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Original Article

Antioxidant effect of apolipoprotein A-I on high-fat diet-induced non-alcoholic fatty liver disease in rabbits

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Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognized condition that encompasses a spectrum of liver abnormalities. It has been suggested that oxidative stress and lipid peroxidation are key pathophysiological mechanisms in NAFLD. Although an antioxidant effect of apolipoprotein A-I (apoA-I) has been reported, its influence on NAFLD has not been reported. The aim of this study was to determine whether apoA-I could improve the biochemical and histological abnormalities associated with high-fat diet-induced NAFLD through its antioxidant actions in rabbits. Liver damage was evaluated by hepatic coefficient, hepatic lipid assay, liver apparent abnormalities as well as hematoxylin–eosin staining of liver sections. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) level in liver. Oxidative stress was assessed by measuring superoxide dismutase (SOD), glutathione peroxidase (GPx), and inducible nitric oxide synthase (iNOS) activities in serum and liver. Also, the mRNA expressions levels of SOD, GPx, and catalase (CAT) were determined by real-time quantitative polymerase chain reaction method. The results showed that apoA-I (20 or 40 mg/kg/w) was effective in reducing hepatic steatosis, inflammation, hepatic coefficient, and liver total cholesterol, triglyceride, low-density lipoprotein-cholesterol, and MDA levels in high-fat diet rabbits. In addition, apoA-I increased SOD and GPx activities while reducing iNOS activity in serum and liver. Moreover, apoA-I significantly increased the mRNA expression levels of SOD, GPx, and CAT in liver. This study showed that apoA-I exerted protective effects against fatty liver disease in rabbits induced by a high-fat diet, possibly through its antioxidant actions.

Keywords apolipoprotein A-I; lipid peroxidation; oxidative stress; non-alcoholic fatty liver

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as hepatic fat accumulation exceeding 5%–10% of liver weight, in the absence of excess alcohol consumption or any other liver disease and other causes of steatosis, such as certain toxins and drugs [1,2]. Regarded as a hepatic manifestation of the metabolic syndrome, NAFLD is strongly associated with obesity, insulin resistance, dyslipidemia, and hypertension [3]. It encompasses a wide spectrum of diseases ranging from simple steatosis characterized by hepatic lipid accumulation in the form of triglyceride (TG) to non-alcoholic steatohepatitis (NASH) characterized by the association of lipid accumulation with evidence of hepatocyte injury, inflammation, and various degrees of fibrosis [4–6].

Although the pathogenesis of steatosis occurrence and progression of NAFLD into NASH are not fully understood, reactive oxygen species (ROS) are thought to be responsible for the progressive replacement of the normal hepatic structure with scar tissue [7–9]. Some studies have found that hepatic and plasma oxidative stress-related parameters are correlated with clinical and histological findings [10,11]. These findings suggest that impaired antioxidant defense mechanisms may be an important factor in the pathogenesis of NAFLD. Antioxidants such as vitamin E, *N*-acetylcysteine, *S*-adenosylmethionine, and others have been suggested to have some beneficial role in the treatment of NAFLD and NASH [12–14]. Recently, more novel antioxidants have been found to be capable of inhibiting NAFLD *in vivo*. For example, Silibinin treatment can counteract the progression of liver injury by modulating lipid homeostasis and suppressing oxidative stress in experimental NASH [15]. Silybin treatment attenuated liver damage in animal models of NASH and showed benefits in patients with liver cirrhosis [16]. In consideration of the complexity of NAFLD and the limitation of existing medicines, developing more safe and effective antioxidants is meaningful and in great need.

As the main protein component of high-density lipoprotein (HDL), apolipoprotein A-I (apoA-I) may be a potential and suitable target, which plays a key role in the biogenesis and functions of HDL [17]. A growing body of evidence suggests that HDL inhibits oxidative events and oxidative stress *in vivo* and *in vitro* by counteracting low-density lipoprotein (LDL) oxidation directly or indirectly [18]. Among HDL components, apoA-I plays the most important role in resisting oxidative stress. HDL obtained from the serum of apoA-I over-expression transgenic animals reduces LDL oxidation two or three times than HDL from wild-type animals. The same effects are observed that apoA-I inhibited the susceptibility of LDL to lipid peroxidation in mice [19]. Moreover, it has been reported that apoA-I renders LDL resistant to oxidation in human [20] and removes oxidation-prone or 'seeding' molecules from human LDL rendering it resistant to oxidation by human artery wall cells [21], which could be related to lipoxigenase [22,23]. Actually, the antioxidant properties of apoA-I have been shown experimentally to protect against atherosclerosis (AS), which may be induced by NAFLD [24]. However, little is known about the antioxidant effects of apoA-I on NAFLD and its mechanisms.

