate the possible gender difference in the subjects examined in the study.

In this study, it was aimed to investigate the aging-induced changes in liver enzymes, plasma lipids and oxidative stress markers in gender-dependent manner, as well as the changes in these parameters with the ER stress inhibitor tauro-ursodeoxycholic acid (TUDCA) treatment.

MATERIALS AND METHODS

Animals and procedure

All animal experiments were carried out in accordance with "Guide for the Care and Use of Laboratory Animals". Ethical approval was obtained from Gazi University Animal Experiments Local Ethics Committee for the experimental protocol of the study (G.Ü.ET-21.058). All animal experiments were performed by the author at Gazi University, Faculty of Pharmacy.

Young (4 months old) and old (24 months old) Wistar albino male and female rats were used in the experiments. TUDCA was applied as an ER stress inhibitor. TUDCA was dissolved in physiological saline and administered for 4 weeks (150 mg/kg/day, ip, 4 weeks). The control groups were injected with physiological saline in the same volume and duration. Animals were divided randomly into 8 groups.

Groups:

- Group 1: Young Female Control Group (n=6)
- Group 2: Old Female Control Group (n=6)
- Group 3: Young Female TUDCA Group (150 mg/kg/day, ip, 4 weeks) (n=8)
- Group 4: Old Female TUDCA Group (150 mg/kg/day, ip, 4 weeks) (n=8)
- Group 5: Young Male Control Group (n=6)
- Group 6: Old Male Control Group (n=6)
- Group 7: Young Male TUDCA Group (150 mg/kg/day, ip, 4 weeks) (n=8)
- Group 8: Old Male TUDCA Group (150 mg/kg/day, ip, 4 weeks) (n=8)

At the end of TUDCA administration, animals were sacrificed under ketamine and xylazine anesthesia. Then blood samples were taken immediately from the heart. The collected blood samples were centrifuged at 3000 rpm for 15 minutes, and plasmas were separated. Plasma samples were stored at -80°C for analysis.

Chemicals

Tauroursodeoxycholic acid (TUDCA) (CAS No. 14605-22-2) was obtained from Zhejiang Multipharma Co., Ltd. company. Rel Assay Diagnostics kits were used in biochemical examinations.

Biochemical analysis

Biochemical analyzes were carried out by experts in the laboratories of Baran Medical Ind. Trade. Co. Ltd. Plasma samples were used in these measurements. Analyses were made using colorimetric kits. The working principles for each parameter are given below.

Aspartate aminotransferase (AST)

A standardized UV test was used. The following reaction is initiated by adding buffers onto the plasma samples. AST is the enzyme that catalyzes the equilibrium reaction.

α-ketoglutarate + L-aspartate ↔ L-glutamate + oxaloacetate

In the second reaction catalyzed by malate dehydrogenase, NADH was oxidized to NAD^+. The rate of reduction in NADH reflects the rate of oxaloacetate formation and thus AST activity. NADH was measured photometrically.

Oxaloacetate + NADH + H^+ ↔ L-Malate + NAD^+

Alanine aminotransferase (ALT)

Optimized UV test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) was used. Transaminase reaction between L-Alanine and 2-oxoglutarate was catalyzed by ALT. Pyruvate was formed and reduced to lactate in the presence of LDH. In the later reaction, NADH was oxidized to NAD. The rate of decrease in NADH was measured photometrically.

Total cholesterol

Cholesterol ester + H_2O → Cholesterol + fatty acids
Cholesterol esters were converted into free cholesterol and fatty acids by the action of cholesterol esterase.
Cholesterol + O_2 → Cholestenone + H_2O_2
$2H_2O_2$ + Phenol + 4-Aminoantipyrine → Quinoneimine dye + 4 H_2O

Cholesterol was converted by cholesterol oxidase to cholestenone and hydrogen peroxide. The hydrogen peroxide formed reacts with 4-aminoantipyrine and phenol under the catalytic effect of peroxidase to form a red dye. The color intensity indicates the cholesterol concentration and can be determined photometrically.

HDL Cholesterol

In the first step, LDL, VLDL, and Chylomicrons are eliminated and converted into non-reactive compounds under special conditions for reaction. In the second step, HDL-Cholesterol color reaction is created, and photometric measurement is performed.

