ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Effect of Fish Oil on Liver MMP-9 and MMP-12 Levels in Hypercholesterolemia Induced Mice

Hiperkolesterolemi İndüklenen Farelerde Balık Yağının Karaciğer MMP-9 ve MMP-12 Düzeyleri Üzerindeki Etkisi

Muge Mavioglu Kaya[1], Tulay Dilan Samanci[2]

[1]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences; [2]Department of Biology, Institute of Science, Kafkas University, Kars, Türkiye

ABSTRACT

Aim: *This study aimed to investigate the effects of fish oil on liver matrix metalloproteinase-9 (MMP-9) and MMP-12 levels in hypercholesterolemic mice.*

Materials and Methods: *For comprehensive evaluation of MMP-9 and MMP-12 levels, total cholesterol (TC), low-density lipoprotein (LDL), total antioxidan status (TAS) and total oxidan status (TOS) levels, as well as liver histopathological status, were assessed. Twenty-eight mice were equally divided into four groups. The control (CNT) group received standard chow and drinking water without any treatment. The fish oil (FO) group received orally 0.1 ml of fish oil daily for 10 weeks. The cholesterol (CHOL) group was fed with a high-cholesterol chow diet. The cholesterol plus fish oil (CHOL+FO) group received a high-cholesterol chow plus an oral FO solution.*

Results: *The MMP-9, TC and TOS levels of the CHOL group were significantly higher, while TAS were lower compared to other groups. While MMP-9 levels decreased in the CHOL+FO and FO groups compared to the CHOL group ($p<0.001$), no significant change was observed for MMP-12 ($p>0.05$). Total oxidan status levels decreased, and TAS levels increased in the CHOL+FO and FO groups compared with the CHOL group, with higher oxidative stress index (OSI) levels ($p<0.05$).*

Conclusion: *It was concluded that combined fish oil administration could reduce the adverse effects of hypercholesterolemia by down-regulating bad cholesterol and oxidative stress in mouse liver and colon tissue, and by reducing MMP-9 protein concentration in colon tissue.*

Key words: *hypercholesterolemia; MMP-9; MMP-12; fish oil; mouse*

ÖZET

Amaç: *Bu çalışmanın amacı, balık yağının hiperkolesterolemil farelerde karaciğer matriks metaloproteinaz-9 (MMP-9) ve MMP-12 düzeyleri üzerindeki etkilerini araştırmaktır.*

Gereç ve Yöntem: *MMP-9 ve -12 düzeylerinin kapsamlı değerlendirilmesi için toplam kolesterol (TK), düşük yoğunluklu lipoprotein (LDL), toplam antioksidan durumu (TAS) ve toplam oksidan durum (TOS) düzeyleri ile karaciğer histopatolojik durumu değerlendirildi. Yirmi sekiz fare dört gruba eşit olarak ayrıldı. Kontrol (CNT) grubuna herhangi bir tedavi uygulanmadan standart yem ve içme suyu verildi. Balık yağı (FO) grubuna 10 hafta boyunca günde 0,1 ml balık yağı oral yolla verildi. Kolesterol (CHOL) grubuna yüksek kolesterollü bir yem diyeti verildi. Kolesterol ve balık yağı (CHOL+FO) grubuna ise yüksek kolesterollü bir yem ve FO solüsyonu verildi.*

Bulgular: *CHOL grubunda MMP-9, TC ve TOS düzeyleri diğer gruplara göre anlamlı olarak yüksek, TAS ise düşük bulundu. MMP-9 düzeyleri CHOL+FO ve FO gruplarında CHOL grubuna göre azalırken ($p<0,001$), MMP-12 için anlamlı bir değişim gözlenmedi ($p>0,05$). Yüksek oksidatif stres endeksi (OSI) düzeylerine sahip CHOL grubuna göre CHOL+FO ve FO gruplarında TOS düzeyleri azalırken, TAS düzeyleri ortalamaları yüksek sevideydi ($p<0,05$).*

Sonuç: *Sonuç olarak, kombine balık yağı uygulamasının, fare karaciğer dokusunda ve kolon dokusunda MMP-9 protein konsantrasyonunu düşürerek kötü kolesterol ve oksidatif stresi azaltarak hiperkolesteroleminin olumsuz etkilerini azaltabileceği kanaatine varıldı.*

Anahtar kelimeler: *hiperkolesterolemi; MMP-9; MMP-12; balık yağı; fare*

İletişim/Contact: *Müge Mavioğlu Kaya, Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Kafkas University, Kars, Türkiye* • ***Tel:*** *0543 878 27 41* • ***E-mail:*** *m.mavioglu@hotmail.com* • ***Geliş/Received:*** *02.06.2025* • ***Kabul/Accepted:*** *07.07.2025*

ORCID: *Müge Mavioğlu Kaya: 0000-0003-1276-3745* • *Tülay Dilan Samancı: 0000-0003-0573-0590*

Kafkas J Med Sci 2025; 15(3):378–386
doi: 10.5505/kjms.2025.54289

Introduction

Cholesterol (C_{27} $H_{46}O$) can be synthesized by nearly all cells, and in humans, approximately 50% of total synthesis occurs in liver cells. Cholesterol homeostasis is of great interest in terms of physiological and pathological conditions because it underlies a growing number of diseases such as inflammation, Alzheimer's, atherosclerosis and various types of cancer[1]. Matrix metalloproteinases (MMPs) are a group of endopeptidase enzymes that are zinc- and calcium-dependent and facilitate the degradation of extracellular matrix (ECM) proteins, thereby playing an important role in human physiology and pathology. Extracellular matrix elements can regulate cellular or tissue development and homeostasis, which are relevant to adverse health conditions such as chronic inflammation, malignant cell proliferation, and apoptosis, which are based on oxidative stress in their pathogenesis[2,3]. Excessive accumulation of reactive oxygen species (ROS) causes oxidative stress and MMP-associated ECM destruction[2]. Excessive accumulation of ROS causes oxidative stress and MMP-associated ECM destruction. Because increased expression levels of MMPs are linked to the pathology of many inflammatory malignant diseases and hypercholesterolemia, MMPs and their tissue inhibitors have become targets for drug discovery[3,4]. MMP-9, also called gelatinase B, is found in very small amounts in healthy tissues and is secreted by dendritic, hematopoietic, macrophage, neutrophil, and fibroblast cells[5]. MMP-12 is an enzyme belonging to the collagenase family ve it also called macrophage metalloelastase. They play a role in ECM degradation by degrading elastin and collagen. Excessive MMP-12 activity has been associated with inflammation and tissue damage[6].

