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Protective effects of docosahexaenoic acid against non-alcoholic hepatic steatosis through activating of JAK2/STAT3 signaling pathway

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Abstract: Non-alcoholic fatty liver disease is the most common cause of hepatic dysfunction. In the present study, human normal hepatocyte L02 cells were treated with 50% fetal bovine serum to induce the formation of hepatic steatosis *in vitro*, and then the cells were treated with docosahexaenoic acid to investigate its protective effect on Non-alcoholic fatty liver disease. Our results showed that 50% of fetal bovine serum significantly induced intracellular lipid accumulation and hepatocyte fatty degeneration within 48 h. The expression level of adipose formation-related genes was significantly up-regulated, such as PPARγ, C/EBPα and SREBP-1; meanwhile, the content of cellular total lipid, total cholesterol and triglycerides were significantly increased after 50% fetal bovine serum treatment. Interestingly, docosahexaenoic acid treatment could inhibit FBS-induced intracellular lipid accumulation in L02 cells and the expression of lipogenic genes. Moreover, docosahexaenoic acid treatment could reduce hepatic steatosis-induced oxidative stress and endoplasmic reticulum stress response, and these responses were shown by the modification of antioxidant enzyme activities and GRP78, CHOP expression. In addition, the results showed that docosahexaenoic acid can activate the JAK2/STAT3 signaling pathway in fatty liver L02 cell; inhibition of JAK2/STAT3 signaling pathway by WP1066 abolished the beneficial effects of docosahexaenoic acid on hepatic steatosis accompanied with the increased expression of lipogenic genes and endoplasmic reticulum stress response. Above all, the present study showed that docosahexaenoic acid can alleviate non-alcoholic hepatic steatosis by activating JAK2/STAT3 signaling pathway.

Introduction

With the development of the economy and the improvement of life quality, the prevalence of various kinds of "disease of affluence" such as obesity and diabetes has increased rapidly; the non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease. It is estimated that 24–42% of the population in Western countries and 5–42% in Asian countries are affected by (Targher *et al.*, 2016; Zheng *et al.*, 2016). The NAFLD is a common liver disease closely linked with the features of metabolic syndrome; the main characteristic of NAFLD is the accumulation of fat in liver cells in the absence of excessive alcohol intake (Merola *et al.*, 2015; Smith and Adams, 2011). The accumulated-lipid droplets led to a liver more sensitive to inflammatory cytokines, oxidative stress, endoplasmic reticulum (ER), or mitochondrial dysfunction, which further induce the development of nonalcoholic steatohepatitis (NASH) (Lim *et al.*, 2010; Luo *et al.*, 2020). However, there is no validated drug therapy at present. The lifestyle interventions designed to reduce body weight remain the first-line treatments, such as diet and exercise, but several studies demonstrated that lifestyle interventions cannot improve the histological features of NAFLD, but only the metabolic parameters and simple steatosis (Nobili *et al.*, 2008; Vilar-Gomez *et al.*, 2015; Zohrer *et al.*, 2017).

The endoplasmic reticulum (ER) is an important organelle that is responsible for proper and posttranslational modification

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of proteins, lipid synthesis, and calcium storage. ER stress, which was caused by the accumulation of unfolded protein and calcium depletion, contributed to unfolded protein response (UPR) and the occurrence of diseases. Hepatocytes contain a large amount of ER to synthesize plasma protein, secrete low-density lipoprotein and metabolize xenobiotics. Therefore, excessive ER stress usually caused the dysfunction of ER and liver diseases, such as NAFLD or liver fibrosis (Rutkowski and Kaufman, 2004). GRP78, ATF4, and SREBP-1C, three markers UPR, which are highly expressed under ER stress, might be involved in the formation and development of NAFLD (Lewis and Mohanty, 2010; Zhang *et al.*, 2011; Yamamoto *et al.*, 2010).

Docosahexaenoic acid (DHA) is the major polyunsaturated fatty acids (PUFA) found in marine fish oil, which is the essential fatty acid of mammals. DHA is the main component of the phospholipid of the cell membrane and cannot be synthesized in the body (Horrocks and Yeo, 1999). Previous studies found that PUFA can regulate lipid metabolization in many kinds of animals (Khan *et al.*, 2002; Peyron-Caso *et al.*, 2003). When NAFLD patients were supplemented with PUFA for 6 months, the alanine aminotransferase and triglyceride levels in the liver were significantly decreased, and the symptoms of NAFLD were also relieved (Spadaro *et al.*, 2008). In addition, DHA also improved insulin sensitivity by regulating lipid-related gene expression and ameliorated hepatic triglycerides accumulation in NAFLD mice (Fedor *et al.*, 2012; Sun *et al.*, 2011). Thereby, supplementation of DHA had a potential therapeutic effect on lipogenesis, fatty acid oxidation, and hepatic lipid metabolism (De Castro *et al.*, 2015; Zhang *et al.*, 2013); it can also attenuate fatty liver-caused damage by inhibiting endoplasmic reticulum stress response (Zheng *et al.*, 2016). However, the possible protective mechanisms of DHA on NAFLD are still not clear.

