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PRIMA1 participates in the inhibition of inflammation and acetylcholinesterase activity in goose fatty liver formation

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ABSTRACT

Goose fatty liver, a product of short-term overfeeding, is notable for its high nutritional value and unique tolerance to severe steatosis without inflammation, in contrast to human nonalcoholic fatty liver disease (**NAFLD**). It is known that choline can alleviate nonalcoholic steatohepatitis and liver cirrhosis, inhibit cell apoptosis, promote lecithin synthesis and fat transportation out of the liver, and relieve cardiovascular disease-related symptoms (e.g., hyperlipidemia and hypercholesterolemia). This study investigates the role of Proline Rich Membrane Anchor 1 (***PRIMA1***) in inhibiting inflammation through choline metabolism during goose fatty liver formation. Overfeeding of geese resulted in increased body and liver weights, elevated fat content, and significantly higher expression of *PRIMA1*, accompanied by decreased **LITAF** and **CRP** (important pro-inflammatory cytokines) expression and reduced acetylcholinesterase (**AchE**) activity, which was assessed by the amount of produced choline. The overexpression of *PRIMA1* in goose primary hepatocytes (**GPHs**) enhanced AchE activity. Consistently, glucose-induced upregulation of *PRIMA1* expression in GPHs was accompanied by increased AchE activity. Further combined treatment with glucose and *PRIMA1* knockdown in GPHs showed that downregulation of *PRIMA1* attenuated the suppression of LITAF and CRP expression caused by glucose addition, but had no effect on the rise in AchE activity. These findings indicate that *PRIMA1* may play a role in promoting choline metabolism and protecting against liver inflammation, offering insights into potential therapeutic targets for NAFLD.

Introduction

Goose fatty liver (commonly known as foie gras) is a premium waterfowl product with a delicate texture, a distinctive flavor, and a high nutritional value. It contains a considerable quantity of unsaturated fatty acids and lecithin, which have been demonstrated to facilitate the softening of blood vessels and the prevention of cardiovascular diseases (Fournier et al., 1997). The production of goose fatty liver is achieved through a short-term overfeeding (3-4 wk) with an energy-rich diet, and the weight of fatty liver can reach 8-10 times that of normal liver (Hermier et al., 1994). The severe steatosis in goose fatty liver does not result in the inflammation, fibrosis or injury that are often observed in human or rodent nonalcoholic fatty liver disease (NAFLD). This feature may be associated with the long-term evolution of goose (Davail et al., 2000).

To meet the energy requirements for a long-distance migration, the ancestors of goose have to deposit large amounts of fat in their livers before migration, thus forming a physiological fatty liver. However, the fatty liver can return to normal after migration, suggesting that geese have developed a mechanism to protect the liver and to make it tolerant to steatosis. Indeed, studies have shown that the goose has some protective components, including increased mitochondrial-related genes, adiponectin receptors and fatty acid desaturases in goose fatty liver, as well as decreased complement genes, endoplasmic reticulum stress-related marker genes and tumor necrosis factor α (TNFα) (Geng, Yang, et al., 2016; Liu et al., 2016). Understanding the mechanisms underlying fatty liver development in geese may provide valuable insights into the pathology of hepatic steatosis and potential therapeutic targets for both animals and humans.

The ingestion of excessive quantities of food causes large influx of

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energy and nutrients into the blood and other tissues, leading to hyperglycemia, hyperlipidemia, cell death and inflammation that are often shown in human nonalcoholic steatohepatitis (**NASH**). It is well established that the dietary intake of choline is closely associated with the incidence of NASH (Sha et al., 2010). Choline, a tetravalent base with a positive charge, has affinity for fat, and thus can promote the transportation of fat out of the liver in the form of phospholipids and prevent abnormal fat accumulation in the liver (Sherriff et al., 2016). Choline deficiency can lead to the development of NASH (Ikawa-Yoshida et al., 2017; Rinella et al., 2008), while choline supplementation can be used to treat NASH and liver cirrhosis, and to inhibit cell apoptosis. Furthermore, choline has been demonstrated to facilitate the synthesis of lecithin and relieve the symptoms of cardiovascular diseases (e.g., hyperlipidemia and hypercholesterolemia) (Ikawa-Yoshida et al., 2017; Rinella et al., 2008). One of the mechanisms for choline to alleviate NASH is to inhibit the production of several pro-inflammatory cytokines (e.g., interleukin 6 and TNFα) (MacDonald and Di Sabatino, 2009). Consequently, choline metabolism may be a significant factor in the prevention of inflammation during the development of goose fatty liver.

Proline-rich membrane anchor 1 (PRIMA1) function as a proline-rich membrane ankyrin, promoting the polymerization of acetylcholinesterase (AchE) and the subsequent localization of the activated AchE to the cell membrane (Dobbertin et al., 2009; Noureddine et al., 2007). AchE is a key enzyme in the regulation of acetylcholine metabolism and homeostasis, as it can degrade acetylcholine into choline and acetyl coenzyme A. Studies have indicated that activation of the vagus nerve can prevent fibrosis in NASH through the choline anti-inflammatory pathway (Morgan et al., 2016). Furthermore, activation of the α7 nicotinic acetylcholine receptor (**α7nAChR**) has been shown to inhibit inflammation (Kimura et al., 2019) and cell apoptosis in NASH by reducing reactive oxygen species (**ROS**) (Ni et al., 2016). In contrast, inhibition of AchE activity has been demonstrated to induce acute fat accumulation and to exacerbate lipid metabolism disorder in the liver (Yokota et al., 2014). Therefore, it is postulated that *PRIMA1* may inhibit inflammation and protect goose fatty liver by promoting AchE activity and choline production.

LITAF is a transcription factor initially identified in response to bacterial lipopolysaccharide (LPS) stimulation. It regulates the expression of various pro-inflammatory cytokines, including TNFα, interleukin-6 (IL-6), and IL-1β, and plays a critical role in the innate immune response and macrophage activation (Hong et al., 2006). *CRP* is an acute-phase protein primarily produced by the liver in response to inflammatory stimuli, such as IL-6. It functions as an opsonin, promoting the clearance of pathogens and damaged cells by enhancing phagocytosis. CRP levels rise rapidly during infection and tissue damage, making it a reliable marker for systemic and hepatic inflammation (Zimmerman et al., 2003). Therefore, LITAF and CRP were selected as representative inflammatory markers in this study to evaluate the hepatic immune status during overfeeding-induced steatosis and *PRIMA1* regulation. Their roles in immune signaling and acute-phase responses provide a broader and more accurate perspective on liver inflammation in the goose model.