Fish oil contains high amounts of polyunsaturated fatty acids (PUFAs) containing 5 or more double bonds and highly unsaturated fatty acids (HUFAs), which are omega-3 (ω-3) PUFAs. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are derived from α-linolenic acid (ALA), the precursor of omega-3 PUFAs. These are abundant in fish, and various studies have shown positive effects on the pathophysiology of diseases such as wound healing, nervous system deformities, and heart and liver disorders, due to their antihypercholesterolemic, antioxidant, and anti-inflammatory properties[7,8]. If the rate of free radicals causing lipid peroxidation in living organisms exceeds the capacity of glutathione and many other antioxidants, oxidative damage originating from oxidative stress can occur in tissues and molecules[9–11]. Assessing TAS and TOS levels is a practical method for determining this rate[12–14]. The literature indicates a lack of studies examining oxidative stress, lipid parameters, and ω-3 and MMP enzyme concentrations simultaneously.

This planned study examines the relationship between MMP-9 and MMP-12 protein expression levels with fish oil as an alternative to statin drugs, which are frequently used in conventional medicine for the treatment of hypercholesterolemia, but are quite expensive and have side effects. Furthermore, a comprehensive assessment aimed at monitoring cholesterol, TOS, and TAS levels.

Material and Methods

Experimental Animals

A total of 28 female Balb/c mice, weighing 20±2 g and aged 10 weeks at the Kafkas University Experimental Animal Research Center in Kars, were housed at 20±2°C, 60–70% relative humidity and under a 12-h day-night cycle. Throughout the experiment, the animals were provided with standard chow (Bayramoğlu Feed Inc., Erzurum, Türkiye) and water. The ethical regulations recommended by the Kafkas University Animal Experimentation Ethics Committee (KAU-HADYEK 2021/188) were complied with at all stages of the experimental study.

High Cholesterol Feed and Fish Oil Application

The mice were divided equally into four groups. The first group was the control (CNT) group, which received no treatment and standard chow and drinking water. The second group received fish oil (FO) for 10 weeks. Fish oil (fish oil from menhaden cas no: 8002-50-4, Sigma-Aldrich) acid content used in the study is as follows: 14:0 myristic acid 6–9%, 16:0 palmitic acid 15–20%, 16:1 palmitoleic acid 9–14%, 18:0 stearic acid 3–4%, 18:1 oleic acid 5–12%, 18:2 linoleic acid <3%, 18:3 linolenic acid <3%, 18:4 octadecatetraenoic acid 2–4%, 20:4 arachidonic acid <3%, 20:5 eicosapentaenoic acid 10–15%, 22:6 docosahexaenoic acid 8–15% (Sigma-Aldrich). Throughout the study, 0.1 ml/mouse/day of fish oil was administered orally to the respective groups at a concentration equivalent to eicosapentaenoic acid and docosahexaenoic acid[15]. The third group (CHOL) was fed a diet containing 2% cholesterol (Alfa Aesar, Karlsruhe, Germany) and 0.5% cholic acid (Thermo Fisher Scientific, Acros Organics, Italy) for 10 weeks to establish a high-cholesterol model[16].

After 10 weeks, blood samples collected after an intraperitoneal injection of 30 mg/kg pentobarbital were intracardially collected with a syringe and placed into heparinized tubes for cholesterol measurements. Then, the animals were euthanized by decapitation and the liver tissues were homogenized with a homogenizer (Isolab, Cat No: 621.11.001) using phosphate buffer (pH: 7.3). Centrifugation (Hettich Zentrifugen Universal 16R, Germany) was performed at +4°C and 2500 rpm for 10 min to create and separate phases from the homogenate samples. The supernatant was kept at -20°C in Eppendorf tubes until analysis. Another portion of the liver tissues was placed in 10% formaldehyde (Sigma-Aldrich) solution for histopathological evaluation.

Lipid Analyses

Total cholesterol (TC) and low-density lipoprotein (LDL) levels were analyzed using a cholesterol-measuring device (Cat No: 02LA10G, SD Biosensor's LipidoCare), which can be activated and produce results from a single drop of blood. Measuring range for TC: 100–450 mg/dL. Low-density lipoprotein was calculated using the Friedewald formula, which uses TC, TG (45–650 mg/dL), and HDL (25–95 mg/dL) levels. A drop of blood was added to the device's application area using an automatic pipette, and the device was allowed to run for 3 minutes. Separate strips (Acc. Accuchek Bulk Accucheck Lancet 25T, Roche) were used for each animal, and the measured values were recorded.

MMP-9 and MMP-12 Analyses

A commercially available ELISA kit (Bioassay Technology Laboratory E0277 and E1518, Mo, China) was used to measure MMP-9 and MMP-12 protein levels in mouse livers. Following the method recommended in the protocol guide, the optical density of each well was measured using a microplate reader (Epoch, Microplate Spectrophotometer) set at 450 nm. A standard curve was then created using the absorbance values from the standards, and the average MMP expression levels of the samples were calculated based on this curve.