Janus kinase 2 -signal transducer and activator of transcription 3 (JAK2/STAT3) signaling pathway are involved in many physiological and pathological regulation processes, such as inflammation and apoptosis. Activation of JAK2/STAT3 signaling pathway induced the expression of HMGB1 and inflammatory reaction, which is associated with the NAFLD; rapamycin can inhibit JAK2/STAT3 signaling pathway to reduce HMGB1 expression, which attenuated the liver injury (Zeng *et al.*, 2014). In the present study, we investigate whether DHA could have a protective effect on non-alcoholic hepatic steatosis, and it is unclear whether the beneficial effect of DHA on NAFLD is regulated by JAK2/STAT3 signaling pathway. Our result demonstrated that supplementation of DHA could attenuate lipid droplets-caused oxidative stress and ER stress response in L02 cells through activating the JAK2/STAT3 signaling pathway.

Materials and Methods

Materials and reagents

Human normal liver cell line (L02 cell), fetal bovine serum (FBS), Dulbecco minimum essential medium (DMEM), penicillin-streptomycin were purchased from KeyGEN Bio TECH (Nanjing, Jiangsu, China), Docosahexaenoic acid (cat: 6217-54-5) was purchased from Tocris Cookson Ltd (USA), and dissolved in anhydrous alcohol. Real-time quantitative PCR reagents were purchased from Ambion (Austin, TX, USA). Triglycerides (TG) (cat: A110-1), aspartate aminotransferase (AST) (cat: C010-2), lactate dehydrogenase (LDH) (A020-2), total cholesterol (TC) (cat: A111-1), malondialdehyde (MDA) (cat: A003-4), glutathione peroxidase (GSH-Px) (cat: A005) and total superoxide dismutase (SOD) (cat: A001-1-1) kits were purchased from Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). PrimeScriptTM RT Master Mix (cat: RR036A) and SYBR® Premix Ex Taq™ (cat: RR420A) were purchased from TaKaRa Biotechnology. C/EBPα (cat: sc-9314) and PPARγ (cat: sc-1981) antibodies were purchased from Santa Cruz Biotechnology (Beijing, China), a-tubulin (cat: 11H10), P-JAK2 (cat: TYR1007/1008), P-STAT3 (cat: D3A7), and STAT3 (cat: 79D7) antibodies were purchased from Cell Signal, JAK2 (cat: 1001-1015) antibody was purchased from Sigma Aldrich (Shanghai, China), GRP78 (cat: 11587-1-AP), GADPH (cat: 60004-1-lg) and CHOP (cat: 15204-1-AP) antibodies were purchased from Proteintech (Wuhan, China).

Cell culture and DHA treatment

L02 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 IU/mL penicillin, 100 μg/mL streptomycin) in 5% CO_2 at 37°C. After the culture reached 80% confluence, cells were seeded in multi-well plates or flasks for 24 h. The cells were then divided into three groups: Control group, FBS-treated groups (24, 48, and 72 h), and DHA-treated group (25 μM). According to previous studies, 50% FBS was able to induce non-alcoholic hepatic steatosis in cell model (Cui *et al.*, 2017; Wu *et al.*, 2010), so the control group cells were cultured in DMEM, and FBS-treated group cells were cultured in 50% FBS medium for 24, 48, and 72 h to induce steatosis. As for the DHA-treated group, the cells first were cultured in 50% FBS medium for 48 h and then cultured in DMEM medium with DHA (25 μM) for 24 h.

Oil red O staining

The accumulation of lipid droplet in L02 cells were visualized and analyzed by Oil Red O staining (Fu *et al.*, 2016). In brief, the cells are grown in 6-wells plates. After treatment, the cells were washed twice with PBS, and then the cells were fixed with 2 mL of 4% (v/v) formaldehyde for 30 min. Then the cells were stained with 0.5 mL of freshly prepared oil red O solution for 30 min at 37°C. After that, the cells were treated with 70% alcohol and observed under a light microscope.

Determination of the contents of total cholesterol, triglycerides and the activities of aspartate aminotransferase and lactate dehydrogenase

The cells were collected and sonicated on ice. The cell lysates were clarified by centrifugation. Then the total cholesterol (TC), triglyceride (TG), and the enzymatic activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were measured by corresponding assay kit according to the instructions (Nanjing Jiancheng Bioengineering Institute, China).

Measurement of anti-oxidative enzyme activates

Malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and total superoxide dismutase (SOD) activities were

measured separately by MDA, GSH-Px, and SOD assay kits (Nanjing Jiancheng Bioengineering Institute, China). The cells were collected and placed in a centrifuge tube for the following experiments. For MDA assay: after adding 500 μL lysis buffer into the cells for 2 min, the cell extraction (100 μL) was mixed with working buffer and incubated at 95°C for 40 min. Then the mixture was centrifuged at 4000 rpm for 10 min, 250 μL of supernatant was added into a 96-well plate, and the absorbance value was measured at 530 nm. MDA content was calculated based on the absorbance value. For GSH-Px assay: After adding the lysis buffer into the tubule, the cell extraction was centrifuged at 3000 rpm for 10 min, and the supernatant was used for measuring GSH-Px. The absorbance value was examined at 412 nm. For SOD assay: the supernatant of cell extraction was added into a 96-well plate and incubated at 37°C for 20 min, then the absorbance value at 450 nm was determined by using Full wavelength marker.

Total RNA extraction and qRT-PCR analysis

The total RNA was extracted using Trizol reagent, and then reverse transcription was performed using a PrimeScriptTM RT Master Mix. The mRNA expression was quantified using qRT-PCR. The expression levels of all target genes were normalized to those of the endogenous reference gene β-actin using an optimized comparative Ct ($2^{-\Delta\Delta Ct}$) value method, where $\Delta\Delta Ct = \Delta Ct_{target} - \Delta Ct_{\beta\text{-actin}}$. Primer sequences are listed in Tab. 1.

