ORIGINAL ARTICLE

Fish oil rich in ω-3 fatty acid alleviates long-term high fructose consumption-induced fatty liver and kidney dysfunction in rats

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Abstract

Excessive fructose intake leads to fatty liver and kidney dysfunction, with associated inflammation and metabolic disturbances. This study evaluated the effects of fish oil rich in ω-3 fatty acid on these conditions in rats fed a high-fructose diet. Male Sprague Dawley rats were divided into three groups: normal diet, high-fructose (HF) diet, and high-fructose diet with 5% fish oil supplementation. The experiment lasted for 21 weeks. Fish oil supplementation significantly reduced blood levels of total cholesterol, triglycerides, and advanced glycation end products (AGEs) in HF-fed rat. It also improved liver function markers and kidney function indicators. Mechanistically, fish oil suppressed fructokinase expression, activated phosphorylated AMP-activated protein kinase (p-AMPK), and decreased peroxisome proliferator-activated receptor (PPAR)-γ expression, reducing triglyceride synthesis-related proteins in the liver. In the kidneys, fish oil supplementation increased p-AMPK and PPARγ, while decreasing inflammatory and oxidative stress markers and fibrosis-related protein α-smooth muscle actin. Histological analysis confirmed that fish oil alleviated liver degeneration and kidney fibrosis. These findings suggest that fish oil not only helps regulate blood lipid levels but also alleviates fructose-induced liver and kidney damage, suggesting its potential as a dietary intervention for metabolic disorders caused by excessive fructose consumption.

Keywords: AMPK, Fatty liver, Fish oil, High-fructose, Kidney dysfunction

1. Introduction

Fructose is rapidly absorbed from the small intestine into the bloodstream via facilitated diffusion through the fructose-specific glucose transporter 5 (GLUT5), then transported to the liver through the portal vein for metabolism [1,2]. Long-term high fructose diet consumption has been shown to induce insulin resistance and to increase endogenous lipid synthesis and the production of ApoB48 in the intestine, indicating that increased fructose absorption stimulates the formation of chylomicrons [2]. Clinical researches have also confirmed that a high-fructose diet elevates blood glucose, triglycerides, triglyceride-rich lipoproteins, and ApoB48 levels [3,4]. Fructose metabolism involves phosphorylation by fructokinase to form fructose-1-phosphate (F-1-P), which is then broken down by aldolase into glyceraldehyde and dihydroxyacetone phosphate (DHAP). These inter-mediates can convert into glycerol-3-phosphate (G-3-P), which, in turn, reacts with Acetyl-CoA to form Acyl-CoA, enhancing endogenous fat synthesis and the formation of long-chain fatty acids and triglycerides (TG) [2,5]. Therefore, prolonged fructose consumption can lead to excessive TG accumulation in the liver,

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causing lipid metabolism disorders [6]. Studies have also suggested that long-term fructose feeding increases the expression of Sterol Regulatory Element-Binding Protein 1 (SREBP-1), Acetyl-CoA Carboxylase (ACC), Fatty Acid Synthase (FAS), and fatty acid transporter CD36 [7]. Furthermore, fructose metabolism reduces liver ATP levels and activates AMP-activated protein kinase (AMPK), impairing insulin signaling and increasing endogenous lipid synthesis [8–10].

In the kidneys, fructose from the diet is fully filtered through the glomerulus and reabsorbed by the proximal tubular epithelial cells via fructose transporters. Dietary fructose or endogenous fructose in pathological conditions acts on renal tubular epithelial cells, endothelial cells, macrophages, and fibroblasts, leading to inflammation and fibrosis [11]. Chronic fructose intake increases uric acid levels, potentially causing insulin resistance, vascular damage, and glomerular hypertension [12]. Normal rats fed a high-fructose diet develop mild renal tubular interstitial inflammation and fibrosis [13]. Research indicates that, under conditions of excessive fructose intake, some of it enters the kidneys through systemic circulation and is excreted in urine. Urinary fructose excretion increases in a dose-dependent manner with fructose intake, which may contribute to early kidney damage in metabolic syndrome patients [13].

Fish oil contains omega-3 fatty acids, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Studies have shown that omega-3 fatty acids can reduce inflammation, lower plasma TG, and improve obesity [14]. They are also beneficial in treating non-alcoholic fatty liver disease (NAFLD) and chronic kidney disease (CKD) [15,16]. Clinical and experimental studies suggest that fish oil can improve NAFLD and non-alcoholic steatohepatitis [17]. Diets rich in omega-3 polyunsaturated fatty acids (PUFAs) are associated with various hepatic mechanisms, including increased fatty acid oxidation, reduced fatty acid synthase gene expression, and inhibition of endogenous lipid synthesis [14,17,18]. Additionally, omega-3 PUFAs improved adipose tissue uncoupling protein-2 (UCP-2), peroxisome proliferator-activated receptor (PPAR) γ, and oxidative stress, preventing dyslipidemia and insulin resistance in sucrose-rich diet-fed mice [18]. Studies also showed that fish oil decreased Malonyl-CoA levels, suppressing SREBP-1c expression and inhibiting the genes of multiple lipogenic enzymes like ACC and FAS, and reducing liver cholesterol by inhibiting SREBP2 [19,20]. Epidemiological research has linked low omega-3 PUFA intake with increased risk of CKD and decreased creatinine clearance [21]. Other studies indicated that fish oil supplementation in rats improved renal tubular interstitial damage and fibrosis by reducing the expression of transforming growth factor (TGF)-β, α-smooth muscle actin (SMA), phosphorylated (p)-extracellular signal-regulated kinase (ERK)1/2, and inflammatory markers monocyte chemoattractant protein (MCP)-1 and cyclooxygenase (COX)-2, inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, and reducing inflammation [22]. A previous study has found that an eight-week fish oil supplementation improved hepatic lipid profile via activating hepatic AMPKα and PPARα in high-fat diet-fed rats [23].

Fructose is a naturally occurring sugar found in honey, fruits, and certain vegetables. Because it is inexpensive and has a high level of sweetness, fructose is widely used in food production, such as in the form of high-fructose corn syrup, which serves as a sweetener in processed foods and sugary beverages. With the increasing prevalence of processed foods, fructose and other refined sugars are becoming more common in diets, making it crucial to address the adverse effects caused by excessive fructose intake. However, the benefits of fish oil on high fructose-induced fatty liver and kidney injury remain unclear. Therefore, this study aimed to investigate the impact of fish oil supplementation on long-term high fructose consumption-induced fatty liver and kidney damage in male Sprague-Dawley rats.

2. Materials and methods

2.1. Chemicals and reagents

The enzymatic kits for plasma cholesterol (#CH7945), triglyceride (#TR213), AST (#AS1267), ALT (#AL1268), and BUN (#UR8334) were purchased from Randox Laboratories Ltd. (Crumlin, UK). A plasma creatinine enzymatic kit (#SKT-217) was purchased from Eagle Biosciences (Amherst, NH, USA). The enzymatic kits for plasma uric acid (#AD803UA), hepatic triglyceride (#AD801T), and hepatic cholesterol (#AD704CH) were obtained from Audit Diagnostics (Cork, Ireland). TRIzol® Reagent (#15596026) was purchased from Invitrogen (Carlsbad, CA, USA). A Trichrome Masson stain kit (#TASS01) was obtained from BioTnA Biotechnology (Kaohsiung, Taiwan). An OxiSelect™ AGE ELISA kit (#STA-817) was purchased from Cell Biolabs (San Diego, CA, USA). These commercial

kits have been well-established. These kit-based assays were evaluated and validated based on the methods of Eurachem Guide (2025) [24].

