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The administration of hepatocyte growth factor prevents total parenteral nutrition-induced hepatocellular injury in a rat model

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Abstract

Purpose: Total parenteral nutrition (TPN) sometimes induces parenteral nutrition-associated liver disease (PNALD). Hepatocyte growth factor (HGF) acts as a potent hepatocyte mitogen anti-inflammatory and antioxidant actions. We aimed to evaluate the effect of HGF on PNALD in a rat model of TPN.

Methods: A catheter was placed in the right jugular vein for seven-day continuous TPN. All rats were divided into 3 groups: TPN alone (TPN group), TPN plus intravenous HGF at 0.3 mg/kg/day [TPN+HGF (Low) group], and TPN plus HGF at 1.0 mg/kg/day [TPN+HGF (High) group]. On day 7, livers were harvested and the histology, inflammatory cytokines and apoptosis were evaluated.

Results: Histologically, lipid droplets were apparent in the TPN group, but decreased in the TPN+HGF (Low) and TPN+HGF (High) groups. The histological nonalcoholic fatty liver disease activity scores in the TPN+HGF (Low) and TPN+HGF (High) groups were significantly lower than that in the TPN group ($p<0.01$). There were no significant differences in the inflammatory cytokine levels of the three groups. The caspase-9 expression levels in the TPN+HGF (Low) and TPN+HGF (High) groups were significantly decreased in comparison to that in the control group ($p<0.05$).

Conclusion: The intravenous administration of HGF attenuated hepatic steatosis induced by 7-day TPN dose-dependently.

Key words: Total parenteral nutrition, Steatosis, Hepatocyte growth factor, PNALD, NAFLD

Introduction

Recently, total parenteral nutrition (TPN) has come to be widely used in the treatment of nutritional disorder [1]. Pediatric patients with gastrointestinal disease causing intestinal failure also require long-term TPN and fasting. However, long-term TPN leads to hepatic complications, such as steatosis, cholestasis, cholelithiasis, and hepatic fibrosis, which are known as the spectrum of parenteral nutrition-associated liver disease (PNALD) [2,3]. Two-thirds of patients with intestinal failure will develop PNALD, and traditionally, 25% would advance to end-stage liver disease. Thus, while the long-term survival rate is 70-90%, PNALD is a feared and life-threatening complication associated with TPN [3]. Evidence that progressive hepatic complications develop in pediatric patients requiring long-term TPN raises a serious dilemma for both pediatric patients and surgeons; thus, established methods of prevention or treatment for PNALD would improve the quality of life of pediatric patients [2,3].

Previously, we used a TPN with small bowel resection (SBR) rat model to study intestinal failure-associated liver disease (IFALD). In our previous studies, fasting rats underwent both TPN and SBR and a histological examination revealed hepatic steatosis and inflammation, as it is called non-alcoholic liver disease (NAFLD). We reported that the exogenous administration of ghrelin, fish oil lipid emulsion, or glucagon-like peptide-2, which have anti-inflammatory effects, were effective for treating or attenuating NAFLD [4-6]. However, although there are few reports on the use of animal models of TPN, the animals

reported by other institutions, which 14-day TPN therapy without bowel resection, showed liver injury, such as hepatic steatosis and cholestasis [7]. We previously had studied using a rat model of 7-day TPN without SBR to evaluate intestinal mucosal atrophy [8]; however, this model has not been used to study liver injury-associated parenteral nutrition. The present study is our first report using an animal with induced PNALD.

Hepatocyte growth factor (HGF) was first purified and isolated as a potent hepatocyte mitogen from the plasma of patients with fulminant hepatic failure [9]. Numerous studies have reported that HGF includes physiologically active peptides with multiple functions such as anti-inflammation, tissue repair, and anti-apoptosis [10-13]. Previously, HGF has been reported to improve liver damage in alcoholic fatty liver and NAFLD in oral high fat diet intake model rats [14-16].

However, no studies have described the effects of HGF on PNALD. The aim of the present study was to evaluate the preventive effect of HGF against PNALD in a TPN and fasting rat model.

Methods

Animal Preparation

Eight-week-old male Sprague-Dawley (SD) rats (body weight, 250-280 g; purchased from Kyudo Co., Ltd., Saga, Japan) were used in this study. The animals were individually

housed in metabolic cages with *ad libitum* access to standard rat chow and water and were acclimatized to their environment for 6 days before the experiments. The animals were maintained under standardized temperature (23 ± 1°C) and humidity (50% ± 10%) and a 12-h light-dark cycle (lights on at 7:00 a.m.). All of the experimental procedures were approved by the Laboratory Animal Committees of Kagoshima University Graduate School and were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals (Approval number: MD20014).