To explore whether apoA-I can inhibit NAFLD through possible antioxidant actions, some important oxidative stress-related parameters draw our close attention. In order to keep the level of ROS under control, living organisms have developed antioxidant systems that consist of enzymatic scavengers like superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) as well as non-enzymatic ones. Hence, antioxidants may be beneficial in patients with NAFLD by strengthening organic antioxidant systems, such as increasing the antioxidant enzymes and inhibiting oxidant enzymes. As the most important antioxidant enzymes, SOD donates a proton to the superoxide anion O^{2-} and converts it to hydrogen peroxide (H_2O_2) and molecular oxygen [25], and then CAT and GPx convert H_2O_2 into water and oxygen [26,27]. On the contrary, nitric oxide synthase (NOS) will aggravate oxidative damage by producing nitric oxide (NO), which inactivates enzymes like SOD and GPx [28]. The most important form of NOS is inducible nitric oxide synthase (iNOS) which can persistently and profusely release NO [29]. Exploration of the effects of apoA-I on these enzymes would be great help to understand the possible influences and antioxidant mechanisms of apoA-I on NAFLD.

In the present study, liver injury and lipid metabolism were evaluated to investigate the effects of apoA-I on NAFLD in high-fat diet rabbits. Oxidative stress-related parameters were also evaluated to explore possible antioxidant mechanisms of apoA-I on NAFLD in high-fat-diet rabbits. We wanted to gain novel insights into apoA-I function, as a possible new target for the treatment of NAFLD.

Materials and Methods

Chemicals and reagents

ApoA-I used in this research was from human serum and provided by RAAS Blood Products Company (Shanghai, China).The kits for determination of total cholesterol (TC), TG, low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) were obtained from Shanghai Rongsheng Bioengineering Company (Shanghai, China). The kits for determination of the activity of SOD, GPx, iNOS, and malondialdehyde (MDA) level were obtained from Jiancheng Bioengineering Institute (Nanjing, China). All other reagents used were of the commercially available grade.

Animals and protocol

Thirty male New Zealand White rabbits weighing 1.8–2.0 kg were provided by the Animal Experimental Center of Fudan University (Shanghai, China). Rabbits were housed individually in metal cages with a constant temperature ($24 \pm 2^{\circ}C$) and relative humidity (50%–60%) in the Animal Experimental Center of Fudan University. All animals were kept under a circadian rhythm of 12 h of light and 12 h of dark. Water was provided *ad libitum*. All animals were fed on a high-lipid diet (standard diet supplemented with 1.0% cholesterol, 30 g/day) and standard diet (120 g/day) for 20 weeks. The rabbits were allocated randomly to the following three groups: model group, low apoA-I dose (20 mg/kg/w) group, and high apoA-I dose (40 mg/kg/w) group. The 10 rabbits of the model group were injected with 5 ml physiological saline once a week. Doses of apoA-I were adjusted according to changes in body weight, which was monitored weekly. The apoA-I-treated groups with 10 rabbits each for low and high doses were injected with pure h-apoA-I once a week. Another 10 rabbits were fed normally to be used in histological research. After 3 months of treatment, rabbits were anesthetized with 10 ml of 20% ethyl carbamate and euthanized with enough air by intravenous injection. Blood was obtained for further examination. The livers were carefully removed from the surrounding tissue, rapidly weighed, and photographed. They were immersed in ice-cold phosphate-buffered saline (PBS), and some were fixed *in situ* with 10% buffered formaldehyde, and then embedded in paraffin. Others were immediately frozen in liquid nitrogen for further analysis. All rabbits were weighed every week and before sacrifice to record body weight variations. All animal experiments were performed with the approval of the Animal Research Committee of Fudan University, and in accordance with the Guidelines for Animal Experiments from the Committee of Medical Ethics, Ministry of Health of China.

Preparation of blood serum and liver homogenates

After the rabbits were euthanized, fasting blood samples were collected from the heart. Whole blood was incubated at room temperature for 30 min, and then centrifuged at 1200 × *g* at 4°C for 10 min to obtain serum for further examination. Segments from the liver were cut into pieces and homogenized in a defined volume of physiological saline. The samples were homogenized at 60 HZ for 30 s using a homogenizer (Jingxin Scientific Company, Shanghai, China). The supernatant was obtained by centrifugation at 1500 × *g* at 4°C for 15 min. Three concentrations (w/v) of liver homogenates (1%, 5%, and 10%) were prepared for different final uses.

Analysis of hepatic homogenate lipids

The TC and TG in 10% liver homogenates were determined by the oxidase–peroxidase method. After a series of reactions between TC or TG in liver and specific reagents, quinine imine is formed. The quinine imine produced by TC and TG can be examined by spectrophotometer at 505 and 546 nm, respectively. LDL-C and HDL-C were determined through the direct measurement method. Briefly, through a series of reactions, HDL-C and LDL-C in liver can be catalyzed specifically by enzyme and react with phenol. The product of a chromogenic reaction can be examined at 546 nm by spectrophotometer.