Fish oil was supplied by Sentosa Co., Ltd. (Taipei, Taiwan): product code: 5015207; concentration of EPA + DHA: 45–50%; EPA content (mg/g) as TG and FFA: 270–330 and 260–320, respectively; DHA content (mg/g) as TG and FFA: 180–220 and 170–210, respectively (more information was shown in Supplementary Information). This fish oil product has been certified as a heath food by Taiwan FDA, Ministry of Health and Welfare (license number: Wei-Shu-Jian-Shih-Kuei-Tze 000008). The fatty acid composition in fish oil was shown in Supplementary Table S1.

2.2. Experimental animals

Twenty-four male Sprague-Dawley (SD) rats with five-week-old (160–175 mg body weight) were purchased from LASCO Biotechnology Co., Ltd. (Taipei, Taiwan). Animals were housed in a controlled environment with a temperature of 23 ± 1 °C, humidity of 40–60%, and a 12-h light/dark cycle (lights on from 6:00 a.m. to 5:59 p.m.; lights off from 6:00 p.m. to 5:59 a.m.). Rats were provided with standard chow (Laboratory Rodent Diet 5001, PMI Nutrition International, St. Louis, MO, USA) and acclimatized for one week. They were then randomly assigned to three groups (n = 8 per group) based on body weight and fed the following diets: (1) N: normal diet; (2) HF: diet with high-fructose 43.1%; (3) HFF: high-fructose diet supplemented with 5% fish oil. The treatment duration was 21 weeks. The composition of experimental diets is detailed in Table 1.

Both food and water were provided ad libitum. Body weight and food intake were recorded weekly. The experiment lasted for 21 weeks. At the end of the study, rats were anesthetized with CO_2/O_2 (70:30), and blood was drawn from the abdominal aorta using heparinized syringes. Blood samples were centrifuged at 3000 rpm for 20 min to obtain plasma. Liver and kidneys were excised and weighed. Portions of liver and kidney tissues were fixed in 10% formalin, and additional liver samples were frozen in liquid nitrogen for subsequent analysis. Prior to blood and tissue collection, all rats were fasted for 12 h overnight with free access to water to minimize the influence of recent food intake on serum biochemical measurements.

All animal procedures were approved by the Institutional Animal Care and Use Committee of National Taiwan Ocean University (IACUC No. 111032).

Table 1. The composition of experimental diets.

Ingredient (%)	N	HF	HFF
Corn starch	63.8	20	20
Fructose		43.1	43.1
Casein	20	20	20
Lard	5	5	
Soybean oil	1	1	1
Vitamin mixture	1	1	1
Mineral mixture	4	4	4
Cholesterol		0.5	0.5
Choline chloride		0.2	0.2
Cholic acid	0.2	0.2	0.2
Cellulose	5	5	5
Fish oil[a]			5
Total calories (kcal/100g)	389.2	386.4	386.4
Carbohydrate (% kcal)	65.57	65.32	65.32
Protein (% kcal)	20.55	20.70	20.70
Fat (% kcal)	13.87	13.98	13.98
Total (% kcal)	100	100	100

N: Normal diet; HF: High fructose diet; HFF: High fructose diet + 5% fish oil.
[a] Fish oil contains 33% EPA and 22% DHA.

2.3. Plasma cholesterol and triglyceride measurement

Ten microliters of plasma were mixed with 1000 μL of either a cholesterol enzymatic kit (#CH7945, Randox Laboratories Ltd.) or triglyceride enzymatic kit (#TR213, Randox Laboratories Ltd.). After mixing, samples were incubated at 37 °C for 5 min in a water bath (B206, FirstTek Scientific, Taipei, Taiwan). Absorbance was measured at 500 nm using a UV/VIS spectrophotometer (UV/VIS-7800, JASCO International Co., Ltd., Tokyo, Japan). Total cholesterol and triglyceride concentrations were determined by comparison to standard curves.

2.4. Plasma creatinine, blood urea nitrogen (BUN), uric acid, and AGEs measurement

Plasma creatinine levels were analyzed using a creatinine enzymatic kit (#SKT-217, Eagle Biosciences), BUN using a BUN enzymatic kit (#UR8334, Randox Laboratories Ltd.), and uric acid using a uric acid enzymatic kit (#AD803UA, Audit Diagnostics). Reactions were conducted at 37 °C, and absorbance was measured with a spectrophotometer (U-2800A, Hitachi Ltd., Tokyo, Japan). Advanced glycation end-products (AGEs) were quantified using an OxiSelect™ AGE ELISA kit (#STA-817, Cell Biolabs).

2.5. Lipoprotein analysis

Plasma lipoproteins were separated and analyzed as previously described [25]. In brief, 110 μL of

plasma was mixed with 110 μL potassium bromide (KBr). The mixtures were centrifuged in an ultracentrifuge (Himac CP-NX, Hitachi, Japan) at 90,000×*g* and 10 °C for 3 h. HDL-C and VLDL-C + LDL-C concentrations were calculated based on total cholesterol levels measured in the supernatant and pellet using enzymatic kits.

2.6. Plasma AST and ALT activity

One hundred microliters of plasma were incubated with 1000 μL of AST enzymatic kit (#AS1267, Randox Laboratories Ltd.) or ALT enzymatic kit (#AL1268, Randox Laboratories Ltd.). Reactions were performed at room temperature for 1 min, and absorbance at 340 nm was recorded every minute for 3 min using a water bath connected to a spectrophotometer (U-2800A, Hitachi Ltd., Tokyo, Japan). AST and ALT activities were calculated accordingly.

2.7. Hepatic triglyceride and total cholesterol measurement

Hepatic triglyceride and total cholesterol levels were determined as previously described [26]. Briefly, 10 μL of liver extract was mixed with 10 μL Triton X-100 in a glass tube and homogenized. Organic solvents were evaporated using a vacuum concentrator. Triglyceride (#AD801T) and cholesterol (#AD704CH) concentrations were then analyzed using commercial enzymatic kits (Audit Diagnostics).

2.8. Preparation of liver cytosolic fraction and determination of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) activity

A modified method based on Nepokroeff et al. was used [27]. Approximately 0.2 g of liver tissue was homogenized in 0.1 mL potassium phosphate buffer (1:5, v/v; pH 7.4) containing 0.1 M dipotassium phosphate, 1 mM dithiothreitol, and 10 mM nicotinamide. The homogenate was first centrifuged at 12,000×*g* for 15 min at 4 °C. The supernatant was then collected and further centrifuged at 10,500×*g* for 60 min at 4 °C using a Hitachi CF 16RXII centrifuge (Hitachi Koki Co., Ltd., Japan). The resulting supernatant, representing the liver cytosolic fraction, was used for determining the activities of FAS and ACC. ACC activity was assessed following a modified protocol from Numa et al. [28]. Enzymatic activity was monitored at 340 nm and 37 °C for 5 min using a microplate reader (VersaMax, Molecular Devices, San Jose, USA), recording absorbance changes every 30 s. Specific activity was calculated as nmol NADPH consumed per minute per mg protein. FAS activity was measured based on a modified method from Nepokroeff et al. [27]. Absorbance at 340 nm was recorded at 37 °C for 5 min with measurements taken every 30 s using a microplate reader (VersaMax, Molecular Devices, San Jose, USA). The specific enzymatic activity was expressed as nmol NADPH oxidized per minute per mg protein.