Study design

The animals were fasted overnight. After jugular vein catheterization, they were randomly divided into the following three treatment groups (n = 8 per group): (1) TPN-alone (TPN group), (2) TPN plus low-dose HGF (0.3 mg/kg/day) [(TPN+HGF (Low) group], and (3) TPN plus high-dose HGF (1.0 mg/kg/day) [TPN+HGF (High) group]. The previous study reported that the intravenous administration of rh-HGF (0.3 mg/kg/day) to rats once a day would treat cirrhosis of the liver, and that rh-HGF (1.0 mg/kg/day) would cause adverse effects in extra-hepatic organs [17]. Thus, we defined the intravenous administration of 0.3 mg/kg/day as "low-dose" and 1.0 mg/kg/day as "High-dose".

rh-HGF (Eisai Co. Ltd., Tokyo, Japan.) [18] was dissolved in phosphate-buffered saline and administered intravenously once a day. On Day 7, the animals were anesthetized,

weighed, and blood was obtained from the heart. Then the animals were sacrificed and the liver tissue was harvested for the analysis of hepatocellular injury. In our previous study, histological findings of liver disease were shown in a TPN-alone rats model infused for 7 days; thus, we determined that the experimental period for this study should be 7 days after catheterization.

The surgical procedure and maintenance methods

For surgery, the animals were anesthetized with isoflurane (1.5% inhalation by mask), and an intravenous catheter was inserted into the right jugular vein. A silastic catheter with an outside diameter of 1.2 mm (NIPRO Co., Ltd., Osaka, Japan) was used, tunneled out of the back, and attached to a standard swivel device (LOMIR BIOMEDICAL INC., Quebec, Canada). The procedures were performed with the aid of an operating microscope. All animals received cefazolin (50 mg/kg per dose, subcutaneously; Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) to prevent postoperative infection and buprenorphine (0.01 mg/kg per dose, subcutaneously; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) for analgesia. They were allowed *ad libitum* access to water immediately after surgery.

TPN was delivered by a multichannel syringe pump (KDS Legato 200 Series Syringe Pump Series, KD Scientific, Inc., Holliston, MA, USA). After catheterization, the animals were maintained with low-concentration NEOPAREN® No. 2 (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) TPN solution (60 ml/day); into which 20% Intralipos® (Otsuka Pharmaceutical

Co., Ltd., Tokyo, Japan) was added. The composition of the TPN solution was as follows (in g/L): amino acids 25, dextrose 145, and soybean oil 33.3. The solution also contained the following electrolytes (final mmol/L): 41.6 Na^+, 22.5 K^+, 41.6 Cl^-, 4.1 Ca^{2+} and 4.1 Mg^{2+}. After 24-h the composition of the TPN solution was switched to amino acids 31.6, glucose 203, and soybean oil 33.3, with similar electrolyte additives. The TPN solution was delivered at the rate of 60 ml/day. This provided equivalent isocaloric/isonitrogenous nutritional support to all TPN-fed animals, consisting of 76.4 kcal/rat/day (1.9 g protein, 2.0 g fat, and 12.2 g carbohydrate).

The rats were anesthetized by isoflurane inhalation after overnight fasting. On day 7, all rats were anesthetized by isoflurane inhalation. Blood was obtained from the heart and immediately centrifuged at 1500×g for 15 min at 4°C. All serum samples were stored at -80°C until use. After blood collection, the animals were euthanized by exsanguination. Liver samples were fixed with 10% formaldehyde for a histological analysis. Paraffin sections of formalin-fixed tissue were cut at a thickness of 3 μm for hematoxylin and eosin (H&E) staining.

The biochemical test of the liver function and lipid metabolites

To evaluate the extent of liver injury, the levels of serum albumin (ALB), aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), cholinesterase (ChE), total bilirubin (T-Bil) and direct bilirubin (D-Bil) were measured. Lipid metabolites, including total cholesterol (TC), free cholesterol (FC), esterified cholesterol (EC), triglyceride (TG),

phospholipid (PL), non-esterified fatty acid (NEFA), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and total bile acid (TBA), were also measured using the Japan Society of Clinical Chemistry standardized matching method. All measurements were performed by Nagahama Life Science Laboratory (ORIENTAL YEAST CO., LTD., Shiga, Japan).

Histological analysis

For the histological analysis of the liver specimens, we evaluated the degree of lipid accumulation (steatosis score), the number of positive macrophages or T lymphocytes in ten randomly selected fields (inflammation score), and the degree of liver cell ballooning injury (ballooning score). Finally, we evaluated the extent of liver dysfunction using the NAFLD activity score, which was calculated from the steatosis score, the inflammation score, and the ballooning score. The three scores were determined based on the histological findings [19], the steatosis score [no lipid droplets (score = 0); lipid droplets in <33% of the hepatocytes (score = 1); lipid droplets in 33-66% of the hepatocytes (score = 2); and lipid droplets in >66% of the hepatocytes (score = 3)], inflammation score [no inflammation (score = 0); <10 inflammatory foci, each consisting of >5 inflammatory cells (score = 1); ≥10 foci (score = 2); or uncountable diffuse or fused inflammatory foci (score = 3)]; and ballooning score [none (score = 0); few balloon cells (score = 1) or many balloon cells/prominent ballooning (score = 2)]. Two

independent observers who were blinded to the physical outcome and other biological and pathological data of each sample evaluated all of the histological slides.