Histopathological examination of liver

Photographs of livers are used for evaluation of the gross appearance of livers. The color, gloss, smoothness, and size of the liver were recorded. A piece of liver tissue dissected from the same part of the liver in each rabbit was fixed in 10% formalin solution and paraffin blocks were subsequently prepared. Sections were cut at a thickness of 4–6 μm and stained with hematoxylin–eosin (HE). Light microscopic examinations were performed in a blinded fashion by two pathologists and histological grade for hepatic steatosis was determined according to Brunt *et al*. [30] as follows: 0, no steatosis, normal liver; I, ≤25% of hepatocytes affected; II, 26%–50% of hepatocytes affected; III, 51%–75% of hepatocytes affected; IV, ≥76% of hepatocytes affected. Additionally, a histological grade for steatohepatitis based on the Brunt's grading system was assigned [30].

Serum activity of SOD, GPx, and iNOS

Serum SOD, GPx, and iNOS activities were used to evaluate the antioxidant status in rabbits as mentioned below. SOD activity was assayed using the xanthine oxidase assay. One unit (U) of SOD activity was defined as the amount of enzyme necessary to make the SOD inhibition rate reach 50% in 1 ml reaction liquid. GPx activity was evaluated by the 5–5′-dithiobis (2-nitrobenzoic acid) (DTNB) method. Units of GPx activity were calculated following GSH oxidation at 412 nm using GSH as the substrate, and one unit of GPx activity was defined as 1 μmol/L GSH consumption/min at pH 7.0 and 37°C. The principle of the iNOS assay is that iNOS catalyses L-arginine (L-Arg) and molecular oxygen to NO; NO and a nucleophilic reagent generate colored substances. The optical absorbance of the colored substances is determined at 530 nm. One unit of iNOS activity was defined as 1 nmol NO generation/min in 1 ml serum.

Activity of SOD, GPx, and iNOS in hepatic homogenate

Liver homogenates, 1%, 5%, and 10%, were used separately to determine the activities of SOD, GPx, and iNOS in liver. The methods were the same as for serum determination. One unit (U) of SOD activity was defined as the amount of enzyme necessary to make the SOD inhibition rate reach 50% per mg liver protein in 1 ml reaction liquid. One unit of GPx activity was defined as 1 μmol/L GSH consumption per minute at pH 7.0 and 37°C. One unit of iNOS activity was defined as 1 nmol NO generation/min/mg liver protein in 1 ml reaction liquid.

Analysis of lipid peroxidation

The level of MDA was used as an indicator of lipid peroxidation. Liver homogenate (10%) was used to determine the level of MDA in liver tissue using the thiobarbituric acid method. MDA levels were expressed as nmol/mg of protein.

Analysis of *SOD*, *GPx*, and *CAT* mRNA expression by real-time reverse transcription polymerase chain reaction

The liver was perfused with nuclease-free PBS and total RNA was extracted from segments of liver using Trizol (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. RNA samples were dissolved in RNase-free water, and the concentration of each sample was determined spectrophotometrically. Equal amounts (1 μg) of total RNA were reverse transcribed in a 20 μl reaction volume using RevertAid™ Premium First Strand cDNA Synthesis Kit (Fermentas, Pittsburgh, USA). Real-time polymerase chain reaction (qRT-PCR) analysis was performed with a SYBR® Premix Ex Taq™ PCR Kit (Takara, Dalian, China) according to the manufacturer's instructions. Detection of *SOD*, *GPx*, *CAT*, and *GAPDH* mRNA levels was made using the qRT-PCR instrument (Bio-Rad, Hercules , USA). The primers for amplification were synthesized by Sangon Gene Company (Shanghai, China), as shown in **Table 1**. A two-step PCR, with denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min for 40 cycles, was conducted to determine the threshold cycle (Ct) value. Expression of antioxidant enzymes was calculated using the $^{\Delta\Delta}$Ct method using

threshold cycles for *GAPDH* as normalization references. All qRT-PCR reactions were carried out at least twice from independent cDNA preparations. The ratio of target genes (*SOD*, *GPx* and *CAT*) to *GAPDH* was the standard for measuring the relative mRNA expression level of the target gene.

Statistical analysis

Data were expressed as the mean ± SEM. One-way analysis of variance was employed. *Post hoc* LSD analysis was used to test the significance of group mean differences. All data were analyzed by the SPSS 11.5 statistical package. $P < 0.05$ was considered significantly difference.

Table 1 Primers used in qRT-PCR

Gene	Primers	
SOD	Sence	5′-GAC TGA CCC GTC GCT ACA TC-3′
	Anti-sence	5′-GCG GTT ACC AAC ACA ACT CC-3′
GPx	Sence	5′-CTC TGC TTG CTG CCA TTC T-3′
	Anti-sence	5′-ATG CTC ACT GAA ACG TGC TC-3′
CAT	Sence	5′-CTG CGG CAG AAA GCA GTT-3′
	Anti-sence	5′-GAA AGT TCT CAG GCC GTCAT-3′
GAPDH	Sence	5′-GGA GCC AAA AGG GTC ATC-3′
	Anti-sence	5′-CCA GTG AGT TTC CCG TTC-3′

Results

Effect of apoA-I on body weight and liver index (hepatic coefficient)

The food intake and body weight gain were not different between groups (data not shown). The hepatic coefficient (the percentage of wet liver weight to body weight) was reduced in both the low and the high apoA-I dose group compared with the model group, by 9.01% and 13.18%, respectively [**Fig. 1(A)**].