Total Antioxidan Status (TAS) and Total Oxidan Status (TOS) Analyses

For analysis, liver tissue samples diluted in phosphate-buffered saline (OXOID, Dulbecco's A, England) were centrifuged at 3500 rpm for 10 cycles, and the supernatants were collected. To determine TAS levels in these supernatants, a measurement method based on the principle of clarification of the specific color of the more stable 2.2'-azino-bis (3-ethylbenz-thiazoline 6-sulfonic acid) (ABTS) radical cation by antioxidants was used[12]. To measure TOS levels, a method based on the principle that oxidant substances in the samples oxidize the ferrous ion-o-dianisidine to ferric ion with the support of glycerol, and that ferric ion forms a colored complex with xylenol in an acidic environment was used[13]. The equation (OSI)=TOS/TAS was used to estimate the oxidative stress index.

Histopathological Analyses

For histopathological evaluation, liver tissue samples from mice were fixed with 10% formaldehyde. After 24 hours, all tissues were placed in tissue-processing cassettes, embedded in paraffin, and 4 μm sections were cut. Sections were stained with hematoxylin and eosin (H&E) using standard histological staining methodology and viewed under a light microscope (Olympus).

Statistical Analyses

Data are presented as mean ± standard deviation (X ± SD). Statistical analyses of data obtained from groups were performed using IBM Statistical Package for Social Sciences (SPSS) program version 20.0 for Windows IBM software. One-way analysis of variance (ANOVA) was performed to determine whether there were statistical differences between group means. The Tukey test, a post hoc multiple-comparison test, was used to determine whether the mean values of each group differed significantly from those of the others.

Results

The toxicological potential of fish oil was evaluated, and no histopathological findings or mucosal or epithelial cell irritation were observed in the mouth, pharynx, or trachea of the mice. The experimental groups were compared for average body weight, and the FO group had the lowest body weight. In contrast, the CHOL+FO group had significantly lower body weight compared to the CNT and CHOL groups. The groups were evaluated for daily feed intake, and the highest intake was in the CHOL group; feed intake was lower in the CHOL+FO, FO, and CNT groups. When the experimental groups were evaluated for water intake, the highest water intake was observed in the CHOL and FO groups, while it was lower in the CHOL+FO and CNT groups (Table 1).

Table 1. *The mean body weight, water and feed consumption rates in mice (n=7)*

	CNT	CHOL	CHOL+FFO	FO
Weight (g/week)	33.25±1.88[b]	33.66±0.92[b]	32.45±0.96[b]	29.74±1.43[a]
Feed consumption (g/day)	26.97±1.84[a]	45.77±4.26[b]	44.48±5.32[b]	31.11±5.42[a]
Water consumption (ml/day)	44.80±9.62[a]	61.82±6.75[b]	47.55±9.28[a]	61.12±7.44[b]

X[a,b]: differences between means with different letters on the same line are significant p<0.001.

Table 2. *The levels of whole blood lipid parameters, liver oxidative status and colon MMP (-9 and -12) in mice (n=7)*

	CNT	CHOL	CHOL+FO	FO	p
TC (mg/dL)	98.26±5.79[a]	145.00±9.53[b]	128.33±15.9[bc]	117.33±0.57[c]	<0.001
LDL (mg/dL)	42.80±4.23	59.20±26.1	49.76±5.09	46.13±6.53	>0.05
TAS (mmol Trolox eq/L)	1.48±0.1[a]	1.24±0.09[b]	1.29±0.08[ab]	1.42±0.17[ab]	<0.05
TOS (µmol H_2O_2 eq/L)	6.79±0.32[ab]	7.67±0.74[b]	6.98±0.46[ab]	6.51±0.52[a]	<0.05
OST (TOS/TAS)	4.62±0.47	6.24±0.73	5.43±0.55	4.65±0.79	<0.05
MMP-9 (ng/mL)	1.51±0.19[ab]	1.65±0.14[b]	1.16±0.17[ab]	1.01±0.25[a]	<0.001
MMP-12 (ng/mL)	10.3±1.39	9.98±3.74	9.06±2.17	9.44±1.86	>0.05

X[a,b,c]: differences between means with different letters on the same line are significant.

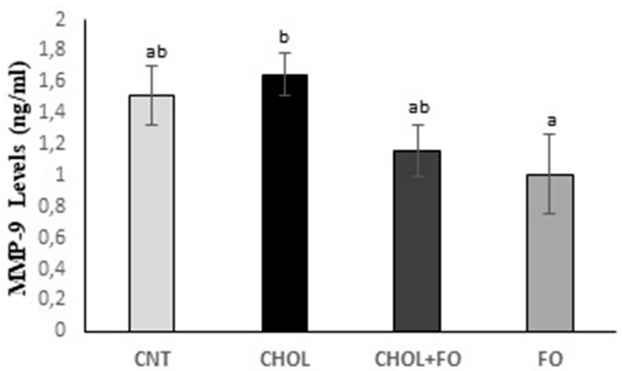


Figure 1. *The liver MMP-9 levels in high-cholesterol and fish oil-fed mice (n=7) (Xa,b: differences between means with different letters on the same line are significant (p<0.001)).*

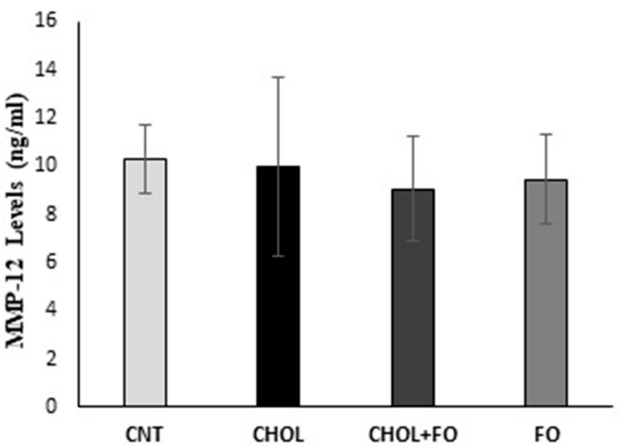


Figure 2. *The liver MMP-12 levels in high-cholesterol and fish oil-fed mice (n=7).*

Whole blood TC concentrations were found to be significantly increased in the CHOL group compared to the CNT group, and significantly decreased in the CHOL+FO group compared to the CHOL group (p<0.001). Low-density lipoprotein levels were numerically higher in the CHOL group than in the CNT group (Table 2).