2.9. Histological analysis

Liver and kidney tissues were fixed in 10% formalin and embedded in paraffin. Sections (4 μm thick) were cut and stained as follows: (1) Hematoxylin and Eosin (H&E) Staining: Sections were stained with H&E, and hepatic lipid vacuoles and renal damage were visualized using a fluorescence upright microscope (U-LH100HG, BX53, Olympus Co., Japan). (2) Masson's Trichrome Staining: Fibrosis was assessed using a commercial Trichrome Masson stain kit (#TASS01, BioTnA Biotechnology). (3) Immunohistochemistry (IHC): Deparaffinized sections were rehydrated through graded ethanol, washed with PBS, and incubated overnight at 4 °C with primary anti-bodies (1:100 dilution) for phosphorylated (p)-AMPK (#2535, Cell Signaling, Boston, MA), PPARγ (#sc-7273, Santa Cruz, Dallas, TX, USA), p-NF-κB (#SAB5700363, Sigma-Aldrich, St. Louis, MO, USA), α-SMA (#ab7817, Abcam, Cambridge, UK), and inducible nitric oxide synthase (iNOS; #ab15323, Abcam). After washing, sections were incubated with biotinylated secondary antibodies and streptavidin-peroxidase complexes, followed by visualization using an HRP/DAB detection kit (#ab64264, Abcam). Sections were counterstained with hematoxylin and observed under the upright fluorescence microscope.

2.10. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from tissues using TRIzol® Reagent (#15596026, Invitrogen) as described previously [23]. Tissues were homogenized in 500 μL TRIzol® and mixed with 200 μL DEPC-treated water. Samples were centrifuged at 12,000×*g* for 15 min, and the supernatant was combined with an equal volume of isopropanol to precipitate RNA. Pellets were washed with 75% ethanol, air-dried, and dissolved in DEPC-treated water. RNA concentrations were measured using a NanoDrop spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA). Complementary DNA (cDNA) was synthesized and used as a template for PCR amplification.

Primer sequences for fructokinase (ketohexokinase; KHK) and GAPDH control were Forword (5′ to 3′)/ Reverse (5′ to 3′) TTCTCTACGACACGAACCTGCC /CAAACACCACCTCTCCATAGCC and ATGACT CTACCCACGGCAAG/GGAAGATGGTGATGGG TTTC, respectively. Quantitative PCR was performed using SYBR Green Master Mix and a StepOnePlus™ Re-al-Time PCR System (Applied Biosystems®, Foster City, CA, USA).

2.11. Statistical analysis

The experimental data were analyzed and plotted using GraphPad Prism 8.0 (GraphPad Software, Boston, MA, USA). One-way ANOVA was used to compare whether there were any differences among groups. Dunnett's test was used for post hoc analysis. When $p < 0.05$, it indicated that there was a significant difference between the two groups.

3. Results

3.1. Effects of fish oil on body weight, food intake, organ weights, and plasma biochemical parameters in HF-fed rats

During the experimental period, the HF group exhibited a significant increase in body weight compared to the N group starting at week 5, followed by a decline at week 14. The HFF group showed a lower body weight than the HF group from week 3, but surpassed the HF group by week 17; however, in general, there were no statistical significance among these three groups (Fig. 1A). No significant differences were observed among groups in food intake and efficiency, water intake, or urine volume (Table 2).

As shown in Table 3, liver and kidney weights, as well as liver-to-body weight and kidney-to-body weight ratios, were significantly elevated in the HF group compared to the N group. The HFF group demonstrated a significant reduction in the liver-to-body weight ratio compared to the HF group, whereas kidney weights (but not relative kidney weights) were higher in the HFF group relative to the HF group.

Plasma glucose levels were comparable among groups (Table 4). The HF group displayed a significant increase in total cholesterol (TC) relative to the N and HFF groups, while no difference was observed between the N and HFF groups. Triglyceride (TG) levels were significantly lower in both the HF and HFF groups compared to the N group, with no difference between the HF and HFF groups. The HF group showed significantly higher levels of VLDL-C + LDL-C and an increased TC/HDL-C

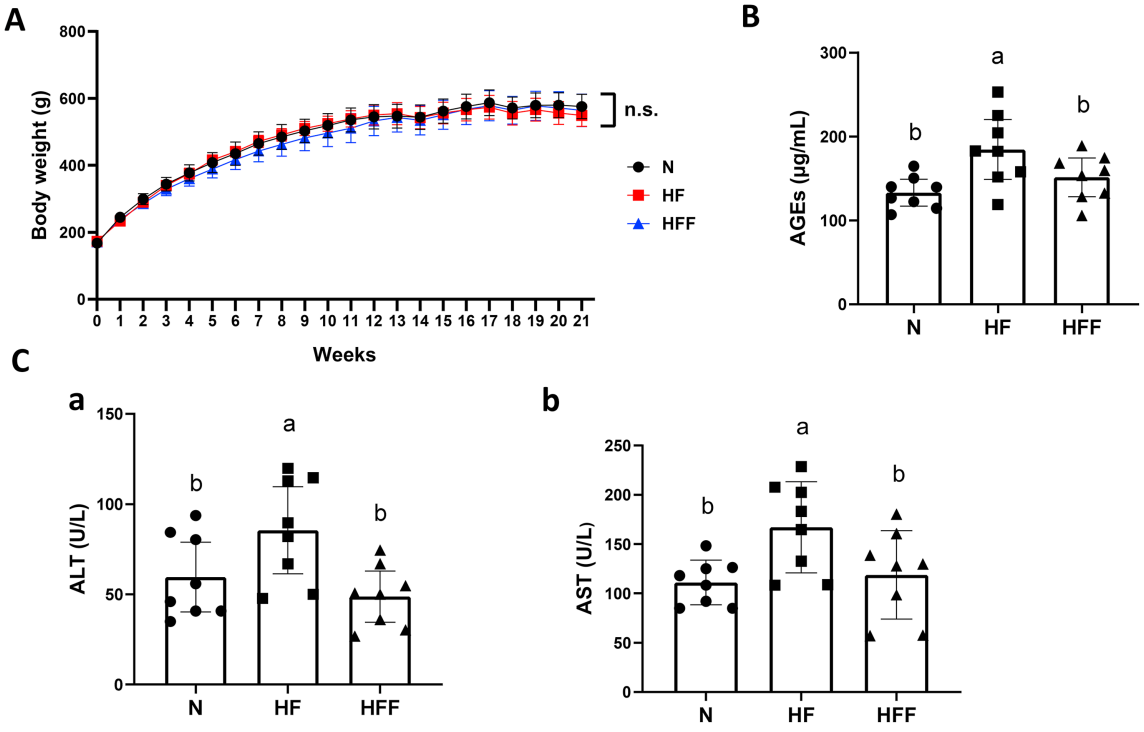

Fig. 1. Effects of fish oil on body weight and plasma biochemical parameters in HF-fed rats. The changes in body weights (A) and plasma levels of AGEs (B), ALT (C-a), and AST (C-b) were shown. Results are expressed as mean with 95% Confidence Interval for each group (n = 8). Different letter indicates statistical significance (p < 0.05). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.

Table 2. The change of body weight, food intake, drinking volume and urine volume in rats fed with different experimental diet after 21 weeks.