Effect of apoA-I on hepatic lipid levels

Liver lipid profiles were determined to address whether the liver protective effect of apoA-I is due to a change in liver cholesterol levels. The liver levels of TC, TG, and LDL-C in the high apoA-I dose (40 mg/kg/w) group were markedly decreased compared with the model group ($P < 0.05$), but the liver levels of TC, TG, HDL-C, and LDL-C were not significantly different between the low apoA-I dose group and the model group [**Fig. 1(B)**]. These findings suggest that the liver protective effect of apoA-I may be related to regulation of TC, TG, and LDL-C levels.

Effect of apoA-I on hepatic lesions

In the photographs of whole liver, normal liver was bright reddish-brown, glossy, and resilient. However, the liver of the model group was dark gray, mottled, and hardened, with a rough and fatty surface. Significant enlargement of the liver was seen for the model group. These apparent abnormalities were significantly improved by apoA-I administration, especially at the higher (40 mg/kg/w) apoA-I dose

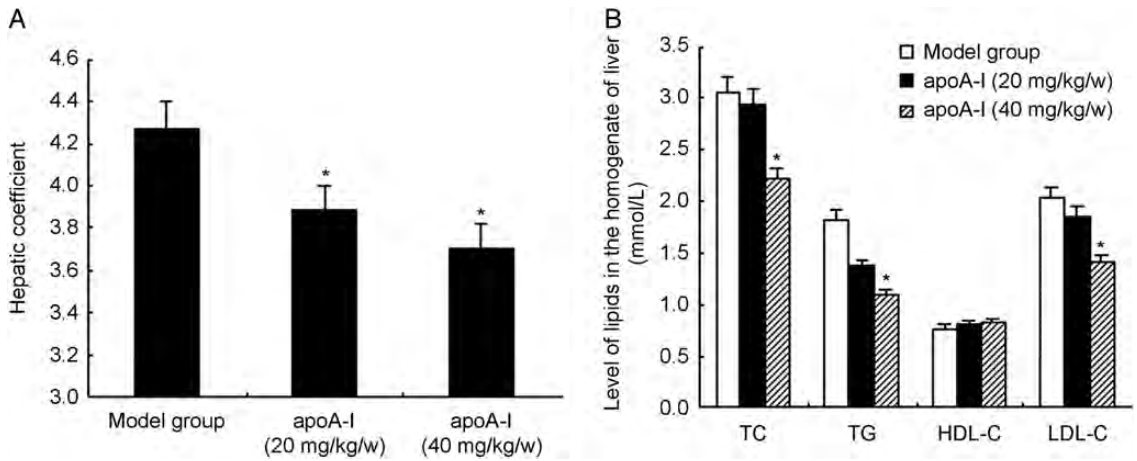

Figure 1 Effect of apoA-I on hepatic coefficient and hepatic lipid levels in rabbits with NAFLD (A) Hepatic coefficient (the percentage of wet liver weight to body weight) was reduced in both apoA-I (20 or 40 mg/kg/w) groups relative to the model group. (B) The liver levels of TC, TG, and LDL-C were markedly decreased in the apoA-I (40 mg/kg/w) group compared with the model group. Values are expressed as mean ± SEM, $n = 10$. *$P < 0.05$, vs. the model group.

(**Fig. 2**). The HE-stained sections showed that no histological abnormalities were observed in the liver of normal rabbits. The hepatic parenchyma manifested normal hepatocytes arranged around the central vein. All rabbits in the model group had grade III–IV hepatic steatosis, and some of them showed steatosis and inflammation, which was significant improved by apoA-I administration, especially at the higher apoA-I dose (**Fig. 3**).

Effect of apoA-I on serum SOD, GPx, and iNOS activity

We determined the serum SOD, GPx, and iNOS activity in rabbits. Treatment with 20 and 40 mg/kg/wk apoA-I caused a significant increase of 7.63% and 19.92% in the SOD activity, respectively, in comparison with the model group [**Fig. 4(A)**]. Treatment with low and high doses of apoA-I caused a significant increase of 20.13% and 33.11% in the GPx activity, respectively, in comparison with the model group [**Fig. 4(B)**]. In contrast, both low and high apoA-I dose groups markedly decreased iNOS activity, by 8.76% and 10.25%, respectively, in comparison with the model group [**Fig. 4(C)**].