This study aimed to investigate the effects of overfeeding on body and liver weight, fat content, and the expression of *PRIMA1* and AchE activity in geese. It was hypothesized that overfeeding would induce fatty liver formation, accompanied by a decrease in choline content and an increase of *PRIMA1* expression. However, the overexpression of *PRIMA1* in goose primary hepatocytes (GPHs) enhanced AchE activity. Consistently, GPHs treated with glucose demonstrated increased *PRIMA1* expression and AchE activity, while the Inflammation-related genes had no significant difference. Furthermore, according to the combined treatment of glucose and *PRIMA1* knockdown, Inflammation-related genes consequently showed significant increases in both mRNA and protein levels due to the knockdown of *PRIMA1* (compared to glucose treatment alone) but this did not affect the AchE activity. The findings of this study provide novel insights into the mechanisms that do not lead to inflammation in the goose fatty liver model and highlight potential targets for mitigating this condition in both avian and human populations.

Materials and methods

Experimental animals

In the overfeeding experiment, twelve 70-day-old healthy Landes geese (provided by Licheng Animal and Poultry Co., Ltd., Huaian, Jiangsu, China) were randomly divided into the two groups: the normal diet group (which had free access to diet and water) and the overfeeding group (which was routinely overfed for 19 days). Each group consisted of 6 geese. The diet consisted of cooked corn plus 1 % vegetable oil and 1 % salt (Zheng et al., 2015). Following overfeeding, the geese were sacrificed, and liver samples were collected. All the samples were snap-frozen in liquid nitrogen and transferred to −70 °C for storage.

Histological examination (HE staining)

Liver tissues were obtained from both the overfeeding group (**OF**) and the normal diet group (**ND**). The liver samples were immediately fixed in 10 % neutral buffered formalin for 24 hours to ensure the preservation of tissue integrity, embedded in paraffin, and sectioned. Tissue sections were first deparaffinized and rehydrated, followed by hematoxylin staining, bluing, and eosin staining. After dehydration and clearing, the samples were mounted for microscopic examination. The production of hematoxylin and eosin (**HE**) staining was conducted by the Servicebio company (Wuhan, China).

Isolation of goose primary hepatocytes and treatment with glucose

The method previously described by Osman (Osman et al., 2016) was employed to isolate GPHs from 23-day-old Landes goose embryos, which were subsequently plated in 12-well plates. Following an 8-hour incubation period, the cells were washed with phosphate buffer saline (**PBS**), after which, the fresh low-sugar Dulbecco's Modified Eagle Medium (**DMEM**) (Sangon Biotech, BBI Life Sciences, Shanghai, China) with 10 % fetal bovine serum (**FBS**) was added to the plates. The cells were incubated in the medium at 38°C, 5 % CO2. Glucose tolerance test was previously used to determine the appropriate dosage of glucose treatment (Geng et al., 2016). For the glucose treatment, the control group received DMEM culture medium containing 10 % FBS without glucose supplementation, while the treatment group received the control medium supplemented with different dosages of glucose (the final concentrations of the supplemented glucose in the media were 200 mmol/L). Following a 24-hour incubation period, the cells were washed with PBS and collected. A total of six replicates were employed for each treatment.

Overexpression of the PRIMA1 gene

Following the construction of the coding sequence of the goose *PRIMA1* gene into the empty vector pcDNA 3.1, the overexpression vector and empty vector were transfected into the GPHs respectively. The jetPRIME universal DNA/RNA transfection reagent was employed under the following protocol: A volume of 0.8 μg of the pcDNA 3.1/*PRIMA1* plasmid DNA was added to 1.6 μL of the jetPRIME transfection reagent. The mixture was vortexed for 10 s and then added to 75 μL jetPRIME buffer. After vortexing for 5 s, the transfection reagent mixture was incubated at room temperature for 10 min. The aforementioned mixture was introduced into the cultured cells, which should be mixed well by cross-shaking. The cells were incubated for a further 24 hours.

Glucose and PRIMA1 knockdown combined treatment

For the combined treatment, the glucose treatment group (**G200**) and the combined treatment group (**GKD**) received the control medium supplemented with 200 mmol/L glucose. After 24 hours, the GKD group was transfected with siRNA-PRIMA1. The jetPRIME universal DNA/RNA transfection reagent was employed under the following protocol: A volume of 306 ng siRNA-PRIMA1 (5′−3′: GCAGACGAAGUGACAGAGATT, 5′−3′: UCUCUGUCACUUCGUCUGCTT) was added to 4 μL of the jetPRIME transfection reagent. The mixture was vortexed for 10 s and then added to 200 μL jetPRIME buffer. After vortexing for 5 s, the transfection reagent mixture was incubated at room temperature for 10 min. The aforementioned mixture was introduced into the cultured cells, which should be mixed well by cross-shaking. The cells were incubated for a further 24 hours.

RNA extraction and cDNA synthesis

According to the manufacturer's instruction for TRIzol (Tiangen Biotech Co., Ltd., Beijing, China), total RNA was extracted from the livers of the geese and the cultured cells. The total RNA samples were diluted with nuclease-free water and treated with DNase to remove any residual genomic DNA. The concentration and quality of total RNA were determined using Nano Drop spectrophotometer (Nano Drop Technologies, Inc. Wilmington, DE, USA). Subsequently, reverse transcription was conducted with the qualified RNA samples in accordance with the instructions for the Hiscriptqrt Super Mix for qPCR reverse transcription kit (Nanjing Vazyme Biology Co., Ltd., Nanjing, China). In brief, 2 μL of RNA, 4 μL of 4 × gDNA wiper mix and 10 μL of RNAse-free ddH2O was combined for each reaction. The mixture was then placed on a PCR machine at 42°C for 2 min, followed by adding 4 μL of 5 × qRT supermix and the PCR was continued at 25°C for 10 min, 42°C for 30 min, and 85°C for 5 min. The cDNA samples were subsequently stored at −20°C until use.