While the liver MMP-9 level increased numerically in the CHOL group compared to the CNT group, the MMP-9 level was significantly lower in the CHOL+FO group compared to the CHOL group (p<0.001) (Fig. 1). Although there was no significant difference in the MMP-12 protein levels in the CHOL group compared to the CNT group, and MMP-12 protein levels decreased numerically in the CHOL+FO group compared to the CHOL group, this difference was not statistically significant (P >0.05) (Fig. 2). Therefore, while a decrease in MMP-9 levels was observed with the use of fish oil in contrast to a high-cholesterol diet, no significant change was detected in MMP-12 protein levels.

No significant morphological findings were observed in the livers of the CNT group. In the CHOL group, the liver lobes had a striking whitish appearance, characteristic of fatty tissue. Macroscopically, changes in liver color and size were evident in the high-cholesterol diet and are represented in Fig. 3.

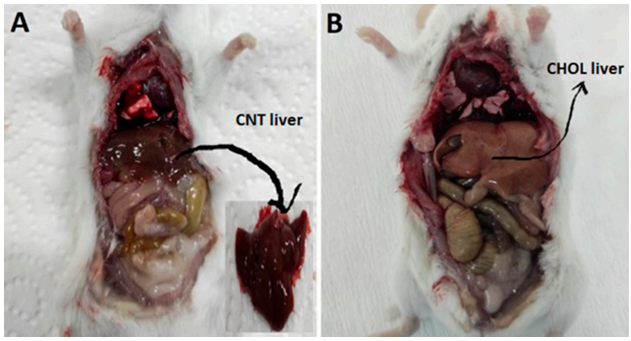


Figure 3. A, B. *The control group (A) and CHOL group (B) mouse liver tissues.*

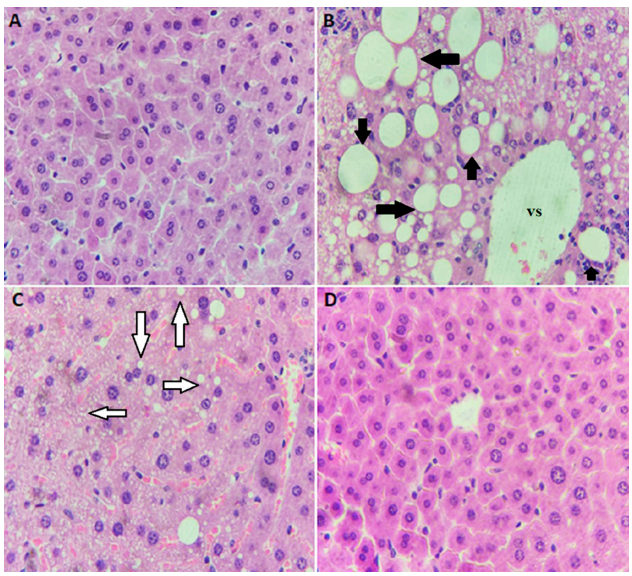


Figure 4. A–D. *CNT (A): the normal liver histology; CHOL (B): the large droplets of steatosis (black arrow) reflecting excessive cholesterol accumulation in the tissue; CHOL+FO (C): the small droplets of steatosis (white arrow); FO (D): the normal liver histological structure, HE×40.*

When the liver histopathology of mice fed a high-cholesterol diet was examined, fatty tissue invasion and hepatocyte steatosis were among the most pronounced findings.

Discussion

Hypercholesterolemia is a pathological inflammatory disorder characterized by excessively elevated cholesterol concentrations in blood plasma and increasingly threatening modern societies[17]. Genetically similar mice and humans, with a few exceptions, share the same set of genes controlling lipoprotein metabolism. This makes mice useful experimental animals for the treatment of hypercholesterolemia and for *in vivo* studies of its molecular biology[18]. Statin and non-statin drugs used in standard hypercholesterolemia treatment are costly and, unfortunately, have serious side effects such as liver enzyme deterioration, skeletal muscle damage, and myopathy[19]. Therefore, in cases of hypercholesterolemia, alternative methods are recommended consumption of foods known to lower cholesterol[16]. It is known that oils containing multiple fatty acids (fish oil, sunflower oil, corn oil, etc.) are more effective in lowering cholesterol concentrations than oils containing monounsaturated fatty acids (olive oil, canola oil, etc.)[20]. Therefore, these data may significantly support the use of fish oil over traditional statin therapy in hypercholesterolemia. Hypercholesterolemia with increased oxidative stress is responsible for hypoxic conditions similar to those in the tumor microenvironment, and for increased inflammatory responses[21,22]. It has been suggested that treatment with fish oil containing high-density lipoprotein may interrupt the link between cholesterol accumulation and inflammation[23].

In the present study, when the fixed liver tissue samples were evaluated macroscopically primarily for initial impression and diagnosis, it was clear that the whitish, swollen appearance of the liver lobes, characteristic of fatty tissue, reflected the pathological condition in the CHOL group. Macroscopically, liver samples from the CNT and FO groups were shiny, brown, and homogeneous, while lipoma-like areas were noted on the serosal surface of one sample from the CHOL+FO group (Fig. 3). When liver tissue histopathology was evaluated, it was determined that the FO groups showed shrinkage of large steatosis droplets in liver tissue sections of experimental animals with hypercholesterolemia compared to the CHOL group (Fig. 4). In a study, it was reported that thymoquinone, as an antioxidant, protected against histological ballooning of hepatocytes in obese rats fed a high-fat diet[24]. When whole blood cholesterol levels were compared between the groups, the CHOL group had the highest mean ($p<0.001$). Whole blood cholesterol levels in the CHOL+FO group were lower than in the CHOL group, while they were similar to those in the CNT and FO groups (Table 2). Circulating whole blood TC levels depend on changes in liver endogenous cholesterol production; the amount of LDL uptake by the liver, the amount stored as cholesteryl esters, the level of very-low-density lipoprotein (VLDL) secretion, and the amount of bile acids and cholesterol excreted in feces[20]. Fish oil may play a role in reducing serum cholesterol levels by inducing hepatic fatty acid oxidation, reducing fatty acid synthesis, exhibiting anti-obesity effects[25] and increasing the fecal excretion of macrophage– and high-density lipoprotein-derived

cholesterol along with bile acids[26]. The findings of this study support previous research.