Protein extraction and Western blot analysis

To extract total protein, cells were treated with lysis buffer and centrifuged for 15 min at 4°C. The protein concentration was quantified using the BC protein assay kit. The Western blot procedures were performed as previously described (Chen *et al.*, 2018). The following primary antibodies were used: Goat monoclonal anti-PPARγ, C/EBPα and SREBP-1 (1:500), P-JAK2, JAK2, P-STAT3, STAT3, GRP78, CHOP, GADPH (1:1000) and α-tubulin (1:2500).

Statistical analysis

All experimental data were expressed as the means ± SEM. Statistical comparisons were made by analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. Statistical significance was shown as $^{*}p < 0.05$, $^{**}p < 0.01$.

Results

Establishment of hepatic steatosis model

Human normal hepatocyte L02 cells were treated with 50% FBS to induce hepatic steatosis, then the cells were stained with Oil Red O to detect the formation of lipid droplets. As shown in Fig. 1, after 50% FBS treatment, there are many small lipid drops were formed in the luminal side of L02 cells (Figs. 1A–1D). Then, the lipid droplets were extracted with isopropyl alcohol, and the absorbance of the extracted solution was measured by a microplate reader at 490 nm. The results showed that intracellular fat content was significantly increased after 50% FBS treatment for 48 and 72 h in L02 liver cells (Fig. 1E).

To further examine the effect of 50% FBS on liver cell steatosis, the intracellular lipogenesis-related indexes TG, TC, and hepatic function-related indexes AST and LDH were examined. The results showed that the contents of TG and TC were significantly increased after 50% FBS treatment compared with the control group in L02 cells ($p < 0.01$) (Figs. 1F–1G). At the same time, 50% FBS treatment also increased the activity of AST and LDH (Figs. 1H–1I), suggesting that the metabolic function has a defect in 50% FBS-treated L02 liver cells. Above all, the accumulation of lipid droplets and the defect of cell metabolism indicated that the hepatic steatosis model was successfully established after high concentration of FBS treatment.

Effect of DHA on lipid accumulation in FBS-induced hepatic steatosis

To detect the effect of DHA on non-alcoholic hepatic steatosis, we first examine its effect on lipid droplet formation in L02 cells. Our result showed that the lipid accumulation was significantly inhibited in DHA-treated cells (Figs. 2A–2C); meanwhile, the absorbance of the extracted solution was also significantly decreased after DHA treatment compared with the group of only 50% FBS treatment (Fig. 2D). These results clearly demonstrated that DHA can diminish the accumulation of lipid droplets, which is accompanied by the decrease of TC and TG contents (Figs. 2E, 2F).

To further examine the effect of DHA on hepatic functions, the activities of AST and LDH were measured after DHA treatment. As shown in Figs. 2G, 2H, 50% FBS treatment can significantly increase the activity of AST and LDH compared with the control group. However, the activities of AST and LDH were significantly decreased after DHA treatment ($p < 0.05$).

To further investigate the mechanism of DHA in lipid droplet formation in NAFLD, the lipogenic genes PPARγ, C/EBPα, and SREBP-1 were examined by qRT-PCR and Western blot. The results showed that the mRNA level and protein expression of PPARγ, C/EBPα, and SREBP-1 were significantly up-regulated in 50% FBS-treated L02 cells;

TABLE 1

Primer sequences of mRNA

Genes	Forward	Reverse
PPARγ	5'-CCACATTACGAAGACATTCCA-3'	5'-CAGGCTCCACTTTGATTGC-3'
SREBP1	5'-ACGGGAGGATGGACTGACTT-3'	5'-AGGCTTCTTTGCTGTGAGATG-3'
C/EBPα	5'-TTGGTGCGTCTAAGATGAGG-3'	5'-TTGGAGCGGTGAGTTTGC-3'
β-Actin	5'-TGACGTGGACATCCGCAAAG-3'	5'-TGGAAGGTGGACAGCGAGG-3'