Analyses of the lipid content of liver tissue

According to the FOLCH methods [20], each snap-frozen tissue specimen was homogenized and extracted using chloroform-methanol, and TC, TG, and FC were measured. All measurements were performed at Skylight Biotech, Inc., Akita, Japan.

Real-time quantitative polymerase chain reaction (qPCR) of IL-6, TNF-α and Caspase-9 in the liver tissue

We evaluated the interleukin-6 (IL-6), tumor necrosis factor-alfa (TNF-α), and Caspase-9 in the liver tissue by real-time qPCR. The first-standard cDNA was synthesized using SuperScript IV® Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA) with oligo (dT) primer. Each cDNA sample was then diluted with RNase/DNase-free water to 1.25 ng template RNA/μL. The expression of each gene was analyzed by qPCR using the Bio-Rad CFX96 system (BioRad Laboratories, Inc., Hercules, CA, USA). Standard DNA was generated by blocks double-stranded DNA fragments synthesis (Integrated DNA Technologies, Inc., Skokie, IL, USA). These measurements were performed by Repertoire Genesis Inc., Osaka, Japan.

Statistical analysis

The data are presented as the mean value ± standard deviation (SD). The statistical analyses of the data were performed using a one-way analysis of variance (ANOVA) followed by Tukey-Kramer's multiple-comparison post hoc test. *P* values of < 0.05 were considered to indicate statistical significance.

Results

The changes in the daily assessment data

The daily stool and urine volume were measured, however, there were no significant differences among the three groups. The body weight at sacrifice did not differ among the three groups.

Histological findings of liver specimens

Representative histological findings of the liver tissue specimens (H&E staining) are shown in Fig. 1. The upper and lower panels show a low-power view (×100) and high-power view (×200), respectively. The examination of H&E-stained sections revealed that the liver specimens of the rats in the TPN group (Fig. 1a, 1b) had an increased number of lipid droplets. The TPN+HGF (Low) group and TPN+HGF (High) group showed moderate or mild steatosis (Fig. 1c, 1d, 1e, 1f).

Fig. 1

Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1d

Fig. 1e

Fig. 1f

The histological analysis and the determination of the NAFLD score

Based on the histological findings, the NAFLD activity score was calculated; the results are shown in Fig. 2. The steatosis score in the TPN group was higher than that in the other two groups [steatosis score: TPN group 2.75 ± 0.46 vs. TPN+HGF (Low) group 1.13 ± 0.64 ($p < 0.001$) vs. TPN+HGF (High) group 0.88 ± 0.64 ($p < 0.001$)] (Fig. 2a). The lobular inflammation score in the TPN group was also higher in comparison to the other groups [lobular inflammation score: TPN group 2.88 ± 0.35 vs. TPN+HGF (Low) group 1.38 ± 0.74 ($p < 0.001$) vs. TPN+HGF (High) 1.14 ± 0.64 ($p < 0.001$)] (Fig.2b). The TPN group showed a higher hepatocyte ballooning score in comparison to the other two groups [hepatocyte ballooning score: TPN group 1.25 ± 0.71 vs. TPN+HGF (Low) 0.63 ± 0.74 ($p = 0.22$) vs. TPN+HGF (High) 0.25 ± 0.71 ($p < 0.05$)] (Fig. 2c). Finally, the NAFLD activity score of the TPN group was significantly higher in comparison to the other groups [NAFLD activity score: TPN group 6.88 ± 1.13 vs. TPN+HGF (Low) 3.13 ± 1.96 ($p < 0.001$), TPN+HGF (High) group 2.25 ± 1.75 ($p < 0.001$)] (Fig. 2d).

Fig. 2 **Fig. 2a** **Fig. 2b** **Fig. 2c** **Fig. 2d**

Serum tests of the liver function

Table 1 showed the serum tests of the liver function. The serum albumin (ALB) level of the TPN group was significantly decreased in comparison to the TPN+HGF (Low) and TPN+HGF (High) groups [ALB (g/dL): TPN group 2.35 ± 0.17 vs. TPN+HGF (Low) group:

Table 1

2.83 ± 0.41 vs. TPN+HGF (High) 2.85 ± 0.34, $p<0.05$, respectively] (Table 1). There were no significant differences in the serum levels of AST, ALT, ChE, T-Bil, and D-Bil.