Effect of apoA-I on liver SOD, GPx, and iNOS activity

In our study, treatment with 20 and 40 mg/kg/wk apoA-I caused an increase of 2.5% and 5.82% in the SOD activity in liver, respectively, in comparison with the model group [**Fig. 4(D)**]. Both low and high apoA-I dose groups had a significantly higher liver GPx activity, of 15.15% and 61.98%, respectively, in comparison with the model group [**Fig. 4(E)**]. In contrast, the iNOS activity was markedly decreased in both low and high apoA-I dose groups, by 15.98% and 47.37%, respectively, in comparison with model group [**Fig. 4(F)**]. Thus, these findings suggest that apoA-I may protect liver by increasing SOD and GPX activity, and decreasing iNOS activity.

Effect of apoA-I on hepatic MDA levels

As indicated in **Fig. 5**, the liver MDA level was increased in both the low and high dose apoA-I-treated rats relative to the model group, by 14.38% and 47.02%, respectively.

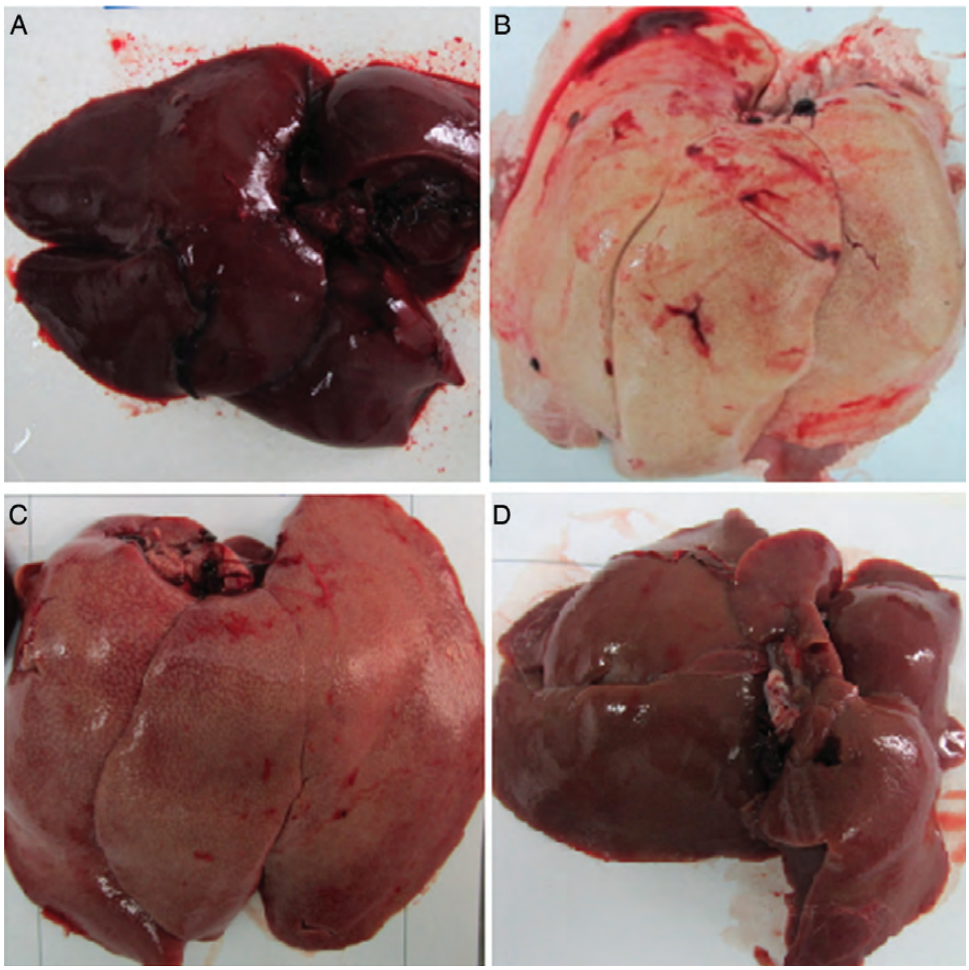

Figure 2 Representative gross morphology of livers from various treatment groups (A) normal rabbits, (B) model group, (**C**) 20 mg/kg/w apoA-I group, (D) 40 mg/kg/w apoA-I group. ApoA-I significantly reversed apparent abnormalities of liver induced by a high-fat diet (C and D).