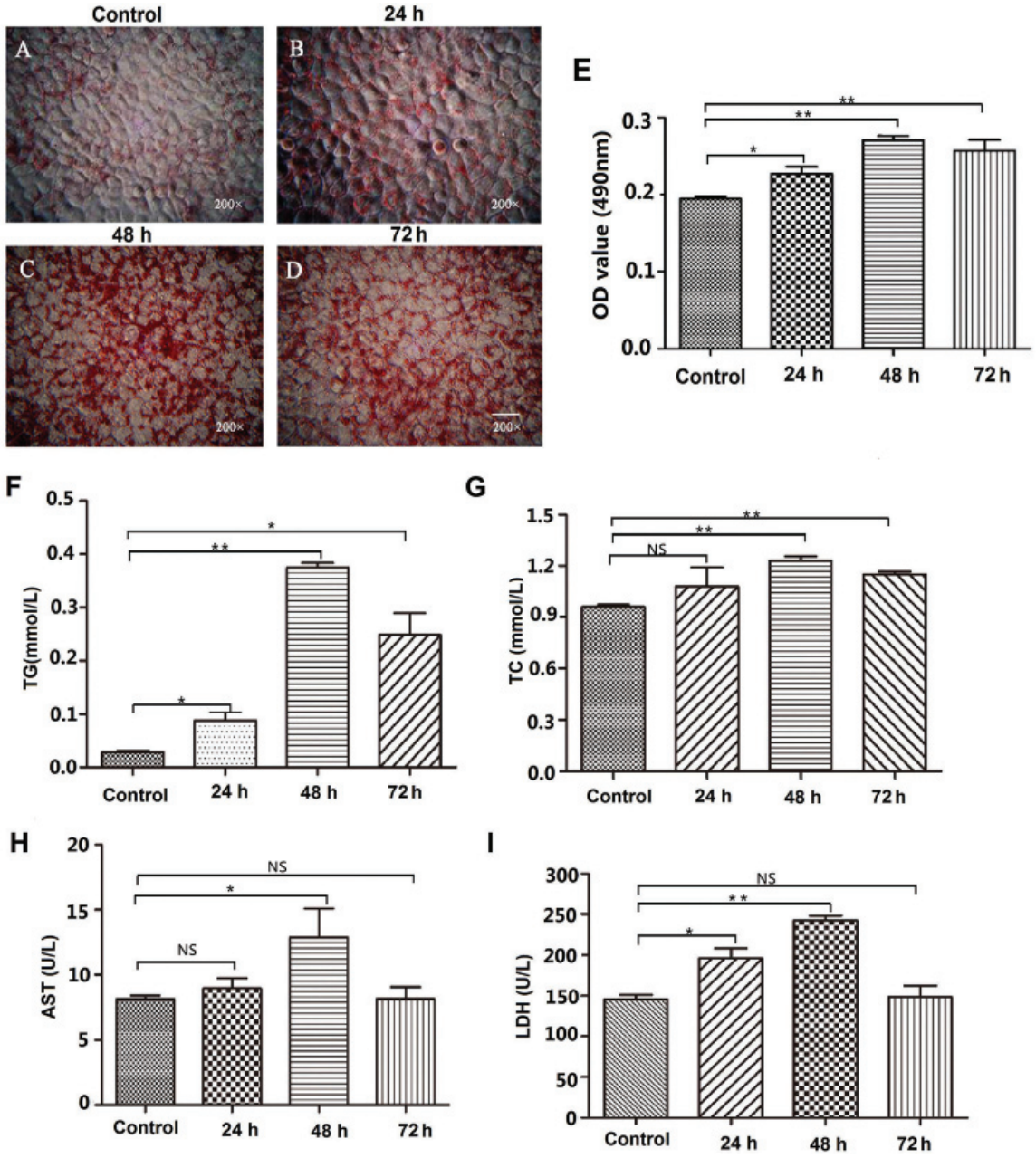

FIGURE 1. Establishment of the hepatic steatosis model in L02 cells.
(A–D) L02 cells were stained by Oil red O working solution. Scale for 100. (E) The intracellular lipids were extracted using isopropanol and then quantified the absorbance at 490 nm. (F–I) The contents of TG, TC, and the activity of AST, LDH were measured by the corresponding kit. These results are presented as the mean ± SE from three independent determinations. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* the control group.

whereas after DHA treatment, it can significantly down-regulated the mRNA level and protein expression of PPARγ, C/EBPα, and SREBP-1 (Figs. 2I–2M).

Effects of DHA on oxidative stress and ER stress response in FBS-induced hepatic steatosis

To further investigate the effect of DHA on hepatic steatosis, the levels of oxidative stress were analyzed. The cells were incubated with 50% FBS and were cultured with DHA (25 μM) medium for 24 h, then the activity of oxidative stress-related enzymes was measured. Our results showed that the contents of GSH-Px and SOD were significantly reduced in 50% FBS-treated L02 liver cells; whereas after DHA treatment, the contents of SOD and GSH-Px were significantly increased (Figs. 3A, 3B). In contrast, DHA can relieve the activity of MDA in FBS-induced hepatic steatosis (Fig. 3C). These results suggested that DHA can prevent the accumulation of oxidative stress products in fatty liver cells.

Glucose-regulated protein 78 (GRP78) and homologous protein (CHOP) are the protein markers of ER stress-induced unfolded protein response. Thereby, we next examined the effect of DHA on the hepatic steatosis-induced ER stress response. As shown in Figs. 3D, 3E, the protein level of GRP78 and CHOP were significantly increased in 50% FBS-treated L02 liver cells; however, DHA treatment can significantly alleviate FBS-induced ER stress by down-regulating GRP78 and CHOP in L02 cells, indicating that DHA could attenuate the hepatic steatosis-induced ER stress response.