It has been reported that decreased expression of sterol regulatory element-binding protein (SREBP)-1 c and fatty acid synthase (FAS) lipogenic genes, and increased fatty acid oxidation via induction of peroxisome proliferator-activated receptor (PPAR-α), are the main mechanisms that reduce circulating lipid concentrations[25]. Dietary fish oil intake increases PPAR-α induction, thereby upregulating genes involved in β-oxidation. This is thought to reduce hepatic triglyceride levels by increasing the expression of β-oxidation-related enzymes in the liver[27].

Docosahexaenoic acid (DHA) reduces apolipoprotein C3 synthesis mediated by the FOX1 transcription factor, leading to excessive hydrolysis of VLDL-c and a much greater conversion of LDL to larger, less atherogenic fragments[28]. Therefore, dose adjustment is important when using unsaturated fatty acid supplements containing DHA or EPA. This may be due to the absence of cholesteryl ester transfer protein (CETP) in mice, a transporter that facilitates the transport of cholesterol esters and triglycerides in humans. Therefore, mice have lower plasma cholesterol levels of 60–100 mg/dl compared to humans, and high-density lipoproteins may be considered the main circulating lipoprotein[29]. Fish oil increases Mdr2 expression in addition to hepatic Srb1, thereby increasing cholesterol secretion into bile, which may lead to increased cholesterol excretion via bile[24]. LDLs deliver cholesterol to inflammatory cells in blood vessels and are associated with diseases requiring cholesterol-lowering therapy[30]. The effects of fish oil on LDL metabolism are highly controversial. Unlike rodents, fish oil can increase LDL concentrations in humans[31] and does not shift circulating LDL particle size to larger particles[32]. In the current study, no significant difference was found between groups in LDL levels ($p>0.05$). However, the numerically highest LDL value was observed in the CHOL group, while LDL values in the CHOL+FO and FO groups were very similar to those in the CNT group (Table 2). Studies in rodents using fish oil compared with a high-cholesterol diet have shown significant reductions in liver LDL levels[33,34]. This is attributed to fish oil's ability to suppress cholesterol synthesis and reduce cholesterol absorption[32].

Studies have shown that MMPs play an important role in regulating cholesterol metabolism and ECM components, which are also affected by transient receptor potential (TRP) proteins that regulate intracellular calcium ions [2,3,35,36]. However, the combined effect of fish oil, which contains abundant unsaturated fatty acids such as EPA or DHA, on ECM modeling, which concerns the lipid-rich cell membrane, remains unknown. Fibroblasts and various immune cells secrete MMP-9, and its effects may vary depending on the stage and etiology of hepatic diseases[37]. Proinflammatory mechanisms such as oxidized low-density lipoprotein (oxLDL) infiltration of monocytes into the arterial wall, macrophage proliferation, and increased foam cell formation lead to elevated serum MMP-9 levels[38]. Furthermore, photosensitizing cholesterol oxidation products induced by ROS generated by UVA irradiation also increase MMP-9 levels[39]. In the current study, liver natural MMP-9 protein expression levels were significantly higher in the CHOL group than in the CNT group, in association with increased LDL levels observed with a high-cholesterol diet (Fig. 2). Statin treatment regulates MMP levels and their tissue inhibitors (TIMPs). While no change in MMP-9 levels was observed after statin treatment, a significant decrease in TIMP-1 levels was reported[40]. In the current study, fish oil exhibited statin-like effects, significantly reducing hepatic MMP-9 protein levels in the CHOL+FO and FO groups, falling below that in the CNT group ($p<0.001$) (Fig. 1 and Table 2). The study in *Mmp9* −/− (MMP-9 knockout) mice also clarified that the MMP system as a whole, and MMP-9 in particular, strongly influences cholesterol homeostasis[41].

MMP-12, secreted primarily by macrophages, can degrade elastin[42]. The development of adipose tissue requires ECM degradation. It is understood that the MMP-12 enzyme reduces plaque stability in blood vessels by causing the atherosclerotic lesion to expand, and this may be an important factor in plaque rupture[43]. This is primarily linked to macrophage migration, which directly regulates MMP-12 activation and expression. MMP-12 mRNA expression levels are significantly increased in obese adipose tissues compared to lean tissues[44]. Treatment of both obese and diabetic mice with polyunsaturated fatty acids suppressed MMP-12 expression in macrophages and adipose cells, leading to remodeling of the degraded ECM and reduced fat cell growth, supporting the relationship between MMP-12 and adipose tissue development[45]. Furthermore, in addition to its ability to degrade elastin, MMP-12 has also been identified as a transcription factor that induces Fabp4 transcription. Therefore, therapeutic approaches targeting MMP-12 would

benefit obesity and related diseases[46]. Statins, known for their cholesterol-lowering effects, increase MMP-12 expression levels[47]. In a study conducted in rabbits with atherosclerosis induced by a 1% cholesterol diet, no significant difference in MMP-12 expression was observed between rabbits and humans[48]. Similarly, in the current study, no statistically significant difference was found between the groups in liver natural MMP-12 protein expression levels ($p>0.5$). Although not significant, a decrease in numerical values was observed in the CHOL+FO group compared with the CHOL group (Fig. 2 and Table 2). MMP-12 activity has fundamental effects on ECM remodeling by reducing excessive tissue infiltration, regulating the activity of anti-inflammatory cytokines and chemokines in macrophages and T cells, stimulating transforming growth factor-beta (TGF-β) action through fibroblast activation, and promoting scar formation[43]. According to both the explanations of previous studies regarding the expansion of atherosclerotic vascular plaque by MMP-12 activity[42–44] and the data we obtained, it can be suggested that the lack of a significant difference in the mean MMP-12 protein levels of the groups may be because hyperlipidemia has not yet progressed chronically.