Real-time fluorescence quantitative PCR (qPCR)

The expression of the *PRIMA1* gene was determined using the Vazyme AceQ qPCR SYBR Green Master Mix kit (Norwegian Biological Company, Nanjing, China) according to the manufacturer's instructions. The qPCR primers for *PRIMA1* were designed based on the sequence, with the forward primer being 5′-AAGCAGGCAGCTTCAG-3′, and the reverse primer being 5′-GGGCACCCAATTCAAACTAA-3′. The forward primer of *LITAF* was 5′-GTATGTGCAGCAACCCGTAG-3′, and the reverse primer was 5′- TGGGCATTGCAATTTGGACA-3′. For *CRP*, the forward primer was 5′-CGTCCAGGAAGGGTTTCGG-3′, and the reverse primer was 5′-TGGATCCAGGCGGTCATTTT-3′. *GAPDH* was employed as the internal reference gene, with the forward primer being 5′-GCATCAATGCTTCAT-3′ and the reverse primer being 5′-CTGGGGTCATCCTCCTG-3′. For qPCR, a total of 20 μL reaction mixture was prepared, comprising 10.0 μL of SYBR Green Master Mix, 0.4 μL of the forward primer (10 μM), 0.4 μL of the reverse primer (10 μM), 0.4 μL of ROX Reference Dye 2, 2.0 μL of the cDNA template, and 6.8 μL of double-distilled water. The reaction conditions were as follow: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 20 s. Three replicates were set for each cDNA sample. The qPCR results were calculated using the $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen, 2001).

Protein extraction and western blot

Total protein was extracted from livers and cells into RIPA buffer according to the manufacturer's instructions (C1055, APPLYGEN, Beijing, China). Relative levels of TNFα protein in individual samples were estimated using Western Blot Analysis method (see protocol on http://biosupport.licor.com). Briefly, protein samples were subjected to electrophoresis on Bis-Tris gels and then transferred to PVDF membranes. Following incubation with primary antibody and secondary antibodies, the membranes were imaged. Scanned images were analyzed with Image Studio Lite Ver 5.2 software. LITAF and CRP antibodies were from Proteintech (16797-1-AP, 83711-1-RR), and TNFα antibody was from Bioss (bs-2081R), the protein level of Tubulin (antibody from Cell Signaling Technology-2144) was used as a loading control.

Measurement of acetylcholinesterase (AchE) Activity

The acetylcholinesterase activity was determined in accordance with the instructions provided in the Acetylcholinesterase Activity Assay Kit (BC2025, Solarbio company, Beijing, China). AchE catalyses the hydrolysis of Ach to form choline, which interacts with disulfidetonitrobenzoic acid (**DTNB**) to form 5-mercapto-nitrobenzoic acid (**TNB**); TNB has an absorption peak at 412 nm, and AchE activity was calculated by determining the rate of increase in absorbance at 412 nm. For *in vitro* studies, the cells were ultrasonically broken in an ice bath (power 300 w, ultrasonic 3 seconds, interval 7 seconds, total time 3 min). The mixture was centrifuged and the obtained supernatant was placed on the ice for measurement. For *in vivo* studies, liver tissues were ground and the homogenates were then centrifuged for 10 min at 8000 g at 4 °C to obtain the supernatant for measurement. The absorbance of samples was detected at 412 nm to calculate AchE activity.

Statistical analysis

All data are presented as the mean ± standard error (**SEM**). A Student's t-test was used to analyze the statistical significance of the difference between the control and treatment groups, and $P < 0.05$ was set as the criteria for statistical significance.

Results

- Overfeeding Led to an Increase in both Body Weight and Liver Weight, Forming Goose Fatty Liver

After 19 days of routine overfeeding, the body weights and liver weights of the overfed group (OF) were compared with those of the normal diet group (ND). The results demonstrated a significant increase in both body weight and liver weight in the OF group compared to the ND group ($P < 0.01$) (Fig. 1- A, B). Correspondingly, the liver-to-body ratio was significantly higher in the OF group compared to the ND group (Fig. 1- C). Additionally, the livers of the OF group exhibited a greater volume and were lighter in color than those of the ND group (Fig. 1- D).

- Fat Content was Increased, while the Expression of Inflammation-related Genes was Reduced in OF compared to ND

Histological examination of the liver revealed a substantial number of white areas, indicating a significantly higher fat content in the livers of the OF group compared to the ND group. However, no lesions or inflammation due to excessive fat content were observed in the OF group (Fig. 2- A). Both the qPCR and Western Blot of Inflammation-related genes even verified that there was no inflammation occurring in the goose fatty liver, and the mRNA and protein levels were even reduced in the OF group compared to the ND group ($P < 0.05$), implying that there is some mechanism to inhibit inflammation during the formation of goose fatty liver to protect it from lesions (Fig. 2-B, C; S1).

- PRIMA1 expression was induced in goose fatty liver

As illustrated by the NCBI database, the expression levels of *PRIMA1* in livers of humans and mice were extremely low or even absent (Fig. 3-A), which was consistent with our observations in normal goose livers.