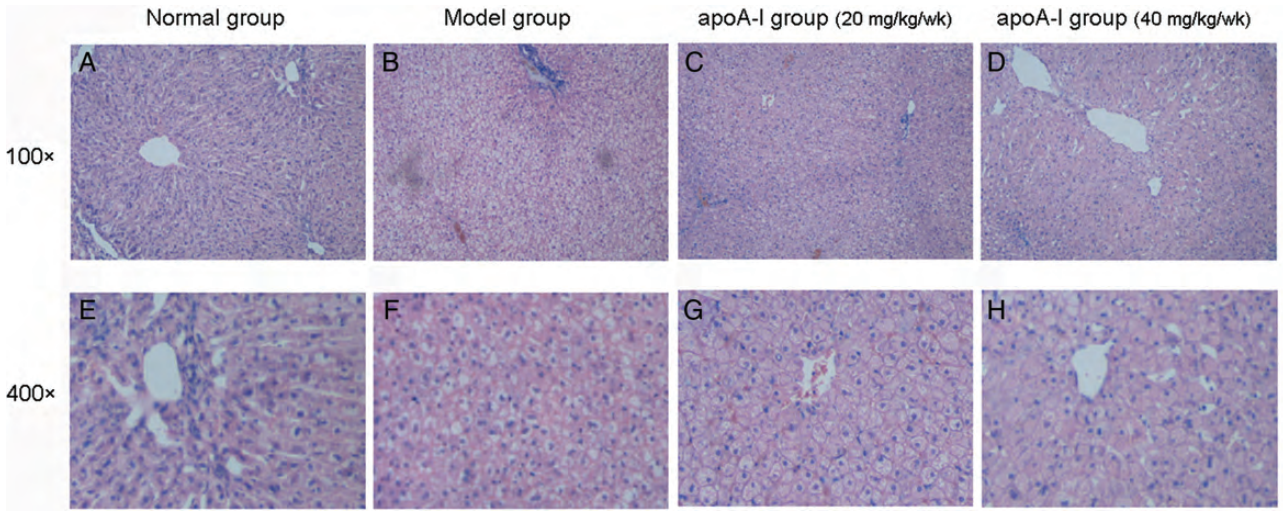

Figure 3 Effect of apoA-I on NAFLD in rats treated with high-fat diets Photographs of livers after HE staining. (A and E) normal group, (B and F) model group, (C and G) 20 mg/kg/wk apoA-I group, (D and H) 40 mg/kg/wk apoA-I group. A, B, C, and D: magnification ×100. E, F, G, and H: magnification ×400.

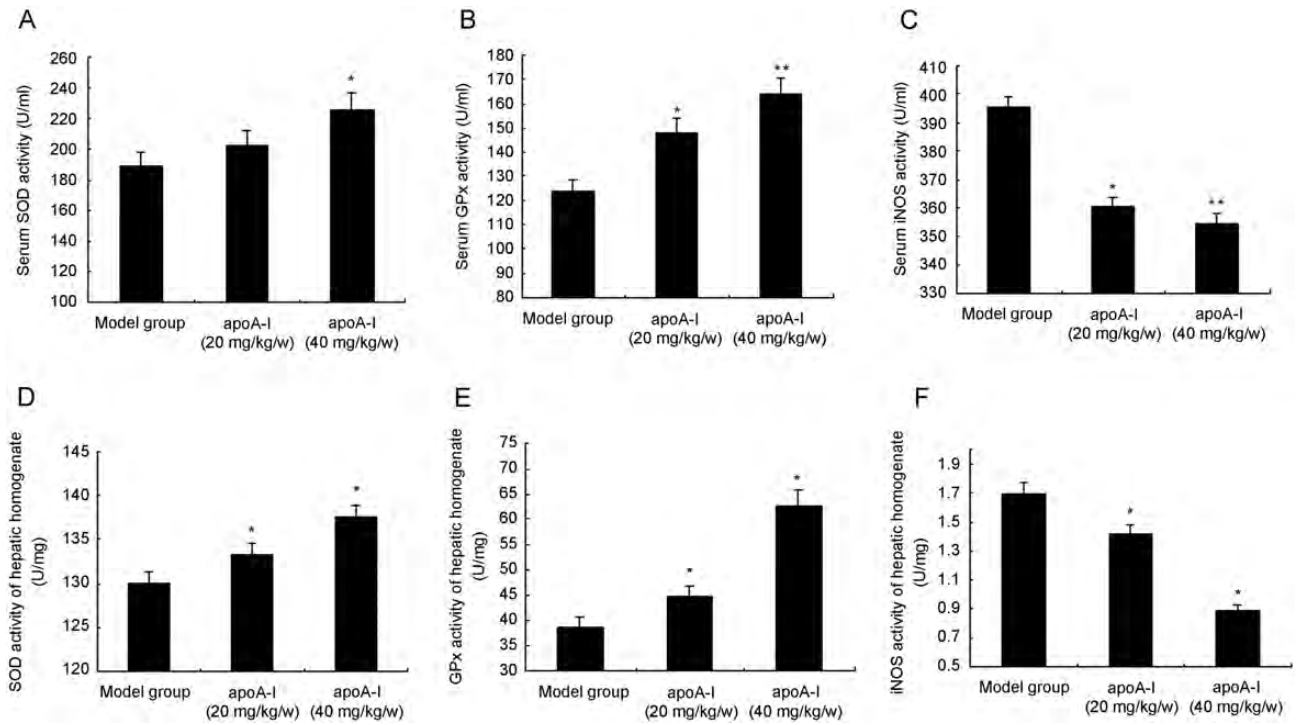

Figure 4 Effect of apoA-I on SOD, GPx, and iNOS activity in serum and liver of rabbits with NAFLD Serum SOD (A) and GPx (B) activity were increased, while iNOS activity (C) was inhibited by apoA-I when compared with the model group. Liver SOD (D) and GPx (E) activity were increased, while iNOS activity (F) was inhibited by apoA-I when compared with the model group. Values are expressed as mean ± SEM, $n = 10$. *$P < 0.05$, **$P < 0.01$ vs. the model group.