LDL Cholesterol

In the first step, HDL, VLDL, and Chylomicrons are eliminated and converted into non-reactive compounds under the specific condition for the reaction. Only LDL-cholesterol color reaction occurs

with the second reagent. The color intensity is proportional to the LDL cholesterol concentration.

Triglyceride

Similar to the measurement of total cholesterol, triglycerides in the sample form colored complexes, which can be measured spectrophotometrically, by cascading reactions.

Malondialdehyde (MDA)

The MDA level was measured by a method based on the reaction with thiobarbituric acid (TBA) at 90-100°C. The reaction was carried out at 90°C for 15 minutes at pH 2-3. One volume of plasma samples and two volumes of cold 10% (w/v) trichloroacetic acid were mixed, and the precipitate protein was centrifuged. The supernatant was mixed with the equal volume of 0.67% (w/v) TBA and induced in a boiling water bath (100°C) for 10 minutes. It was allowed to cool, then absorbance was read at 532 nm.

Myeloperoxidase (MPO)

In the kit used, MPO catalyzes the o-dianisidyl to the colored o-dianisidyl radical using H_2O_2. Increased absorbance was monitored at 412 nm, and activity was measured kinetically.

Statistical analysis

Statistical power analysis was performed with the G*Power Version 3.1.9.4 (2019, Germany) program to determine the sample size based on the data obtained from published studies. In this study, the minimum number of samples in each required group was calculated as 6 by taking the effect size of 0.80 (using the Cohen criteria), alpha=0.05 and power=0.95.

Normally distributed data are shown as mean ± standard error (SEM). Before performing the test, it was checked by the histograms of the data whether the samples were normally distributed. GraphPad 5.01 (GraphPad Software, Inc., La Jolla, USA) program was used for the histogram analyses. There are no outliers and the data had normal distribution. Therefore, the student t-test was found appropriate. The unpaired test was chosen because the groups were independent. An unpaired t-test (also known as an independent t-test) is a statistical procedure that compares the means of independent groups to determine if there is a significant difference between them. Unpaired Student's t-test was applied using GraphPad 5.01 (GraphPad Software, Inc., La Jolla, USA) program for the statistical analysis of the results. P-values less than 0.05 ($P<0.05$) were accepted as statistically significant.

RESULTS

Two graphs are given for each parameter so that the comparison of control and TUDCA, comparison of old and young can be clearly seen.

AST values in young groups were not affected by TUDCA administration. In elderly female and male rats, TUDCA treatment significantly decreased AST values ($p<0.05$). When we looked at the effect of aging, it was observed that AST levels increased with age in both female and male control groups ($p<0.05$). In addition, elderly female rats had higher plasma AST levels than male rats ($p<0.05$) (Figure 1).

ALT plasma levels did not change with TUDCA administration. However, ALT levels were found to be higher in older female rats and male rats than in younger ones. The values in the female old TUDCA group were also higher than the young group ($p<0.05$) (Figure 1).

TUDCA administration significantly decreased plasma LDL concentrations in young females and males ($p<0.05$). In aged female rats, LDL levels were higher in the TUDCA-administered group ($p<0.05$). Considering the changes that occur with aging, it was observed that LDL levels were higher in both elderly female rats and elderly male rats who were treated with TUDCA compared to the young groups ($p<0.05$). LDL levels were also found to vary according to gender. LDL values in the old and young male control groups were significantly higher than in the females ($p<0.05$) (Figure 2).

HDL cholesterol values did not change with TUDCA administration in young animals. TUDCA treatment increased HDL levels in aged female rats ($p<0.05$). Again, HDL values were higher in the older female TUDCA group than in the younger female rats ($p<0.05$) (Figure 2).