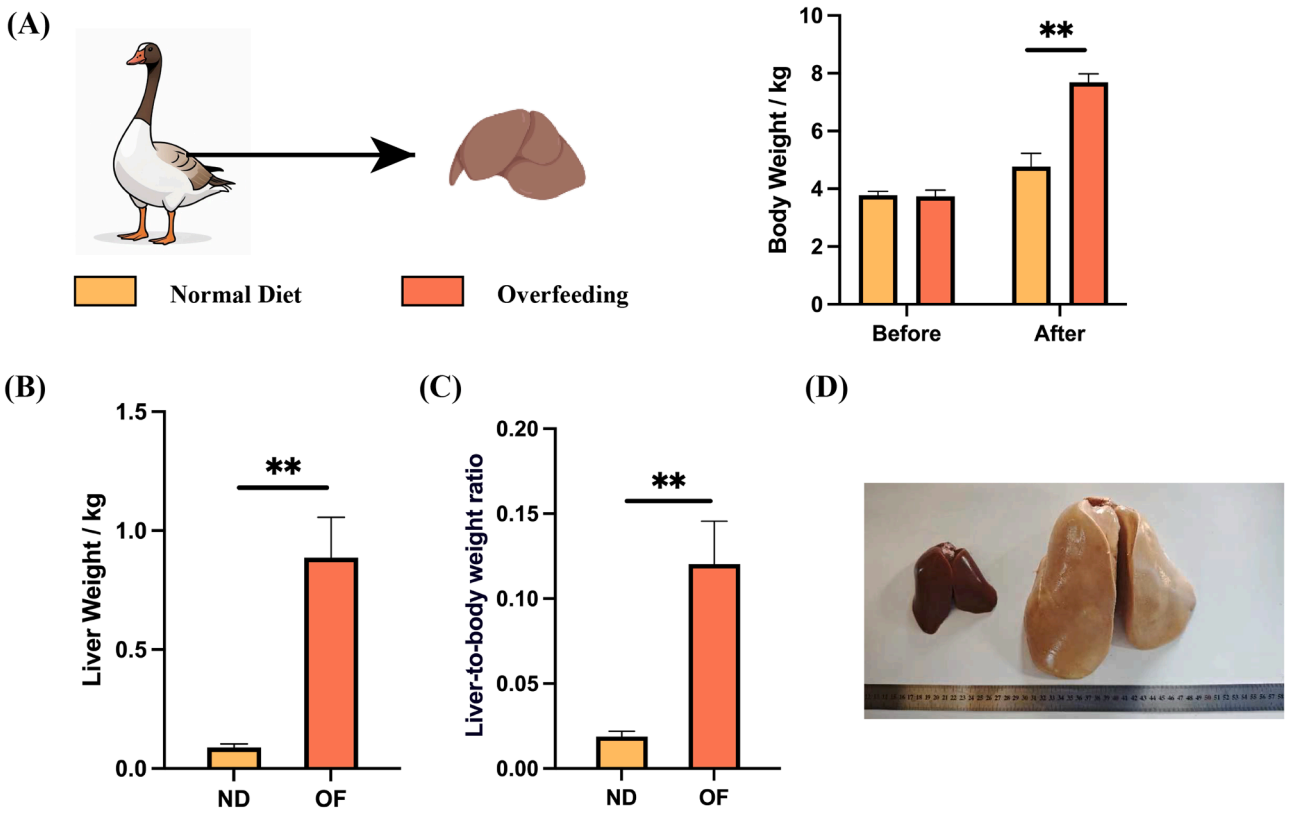


Fig. 1. Overfeeding led to increase in body weight and liver weight to form goose fatty liver. A. Different feeding diets for geese and body weight comparison between OF and ND groups. The body weight of the overfed geese (the OF group, the geese overfed for 19 days) is presented as the fold change over the normally fed geese (the ND group). The geese were $n = 6$. ** denotes $P < 0.01$ vs. control. All the data are shown as the means ± SEM. B. Liver weight comparison between OF and ND groups. The liver weight of the OF group is presented as the fold change over the ND group. The geese were $n = 6$. ** denotes $P < 0.01$ vs. control. All the data are shown as the means ± SEM. C. Liver-to-body ratio comparison between OF and ND groups. D. Liver morphology and comparison. The geese were $n = 6$. ** denotes $P < 0.01$ vs. control.

However, it is noteworthy that *PRIMA1* expression was elevated in goose fatty livers caused by overfeeding (Fig. 3- B), while AchE activity showed a significant decrease ($P < 0.05$) (Fig. 3- C).

- Overexpression of PRIMA1 Promoted Choline Production in Goose Primary Hepatocytes

According to the NCBI database (Taihu_goose_T2T_genome), goose *PRIMA1* (XM_066998283.1, 5091 bp) comprises four exons (Fig. 4- A). Research in mice has shown that *PRIMA1* can facilitate the binding of AchE tetramers to the cell membrane, thereby enabling their functional activity and preventing their escape from the cells in nervous tissue (Fig. 4- B) (Montenegro et al., 2014). In the present study, the *PRIMA1* gene was overexpressed in GPHs (Fig. 4- C), and the resulting cells were analyzed for AchE activity. The AchE activity was found to be elevated in GPHs overexpressing *PRIMA1* (OE) in comparison to normal cells (NC) ($P < 0.05$) (Fig. 4- D), indicating an increase in choline content following *PRIMA1* overexpression.

- Knockdown of *PRIMA1* in glucose-treated hepatocytes results in upregulation of inflammatory factor expression

Given that the development of goose fatty liver is accompanied by hyperglycemia (Geng, Zhao, et al., 2016), GPHs were treated with glucose. The western blot results showed that Inflammation-related genes were somehow suppressed under 200 mmol/L glucose treatment (Fig. 5-A, B; S1), while the expression of the *PRIMA1* gene was significantly elevated (Fig. 5- C). This expression pattern was similar to that observed in the liver, indicating that there were identical protective mechanisms between the two and also suggesting that glucose treatment could be a good cellular model to mimic some of the mechanisms associated with goose fatty liver. Furthermore, *PRIMA1* was down-regulated to a normal level through RNAi in glucose-treated cells (Fig. 5- C), and the results demonstrate that the expression of Inflammation-related genes exhibited a recovery tendency in the GKD group, indicating the role of *PRIMA1* in suppressing inflammation (Fig. 5-A, B; S1). However, there was no significant effect on the elevation of glucose-induced AchE activity when *PRIMA1* gene expression was diminished to its normal level ($P < 0.05$) (Fig. 5- C, D).

Discussion

Goose fatty liver, a physiological form of fatty liver, is characterized by severe steatosis without accompanying inflammation or fibrosis, suggesting the presence of protective mechanisms (Fournier et al., 1997). This study aimed to explore the potential protective role of *PRIMA1* in the formation of goose fatty liver. We hypothesized that *PRIMA1* enhances AchE activity and choline production, promoting lecithin synthesis and facilitating fat transport out of the liver. The findings strongly support this hypothesis, providing a basis for further investigation into the role and underlying mechanisms of the *PRIMA1* gene in the development of goose fatty liver.