Diet	N	HF	HFF
Initial body weight (g)	244.5 ± 15.0 (95% CI: 232.0–257.0) **a**	233.7 ± 13.0 (95% CI: 222.9–244.6) **a**	238.0 ± 12.5 (95% CI: 227.6–248.5) **a**
Final body weight (g)	575.7 ± 43.4 (95% CI: 539.4–612.0) **a**	549.3 ± 39.5 (95% CI: 516.3–582.3) **a**	564.0 ± 57.8 (95% CI: 515.7–612.3) **a**
Body weight gain (g)	331.2 ± 35.1 (95% CI: 301.8–360.6) **a**	315.6 ± 38.2 (95% CI: 283.6–347.5) **a**	326.0 ± 51.2 (95% CI: 283.1–368.8) **a**
Food intake (g/day)	21.9 ± 2.1 (95% CI: 20.1–23.6) **a**	22.5 ± 4.0 (95% CI: 19.2–25.9) **a**	21.2 ± 3.2 (95% CI:18.6–23.9) **a**
Feed efficiency (%)[1]	6.6 ± 0.5 (95% CI: 6.2–7.0) **a**	7.3 ± 1.8 (95% CI: 5.8–8.7) **a**	6.6 ± 1.1 (95% CI: 5.7–7.5) **a**
Drinking water (mL/day)	21.8 ± 4.2 (95% CI: 18.4–25.3) **a**	30.7 ± 9.0 (95% CI: 23.1–38.2) **a**	27.4 ± 13.6 (95% CI: 16.0–38.7) **a**
Urine volume (mL/day)	12.9 ± 2.1 (95% CI: 11.2–14.7) **a**	21.1 ± 7.9 (95% CI: 14.5–27.7) **a**	18.2 ± 8.7 (95% CI: 11.0–25.5) **a**

Results are expressed as mean ± S.D. and 95% Confidence Interval for each group (n = 8). Different letter (e.g. a vs b) indicates statistical significance ($p < 0.05$). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.
[1] Feed efficiency (%): [food intake (g/day)/body weight gain (g)] × 100%.

Table 3. The change of tissue weight in rats fed with different experimental diet after 21 weeks.

Diet	N	HF	HFF
Liver weight (g)	14.8 ± 1.7 (95% CI: 13.4–16.2) **a**	33.7 ± 4.3 (95% CI: 30.0–37.3) **b**	29.9 ± 3.2 (95% CI: 27.3–32.6) **b**
Relative liver weight (g/100 g B.W.)	2.6 ± 0.2 (95% CI: 2.4–2.8) **a**	6.1 ± 0.7 (95% CI: 5.5–6.7) **b**	5.1 ± 0.3 (95% CI: 4.8–5.4) **c**
Kidney weight (g)	2.9 ± 0.1 (95% CI: 2.8–3.0) **a**	3.3 ± 0.2 (95% CI: 3.1–3.4) **b**	3.7 ± 0.3 (95% CI: 3.5–4.0) **c**
Relative kidney weight (g/100 g B.W.)	0.51 ± 0.03 (95% CI: 0.48–0.53) **a**	0.60 ± 0.04 (95% CI: 0.56–0.63) **b**	0.63 ± 0.05 (95% CI: 0.59–0.67) **b**

Results are expressed as mean ± S.D. and 95% Confidence Interval for each group (n = 8). Different letter (e.g. a vs b or a vs c or b vs c) indicates statistical significance ($p < 0.05$). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.

Table 4. The change of plasma lipids and lipoproteins in normal and high fructose rats fed fish oil for 21 weeks.

Diet	N	HF	HFF
Glucose (mg/dL)	214.5 ± 16.4 (95% CI: 200.7–228.2) **a**	216.6 ± 31.7 (95% CI: 190.1–243.1) **a**	219.4 ± 18.0 (95% CI: 204.3–234.4) **a**
Total cholesterol (mg/dL)	157.7 ± 34.8 (95% CI: 128.6–186.8) **a**	324.5 ± 67.9 (95% CI: 267.8–381.3) **b**	130.7 ± 25.4 (95% CI: 109.4–151.9) **a**
Triglyceride (mg/dL)	112.5 ± 18.3 (95% CI: 97.1–127.8) **a**	59.6 ± 13.5 (95% CI: 48.3–70.8) **b**	42.8 ± 7.5 (95% CI: 36.5–49.1) **b**
HDL-C (mg/dL)	104.5 ± 18.4 (95% CI: 89.1–119.9) **a**	61.8 ± 13.5 (95% CI: 50.6–73.1) **b**	44.6 ± 6.0 (95% CI: 39.7–49.6) **c**
VLDL-C + LDL-C (mg/dL)	58.1 ± 18.3 (95% CI: 42.8–73.4) **a**	239.7 ± 63.2 (95% CI: 186.9–292.5) **b**	71.1 ± 16.6 (95% CI: 57.2–84.9) **a**
TC/HDL-C ratio	1.57 ± 0.15 (95% CI: 1.45–1.70) **a**	4.58 ± 0.94 (95% CI: 3.80–5.37) **b**	2.54 ± 0.38 (95% CI: 2.22–2.86) **c**
HDL-C/VLDL-C + LDL-C ratio	1.86 ± 0.51 (95% CI: 1.44–2.29) **a**	0.30 ± 0.09 (95% CI: 0.23–0.37) **b**	0.69 ± 0.18 (95% CI: 0.53–0.84) **b**

Results are expressed as mean ± S.D. and 95% Confidence Interval for each group (n = 8). Different letter (e.g. a vs b or a vs c or b vs c) indicates statistical significance ($p < 0.05$). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.

ratio relative to the N and HFF groups. Notably, supplementation with fish oil in the HFF group resulted in marked improvements in these parameters, although HDL-C concentrations were significantly reduced.

As shown in Table 5, plasma creatinine levels did not differ significantly among groups. Uric acid levels were elevated in the HF group compared to the N group, though the difference was not statistically significant. However, fish oil supplementation in the HFF group significantly lowered uric acid levels. Blood urea nitrogen (BUN) was significantly reduced in the HFF group relative to the HF group, with no differences observed between the N group and either the HF or HFF groups.

Plasma advanced glycation end-products (AGEs) were significantly elevated in the HF group compared to the N group and were markedly reduced in the HFF group following fish oil supplementation (Fig. 1B). The HF group exhibited significantly increased ALT and AST activities compared to the N group, indicating hepatic injury. Fish oil

Table 5. The change of plasma creatinine, BUN and uric acid in rats fed with different experimental diet after 21 weeks.

Diet	N	HF	HFF
Creatinine (mg/dL)	0.47 ± 0.06 (95% CI: 0.42–0.52) **a**	0.49 ± 0.04 (95% CI: 0.45–0.52) **a**	0.50 ± 0.10 (95% CI: 0.41–0.58) **a**
BUN (mg/dL)	50.5 ± 46.8 (95% CI: 11.3–89.7) **ab**	94.3 ± 83.1 (95% CI: 24.9–163.7) **a**	16.8 ± 3.9 (95% CI: 13.6–20.1) **b**
Uric acid (mg/dL)	2.17 ± 0.45 (95% CI: 1.80–2.54) **ab**	2.72 ± 0.45 (95% CI: 2.34–3.09) **a**	2.11 ± 0.46 (95% CI: 1.72–2.50) **b**

Results are expressed as mean ± S.D. and 95% Confidence Interval for each group (n = 8). Different letter (e.g. a vs b) indicates statistical significance ($p < 0.05$). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.

supplementation in the HFF group significantly attenuated these elevations, restoring enzyme activities toward levels observed in the N group (Fig. 1C).

3.2. Effects of fish oil on changes in hepatic indicators and histological analysis in HF-fed rats

As shown in Fig. 2A and B, hepatic TC and TG levels were markedly increased in the HF group, whereas these parameters were significantly decreased by fish oil supplementation in the HFF group.

Hepatic ACC activity was significantly elevated in the HF group relative to the N group (Fig. 2C), and FAS activity showed an increasing trend (Fig. 2D). In contrast, the HFF group exhibited significantly reduced ACC and FAS activities compared to the HF group.