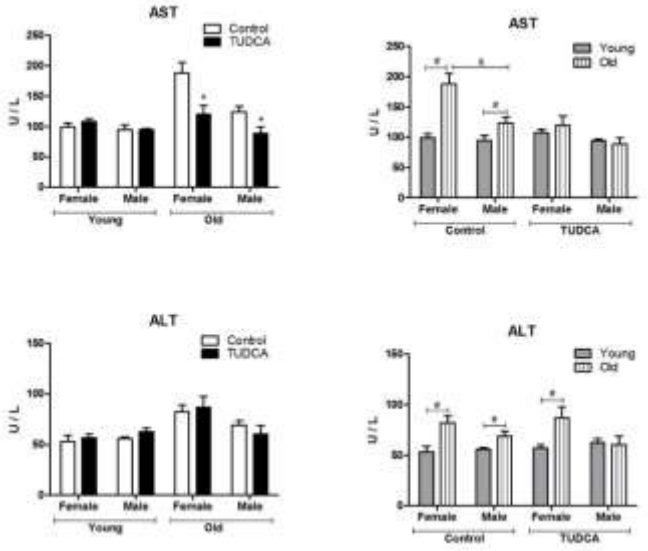

Figure 1. Change of AST and ALT values with TUDCA treatment according to aging and gender.

All results are shown as mean ± standard error (SEM). *indicates statistically significant from control ($p<0.05$); #indicates statistically significant from the young group receiving the same treatment ($p<0.05$); &indicates statistically significant from the female group of the same age ($p<0.05$).

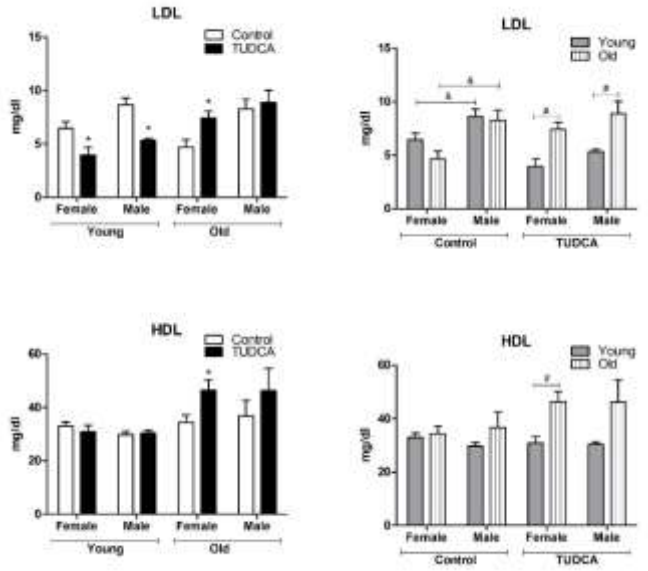

Figure 2. Change of LDL and HDL values with TUDCA treatment according to aging and gender.

All results are shown as mean ± standard error (SEM). *indicates statistically significant from the control group ($p<0.05$); #indicates statistically significant from the young group receiving the same treatment ($p<0.05$); & indicates statistically significant from the female group of the same age ($p<0.05$).

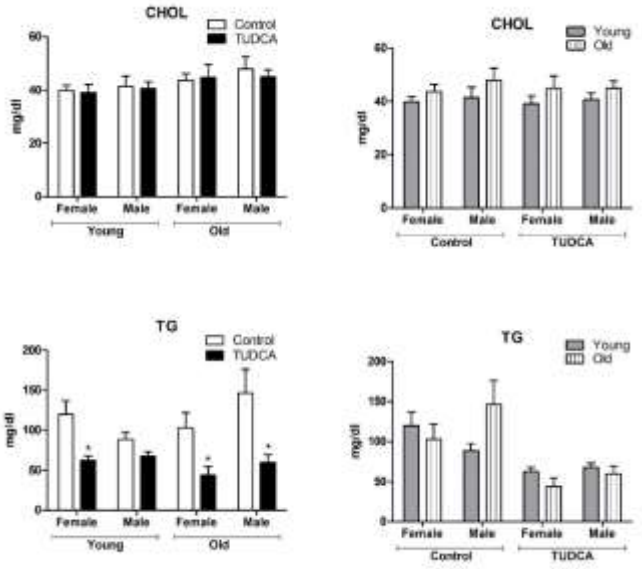

Figure 3. Change of total cholesterol and TG values with TUDCA treatment according to aging and gender.
All results are shown as mean ± standard error (SEM). *indicates statistically significant from the control group ($p<0.05$).