The expression of the *PRIMA1* gene in goose fatty liver was significantly higher than in normal liver tissue, indicating its involvement in fatty liver development. Overfeeding-induced goose fatty liver is accompanied by elevated blood glucose levels, particularly postprandial. Previous studies have demonstrated that blood glucose levels in geese with fatty liver were significantly elevated compared to those

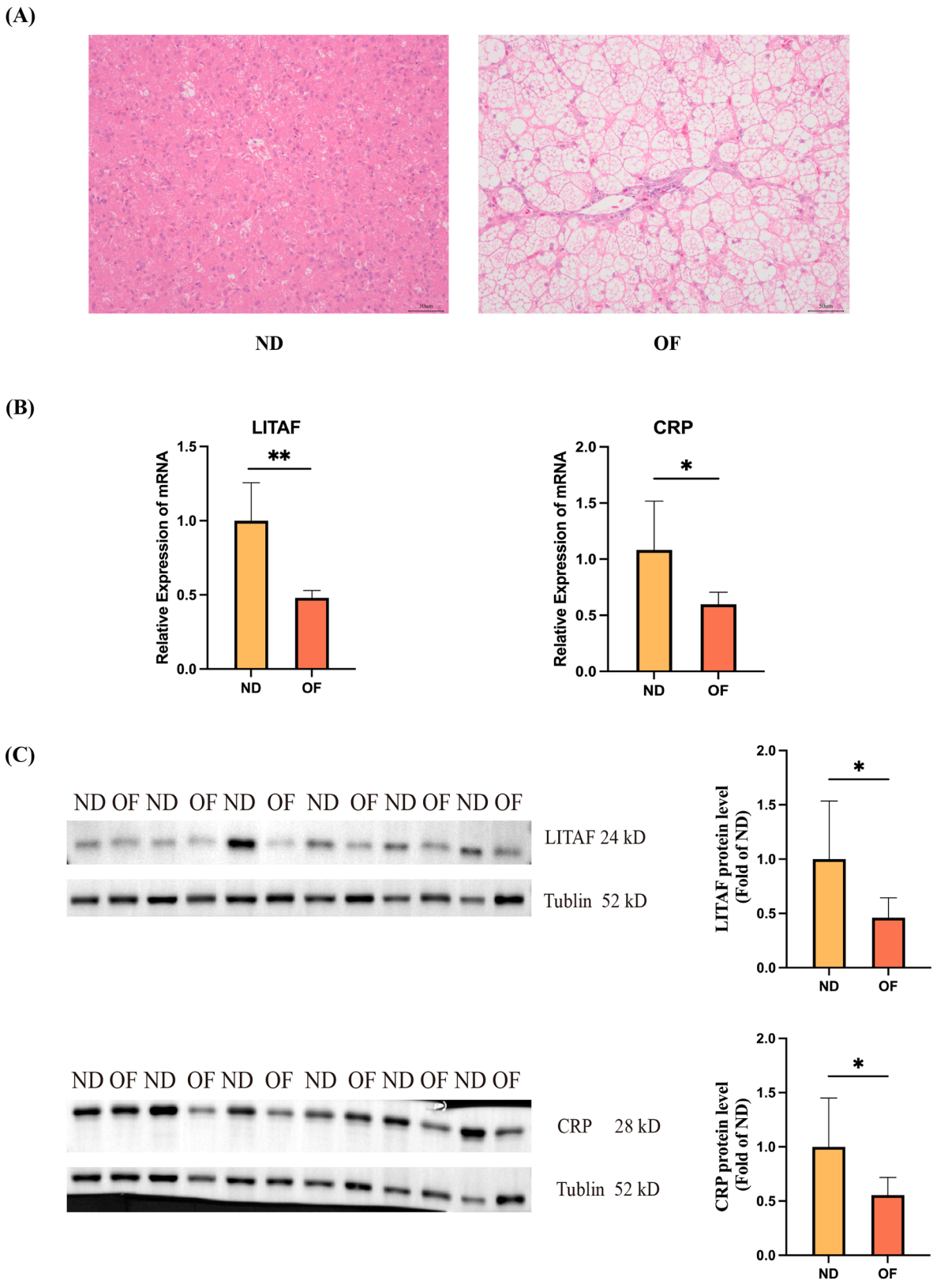
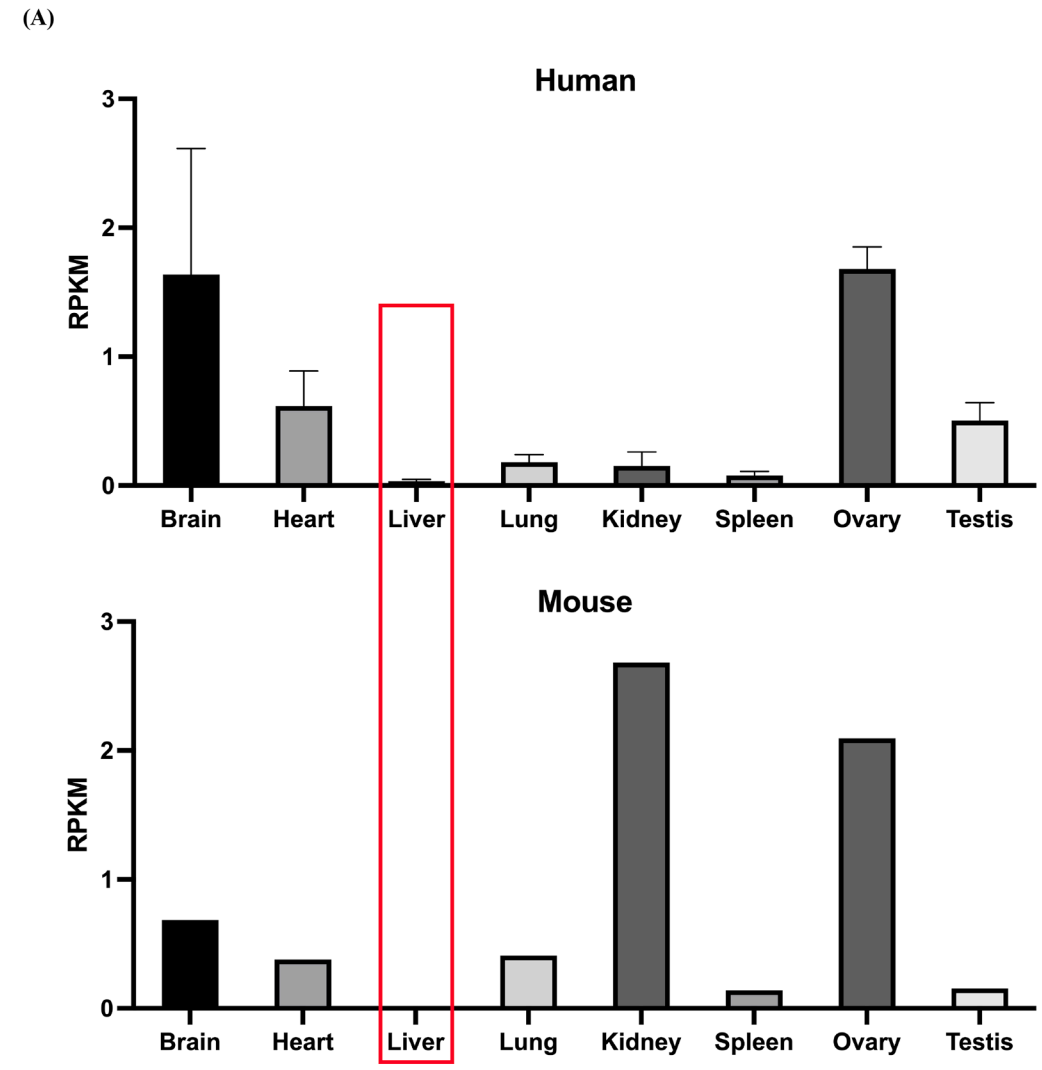
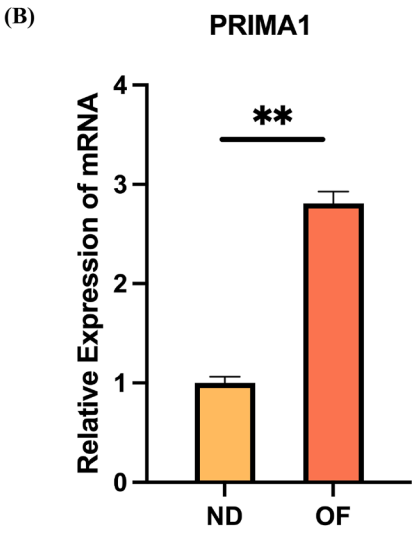