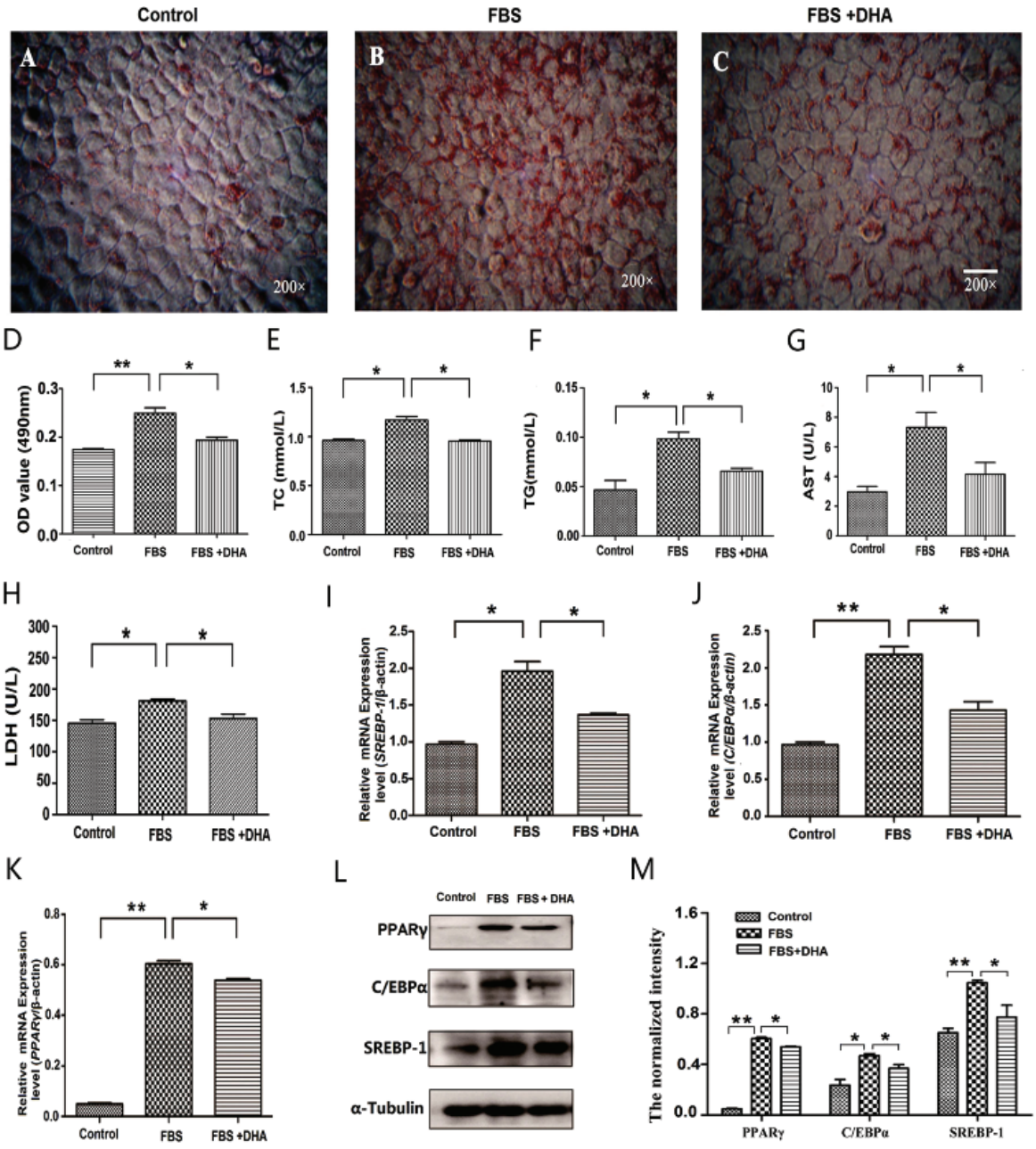

FIGURE 2. Effect of DHA on lipid accumulation in FBS-induced hepatic steatosis.
(A–C) The cells were stained with Oil red O working solution after DHA treatment. Scale for 100. (D) The intracellular lipids were extracted using isopropanol and then quantified the absorbance at 490 nm in different groups. (E–H) The contents of TG, TC, and the activity of AST, LDH were measured after DHA treatment. (I–K) The mRNA levels of SREBP-1, C/EBPα and PPARγ were determined by qRT-PCR. (L) SREBP-1, C/EBPα and PPARγ protein levels were measured by western blot. (M) The normalized bands' intensity was assayed by Image J software. The results are presented as the Mean ± SE from three independent determinations. $^{*}p < 0.05$, $^{**}p < 0.01$.

DHA attenuate hepatic steatosis through mediating JAK2/STAT3 signaling pathway

To further investigate the protective mechanism of DHA in hepatic steatosis-induced oxidative stress and ER stress, we examined whether the JAK2/STAT3 signaling pathway involved in this process by investigating the expression of JAK2/STAT3 and the phosphorylation of JAK2/STAT3. Our result showed that the phosphorylation of JAK2/STAT3 was significantly decreased in 50% FBS-treated L02 cells compared with the control group. Whereas DHA treatment restored the phosphorylation level of JAK2 and STAT3 (Figs. 4A–4C). These results implied that DHA could attenuate hepatic steatosis-induced injury through activating JAK2/STAT3 signaling pathway.

Inhibition of JAK2/STAT3 signaling pathway reversed the beneficial effect of DHA on hepatic steatosis

WP1066 is considered as a specific inhibitor of the JAK2/STAT3 signaling pathway. As shown in Figs. 4A–4C, WP1066 treatment significantly inhibited the phosphorylation of JAK2 and STAT3, while it also blocked the effect of DHA on the phosphorylation of JAK2 and STAT3, which further demonstrated that DHA could attenuate FBS-induced steatosis hepatocytes via mediating JAK2/STAT3 signaling pathway.