Histological examination of H&E-stained liver sections revealed substantial lipid accumulation in the HF group, as reflected by significantly larger vacuolated areas relative to the N and HFF groups. The HFF group showed significantly reduced lipid ac-cumulation compared to the HF group, though levels remained higher than in the N group (Fig. 3A). Moreover, hepatic fructokinase (ketohexokinase) mRNA expression was significantly upregulated in the HF group compared to the N group, but was significantly downregulated in the HFF group (Fig. 3B).

Immunohistochemical staining for hepatic phosphorylated (p)-AMPK demonstrated significantly decreased expression in the HF group compared to the N and HFF groups (Fig. 4A). The HFF group showed significantly higher p-AMPK levels than both the N and HF groups. Hepatic PPARγ expression was elevated in the HF group relative to the N and HFF groups, with no significant difference between the N and HFF groups (Fig. 4B). In

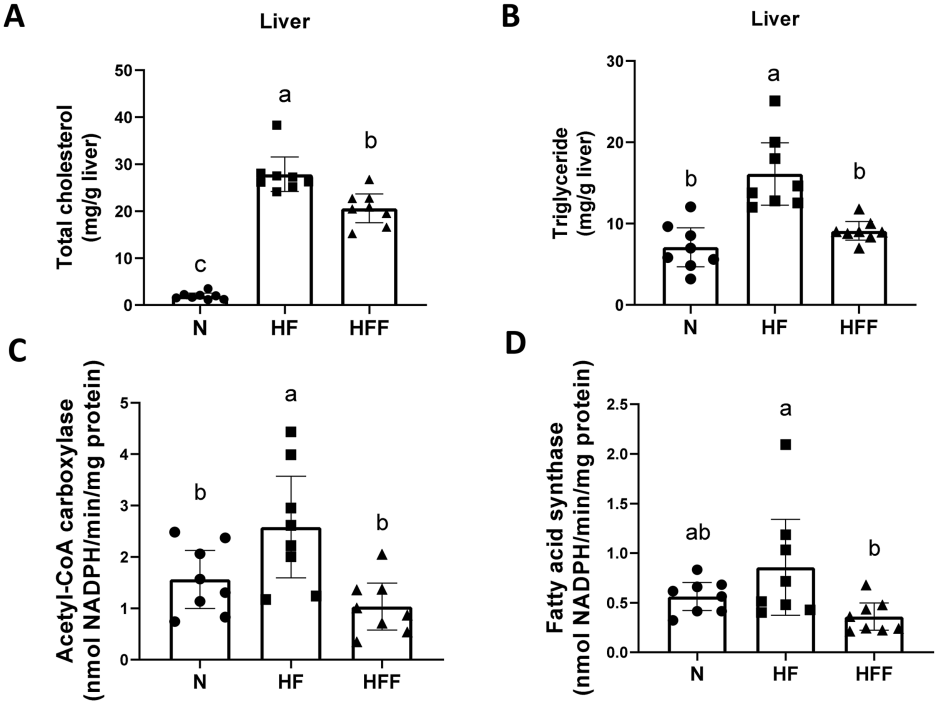

Fig. 2. Effects of fish oil on changes in hepatic indicators in HF-fed rats. The changes in hepatic TC (A) and TG (B) levels and ACC (C) and FAS (D) activities in HF-fed rats. Results are expressed as mean with 95% Confidence Interval for each group (n = 8). Different letter indicates statistical significance ($p < 0.05$). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.

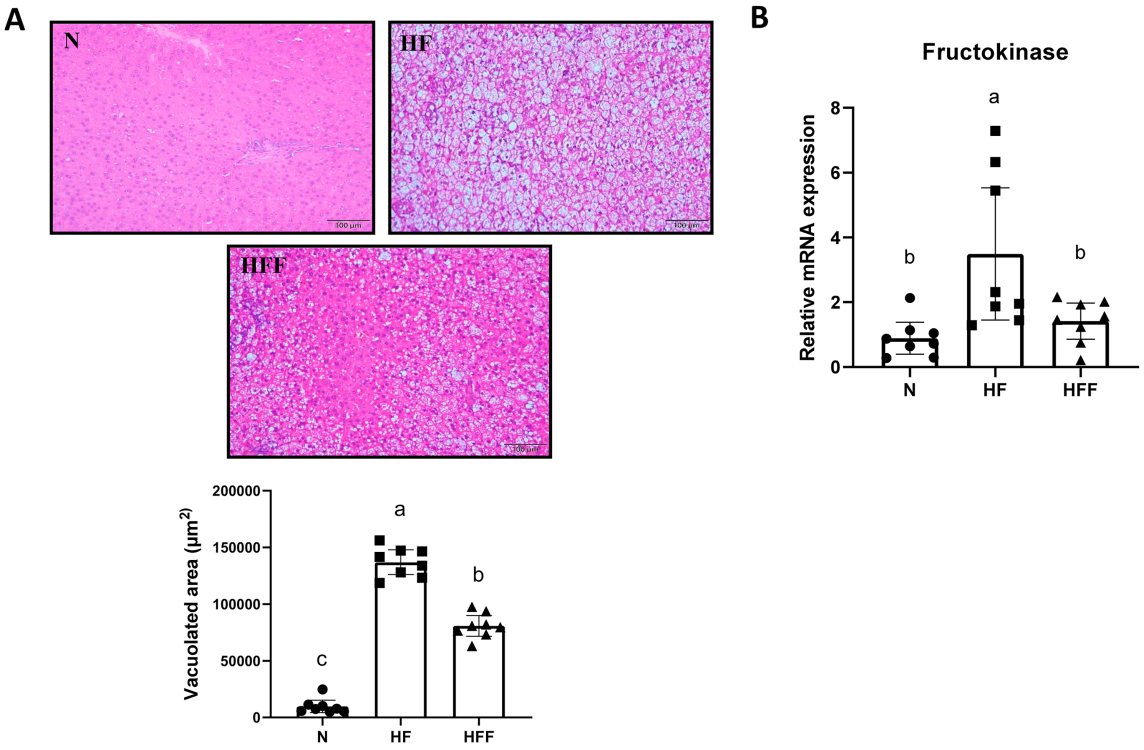

Fig. 3. Effects of fish oil on changes in hepatic histology and fructokinase mRNA expression in HF-fed rats. The changes in hepatic histologically vacuolated areas (A) and fructokinase mRNA expression (B) were shown. Results are expressed as mean with 95% Confidence Interval for each group (n = 8). Different letter indicates statistical significance (p < 0.05). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.