Fig. 2. HE sections and expression of LITAF and CRP in OF vs. ND. A. HE section of OF and ND groups, scale bar = 50 μm for all the panels. B. The relative expression of *LITAF* and *CRP* were determined by quantitative PCR. The expression of *LITAF* and *CRP* in the livers of the OF group are presented as the fold change over the ND group. The geese were $n = 6$. * denotes $P < 0.05$ vs. control. ** denotes $P < 0.01$ vs. control. All the data are shown as the means ± SEM. C. LITAF and CRP protein level comparisons between OF and ND groups. Protein levels were determined with Western Blot. The protein level in the livers of the OF group is presented as the fold change over the ND group. The geese were $n = 6$. * denotes $P < 0.05$ vs. control. All the data are shown as the means ± SEM.





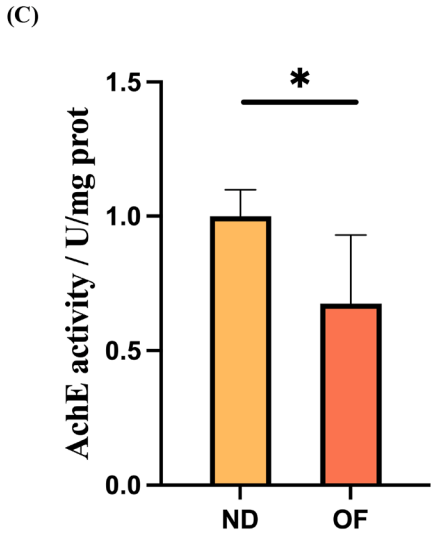


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Fig. 3. Expression of *PRIMA1* gene in livers of different species. A. The Reads Per Kilobase per Million (RPKM) of *PRIMA1* gene in 8 tissues of human and mouse. B. The relative expression of *PRIMA1* gene was determined by quantitative PCR. The expression of *PRIMA1* in the livers of the OF group is presented as the fold change over the ND group. The geese were $n = 6$. ** denotes $P < 0.01$ vs. control. All the data are shown as the means ± SEM. C. AchE activity comparison between OF and ND groups. The AchE activity in the livers of the OF group is presented as the fold change over the ND group. The geese were $n = 6$. * denotes $P < 0.05$ vs. control. All the data are shown as the means ± SEM.