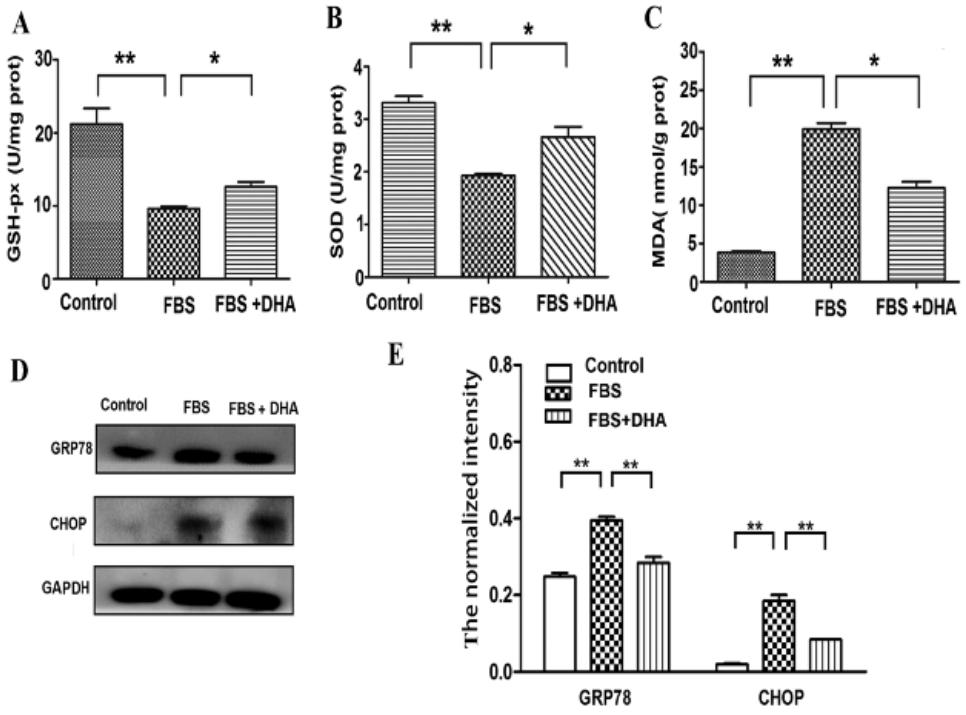

FIGURE 3. Effects of DHA on oxidative stress and ER-stress response in FBS-induced hepatic steatosis. (A–C) MDA, GSH-Px and SOD levels were measured using oxidative enzymatic kits. (D) GRP78 and CHOP protein levels were measured by western blot. (E) The normalized bands' intensity was assayed by Image J software. These results are presented as the mean ± SE from three independent determinations. $^{*}p < 0.05$, $^{**}p < 0.01$.

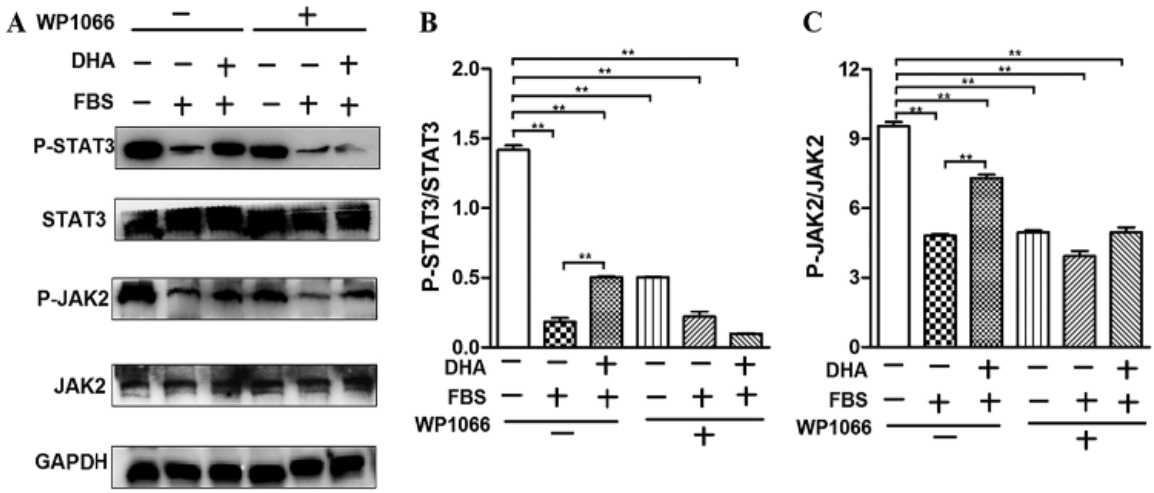

FIGURE 4. DHA attenuates hepatic steatosis by mediating JAK2/STAT3 signaling pathway. (A) P-STAT3, STAT3, P-JAK2 and JAK2 protein levels were measured by western blot. (B, C) The normalized bands' intensity was assayed by Image J software. These results are presented as the mean ± SE from three independent determinations. $^{*}p < 0.05$, $^{**}p < 0.01$.

To further confirm the protective role of DHA on the hepatic steatosis-induced injury was associated with JAK2/STAT3 signaling pathway, we treated the cells with WP1066 to determine the effect of DHA on the expression of lipogenic genes and ER stress response. Our results showed that WP1066 treatment can reverse the effect of DHA on PPARγ, C/EBPα, and SREBP-1 expression in FBS-induced hepatic steatosis (Figs. 5A–5E). Meanwhile, WP1066 treatment also blocked the restoration of DHA in FBS-induced ER stress response (Figs. 5F–5G).

Discussion

In the present work, we treated the L02 cells with 50% FBS to induce hepatic steatosis, which mimics the feature of NAFLD *in vitro* to study the protective effect of DHA on non-alcoholic hepatic steatosis. The present study provides mechanistic insights into how DHA alleviate the injury of hepatic steatosis in L02 cells. We demonstrated that DHA can reduce the accumulation of lipid droplets in hepatic steatosis and also reduced the hepatic steatosis-induced oxidative stress and ER stress response through activating the JAK2/STAT3 signaling pathway.