Table 1

Serum tests of the lipid metabolite

Table 2 showed the serum tests of the lipid metabolite. The serum total cholesterol levels in the TPN+HGF (Low) and TPN+HGF (High) groups were increased in comparison to that in the TPN group [TC (mg/dL): TPN group 54.13 ± 5.38 vs. TPN+HGF (Low) group 79.00 ± 14.76 ($p<0.05$), TPN+HGF (High) group 75.13 ± 22.94 ($p<0.05$)]. The serum free cholesterol and esterified cholesterol, phospholipid, non-esterified fatty acid and LDL-cholesterol displayed the same tendency (Table 2) However, the serum triglyceride levels did not differ to a statistically significant extent among the three groups.

Table 2

Table 2

Lipid content of the liver tissue

The TG level in the hepatic tissue of the TPN group was significantly higher than that in the HGF-treated groups (TG (mg/g liver): TPN group 82.91 ± 17.10 vs. TPN+HGF (Low) group 41.95 ± 19.61 ($p < 0.001$) vs. TPN+HGF (High) group: 53.51 ± 14.72 ($p < 0.01$); (Fig. 3a). The levels of TC and FC did not differ to a statistically significant extent among the three groups (Fig. 3b, 3c).

Fig. 3a

Fig. 3b

Fig. 3c

The expression of IL-6 and TNF-α in the liver tissue

The IL-6 and TNF-α expression levels in the liver tissue did not differ to a statistically significant extent among the three groups (Table 3).

Table 3

The Caspase-9 expression in the liver tissue

The Caspase-9 expression in the liver tissue of the TPN group was significantly higher than that in the TPN+HGF (Low) and TPN+HGF (High) groups (Fig. 4).

Fig. 4

Discussion

Patients with gastrointestinal disorders that cause intestinal failure, such as Hirschsprung's disease, midgut volvulus, necrotizing enterocolitis and inflammatory bowel disease, require TPN for the provision of fluids and nutrients. However, TPN sometimes induces PNALD, which is one of the pathological conditions of IFALD and its clinical spectrum includes hepatic steatosis, cholestasis and fibrosis. In our previous study, TPN with SBR induced the hepatic steatosis. Several studies have shown that HGF was effective against the hepatic steatosis induced by a high fat diet, as it is called NAFLD. No studies have evaluated the effect of HGF on the PNALD induced by TPN. Thus, we conducted the present study.

The major findings of the present study were as follows. (1) PNALD induced by 7-day TPN without SBR resulted in the hepatic steatosis histologically. (2) The NAFLD activity

scores in the TPN+HGF (Low) and TPN+HGF (High) groups were significantly lower in comparison to that in the TPN group. (3) The serum albumin and serum lipid (total cholesterol, free cholesterol and LDL cholesterol) levels in the TPN+HGF (Low) and TPN+HGF (High) groups were significantly higher in comparison to the TPN group. (4) The triglyceride content of the liver tissue in the TPN+HGF (Low) and TPN+HGF (High) groups was significantly lower in comparison to that in the TPN group. (5) The expression of caspase-9 in the TPN+HGF (Low) and TPN+HGF (High) groups was significantly decreased in comparison to that in the TPN group. (6) There were no significant differences in the expression of IL-6 and TNF-α among three groups.

Total parenteral nutrition sometimes induces PNALD, which is one of the pathological conditions of intestinal IFALD and its clinical spectrum includes hepatic steatosis, cholestasis, and hepatic fibrosis. In the present study, severe hepatic steatosis without cholestasis was induced by 7-day TPN without bowel resection. Therefore, we evaluated the histological findings using NAFLD activity score, and NAFLD activity score in the TPN group was significantly increased compared to the HGF treated groups. Cao et al. induced hepatic cholestasis by 14-day TPN without bowel resection [7]. In their study, in terms of histological hepatic steatosis, though hepatocyte ballooning was obvious, lipid droplets were hardly observed. Hence, there was histological discrepancy between our study and Cao's study. The total administered calorie in our study was 320 kcal/kg/day and that in Cao's study was 205

kcal/kg/day. We speculated that the difference of the administered total calorie would be associated with histological findings in the liver.

Ishii et al. presented that HGF, which has the effect of a hepatocyte mitogen, stimulated liver regeneration and increased the level of albumin [21]. In their study, they showed that the stimulation of the serum albumin level seemed to result from the direct effect of HGF on the protein production in hepatocytes, as they demonstrated that the mRNA content of albumin in the liver was increased by HGF administration. In present study, we hypothesized that the same direct effect of HGF induced the increase in the serum albumin level.