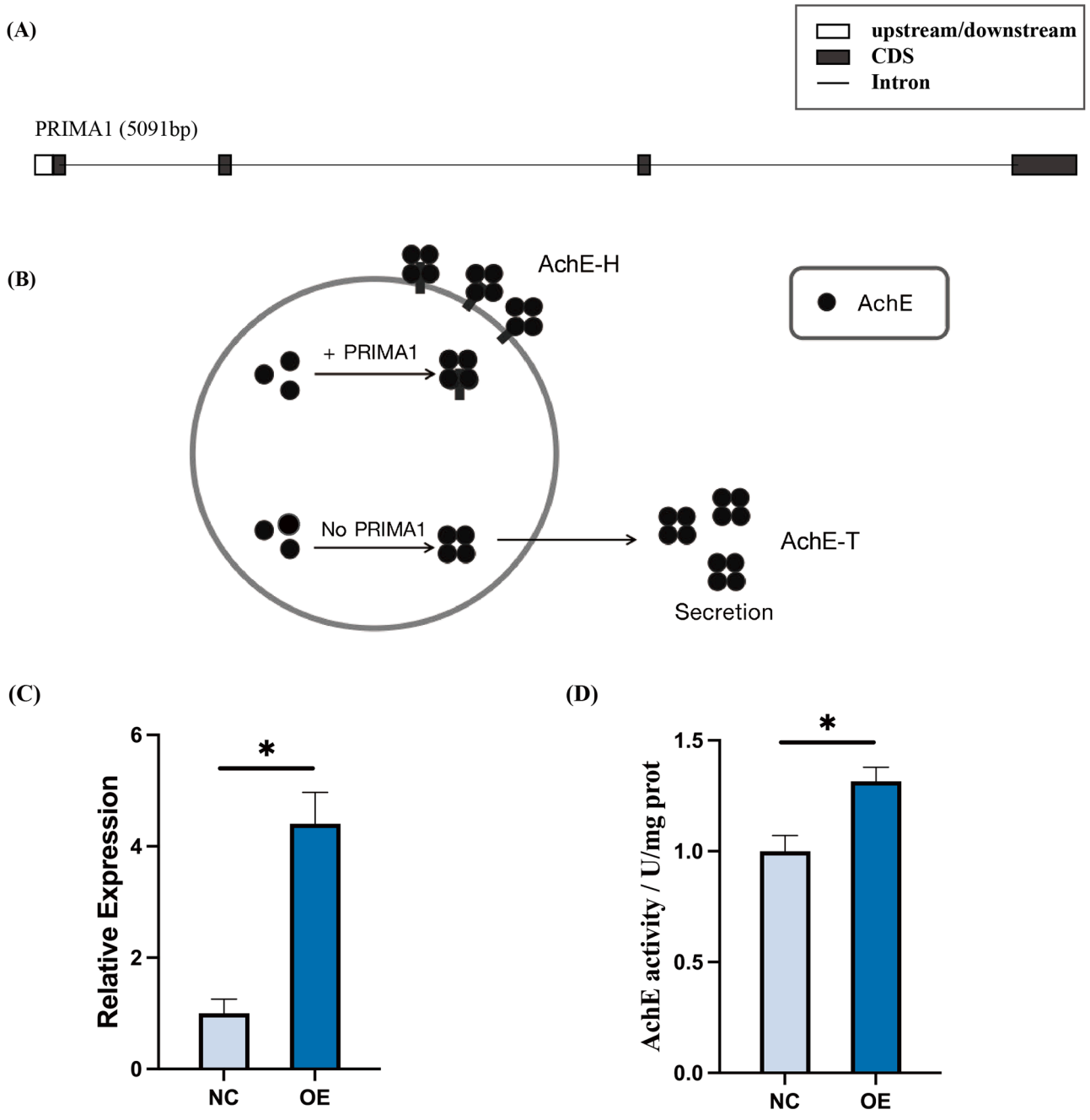

Fig. 4. Overexpression of PRIMA1 elevated AchE activity in goose primary hepatocytes. A. Scheatic depicting RNA sequences of goose PRIMA1. B. Hypothetical diagram of the role of *PRIMA1* in goose fatty liver. C. The relative expression of PRIMA1 gene in goose primary hepatocytes was determined by quantitative PCR. The expression of PRIMA1 in the OE group is presented as the fold change over the NC group. D. AchE activity in PRIMA1 overexpressing hepatocytes vs. control. The AchE activity in the PRIMA1 overexpressing hepatocytes (the OE group) is presented as the fold change over the control (the NC group). The geese were $n = 6$. * denotes $P < 0.05$ vs. control. All the data are shown as the means ± SEM.

with normal liver (Geng, Zhao, et al., 2016). This study investigated the effects of glucose on *PRIMA1* expression in GPHs. The results revealed that glucose significantly induced *PRIMA1* expression, providing a mechanistic explanation for the elevated levels of *PRIMA1* in fatty liver compared to normal liver. Thus, the increased expression of *PRIMA1* in fatty liver can, at least partially, be attributed to hyperglycemia associated with overfeeding.

Previous studies have shown that *PRIMA1* promotes the tetramerization and membrane localization of AchE, enhancing its activity and increasing choline production (Xie et al., 2010). Choline plays a vital