The liver is an important organ for maintaining the metabolism of the human body. It plays an important role in the metabolism of various nutrients and drugs and is also the central hub of lipid metabolism in the human body (Hallsworth *et al.*, 2013). Fatty liver has become an important liver disease in the Asia-pacific region, especially the NAFLD has become the major form of chronic liver disease that is characterized by the formation of steatosis, inflammation, and different degree of fibrosis in liver tissue

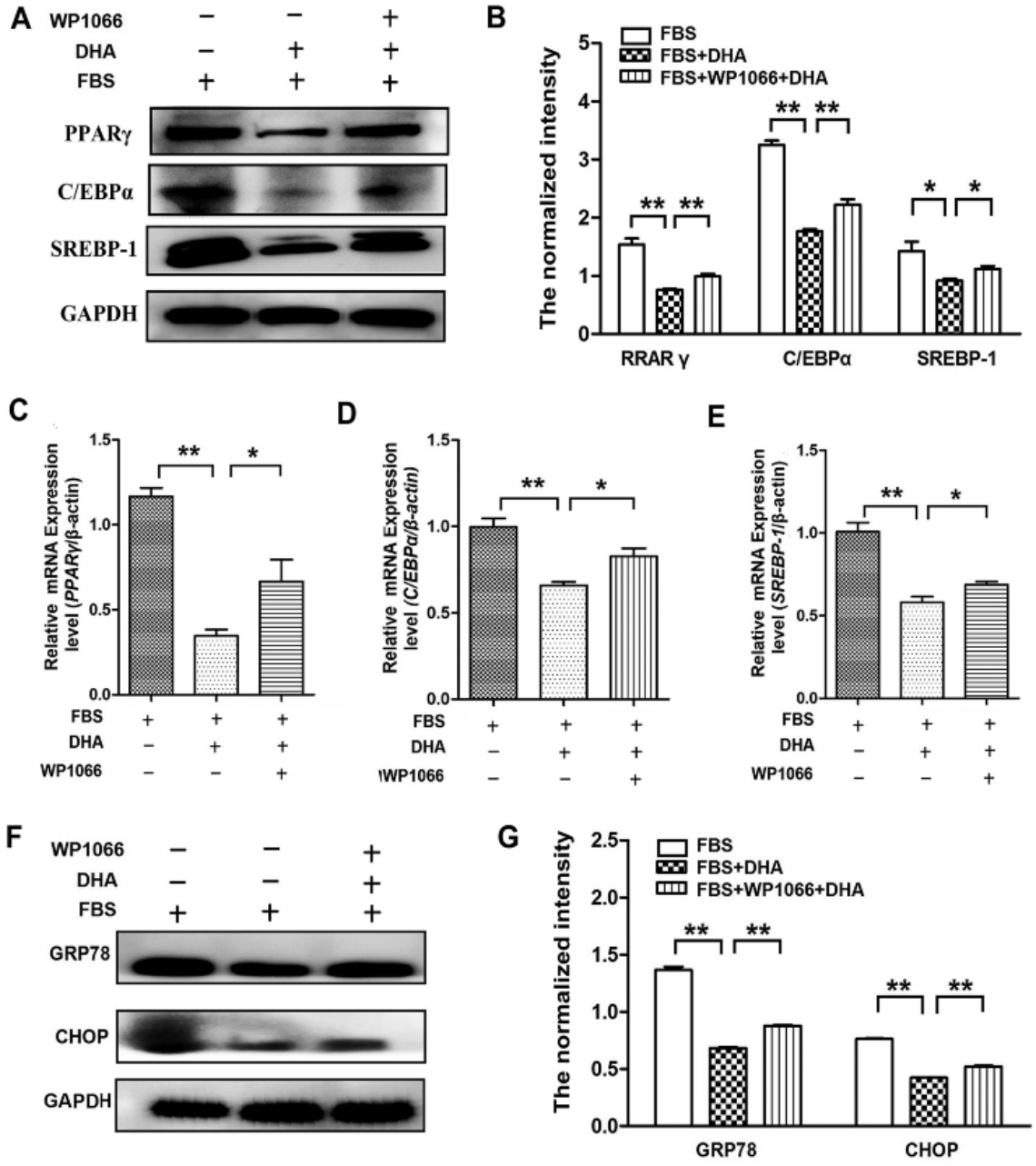

FIGURE 5. Inhibition of JAK2/STAT3 signaling pathway reversed the beneficial effect of DHA on hepatic steatosis.
(A) The protein levels of PPARγ, C/EBPα and SREBP-1 were measured by western blot. (B) The bands' intensity was assayed by Image J software. (C–E) The mRNA level of SREBP-1, PPARγ and C/EBPα were analyzed by qRT-PCR. (F) The protein levels of GRP78 and CHOP were measured by western blot. (G) The normalized bands' intensity was assayed by Image J software. Data are expressed as the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$.

(Ahmed *et al.*, 2010; Fassio *et al.*, 2004). It was well known that excessive accumulation of TC and TG in hepatocytes are the main factors of NAFLD (Wang *et al.*, 2012). Meanwhile, AST and LDH are often used as sensitive and fairly specific biomarkers to evaluate drug-induced hepatocellular injury in preclinical and clinical studies (Schurr and Payne, 2007). Previous studies have shown that supplementation of DHA can inhibit lipogenesis and has a beneficial effect on hepatic lipid metabolism (De Castro *et al.*, 2015; Devarshi *et al.*, 2013). The present study showed that DHA can significantly reduce hepatic lipid accumulation through inhibiting the accumulation of TC and TG and also reducing the activities of AST and LDH. In addition, supplementation of DHA also inhibited the expression of lipogenic genes (such as PPARγ, C/EBP, SREBP-1), suggesting that DHA has anti-hepatic steatosis function in our NAFLD model.