Effect of apoA-I on liver *SOD*, *GPx*, and *CAT* mRNA expression

We then explored whether apoA-I increases the expression of hepatic antioxidant enzymes. Earlier studies presented evidence that the level of liver antioxidant enzymes such as SOD, GPx, and CAT had an inverse relationship to the severity of fatty liver disease. Hence, a potential mechanism contributing to expression of antioxidant enzymes (SOD, GPx, and CAT) in liver was determined. RT-PCR analyses showed that the mRNA expression levels of *SOD*, *GPx*, and *CAT* were significantly higher in the apoA-I-treated groups compared with the model group (**Fig. 6**). The higher ApoA-I dose (40 mg/kg/wk) had the best effects; compared with the model group, *SOD*, *GPx*, and *CAT* mRNA

expression levels were significantly enhanced by 245%, 280%, and 119%, respectively.

Discussion

NAFLD is recognized as the most important liver disease in Western societies, but its progression mechanism is still not fully understood. Owing to the effects of increased lipid peroxidation, and activated Kupffer cells which lead to production of inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6 [31], oxidative stress is believed to play a pivotal role in the pathogenesis of NAFLD. Consequently, antioxidant supplements have become a common therapeutic approach for the treatment of NAFLD. The antioxidant effects of apoA-I have been demonstrated in many studies, which makes apoA-I another potential treatment for NAFLD. ApoA-I renders LDL resistant to oxidation by lipoxygenase, an enzyme that participates in the oxidation of fatty acids [22,23]. Also, apoA-I removes oxidation-prone or 'seeding' molecules from human LDL, rendering it resistant to oxidation by human artery wall cells *in vitro*. The 'seeding' molecules are susceptible to oxidation under conditions of oxidative stress [20]. Furthermore, Garner *et al*. [32] demonstrated that apoA-I not only reduced peroxides of phospholipids and cholesteryl esters, but also removed hydroperoxy-eicosatetraenoic acid and hydroperoxy-octadecadienoic acid from native LDL, which are products of 12-lipoxygenase and are necessary for induction of the non-enzymatic oxidation of lipoprotein phospholipids.

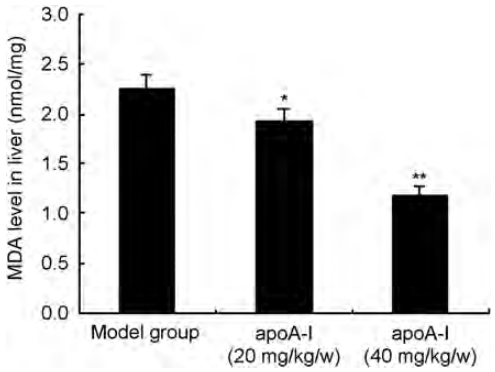

Figure 5 Effect of apoA-I on MDA levels in the liver of rabbits with NAFLD The MDA level was decreased in both apoA-I groups compared with the model group. Values are expressed as mean ± SEM, $n = 10$. *$P < 0.05$, **$P < 0.01$ vs. the model group.

Although the antioxidant effects of apoA-I have been reported, little is known about its influence on NAFLD and possible mechanisms for these effects. In this context, we studied the relationship between apoA-I and NAFLD in high fat-fed New Zealand white rabbits, a common method to establish this NAFLD animal model [33]. The experimental animals developed significant NAFLD, as well as an increase in TC and TG in the serum and liver. Our findings demonstrated, for the first time, that apoA-I supplementation reduced NAFLD-induced abnormalities in livers of rabbits. Although no significant difference in food intake or weight gain was found, the hepatic coefficient (percentage liver weight to body weight) was decreased by apoA-I administration, which indicates that apoA-I most likely reduces fat deposition in internal organs. At the same time, apparent abnormalities in liver morphology, such as changes in liver color, gloss, smoothness, and size, were alleviated by apoA-I administration. ApoA-I also relieved much of the hepatic steatosis and inflammatory cell infiltration.

Encouraged by the inhibitory effects of apoA-I on NAFLD, we explored the possible mechanisms for the effects of apoA-I in this process. Oxidative stress is defined as an imbalance between pro-oxidant and anti-oxidant effects in favor of the former, leading to potential damage. Both increased ROS or reactive nitrogen species production and decreased anti-oxidants can result in oxidative stress. Over-accumulation of lipids can increase the level of fatty