The serum cholesterol levels in the HGF-treated groups were increased in comparison to the TPN group. We hypothesized that this was due to lipid metabolism. In a study using cultured hepatocytes, Kaibori et al. reported that HGF stimulated the level of very low-density lipoprotein (VLDL) [22]. Since VLDL has the role of transporting hepatic lipids, such as free cholesterol and cholesterol ester, from the liver to peripheral cells, the increase of VLDL induced by the administration of HGF may induce the transference of cholesterol ester in the liver to the blood, and eventually serum cholesterol may increase. Further studies are required to clarify this mechanism.

In the present study, the administration of HGF attenuated the hepatic steatosis induced by 7-day continuous TPN. He et al. showed that HGF alleviated the hepatic steatosis induced by a high fat and cholesterol diet in a rat model [23]. Using TUNEL staining, which was the

histological endpoint of apoptosis, they demonstrated that HGF had the effect of protecting hepatocytes from apoptosis. In our study, we evaluated the expression of the caspase-9 as the endpoint of apoptosis. The expression of caspase-9 in the HGF-treated groups were significantly decreased in comparison to that in the TPN group. We hypothesized that HGF suppressed apoptosis and resulted in the attenuation of hepatic steatosis in the present study.

In our previous studies of NAFLD induced by TPN in a rat model with massive bowel resection, inflammatory cytokines (e.g., IL-6 or TNF-α) were significantly increased in the groups with the severe hepatic steatosis [4-6]. Treatment agents (e.g., GLP-2, ghrelin, or fish oil lipid emulsion) attenuated the hepatic steatosis and reduced the inflammatory cytokine levels. In present study, however, the expression levels of IL-6 and TNF-α in the liver did not differ to a statistically significant extent among the three groups. Histologically, inflammation score on the basis of NAFLD activity score, was significantly decreased in the HGF-treated groups. Some previous studies reported that IL-6 or TNF-α were key cytokines for the formation of hepatic steatosis [4-6]. We did not understand this discrepancy between the histological findings and the cytokine expression. Since Tilg et al. hypothesized that NAFLD would be induced by some factors, such as inflammatory cytokines, adiponectin, leptin, toll like receptors, and they stated the multiple parallel hits model [24], further studies should be performed to clarify the mechanism.

Conclusion

Seven-day TPN without bowel resection induced the hepatic steatosis, and which was attenuated histologically by the intravenous administration of HGF in a dose-dependent manner. HGF may therefore be effective for preventing the pathogenic PNALD. Further studies are needed to clarify the appropriate dose of HGF and the mechanism underlying the suppression of hepatic steatosis in PNALD.

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Conflict of interest

The authors declare no conflicts of interest in association with the present study.

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Figure/Table Legends

Table 1 **Serum tests of the liver function**

The values represent the mean ± S.D.

ALB, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ChE, cholinesterase; T-Bil, total bilirubin; D-Bil, direct bilirubin.

Table 2 **Serum tests of the lipid metabolite**

TC, total cholesterol; FC, free cholesterol; EC, esterified cholesterol; TG, triglyceride; PL, phospholipid; NEFA, non-esterified fatty acid; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TBA total bile acid.

Table 3 **The expression of IL-6 and TNF-α in the liver tissue (RT-qPCR)**

The values represent the mean ± S.D.

Figure 1. **Representative histological findings of the liver tissue specimens (H&E staining)**

The upper panels show a low-power view (×100).

The lower panels show a high-power view (×200).

H&E-stained liver sections from rats in the TPN group (a, b), TPN+HGF (Low) group (c, d), and TPN+HGF (High) group (e, f).

Figure 2. **Summary of NAFLD activity score**

The values represent the mean ± SD. The NAFLD activity score was calculated as the total of the steatosis score, the inflammation score, and the ballooning score.

Figure 3. **The lipid content analysis of liver tissue**

The levels of TG, TC, and FC in hepatic tissue from rats in the three groups.

Figure 4. **The caspase9 expression in liver tissue**

The values represent the mean ± S.D.

Table 1. Serum tests of the liver function

	TPN group	TPN+HGF (Low) group	TPN+HGF (High) group
ALB (g/dL)	2.35 ± 0.17	2.83 ± 0.41†	2.85 ± 0.34†
AST (IU/L)	139.75 ± 72.06	140.88 ± 44.19	120.63 ± 42.72
ALT (IU/L)	16.63 ± 6.97	16.63 ± 4.60	14.50 ± 5.04
ChE (IU/L)	8.71 ± 4.54	7.29 ± 1.50	12.20 ± 5.26
T-Bil (mg/dL)	0.031 ± 0.034	0.051 ± 0.045	0.091 ± 0.077
D-Bil (mg/dL)	0.024 ± 0.024	0.031 ± 0.023	0.058 ± 0.051

Values: means ± S.D. †$p<0.05$ vs. TPN,
ALB albumen. *AST* aspartate aminotransferase. *ALT* alanine aminotransferase.
ChE cholinesterase. *T-Bil* total bilirubin. *D-Bil* direct bilirubin.