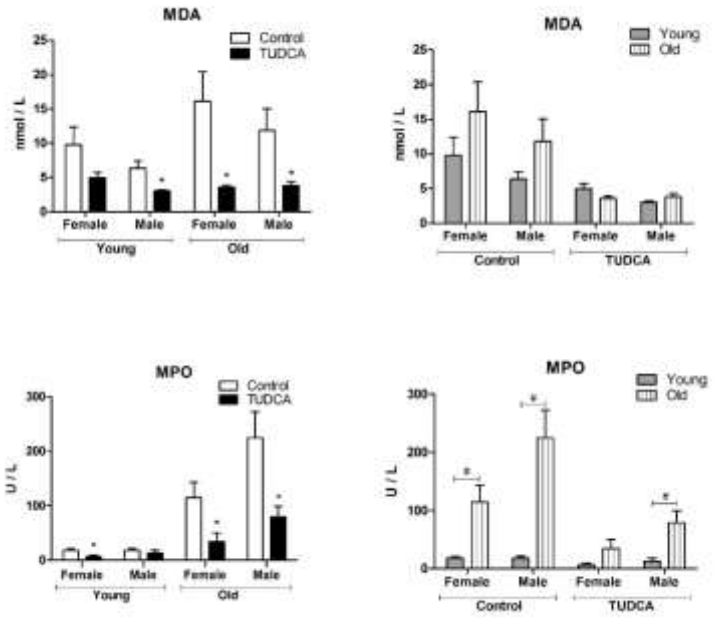

Figure 4. Change of MDA and MPO values with TUDCA treatment according to aging and gender.
All results are shown as mean ± standard error (SEM). *indicates statistically significant from the control group ($p<0.05$); #indicates statistically significant from the young group receiving the same treatment ($p<0.05$).

TUDCA administration did not change the total cholesterol values in either the young or the elderly rats. In addition, there was no change in total cholesterol values depending on gender and aging (Figure 3).

TUDCA application caused a decrease in TG values in all groups. This decrease was statistically significant in young female rats, old female rats, and old male rats ($p<0.05$) (Figure 3).

It was observed that TUDCA treatment decreased MDA concentrations in all groups. This decrease was found to be significant in the young male, old female, and old male groups ($p<0.05$). MDA levels of female and male animals showed a tendency to increase in the elderly groups, but there was no statistically significant difference (Figure 4).

Plasma MPO levels also decreased as a result of TUDCA administration. MPO values of young female, aged female, and aged male TUDCA groups were significantly lower than control groups ($p<0.05$). MPO values also showed changes with aging. MPO values in the female control, male control, and male TUDCA groups were higher in the elderly than in the young animals ($p<0.05$) (Figure 4)

DISCUSSION

In this study, it has been shown for the first time that ER stress inhibition with TUDCA has a beneficial effect on liver function markers, plasma lipid profile, and oxidative stress biomarkers in the elderly, and these effects may vary depending on gender.

The aging process predisposes the liver to functional and structural deterioration and metabolic risk[13]. Peripheral blood levels of biochemical markers such as albumin, ALT, AST, and total bilirubin are measured to evaluate liver function. Although ALT and AST are used to measure liver injury in general clinical practice, these enzymes are also the factors associated with cardiovascular and metabolic diseases[14,15]. Liver dysfunctions are associated with the development of various metabolic diseases through disturbances in glucose and lipid metabolism, which are the basic physiological functions of the liver[16,17]. Some clinical studies show reference ranges for ALT and AST values according to age. While the upper limit of ALT increases with age, it decreases for AST[18]. There are also studies showing that ALT levels decrease with aging[19]. In the previous clinical studies, it was observed that AST and ALT enzyme levels change with age, while these changes increase or decrease according to parameters such as gender, age, and health status of the individual[20]. In a previous study, both ALT and AST values increase with aging, similar to our results[21]. In our study, aminotransferases were used as biomarkers of liver metabolic function. The results showed that AST and ALT plasma levels showed an increase with aging in both male and female control groups, and AST levels are higher in the older female group than that of males.

In addition, we found that TUDCA treatment reduced the increased AST levels in the elderly, but did not make a difference in the young group. The beneficial effect of TUDCA on liver functions in different disease models has been demonstrated in previous studies. Administration of TUDCA has decreased ALT and AST levels in chemically induced hepatotoxicity[22]. TUDCA treatment has also been shown to decrease ALT and AST values in patients with liver cirrhosis[23]. According to the results obtained, it can be suggested that aging causes deterioration in liver functions in male and female rats in different degrees, while TUDCA treatment has a curative effect.