Hypercholesterolemia may also increase atherogenic biomolecules in the vascular or circulatory system by increasing the production of reactive oxygen species, thereby increasing oxidative stress through decreased antioxidant levels. It has been reported that blocking reactive oxygen species with factors or antioxidant agents such as green tea, probiotics, pioglutazone or ellagic acid can reduce the development of hypercholesterolemic atherosclerosis or similar disorders by decreasing the rate of oxidative stress[49–53]. If oxidative stress cannot be blocked, it can also affect the ECM, leading to fatty liver and vascular dysfunction, including the accumulation of bad cholesterol. According to significant findings ($p<0.001$) obtained from ox-LDL concentrations in humans, a positive correlation may exist between hypercholesterolemia and oxidative stress parameters[54]. Nicotinamide adenine dinucleotide phosphate (NADPH) levels, which reflect oxidative stress, have been reported to increase in mice on a high-fat diet[55]. It has been suggested that oral administration of omega-3 fish oil may reduce cholesterol levels and consequently oxidative stress[56]. One study found that oxidative stress was significantly reduced in male rats supplemented with 0.4 g/kg of fish oil, suggesting that fish oil, rich in EPA and DHA, has free radical-scavenging activity against oxidative stress[57]. Another study reported that fish oil and krill oil, when administered to ulcer-induced rats, reduced ROS formation and lipid peroxidation, thereby inhibiting increased ROS and lipid peroxidation[58]. Higher TOS levels than TAS levels in organisms indicate the presence of oxidative stress. The current study showed a significant increase in oxidative stress levels in the CHOL group fed a high-cholesterol diet compared to the CNT and CHOL+FO groups. Furthermore, TOS levels decreased, and TAS levels increased in the CHOL+FO and FO groups receiving fish oil supplements, and fish oil consumption improved these values compared to the CHOL group. These findings are consistent with previous studies, suggesting that fish oil supplementation may be important in reducing oxidative stress in mice with induced hypercholesterolemia.

Conclusion

The results of this study showed that MMP-9 and -12 were increased in the liver tissue of hypercholesterolemia-induced mice, and oxidative stress was reduced by 10 weeks of fish oil supplementation. Therefore, it can be hypothesized that fish oil treatment may help treat hypercholesterolemia by reducing free radicals, inflammation, and MMP (MMP-9 and -12) protein expression. In addition to demonstrating the expression levels of MMP-9 and -12 in hepatocytes, these results also highlight the potential of fish oil as a candidate therapeutic agent for the treatment of many diseases associated with hypercholesterolemia and inflammation by acting as a non-synthetic MMP inhibitor analog.

Acknowledgement

This study was supported by the Scientific Research Project Coordinator of Kafkas University (*Project No: 2022-FM-15*), and the authors express their gratitude to the institute.