Table 2. Serum tests of the Lipid metabolite

	TPN group	TPN+HGF (Low) group	TPN+HGF (High) group
TC (mg/dL)	54.13 ± 5.38	79.00 ± 14.76†	75.13 ± 22.94†
FC (mg/dL)	17.75 ± 3.96	26.50 ± 5.58††	25.13 ± 5.51†
EC (mg/dL)	36.38 ± 2.50	52.50 ± 10.89†	50.00 ± 17.76
TG (mg/dL)	55.13 ± 16.46	75.75 ± 26.69	72.88 ± 25.33
PL (mg/dL)	110.50 ± 9.81	140.75 ± 29.18†	139.62 ± 26.17
NEFA (μEq/L)	258.00 ± 53.21	312.88 ± 110.84†	469.13 ± 223.58†
LDL-C (mg/dL)	10.50 ± 3.30	16.13 ± 5.00†	16.13 ± 3.94†
HDL-C (mg/dL)	16.25 ± 1.83	21.00 ± 5.45	20.00 ± 6.19
TBA (mg/dL)	7.13 ± 6.98	13.00 ± 5.21	20.12 ± 24.43

Values: means ± S.D. †$p < 0.05$ vs. TPN. †† $p < 0.01$ vs. TPN.
TC total cholesterol. *FC* free cholesterol. *EC* esterified cholesterol. *TG* triglyceride. *PL* phospholipid.
NEFA non-esterified fatty acid. *LDL-C* low density lipoprotein cholesterol. *HDL-C* high density lipoprotein cholesterol.
TBA total bile acid.

Table 3. The expression of IL-6 and TNF-α in the liver tissue (RT-qPCR)

	TPN group	TPN+HGF (Low) group	TPN+HGF (High) group	*p* value
IL-6	17.08 ± 25.49	27.91 ± 16.53	121.77 ± 302.52	0.437
TNF-α	1.30 ± 0.26	0.97 ± 0.38	1.58 ± 0.96	0.160

Values: means ± S.D.