Multiple dysfunctions that occur with aging are accompanied by lipid metabolism disorders[2]. Although studies have reported some potential mechanisms based on animal experiments, the underlying mechanisms of this age-related increase in dyslipidemia are still unclear. Aging and gender are two physiological factors that have a significant impact on plasma lipid levels in various species[24,25]. In this study, it was found that aging did not significantly change LDL, HDL, TG, and total cholesterol levels, while LDL levels in the elderly male control group were significantly higher than in the female group.

The hepatocytes responsible for lipogenesis and cholesterol biosynthesis are enriched with ER to perform their numerous metabolic functions. The ER in hepatocytes has an important capacity to adapt to extracellular and intracellular changes, which ensures the maintenance of vital hepatic metabolic functions[26]. Diseases such as hyperlipidemia and inflammation may play a role in the dysregulation of hepatic lipid metabolism by disrupting hepatocyte ER homeostasis[27]. It has been shown that there is a direct link between ER homeostasis and the transcriptional regulation of metabolism, and ER stress is one of the mechanisms underlying fatty liver disease[28]. It has been suggested that ER stress is the cause or

consequence of impaired lipid and glucose signaling, inflammatory and cell death pathways, thus playing a role in the development of different liver diseases[27,29]. On the other hand, the effects of ER stress and ER stress inhibition on lipid metabolism and lipid profile in the aging have not been clarified yet. In this study, the effect of ER stress inhibition by TUDCA in the young and older rats showed differences according to gender. TUDCA reduced LDL cholesterol levels in young female and male rats; in addition, it reduced TG levels in the young female group. TUDCA increased HDL and LDL values in the aged female group but decreased TG values in both genders. When all the results are considered together, TUDCA treatment appears to have positive effects on some plasma lipids in both aged and young animals. Moreover, these effects show differences depending on gender.

Oxidative stress, which increases with aging, has been known for many years and studied by many researchers[30-32]. It is known that oxidative stress is involved in the pathogenesis of many diseases including aging-induced diseases. Interest in searching for new antioxidant treatments in aging remains up to date[33]. There are many biomarkers used to measure the amount of oxidative stress[34,35]. MDA and MPO were chosen as oxidative stress markers in this study. The oxidative stress hypothesis of aging is based on the premise that age-related functional losses result from the accumulation of RONS-induced oxidative damage to macromolecules (lipid, DNA, and protein), resulting in structural damage[6,36]. The enzymes such as MPO, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and lipoxygenase are endogenous sources of RONS[6]. Polyunsaturated fatty acids (PUFAs), such as linoleic and arachidonic acids, are particularly subject to lipid peroxidation mediated by hydroxyl-peroxyl radicals. Different reactive aldehydes such as MDA, trans-4-hydroxy-2-nonenal (4-HNE), and isoprostanes are produced depending on the type of PUFAs[37,38]. In this study, MDA levels did not change with aging, but plasma MPO levels were found to be significantly higher in both female and male old groups compared to young groups. The increase in MPO value was interpreted as an increase in oxidative stress due to aging. On the other hand, ER stress inhibition by TUDCA decreased both MPO and MDA concentrations in the elderly rats of both genders. TUDCA significantly decreased MPO levels in young female rats and MDA levels in young male rats. Our results indicate that ER stress inhibition has a reducing effect on oxidative stress in both the aged and young rats. There are a limited number of studies pointing out the relationship between ER stress and oxidative stress[39,40]. Further research is needed to elucidate the mechanisms underlying this interaction in aging.

As a conclusion, it has been demonstrated that the inhibition of ER stress with TUDCA has beneficial effects on liver function and oxidative stress parameters in the elderly, and these effects may differ depending on gender. It is known that oxidative stress and metabolic disorders play a role in the formation of many age-related diseases. Therefore, it can be thought that the results obtained from this study may contribute to the new approaches in the treatment of ER stress. Furthermore, considering the gender factor in the older population may develop treatment strategies.

Limitations: In this study, new findings were revealed, but these parameters could not be examined in detail. Further research is needed to elucidate the mechanisms underlying the effects of ER stress inhibition by TUDCA.