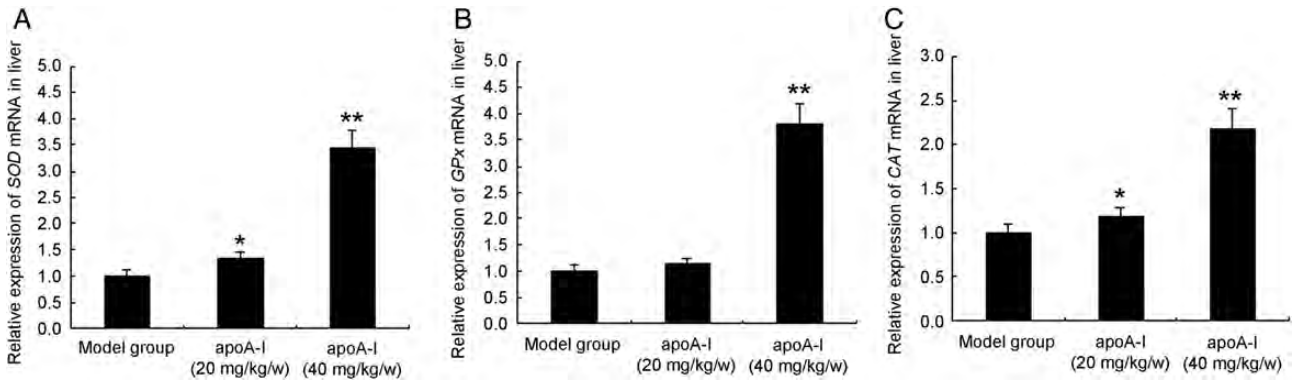

Figure 6 Effect of apoA-I on *SOD*, *GPx*, and *CAT* mRNA expression levels in the liver of rabbits with NAFLD The mRNA expression level of *SOD* (A), *GPx* (B), and *CAT* (C) was significantly increased in both the apoA-I groups compared with the model group. Values are expressed as mean ± SEM, $n = 10$. *$P < 0.05$, **$P < 0.01$ vs. the model group.

acids, and the oxidation of fatty acids is an important source of ROS in fatty liver [34]. ROS attack polyunsaturated fatty acids and initiate lipid peroxidation within the cell, which results in the formation of aldehyde by-products such as MDA. Thus, MDA is widely used as a marker of lipid oxidation that reflects the level of oxidative stress. These molecules have the potential to diffuse from their site of origin to reach distant intracellular and extracellular targets, thereby amplifying the effects of oxidative stress [35]. Given all this, lipid over accumulation is the most important risk factor in the formation and development of oxidative stress. Therefore, the levels in liver of lipid (TC, TG, LDL-C, and HDL-C) and the lipid peroxidation product MDA were investigated. In our study, the elevated levels of TC, TG, and LDL-C among rabbits with NAFLD were reduced following treatment with apoA-I (40 mg/kg/wk), and the concentration of MDA was also reduced in rabbits of both the apoA-I groups (20 or 40 mg/kg/wk). Our results indicate that apoA-I might reduce the formation and development of oxidative stress through decreasing lipid levels, which is beneficial for prevention and treatment of NAFLD.

Enhancement of organic antioxidant levels can resist oxidative stress effectively. In order to investigate any antioxidant effect of apoA-I in NAFLD, the levels of SOD, GSH-Px, CAT, and iNOS were measured. SOD, GSH-Px, and CAT are three vital antioxidant enzymes, which play important roles in reducing lipid peroxides as well as hydrogen peroxide and maintaining the structure and function of biological membranes. In contrast, iNOS can persistently and profusely release NO, which can lead to an imbalance between the production of free radicals and the antioxidant level, and aggravate oxidative damage. Our results revealed that apoA-I (20 or 40 mg/kg/wk) markedly increased SOD and GPx activity in serum and liver of high fat-fed rabbits, while apoA-I reduced iNOS activity in the liver of rabbits on a high-fat diet. At the same time, apoA-I (20 or 40 mg/kg/wk) enhanced liver *SOD*, *GPx*, and *CAT* mRNA expression levels in high fat-fed rabbits. These results indicated that apoA-I could resist oxidative stress through enhancement of organic antioxidant levels, which could alleviate the symptoms of NAFLD.

In addition to oxidative stress, inflammation is often seen in the pathogenesis of NAFLD. The anti-inflammatory effects of apoA-I have been confirmed, both *in vitro* and *in vivo*, by many studies. ApoA-I has been used in the treatment of some diseases related to inflammation, such as AS, which is closely associated with NAFLD. Therefore, the anti-inflammatory effects of apoA-I may also have contributed to the alleviation of NAFLD, but further research is needed to confirm this hypothesis.

In conclusion, apoA-I improved the pathological abnormalities and decreased the concentration of MDA, TC, TG, and LDL-C in the liver of rabbits fed a high-fat diet. The activity of iNOS was reduced while SOD and GPx activity was increased in serum and liver of rabbits given this diet and supplemented with apoA-I. Also, apoA-I enhanced mRNA expression levels of *SOD*, *GPx*, and *CAT* in the liver of high fat-fed rabbits. These findings suggest that apoA-I probably exerts its protective effects against NAFLD in rabbits induced by high-fat diets through its antioxidant actions, which might provide an insight into further research on the effects of apoA-I on NAFLD and NASH.

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