References

1. Luo J, Yang H, Song BL. Mechanisms and regulation of cholesterol homeostasis. Nature reviews Molecular Cell Biology. 2020; 21(4):225-245.
2. Bai, X., Yao, M., Zhu, X., Lian, Y., Zhang, M. (2023). Baicalin suppresses interleukin-1β-induced apoptosis, inflammatory response, oxidative stress, and extracellular matrix degradation in human nucleus pulposus cells. Immunopharmacology and Immunotoxicology *45*(4), 433-442.
3. Wang K, Zhang Y, Si C, Cao Y, Shao P, Zhang P, et al. Cholesterol: The driving force behind the remodeling of tumor microenvironment in colorectal cancer. Heliyon. 2024;10(23).
4. Khotimchenko YS, Silachev DN, Katanaev VL. Marine natural products from the Russian Pacific as sources of drugs for neurodegenerative diseases. Marine Drugs. 2022;20(11):708.
5. Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global prevalence of periodontal disease and lack of its surveillance. The Scientific World Journal. 2020;2020(1):2146160.
6. Chuliá-Peris L, Carreres-Rey C, Gabasa M, Alcaraz J, Carretero J, Pereda J. Matrix metalloproteinases and their inhibitors in pulmonary fibrosis: EMMPRIN/CD147 comes into play. International Journal of Molecular Sciences 2022; 23(13):6894.
7. Karapehlivan M, Ogun M, Kaya I, Ozen H, Deveci HA, Karaman M. Protective effect of omega-3 fatty acid against mercury chloride intoxication in mice. Journal of Trace Elements in Medicine and Biology 2014;28(1):94-99.
8. Chen J, Jayachandran M, Bai W, Xu B. A critical review on the health benefits of fish consumption and its bioactive constituents. Food Chemistry 2022;369:130874.
9. Kaya I, Citil M, Sozmen M, Karapehlivan M, Cigsar G. Investigation of protective effect of L-carnitine on L-asparaginase-induced acute pancreatic injury in male Balb/c mice. Digestive Diseases and Sciences 2015;60:1290-1296.
10. Çenesiz S. The role of oxidant and antioxidant parameters in the infectious diseases: A systematic literature review. Kafkas Universitesi Veteriner Fakultesi Dergisi. 2020;*26*(6).
11. Kaya, MM. The roles of matrix metalloproteases in different stages of cancer. In: Kaya I. Current Researches in Cancer Biology. Livre de Lion. 2023;27.
12. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry 2004; 37:277-285.
13. Erel O. A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry 2005;38:1103-11.
14. Deveci HA, Unal S, Karapehlivan M, Ayata MK, Gaffaroglu M, Kaya I, Yilmaz M. Effects of glyphosate (herbicide) on serum paraoxonase activity, high density lipoprotein, total antioxidant and oxidant levels in Kars Creek Transcaucasian Barbs (*Capoeta capoeta* [Guldenstaedt, 1773]) Fresenius Environmental Bulletin (2017); 3514-3518.
15. Chacon AC, Phillips BE, Chacon MA, Brunke-Reese D, Kelleher SL, et al. Oral omega-3 fatty acids promote resolution in chemical peritonitis. Journal of Surgical Research 2016; 206(1):190-198.
16. Penumathsa SV, Thirunavukkarasu M, Koneru S, Juhasz B, Zhan L, Pant R, et al. Statin and resveratrol in combination induces cardioprotection against myocardial infarction in hypercholesterolemic rat. Journal of Molecular and Cellular Cardiology. 2007;42(3):508-516, 2007.
17. Du Y, Su J, Yan M, Wang Q, Wang T, Gao S, et al. Polymethoxyflavones in citrus extract has a beneficial effect on hypercholesterolemia rats by promoting liver cholesterol metabolism. Journal of Ethnopharmacology. 2024;322:117644.
18. Hofker MH, van Vlijmen BJ, Havekes LM. Transgenic mouse models to study the role of APOE in hyperlipidemia and atherosclerosis. Atherosclerosis1998;137(1):1-11.
19. Al-Mansoub MA, Asmawi MZ, Murugaiyah V. Effect of extraction solvents and plant parts used on the antihyperlipidemic and antioxidant effects of Garcinia atroviridis: a comparative study. Journal of the Science of Food and Agriculture 2014;94(8):1552-1558.
20. Mjaatveit M, Oldernes H, Gudbrandsen OA. Effects of diets containing fish oils or fish oil concentrates with high cetoleic acid content on the circulating cholesterol concentration in rodents. A systematic review and meta-analysis. British Journal of Nutrition. 2024;131(4):606-621.
21. Mildner-Szkudlarz S, Bajerska J. Protective effect of grape by-product-fortified breads against cholesterol/cholic acid diet-induced hypercholesterolaemia in rats. Journal of the Science of Food and Agricultur. 2013; 93(13):3271-3278.
22. Leopold JA, Loscalzo J. Oxidative risk for atherothrombotic cardiovascular disease. Free Radical Biology and Medicine. 2009; 47(12):1673-1706.
23. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. Nature Reviews Immunology 2015; 15(2):104-116.
24. Alkan I, Bakırhan EG, Tüfek NH, Arslan G, Altunkaynak BZ, Kaplan S. Investigation of the protective effect of thymoquinone on the livers of the obese rats ınduced by a high-fat diet. Kafkas Journal of Medical Sciences 2024;14(3):252-259.
25. Kamisako T, Tanaka Y, Ikeda T, Yamamoto K, Ogawa H. Dietary fish oil regulates gene expression of cholesterol and bile acid transporters in mice. Hepatology Research. 2012; 42(3):321-326.
26. Nishimoto T, Pellizzon MA, Aihara M, Stylianou IM, Billheimer JT, Rothblat G, et al. Fish oil promotes macrophage reverse cholesterol transport in mice. Arteriosclerosis, Thrombosis, and Vascular Biology. 2009;29(10):1502-1508.
27. Hirako S, ju Kim H, Arai T, Chiba H, Matsumoto A. Effect of concomitantly used fish oil and cholesterol on lipid metabolism. The Journal of Nutritional Biochemistry 2010;21(7):573-579.
28. Sales ALC, Primo MG, Mello Neto RS, Mendes AVS, Furtado MM, Rocha JÉL, et al. A Preliminary study on hepatoprotective, hypolipidemic and aortic morphometric effects of omega-3-rich fish oil in hypercholesterolemic mice. Pharmaceuticals. 2024;17(1):72.
29. Lee YT, Lin HY, Chan YWF, Li KHC, To OTL, Yan BP, et al. Mouse models of atherosclerosis: a historical perspective and recent advances. Lipids in health and disease. 2017;16:1-11.
30. Rader DJ, Tall AR. Is it time to revise the HDL cholesterol hypothesis?. Nature medicine. 2012; 18(9):1344-1346.