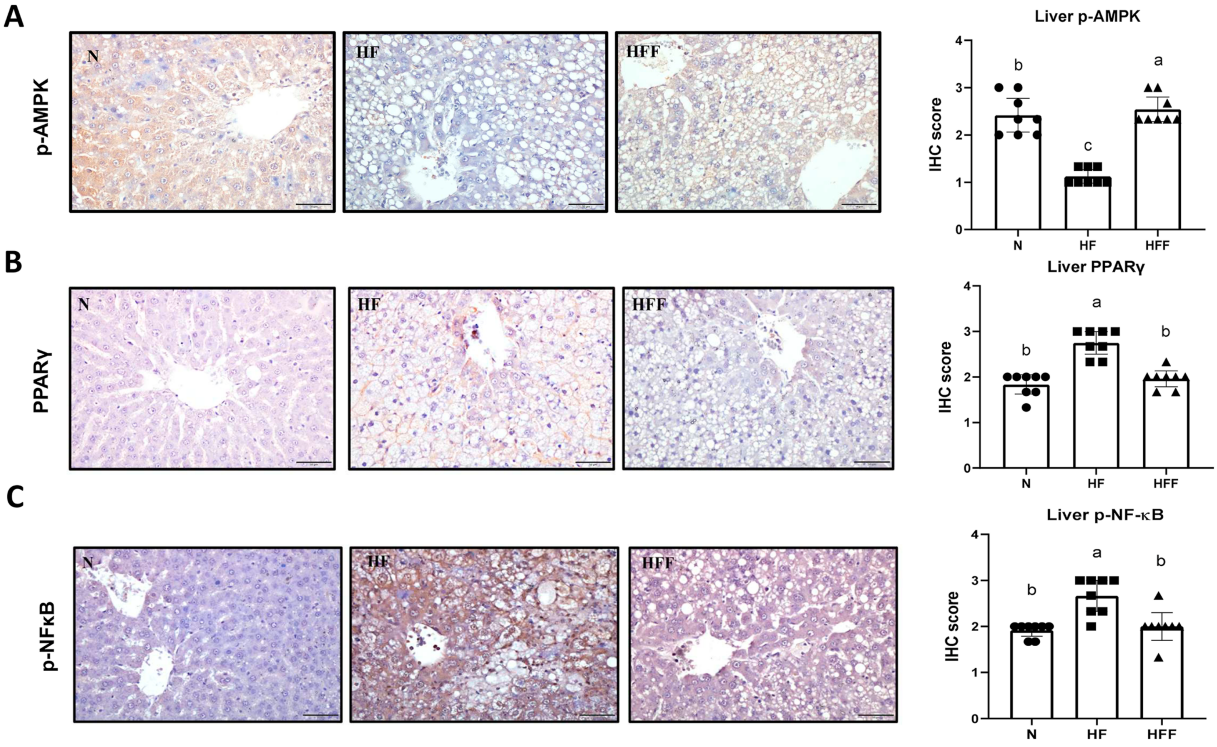

Fig. 4. Effects of fish oil on changes in hepatic immunohistochemistry for p-AMPK, PPARγ, and p-NF-κB expression in HF-fed rats. The changes in immunohistochemical stains for p-AMPK (A), PPARγ (B), and p-NF-κB (C) expression were shown. Results are expressed as mean with 95% Confidence Interval for each group (n = 8). Different letter indicates statistical significance (p < 0.05). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.

contrast, hepatic p-NFκB expression was increased in the HF group compared to the N and HFF groups, while no difference was observed be-tween the N and HFF groups (Fig. 4C).

3.3. Effects of fish oil on renal histopathological changes in HF-fed rats

As shown in Fig. 5A, H&E staining revealed pronounced renal damage in the HF group compared to the N and HFF groups, with no significant difference between the N and HFF groups. Moreover, Masson's trichrome staining showed increased renal fibrosis in the HF group relative to the N and HFF groups (Fig. 5B).

We next investigated the changes of signaling molecules in the kidneys using immunohistochemistry. Renal α-smooth muscle actin (α-SMA) expression was significantly higher in the HF group than in the N and HFF groups, with no difference observed between the N and HFF groups (Fig. 6A). Immunohistochemical staining for renal p-AMPK revealed markedly increased expression in the HFF group compared to both the N and HF groups (Fig. 6B). Similarly, renal PPARγ expression was elevated in the HFF group relative to the N and HF groups (Fig. 6C). Renal p-NF-κB expression was significantly higher in the HF group than in the N and HFF groups (Fig. 6D). Renal iNOS expression was also elevated in the HF group compared to the N and HFF groups, with no significant difference between the N and HFF groups (Fig. 6E).

4. Discussion

In the present study, no significant differences in body weight were observed among groups throughout the experimental period. This finding aligns with some previous studies such as Liu et al. [29]. Liu et al. reported significant weight gain in rats fed a fructose diet [30]. Conversely, Tranchida et al. found that rats fed a 61.7% fructose diet for 30 weeks exhibited a decline in body weight [31]. These discrepancies suggest that the effects of high-fructose intake on body weight may not follow a linear relationship but could depend on factors such as feeding duration, metabolic adaptations, and fructose dosage. Moreover, Benado et al. proposed that high-fructose consumption may increase energy intake [32], yet body weight gain is often lower than expected, potentially due to fructose's thermogenic effects. Fructose metabolism promotes gluconeogenesis and de novo lipogenesis, processes that demand substantial ATP, which subsequently stimulates adenosine monophosphate (AMP) deaminase (AMPD) activation to enhance uric acid levels, and may elevate resting energy expenditure [33]. This metabolic energy loss could partly explain the absence of significant weight gain in the high-

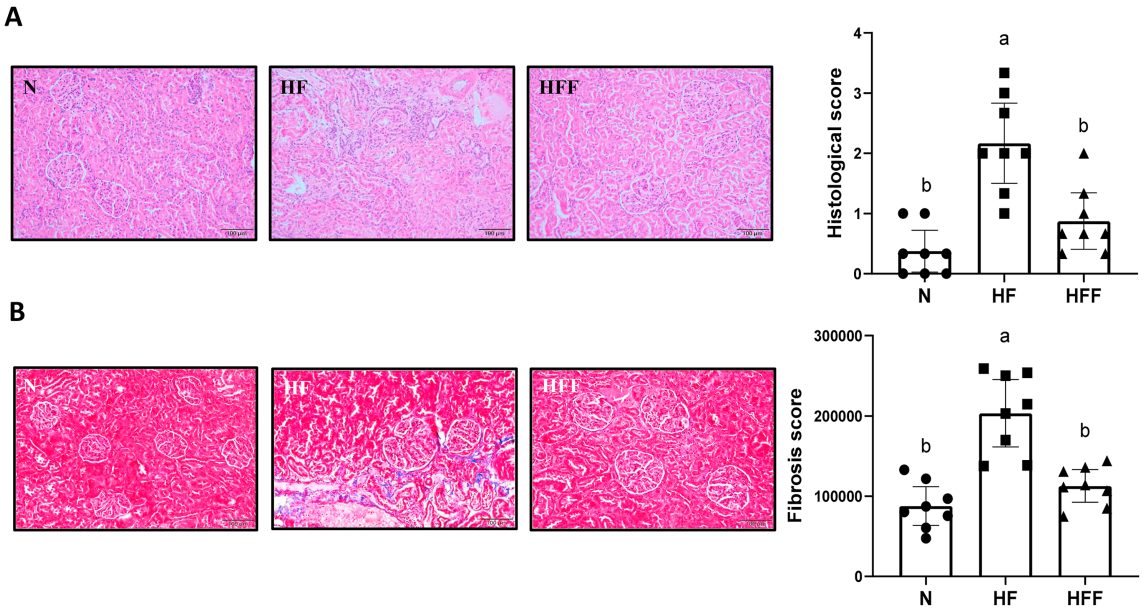

Fig. 5. Effects of fish oil on changes in renal histology and fibrosis in HF-fed rats. The changes in H&E staining for renal histology (A) and Masson's trichrome staining for renal fibrosis (B) were shown. Results are expressed as mean with 95% Confidence Interval for each group (n = 8). Different letter indicates statistical significance (p < 0.05). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.