The excessive accumulation of lipid droplets in the liver was associated with the dysfunction of organelles, such as mitochondria and ER. The overloaded lipid increased the synthesis of acetyl-CoA and disturbed the function of the tricarboxylic acid (TCA) cycle during mitochondrial respiration, which increased the reactive oxygen species (ROS) formation. The defect of mitochondrial morphology, electron transport chain, and ATP generation have been shown in NAFLD accompanied by the high level of ROS and inflammation (Sunny *et al.*, 2017; Wang *et al.*, 2020). Therefore, oxidative stress has been considered as one of the major pathogenic mechanisms for the progression of

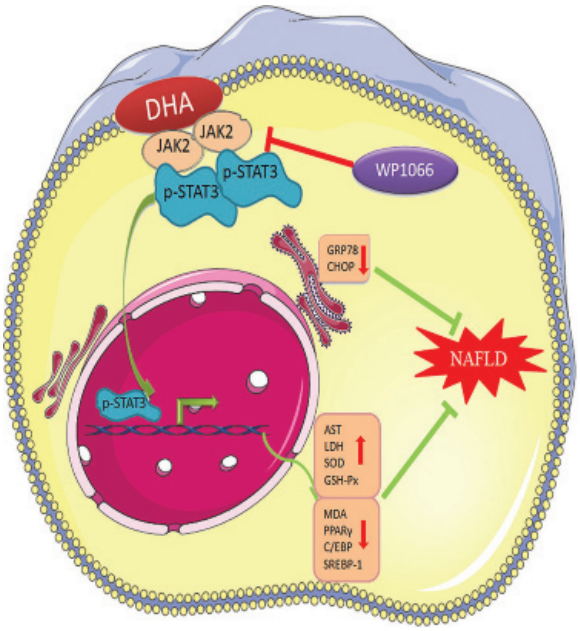

FIGURE 6. The potential functional mechanism of DHA in non-alcoholic hepatic steatosis.

NAFLD (Madan *et al.*, 2006). Different levels of intracellular GSH-Px, SOD, and MDA are important factors to evaluate antioxidant ability. The result showed that the anti-oxidative markers SOD and GSH-Px were significantly increased after DHA treatment, whereas the MDA content was decreased, suggesting that DHA has anti-oxidative functions to relieve the hepatic steatosis-induced oxidative stress.

Under environmental or physiological conditions, when the unfolded protein response (UPR) is not sufficient to maintain normal hepatocellular function, which can induce ER stress and disrupt the ER-dependent liposome homeostasis, thereby stimulating the development of steatosis (Bozaykut *et al.*, 2016). Previous studies showed that the URP was activated in NAFLD patients, accompanied by the increased expression of CHOP and GRP78 (Lee *et al.*, 2017; Zhou *et al.*, 2017). The present study showed that supplementation of DHA significantly alleviated hepatic steatosis-induced ER stress response by down-regulating GRP78 and CHOP. These results indicated that DHA could directly or indirectly target GRP78 and CHOP to inhibit liver lipid accumulation and cell inflammation, which further ameliorate DAFLD development.

JAK-STAT pathway was found in recent years that involved in colonization of the cell increases, differentiation, death depth, and immune regulation and many important biological processes (Zhao *et al.*, 2013). In the high-energy-induced fatty liver rats model, kefir peptides or puerarin can effectively attenuate the symptoms of NAFLD by enhancing the phosphorylation of JAK2 and STAT3, which suggested that the JAK-STAT signaling pathway plays critical roles in NAFLD (Chen *et al.*, 2016). DHA, a natural ligand of PPARγ (Neschen *et al.*, 2007), may activate the JAK2/STAT3 signaling pathway by inhibiting PPARγ (Hwang *et al.*, 2017), thereby alleviating nonalcoholic fatty liver disease. In the present study, we found that the phosphorylation of JAK2 and STAT3 were increased after DHA treatment; meanwhile, WP1066 treatment significantly inhibited the phosphorylation of DHA on JAK2 and STAT3, further suggesting that DHA mediated the JAK2/STAT3 signaling pathway attenuated FBS-induced steatotic hepatocytes. To further demonstrate the effect of DHA on lipid gene expression and endoplasmic reticulum stress via the JAK2/STAT3 pathway. We treated the cells with WP1066, and the results showed that WP1066 treatment could offset the decreased expressions of PPAR, C/EBP, and SREBP-1 in FBS-induced hepatic steatosis induced by DHA. Meanwhile, WP1066 treatment also prevented DHA recovery from FBS induced stress response. The results indicated that DHA attenuated the hepatic steatosis-induced oxidative stress and ER stress through activating the JAK2/STAT3 signaling pathway in the NAFLD.

Conclusions

Our study demonstrated that a high concentration of FBS can cause hepatic steatosis, which can be used to build the *in vitro* NAFLD model. Meanwhile, DHA could attenuate lipid droplets-caused oxidative stress and ER stress response in L02 cells through activating the JAK2/STAT3 signaling pathway (Fig. 6), which indicated that the JAK2/STAT3 signaling pathway might be an important molecular target of DHA for alleviating NAFLD.

Ethics Approval: Not applicable.

Availability of Data and Material: All data generated or analyzed during this study are included in this published article.

Author Contribution: YW and YPD conceptualized the study, participated in its design and research. KLC carried out the molecular studies and sample collection. HXL and YQ analyzed data and drafted the manuscript. All authors read and approved the final manuscript for publication.

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Conflicts of Interest: The authors declare that there is no conflict of interests.

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