Yazar Katkıları: Çalışma konsepti/Tasarımı: SH; Veri toplama: SH; Veri analizi ve yorumlama: SH; Yazı taslağı: SH; İçeriğin eleştirel incelenmesi: SH; Son onay ve sorumluluk: SH; Teknik ve malzeme desteği: SH; Süpervizyon: SH; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Gazi Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu Başkanlığı'nın 02.09.2021 tarih ve E-66332047-604.01.02-156111 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.
Yazarın Notu: Gazi Üniversitesi Akademik Yazı Uygulama ve Araştırma Merkezi'ne bu yazının revize edilmesindeki desteklerinden dolayı teşekkür ediyorum.

Author Contributions: Concept/Design : SH; Data acquisition: SH; Data analysis and interpretation: SH; Drafting manuscript: SH; Critical revision of manuscript: SH; Final approval and accountability: SH; Technical or material support: SH; Supervision: SH; Securing funding (if available): n/a.
Ethical Approval: For this study, the Department of Animal Experiments of Gazi University Local Ethics Committee dated 02.09.2021 and E-66332047-604.01.02-156111 ethical approval has been obtained with the numbered decision.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support
Acknowledgement: I would like to thank Gazi University Academic Writing Application and Research Center for their support in revising this manuscript.

REFERENCES

1. Costantino S, Paneni F, Cosentino F. Ageing, metabolism and cardiovascular disease. J Physiol. 2016;594:2061-73.
2. Liu HH, Li JJ. Aging and dyslipidemia: a review of potential mechanisms. Ageing Res Rev. 2015;19:43-52.

3. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). Eur Heart J. 2020;41:111-188.
4. Yandrapalli S, Gupta S, Andries G, Cooper HA, Aronow WS. Drug therapy of dyslipidemia in the elderly. Drugs Aging. 2019;36:321-40.
5. Acharya P, Talahalli RR. Aging and hyperglycemia intensify dyslipidemia-induced oxidative stress and inflammation in rats: assessment of restorative potentials of ALA and EPA + DHA. Inflammation. 2019;42:946-952.
6. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757-72.
7. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov. 2021;20:689-709.
8. Taylor RC. Aging and the UPR(ER). Brain Res. 2016;1648:588-93.
9. Naidoo N. ER and aging-Protein folding and the ER stress response. Ageing Res Rev. 2009;8:150-9.
10. Brown MK, Naidoo N. The endoplasmic reticulum stress response in aging and age-related diseases. Front Physiol. 2012;3:263.
11. Murray HC, Dieriks BV, Swanson MEV, Anekal PV, Turner C, Faull RLM et al. The unfolded protein response is activated in the olfactory system in Alzheimer's disease. Acta Neuropathol Com. 2020;8.
12. Sreedhar R, Giridharan VV, Arumugam S, Karuppagounder V, Palaniyandi SS, Krishnamurthy P et al. Role of MAPK-mediated endoplasmic reticulum stress signaling in the heart during aging in senescence-accelerated prone mice. Biofactors. 2016;42:368-75.
13. Sheedfar F, Di Biase S, Koonen D, Vinciguerra M. Liver diseases and aging: friends or foes? Aging Cell. 2013;12:950-54.
14. Nho K, Kueider-Paisley A, Ahmad S, MahmoudianDehkordi S, Arnold M, Risacher SL et al. Association of altered liver enzymes with Alzheimer disease diagnosis, cognition, neuroimaging measures, and cerebrospinal fluid biomarkers. JAMA Netw Open. 2019;2:e197978.
15. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. Can Med Assoc J. 2005;172:367-79.
16. Bedogni G, Gastaldelli A, Tiribelli C, Agosti F, De Col A, Fessehatsion R et al. Relationship between glucose metabolism and non-alcoholic fatty liver disease severity in morbidly obese women. J Endocrinol Invest. 2014;37:739-44.
17. Katsiki N, Perez-Martinez P, Anagnostis P, Mikhailidis DP, Karagiannis A. Is nonalcoholic fatty liver disease indeed the hepatic manifestation of metabolic syndrome? Curr Vasc Pharmacol. 2018;16:219-27.
18. Adeli K, Higgins V, Nieuwesteeg M, Raizman JE, Chen Y, Wong SL et al. Biochemical marker reference values across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian Health Measures Survey. Clin Chem. 2015;61:1049-62.
19. Le Couteur DG, Blyth FM, Creasey HM, Handelsman DJ, Naganathan V, Sambrook PN et al. The association of alanine transaminase with aging, frailty, and mortality. J Gerontol A Biol Sci Med Sci. 2010;65:712-7.
20. Edvardsson M, Sund-Levander M, Milberg A, Wressle E, Marcusson J, Grodzinsky E. Differences in levels of albumin, ALT, AST, gamma-GT and creatinine in frail, moderately healthy and healthy elderly individuals. Clin Chem Lab Med. 2018;56:471-78.
21. Azman KF, Safdar A, Zakaria R. D-galactose-induced liver aging model: Its underlying mechanisms and potential therapeutic interventions. Exp Gerontol. 2021;150:111372.
22. Fu J, Zhang X, Chen P, Zhang Y. Endoplasmic reticulum stress is involved in 2,4-dichlorophenol-induced hepatotoxicity. J Toxicol Sci. 2016;41:745-56.
23. Pan XL, Zhao L, Li L, Li AH, Ye J, Yang L et al. Efficacy and safety of tauroursodeoxycholic acid in the treatment of liver cirrhosis: a double-blind randomized controlled trial. J Huazhong Univ Sci Technolog Med Sci. 2013;33:189-94.
24. Walter M. Interrelationships among HDL metabolism, aging, and atherosclerosis. Arterioscl Throm Vas. 2009;29:1244-50.
25. Ferrara A, Barrett-Connor E, Shan J. Total, LDL, and HDL cholesterol decrease with age in older men and women. The Rancho Bernardo Study 1984-1994. Circulation. 1997;96:37-43.
26. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature. 2016;529:326-35.
27. Lebeaupin C, Vallee D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B. Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. J Hepatol. 2018;69:927-47.
28. Rutkowski DT, Wu J, Back SH, Callaghan MU, Ferris SP, Iqbal J et al. UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. Dev Cell. 2008;15:829-40.
29. Wang SY, Kaufman RJ. How does protein misfolding in the endoplasmic reticulum affect lipid metabolism in the liver? Curr Opin Lipidol. 2014;25:125-32.
30. Moldogazieva NT, Mokhosoev IM, Mel'nikova TI, Porozov YB, Terentiev AA. Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in Aging and Age-Related Diseases. Oxid Med Cell Longev. 2019;2019:3085756.
31. Papaconstantinou J. The role of signaling pathways of inflammation and oxidative stress in development of