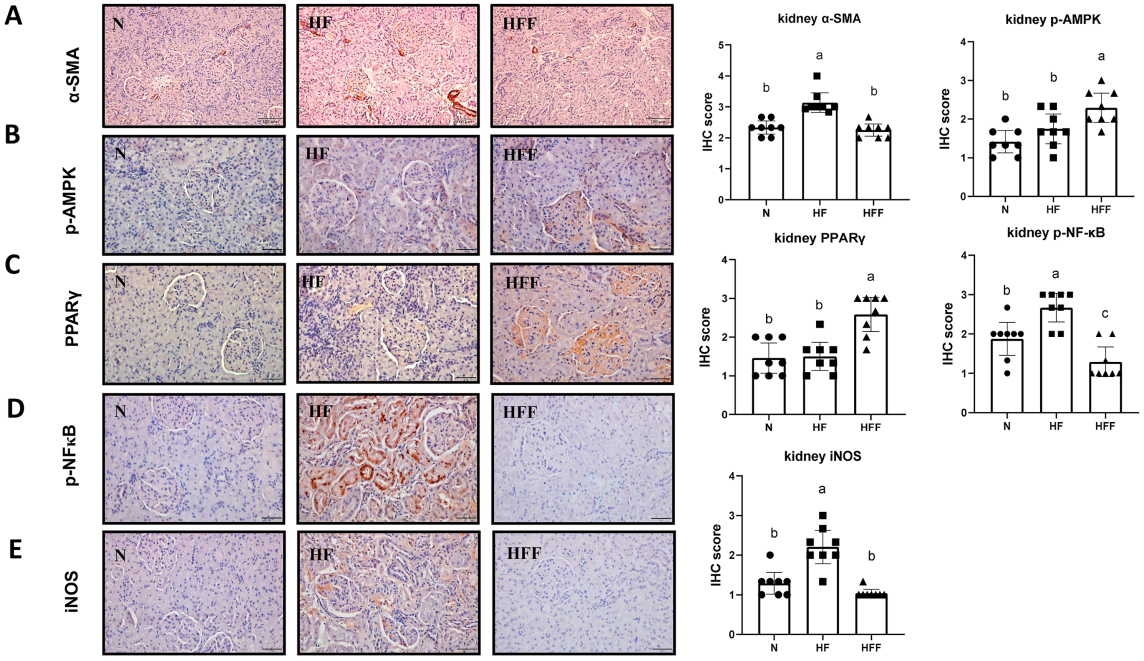

Fig. 6. Effects of fish oil on changes in renal immunohistochemistry for α-SMA, p-AMPK, PPARγ, p-NF-κB, and iNOS expression in HF-fed rats. The changes in immunohistochemical stains for α-SMA (A), p-AMPK (B), PPARγ (C), p-NF-κB (D), and iNOS (E) expression were shown. Results are expressed as mean with 95% Confidence Interval for each group (n = 8). Different letter indicates statistical significance (p < 0.05). N: Normal group. HF: High fructose group. HFF: High fructose + fish oil group.

fructose group in our study. Interestingly, the group receiving fish oil supple-mentation showed no significant changes in body weight or metabolic parameters compared to the high-fructose group, indicating that under the present experimental conditions, fish oil had no notable regulatory effect on fructose-induced metabolic alterations.

In the current study, our primary objective was to evaluate whether fish oil supplementation could ameliorate metabolic and organ dysfunctions induced by excessive fructose intake, rather than to assess its effects under normal conditions. Moreover, according to literature reports, studies have shown that feeding rats a normal (control) diet supplemented with higher concentrations of fish oil (25% and 75% of fat replaced by fish oil) compared with a control diet alone results in no significant differences in most measured parameters [34,35]. In the present study, we used only 5% fish oil in diet, so we hypothesized that 5% fish oil alone would not affect normal physiological functions in normal rats. Therefore, based on the aforementioned factors and the 3Rs principle of experimental animal use, which aims to minimize animal use, this study did not include a control diet +5% fish oil group. However, although the present study primarily aimed to evaluate whether fish oil supplementation could ameliorate metabolic and organ dysfunctions induced by excessive fructose intake, the absence of a normal diet + fish oil group may limit the ability to distinguish the direct physiological effects of fish oil from its protective role under metabolic stress. This is acknowledged as a limitation of the study. Future investigations including a normal diet + fish oil group are warranted to clarify the baseline metabolic influence of fish oil and to confirm that the observed benefits are specifically attributable to the mitigation of fructose-induced metabolic alterations.

Although the present study demonstrated the beneficial effects of fish oil supplementation during a 21-week experimental period, the relatively limited duration restricts our ability to evaluate the long-term efficacy and safety of continuous fish oil intake. The primary focus of this research was to investigate the protective role of fish oil against high-fructose-induced metabolic disturbances, rather than to assess its chronic metabolic, hepatic, or renal safety. Nevertheless, prolonged administration may lead to additional physiological adaptations or potential adverse effects that were not captured within the current experimental timeframe. Future studies extending the intervention period to 6 months or longer are warranted to further evaluate the long-term metabolic impact and systemic safety profile of fish oil supplementation.

The composition and source of fish oil can substantially influence its biological activity and

metabolic effects. Differences in EPA/DHA ratio, degree of triglyceride versus free fatty acid content, and purification process may all contribute to variable physiological responses. For example, fish oil preparations with a higher EPA proportion have been shown to more effectively reduce hepatic lipogenesis, triglyceride accumulation, and systemic inflammation, whereas those with a higher DHA content tend to enhance membrane fluidity and improve insulin sensitivity through modulation of PPARγ and adiponectin signaling pathways [36,37]. In this study, we used a commercially standardized fish oil (Sentosa), which has been certified as a health food by the Taiwan FDA, with a total EPA + DHA concentration of 45–50%. While this ensured compositional consistency, we acknowledge that fish oils derived from other marine sources or with different EPA/DHA ratios might exhibit distinct metabolic outcomes. Future comparative studies will therefore investigate whether these compositional differences modulate the efficacy of fish oil in ameliorating fructose-induced metabolic disturbances.

In contrast, high-fructose feeding significantly affected liver and kidney weights. The HF group exhibited elevated liver weight and liver-to-body weight ratios, suggesting hepatic lipid accumulation. This finding aligns with de Castro et al. who demonstrated that high fructose intake enhances the production of pyruvate and glycerol-3-phosphate, further stimulating de novo lipogenesis and upregulating lipo-genic enzymes [38]. Consequently, cholesterol and fatty acid synthesis, secretion, and deposition in hepatocytes increase, leading to hepatomegaly. For the kidneys, the HF group displayed significantly higher kidney weight and kidney-to-body weight ratios compared to the N group. This may reflect fructose-induced renal hypertrophy. Kretowicz et al. suggested that fructose metabolism elevates uric acid production, which can cause afferent arteriolar injury, glomerular hypertension, and structural renal changes [39]. Additionally, fructose filtered into urine and reabsorbed in the S3 segment of renal tubules may locally increase uric acid levels, driving oxidative stress and inflammation, contributing to renal enlargement. Notably, fish oil supplementation significantly reduced the liver-to-body weight ratio in the HFF group, suggesting an ameliorative effect on hepatic lipid accumulation. However, no significant changes in kidney tissue weights were observed, indicating potential organ-specific effects of fish oil.