Figure 1. Representative histological findings of the liver tissue specimens

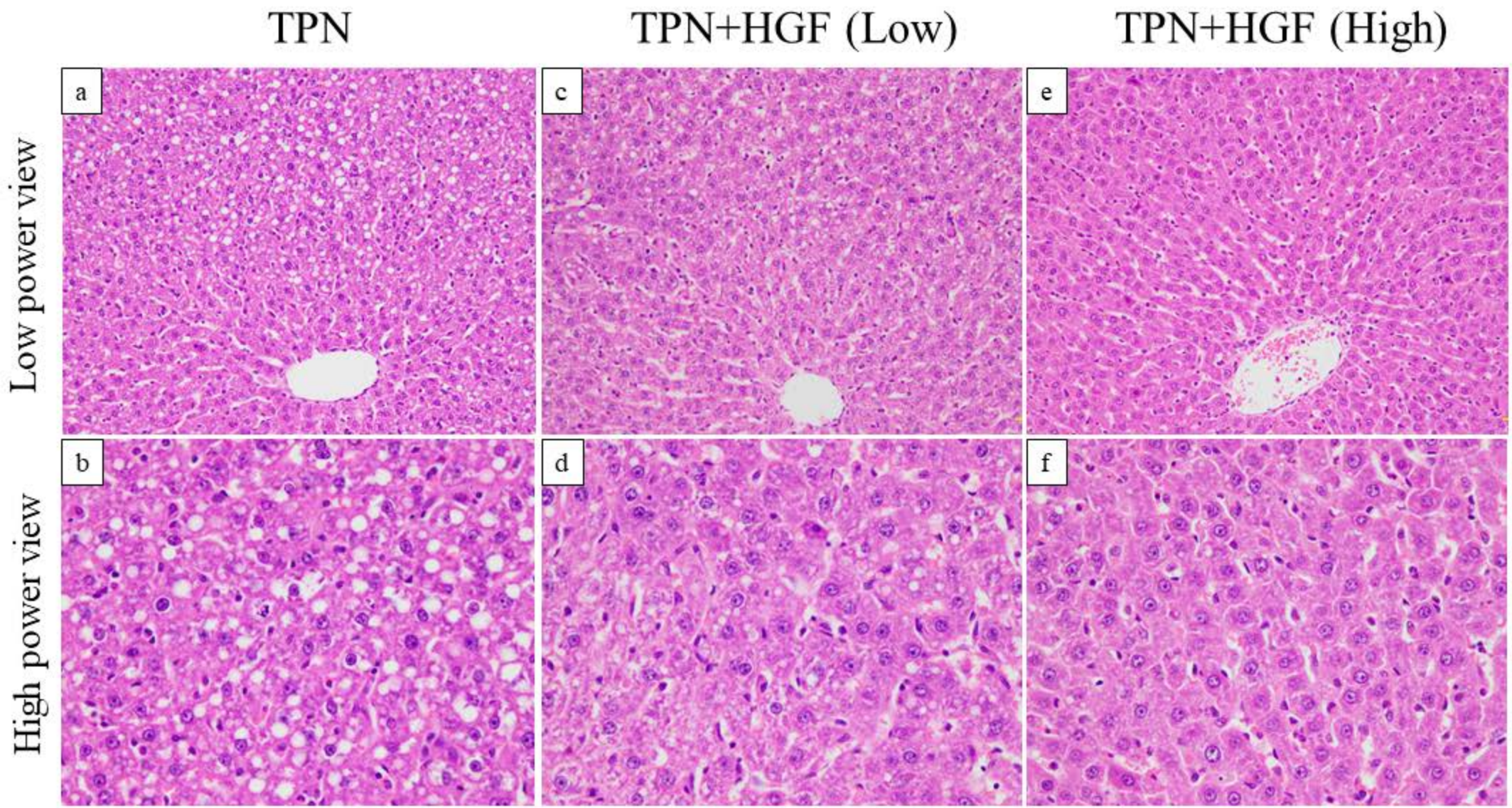

Figure 2. Summary of NAFLD activity score

$^{**}p < 0.01$
$^{*}\ p < 0.05$