31. Nestel PJ. Fish oil and cardiovascular disease: lipids and arterial function. The American Journal of Clinical .000; 71(1):228S-231S.
32. Oelrich B, Dewell A, Gardner CD. Effect of fish oil supplementation on serum triglycerides, LDL cholesterol and LDL subfractions in hypertriglyceridemic adults. Nutrition, metabolism and cardiovascular diseases. 2013;23(4):350-357.
33. Komprda T, Škultéty O, Křížková S, Zorníková G, Rozíková V, Krobot R. Effect of dietary *Schizochytrium microalga* oil and fish oil on plasma cholesterol level in rats. Journal of animal physiology and animal nutrition. 2015;99(2):308-316.
34. Shang T, Liu L, Zhou J, Zhang M, Hu Q, Fang M, et al. Protective effects of various ratios of DHA/EPA supplementation on high-fat diet-induced liver damage in mice. Lipids in Health and Disease. 2017;16:1-13.
35. Kaya İ, Dağ S, Mavioğlu Kaya, M, Tanrıverdi EA, Beşeren H, Aşasın G. Modulatory effect of pomegranate extract on TRPA1, TRPM2 and caspase-3 expressions in colorectal cancer induction of mice. Turkish Journal of Biochemistry 2022;47(5):612-619.
36. Wang Q, Ji C, Smith P, McCulloch CA. Impact of TRP channels on extracellular matrix remodeling: focus on TRPV4 and collagen. International Journal of Molecular Sciences 2024;25(7):3566.
37. Balkrishna A, Paliwal R, Singh S, Singh R, Gohel V, Dev R, et al. Livogrit mitigates ANIT-induced cholestasis-like symptoms in an *in vivo* model by curbing hepatic inflammation and regulating BAX, TGF-β, MMP-9 and α-SMA gene expression. Heliyon 2025;11(3).
38. Kalela A, Koivu TA, Höyhtyä M, Jaakkola O, Lehtimäki T, Sillanaukee P, et al. Association of serum MMP-9 with autoantibodies against oxidized LDL. Atherosclerosis 2002; 160(1):161-165.
39. Minami Y, Kawabata K, Kubo Y, Arase S, Hirasaka K, Nikawa, et al. Peroxidized cholesterol-induced matrix metalloproteinase-9 activation and its suppression by dietary β-carotene in photoaging of hairless mouse skin. The Journal of Nutritional Biochemistry. 2009;20(5):389-398.
40. Ferretti G, Bacchetti T, Banach M, Simental-Mendia LE, Sahebkar A. Impact of statin therapy on plasma MMP-3, MMP-9, and TIMP-1 concentrations: a systematic review and meta-analysis of randomized placebo-controlled trials. Angiology 2017;68(10):850-862.
41. Hernandez-Anzaldo S, Brglez V, Hemmeryckx B, Leung D, Filep JG, Vance JE, et al. Novel role for matrix metalloproteinase 9 in modulation of cholesterol metabolism. Journal of the American Heart Association 2016;5(10), e004228.
42. Sabir U, Gu HM, Zhang DW. Extracellular matrix turnover: Phytochemicals target and modulate the dual role of matrix metalloproteinases (MMPs) in liver fibrosis. Phytotherapy Research. 2023;37(11):4932-4962.
43. Johnson JL, George SJ, Newby AC, Jackson CL. Divergent effects of matrix metalloproteinases 3, 7, 9, and 12 on atherosclerotic plaque stability in mouse brachiocephalic arteries. Proceedings of the National Academy of Sciences 2005;102(43):15575-15580.
44. Malaguarnera M, Cauli O, Cabrera-Pastor A. Obesity and adipose-derived extracellular vesicles: implications for metabolic regulation and disease. Biomolecules 2025;15(2):231.
45. Bauters D, Van Hul M, Lijnen HR. Macrophage elastase (MMP-12) in expanding murine adipose tissue. Biochimica et Biophysica Acta (BBA)-General Subjects 2013;1830(4):2954-2959.
46. Song, M., Zhang, S., Tao, Z., Li, J., Shi, Y., Xiong, Y., ... & Chen, S. (2021). MMP-12 siRNA improves the homeostasis of the small intestine and metabolic dysfunction in high-fat diet feeding-induced obese mice. Biomaterials 278, 121183.
47. Arikan MC, Shapiro SD, Mariani TJ. Induction of macrophage elastase (MMP-12) gene expression by statins. Journal of Cellular Physiology. 2005;204(1):139-145.
48. Yamada S, Wang KY, Tanimoto A, Fan J, Shimajiri S, Kitajima S, et al. Matrix metalloproteinase 12 accelerates the initiation of atherosclerosis and stimulates the progression of fatty streaks to fibrous plaques in transgenic rabbits. The American Journal of Pathology. 2008; 172(5):1419-1429.
49. Prasad K, Mishra M. Mechanism of hypercholesterolemia-induced atherosclerosis. Reviews in Cardiovascular Medicine. 2022;23(6):212.
50. Kara AB. Pioglitazone Reduces Oxidative Stress in Kidney Against High Sucrose Diet-Induced Metabolic Syndrome in Rats. Kafkas Journal of Medical Sciences 2018;8(3):165-171.
51. Prasad K. Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed. Circulation. 1999;99(10):1355-1362.
52. Karaca T, Bayıroğlu F, Cemek M, Comba B, Ayaz A, Karaboğa İ. Effects of green tea extract and lactobacillus casei strain shirota on levels of serum minerals, cholesterol, triglycerides, glucose and lactate in rats fed on high-carbohydrate and high-lipid diets. Kafkas Journal of Medical Sciences 2013;(1): 1-7.
53. Kaya I, Deveci H, Ekinci U, Karapehlivan M, Kaya M, Alpay M. The Effect of ellagic acid and sodium fluoride ınтake on total sialic acid levels and total oxidant/antioxidant status in mouse testicular tissue. Annual Research & Review in Biology 2015;7(5):329-335.
54. Duarte MM, Rocha JB, Moresco RN, Duarte T, Da Cruz IB, Loro VL, et al Schetinger, M. R. Association between ischemia-modified albumin, lipids and inflammation biomarkers in patients with hypercholesterolemia. Clinical Biochemistry 2009;42(7-8):666-671.
55. Uetake Y, Ikeda H, Irie R, Tejima K, Matsui H, Ogura S, et al. High-salt in addition to high-fat diet may enhance inflammation and fibrosis in liver steatosis induced by oxidative stress and dyslipidemia in mice. Lipids in Health and Disease 2015;14(1):6.
56. Moghadamnia D, Mokthari M, Khatamsaz S. Comparison of protective effects of omega3 fish oil and aqueous extract of glycyrrhiza glabra root on thioacetamide ınduced lipid toxicity in male rats. Biomedical and Pharmacology Journal 2016; 9(1):111-119.
57. Erdoğan H, Fadıllıoglu E, Özgöçmen S, Söğüt S, Özyurt B, Akyol O, et al. Effect of fish oil supplementation on plasma oxidant/antioxidant status in rats. Prostaglandins, Leukotrienes and Essential Fatty Acids 2004;71(3):149-152.
58. Sarıyer ET, Baş M, Çolak H, Özkan Yenal N, Unay Demirel Ö, Yüksel M. Comparison of dietary supplementation with krill oil, fish oil, and astaxanthin on an experimental ethanol-ınduced gastric ulcer model: a biochemical and histological study. Nutrients. 2024; 16(20):3426.