Although high-fructose diets are typically associated with hypertriglyceridemia, the HF group in this study paradoxically exhibited significantly lower plasma triglyceride levels than the N group. This might be due to hepatic triglyceride accumulation limiting further VLDL-TG secretion. Additionally, the HF group had increased VLDL-C + LDL-C levels and reduced HDL-C levels. Kaser et al. reported that suppressed lipoprotein lipase (LPL) activity promotes cholesterol ester transfer from HDL to VLDL, increasing triglyceride content in HDL particles and consequently lowering HDL-C concentrations [40]. Interestingly, plasma triglyceride levels in the HF group were lower than those in the control group, which differs from the typical hypertriglyceridemic response reported for high-fructose diets. However, a systematic review and meta-analysis by Evans et al. indicated that chronic fructose substitution for glucose or sucrose has little to no effect on fasting triglyceride levels, suggesting that the lipid response to fructose varies depending on dose, duration, and metabolic context [41]. Moreover, a previous study has shown that prolonged fructose exposure can induce hepatic triglyceride retention and adaptive regulation of VLDL secretion, leading to lower circulating TG concentrations despite increased hepatic lipogenesis [42]. These findings suggest that the TG reduction observed in this study likely reflects hepatic lipid retention and metabolic adaptation rather than a contradictory response. Fish oil supplementation markedly reduced plasma TC and TG levels, and significantly decreased VLDL-C + LDL-C concentrations, suggesting an important regulatory role in lipid metabolism. Fish oil may influence VLDL dynamics via multiple mechanisms, including reduced VLDL-TG synthesis and secretion, altered apoB secretion, diminished triglyceride transport leading to smaller VLDL particles, and enhanced VLDL clearance [43]. These changes could promote VLDL conversion to LDL, improving plasma lipid profiles. Although previous studies have reported minimal effects of fish oil on LPL activity [43], our findings support its efficacy in reducing TG and TC levels. Pitha et al. proposed that ω-3 fatty acids could reduce the concentrations of TG and LDL-C by enhancing lipolysis, reducing hepatic lipid synthesis and VLDL production [44]. However, fish oil also reduced HDL-C concentrations, potentially through inhibition of cholesteryl ester transfer protein (CETP) activity and decreased cholesterol content in HDL particles [45], further influenced by reduced TG levels.

Approximately 70% of fructose is metabolized in the liver. Fructose-rich diets promote de novo lipogenesis, hepatic triglyceride accumulation, and progression to non-alcoholic fatty liver disease

(NAFLD) and non-alcoholic steatohepatitis (NASH) [1,5,6]. Ketohexokinase (KHK; fructokinase), particularly the KHK-C isoform, plays a central role in hepatic fructose metabolism. KHK-C rapidly phosphorylates fructose to fructose-1-phosphate without significant negative feedback, depleting ATP and generating AMP, which activates AMPD, lowers intra-cellular AMP levels, and suppresses AMPK activation [1,8,33]. Muriel et al. demonstrated that KHK knockout mice fail to upregulate lipogenic enzymes such as ACC and FAS under high-fructose feeding, underscoring KHK's role in hepatic lipid biosynthesis [6]. In our study, fish oil supplementation reduced hepatic TC and TG levels, consistent with its reported ability to induce hepatic Cyp27a1 expression and activate Abcg5/Abcg8 through the LXRα pathway, promoting cholesterol excretion [46]. Furthermore, we also found that fish oil significantly downregulated hepatic KHK mRNA expression, potentially preserving AMP levels and activating p-AMPK. Activated AMPK is known to suppress PPARγ and downstream lipogenic enzymes, including ACC and FAS, there-by mitigating hepatic lipid accumulation. While the present study highlights the potential involvement of the AMPK-PPARγ signaling axis in mediating the protective effects of fish oil against fructose-induced metabolic dysfunction, the absence of direct measurements of phosphorylation levels or downstream target proteins limits the mechanistic interpretation. Future investigations will incorporate Western blot analyses to evaluate the phosphorylation status of AMPK and PPARγ, as well as their downstream effectors such as ACC, CPT1, and SREBP-1c. These studies will help elucidate how fish oil regulates lipid metabolism and energy homeostasis at the molecular level and provide stronger mechanistic evidence for its protective role against fructose-induced metabolic disturbances.

Histological analyses confirmed reduced lipid droplet accumulation in the HFF group compared to the HF group, supporting fish oil's beneficial effect on fructose-induced hepatic steatosis. Elevated plasma ALT and AST activities in the HF group reflected hepatic injury, which was significantly attenuated in the HFF group. These findings align with reports demonstrating that omega-3 polyunsaturated fatty acids reduce hepatic steatosis, fibrosis, and inflammation [47].

Fructose overconsumption is known to increase plasma uric acid levels, contributing to hyperuricemia, a recognized risk factor for metabolic, cardiovascular, and renal diseases [48]. In our study, the HF group showed trends toward increased uric acid and BUN levels, which were significantly improved by fish oil supplementation. AGEs accumulation is another consequence of high-fructose feeding [49]. AGEs interact with their receptor (RAGE) to activate NF-κB signaling, triggering proinflammatory cytokine production and ROS generation, contributing to renal fibrosis, glomerulosclerosis, and ultimately renal dysfunction [50]. AMPK dysregulation has been implicated in obesity- and diabetes-related kidney disease. LKB1, a major upstream kinase of AMPK, plays a key role in renal tubular epithelial physiology. LKB1 deficiency leads to tubular dedifferentiation, fibrosis, and inflammation, processes mitigated by AMPK activation. Studies have shown that AMPK activators alleviate diabetic nephropathy and high-fat diet-induced renal injury [51]. Moreover, PPARγ is highly expressed in renal medullary collecting ducts and urothelial cells. PPARγ activation exerts renoprotective effects by inhibiting mesangial cell proliferation and apoptosis, suppressing NF-κB activation and proinflammatory cytokine production, and attenuating TGF-β/SMAD signaling involved in renal fibrosis [52]. In this study, fish oil supplementation reduced plasma and renal AGEs, enhanced AMPK and PPARγ expression, and lowered NF-κB and iNOS levels. These changes were accompanied by reduced α-SMA expression and improved histological indicators of renal tubular degeneration and fibrosis. These findings suggest that fish oil may exert renoprotective effects via AMPK activation, ultimately suppressing inflammation and fibrosis.

5. Conclusion

Collectively, our findings indicate that fish oil supplementation alleviates high-fructose diet-induced dyslipidemia, hepatic steatosis, and renal dysfunction, highlighting its potential as a dietary intervention to mitigate metabolic and organ-specific complications.

CRediT authorship contribution statement

Conceptualization, M.-T.C. and S.-H.L.; methodology, T.-Y.C., R.-W.H., M.-T.C. and S.-H.L.; formal analysis, R.-W.H.; acquisition of data, R.-W.H.; resources, M.-T.C. and S.-H.L.; writing—original draft preparation, T.-Y.C. and S.-H.L.; writing—review and editing, M.-T.C. and S.-H.L.; project administration, M.-T.C.; funding acquisition, M.-T.C.; supervision, M.-T.C. and S.-H.L. All authors have

read and agreed to the final manuscript. All authors have read and agreed to the published version of the manuscript.

Data availability

The data presented in this study are available from the corresponding author upon reasonable request.

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Conflicts of interest

The authors declare no conflicts of interest.

Supplementary material

Table S1. Fatty acid composition in fish oil.

Fatty acid	Fish oil (%)
C14:0 (SFA)	2.27
C16:0 (SFA)	5.58
C18:0 (SFA)	3.11
C20:0 (SFA)	1.54
C22:0 (SFA)	1.95
C16:1 (MUFA)	3.02
C18:1 (MUFA)	8.13
C18:2 (PUFA)	0.76
C18:3 (PUFA)	2.11
C20:3 (PUFA)	1.62
C20:4 (PUFA)	3.30
C20:5 (PUFA)	30.58
C22:6 (PUFA)	19.18

SFA, Saturated fatty acid.
MUFA, Monounsaturated fatty acid.
PUFA, Polyunsaturated fatty acid.
As previously described by Chiu et al. [23].

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