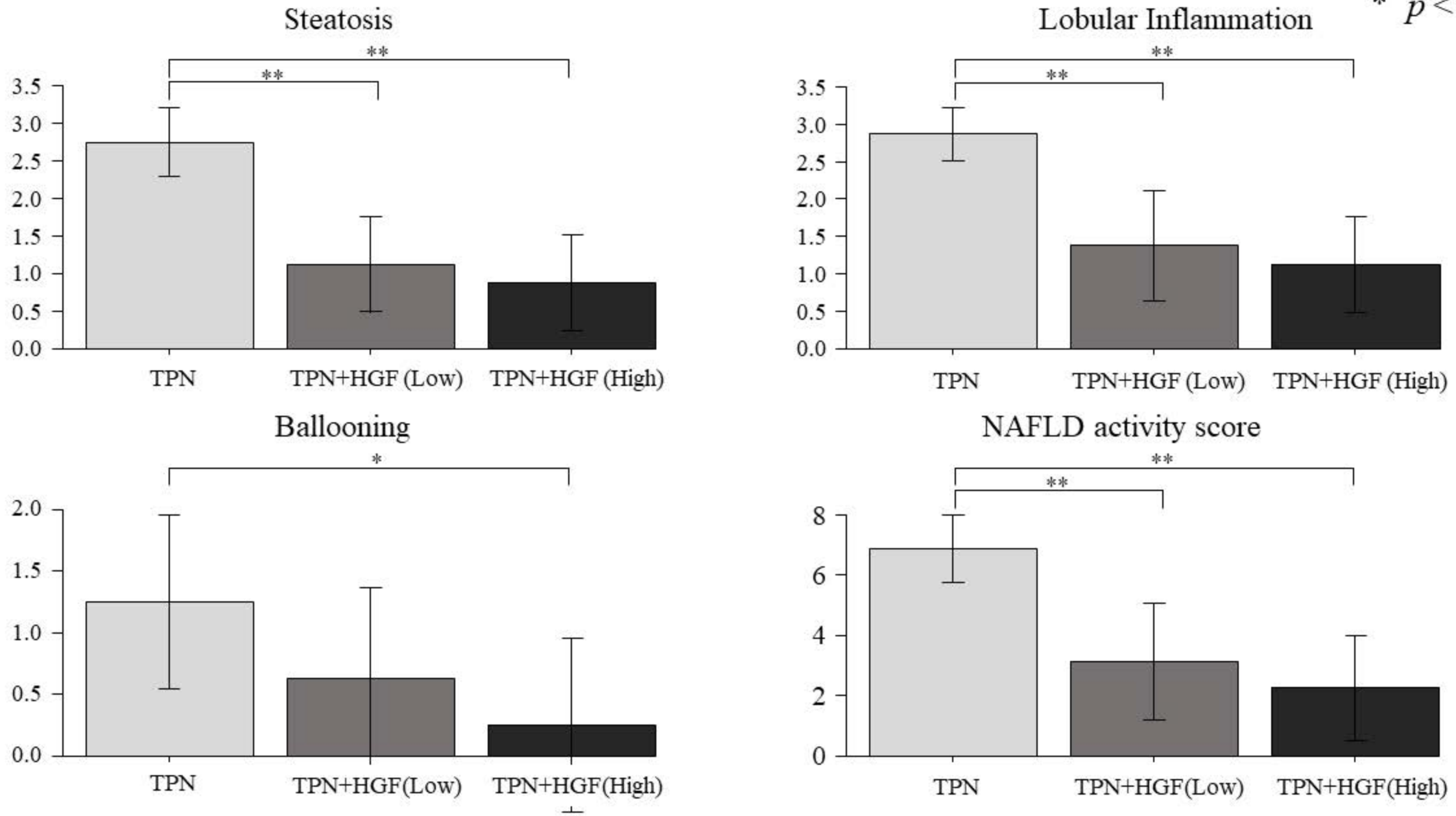

Figure 3. Lipid content of the liver tissue

** $p < 0.01$

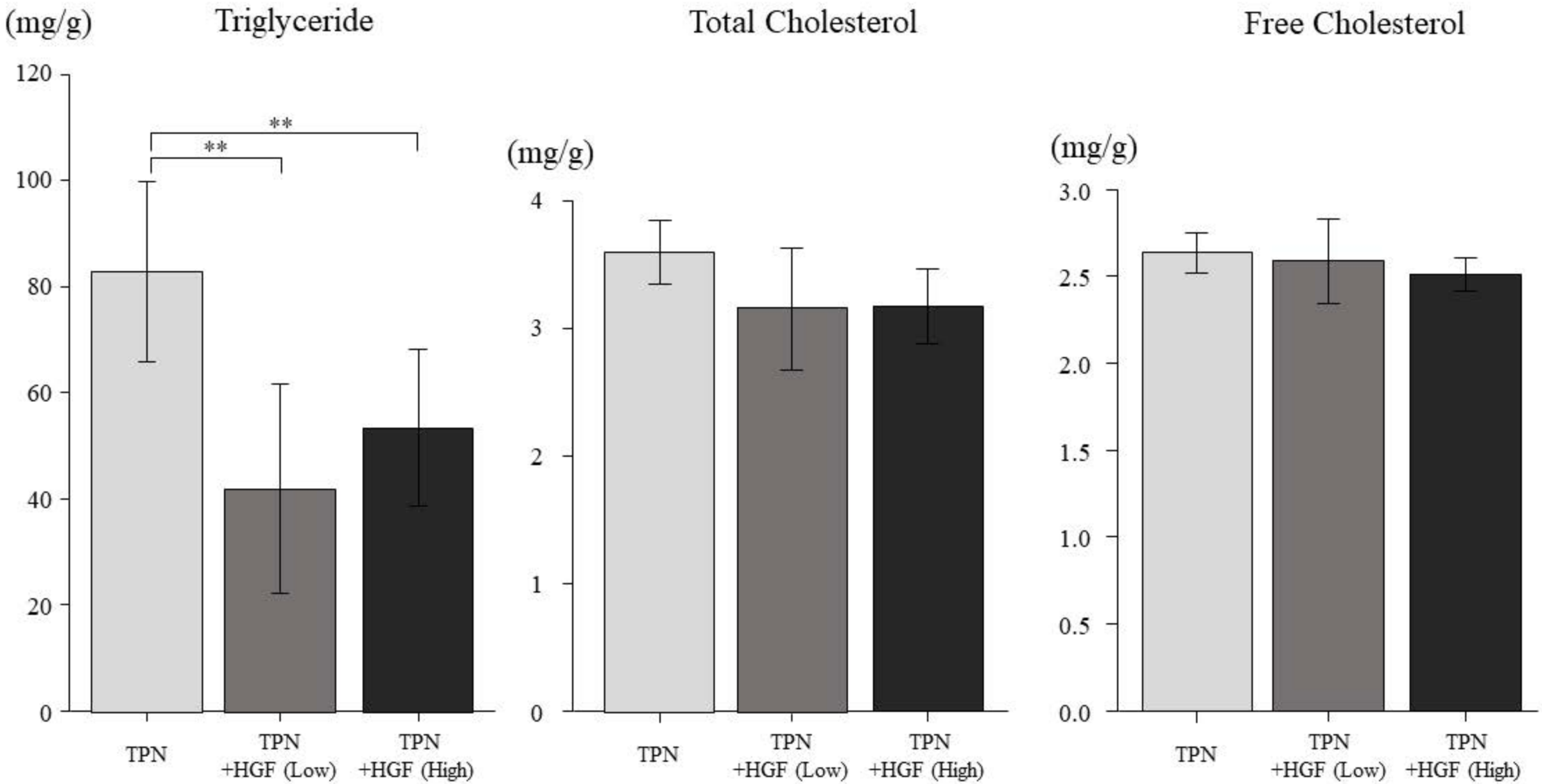

Figure 4. The expression of Caspase-9 in the liver tissue (RT-qPCR)