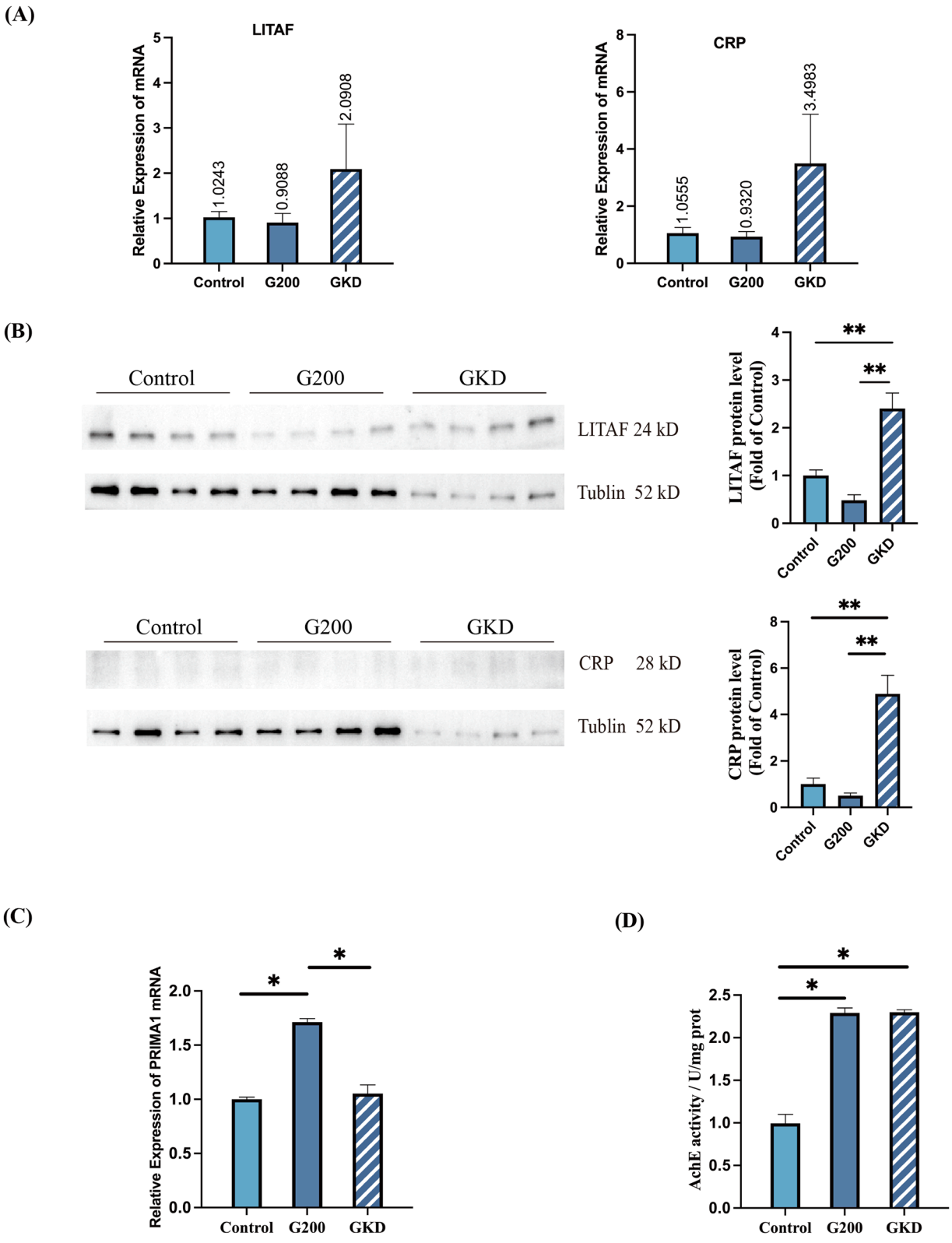

Fig. 5. Knockdown of PRIMA1 in glucose-treated hepatocytes. A. The relative expression of *LITAF* and *CRP* in goose primary hepatocytes with different treatments were determined by quantitative PCR. The expression in G200 and GKD groups are presented as the fold change over the control group. $n = 6$. All the data are shown as the means ± SEM. B. LITAF and CRP protein level comparisons between control (C), 200 mmol/L glucose treatment (G200), and PRIMA1 Knockdown after 200 mmol/L glucose treatment (GKD) groups. The protein levels of G200 and GKD groups are presented as the fold change over the control group. The geese were $n = 4$. ** denotes $P < 0.01$ vs. control. All the data are shown as the means ± SEM. C. The relative expression of *PRIMA1* in goose primary hepatocytes with different treatments was determined by quantitative PCR. The expression of *PRIMA1* in G200 and GKD group is presented as the fold change over the control group. $n = 6$. * denotes $P < 0.05$ vs. control. All the data are shown as the means ± SEM. D. AchE activity in G200 and GKD vs. control. The AchE activity in G200 and GKD group is presented as the fold change over the control. The geese were $n = 6$. * denotes $P < 0.05$ vs. control. All the data are shown as the means ± SEM.

role in lipid excretion from the liver as phospholipids and exhibits anti-inflammatory properties, which have been leveraged in the treatment of non-alcoholic steatohepatitis (NASH) and liver cirrhosis (Corbin and Zeisel, 2012). Consistent with this, our study demonstrated increased AchE activity following *PRIMA1* overexpression in GPHs. However, AchE activity was reduced in goose fatty liver tissues compared to normal liver tissues.

We hypothesize that the *PRIMA1*-induced increase in AchE activity represents an initial stress response to hyperglycemic conditions. However, with prolonged stimulation, the cellular regulatory mechanisms may suppress choline production to avoid cellular damage caused by excessive AchE activation. This hypothesis aligns with the observation that AchE activity increased in both PRIMA1-overexpression and glucose-treated hepatocytes but decreased in fatty livers formed after 19 days of overfeeding. This regulatory mechanism suggests that *PRIMA1* might exert anti-inflammatory effects by modulating choline metabolism, thereby protecting the goose fatty liver from inflammation and injury.

The role of *PRIMA1* in suppressing inflammation was further confirmed through combined treatments. First, *PRIMA1* knockdown resulted in an increasing trend in Inflammation-related gene expression, implicating the involvement of *PRIMA1* in the suppression of inflammation in GPHs. Additionally, GPHs treated with 200 mmol/L glucose exhibited a relatively lower expression of the Inflammation-related genes, indicating the activation of protective mechanisms against inflammation in a high-glucose environment. Interestingly, AchE activity did not decrease following *PRIMA1* knockdown in glucose-treated hepatocytes, maintaining levels comparable to those observed in the high-glucose group. This could be attributed to two reasons: (1) the 24-hour knockdown duration may be insufficient for downstream AchE activity to exhibit drastic changes, allowing the system to maintain homeostasis and avoid adverse effects of sudden choline fluctuations; (2) the production of choline in excess due to increased AchE activity may not have been metabolised yet and the accumulation of choline may have masked any immediate changes caused by *PRIMA1* knockdown.

While our use of GPHs provides valuable insights into *PRIMA1* function, in vitro models have inherent limitations in replicating in vivo conditions. Hepatic metabolism in vivo is influenced by complex systemic factors, including hormonal regulation, immune interactions, and organ crosstalk, which are absent in isolated hepatocytes. Additionally, while we used glucose treatment to mimic hyperglycemia observed in overfed geese, this approach does not fully capture the systemic metabolic dysregulation, such as insulin resistance and impaired glucose uptake, that contributes to hyperglycemia in vivo. Furthermore, our in vitro experiments reflect acute cellular responses, whereas goose fatty liver develops over weeks, meaning long-term regulatory effects of PRIMA1 may require further validation in whole-animal models. Despite these constraints, our in vitro model effectively isolates hepatocyte-specific mechanisms, supporting the role of *PRIMA1* in regulating AchE activity and inflammation.

Our findings underscore the critical role of *PRIMA1* in regulating choline metabolism and inflammation in goose fatty liver. By enhancing AchE activity and choline production, *PRIMA1* helps suppress inflammatory responses, contributing to the unique resistance of goose fatty liver to inflammation and fibrosis. A deeper understanding of the molecular mechanisms underlying these effects could identify new therapeutic targets for the treatment of NAFLD in other species.

Further research is warranted to clarify how *PRIMA1* regulates lipid and choline metabolism, as well as its interaction with other genes and proteins involved in liver health. It is also essential to explore whether the observed mechanisms are unique to geese or conserved across other species.

Conclusion

In conclusion, although there are still numerous unanswered questions regarding *PRIMA1*, this study offers valuable insights that can be used to further investigate the function of the *PRIMA1* gene in goose fatty liver. These insights include the observation that overfeeding-induced fatty liver in geese is characterized by significant alterations in body and liver weights, elevated fat content, and enhanced *PRIMA1* expression. The enhancement of choline metabolism and the suppression of inflammation by *PRIMA1* suggest a protective mechanism that enables geese to tolerate hepatic steatosis. These insights provide a valuable foundation for future research aimed at understanding and treating fatty liver disease in both avian and human populations.

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Institutional review board statement

The animal study protocol was approved by the Animal Welfare and Use Committee of Yangzhou University [permission number: SYXK(Su) IACUC 2012–0029].

Declaration of competing interest

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105219.

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