senescence and aging phenotypes in cardiovascular disease. Cells. 2019;8.
32. Singh A, Kukreti R, Saso L, Kukreti S. Oxidative stress: a key modulator in neurodegenerative diseases. Molecules. 2019;24.
33. Vatner SF, Zhang J, Oydanich M, Berkman T, Naftalovich R, Vatner DE. Healthful aging mediated by inhibition of oxidative stress. Ageing Res Rev. 2020;64:101194.
34. Marrocco I, Altieri F, Peluso I. Measurement and clinical significance of biomarkers of oxidative stress in humans. Oxid Med Cell Longev. 2017;2017:6501046.
35. Ore A, Akinloye OA. Oxidative stress and antioxidant biomarkers in clinical and experimental models of non-alcoholic fatty liver disease. Medicina (Kaunas). 2019;55.
36. Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev. 1998;78:547-81.
37. Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D et al. Clinical relevance of biomarkers of oxidative stress. Antioxid Redox Signal. 2015;23:1144-70.
38. Kimura M, Yokoyama A, Higuchi S. Aldehyde dehydrogenase-2 as a therapeutic target. Expert Opin Ther Tar. 2019;23:955-66.
39. Zhao T, Wu K, Hogstrand C, Xu YH, Chen GH, Wei CC et al. Lipophagy mediated carbohydrate-induced changes of lipid metabolism via oxidative stress, endoplasmic reticulum (ER) stress and ChREBP/PPARgamma pathways. Cell Mol Life Sci. 2020;77:1987-2003.
40. Liu X, Zhang R, Huang L, Zheng Z, Vlassara H, Striker G et al. Excessive oxidative stress contributes to increased acute ER stress kidney injury in aged mice. Oxid Med Cell Longev. 2019;2019:2746521.