Research Article

Gualou Xiebai Banxia decoction ameliorates Poloxamer 407-induced hyperlipidemia

Mingzhu Luo1,2,*, Rong Fan2,3,*, Xiaoming Wang2,*, Junyu Lu2,*, Ping Li2,4, Wenbin Chu2, Yonghe Hu5 and Xuewei Chen2

1Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu 611137, Sichuan, China; 2Department of Operational Medicinal Research, Tianjin Institute of Environmental and Operational Medicine, Tianjin 300050, China; 3Central Laboratory, Tianjin Xiqing Hospital, Tianjin 300380, China; 4Department of Pharmacology Research, Southwest Medical University, Luzhou 646000, Sichuan, China; 5Department of Traditional Chinese Medicine, The General Hospital of Western Theater Command, Chengdu 611137, Sichuan, China

Correspondence: Yonghe Hu (huyonghe@vip.126.com) or Xuewei Chen (chenxuewei11@sina.com)

Ethnopharmacological relevance: Gualou Xiebai Banxia (GLXBBX) decoction is a well-known traditional Chinese herbal formula that was first discussed in the Synopsis of the Golden Chamber by Zhang Zhongjing in the Eastern Han Dynasty. In traditional Chinese medicine, GLXBBX is commonly prescribed to treat cardiovascular diseases, such as coronary heart disease and atherosclerosis.

Objective: The present study aimed to examine GLXBBX's preventative capacity and elucidate the potential molecular mechanism of Poloxamer 407 (P407)-induced hyperlipidemia in rats.

Materials and methods: Both the control and model groups received pure water, and the test group also received a GLXBBX decoction. For each administration, 3 ml of the solution was administered orally. To establish hyperlipidemia, a solution mixed with 0.25 g/kg P407 dissolved in 0.9% normal saline was injected slowly into the abdominal cavity. At the end of the study, the rats' plasma lipid levels were calculated using an automatic biochemical analyzer to evaluate the preventative capability of the GLXBBX decoction, and the serum and liver of the rats were collected.

Results: The GLXBBX decoction significantly improved P407-induced hyperlipidemia, including increased plasma triglycerides (TGs), aspartate aminotransferase (AST) elevation, and lipid accumulation. Moreover, GLXBBX decoction treatment increased lipoprotein lipase (LPL) activity and mRNA expression of LPL. Furthermore, GLXBBX significantly suppressed the mRNA expression of stearoyl-CoA desaturase (SCD1).

Conclusion: GLXBBX significantly improved P407-induced hyperlipidemia, which may have been related to enhanced LPL activity, increased LPL mRNA expression, and decreased mRNA expression of SCD1.

Introduction

Abnormal blood lipid metabolism is very common and a major risk factor for cardiovascular disease, though it is also involved in the development of a variety of diseases [1]. Hyperlipidemia involves abnormal blood lipid metabolism, which includes elevated levels of serum triglycerides (TGs), total cholesterol (TC), low-density lipoprotein (LDL), cholesterol, and free fatty acids and decreased levels of serum high-density lipoprotein cholesterol [2]. According to epidemiological investigations, both elevated plasma TG and TC are emerging as independent risk factors for metabolic syndrome, type 2 diabetes, hepatic steatosis, and atherosclerotic cardiovascular disease [3,4]. Hypertriglyceridemia is generated by a complex interplay among several genetic susceptibilities and environmental factors that affect the production and clearance...
of TG-rich lipoproteins. Among these factors, the etiopathogenesis of hyperlipidemia consists of the dysregulation of gene expression during the synthesis and decomposition of TGs. Plasma TG levels increase as a result of both the absorption of dietary fat by intestinal cells and the restricted catabolism of TGs. The catabolism of TGs is mainly regulated by plasma lipoprotein lipase (LPL), which gradually hydrolyzes TGs into free fatty acids and glycerol, which are released into the bloodstream for tissue oxidation [5]. While adipocytes and myocytes are the main source of LPL, the vast majority of LPL activity occurs in capillary endothelial cells. A partial or complete LPL deficiency results in an inability to hydrolyze TGs in chylomicrons and very-LDLs, causing mild to severe hypertriglyceridemia [6,7]. Poloxamer 407 (P407) has been shown to induce massive hyperlipidemia by directly inhibiting the heparin-releasable fraction of LPL [8,9].

Hyperlipidemia, which makes the liver more susceptible to damage, is the first step in hepatic lipid accumulation. The synthesis of hepatic TGs is mainly regulated by the combined action of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and stearoyl-CoA desaturase (SCD1) [10–12]. In addition, inflammatory cytokines, oxidative stress, and anti- and pro-inflammatory molecules play important roles in lipid metabolic disorders.

Effective lipid-lowering therapy is essential for hyperlipidemia. Mild hyperlipidemia is usually treated through the introduction of lifestyle modifications, and pharmacotherapy is indicated for more severe cases of hyperlipidemia. Traditional Chinese medicine is a classic and effective therapeutic approach that is both diverse and unique. Gualou Xiebai Banxia (GLXBBX) decoction was first discussed in the Synopsis of the Golden Chamber written by Zhang Zhongjing. It mainly treats phlegm and stasis obstructing chest discomfort syndrome. Herbal components of GLXBBX have been identified as Trichosanthes, Allium macrostemon, Pinellia ternata, and rice wine. GLXBBX is effective for cardiovascular diseases and is widely used during clinical surgery in China to treat coronary heart disease and atherosclerosis and has a good curative effect [13–15]. GLXBBX appears to have beneficial effects on the electrocardiogram and a reduction in angina symptoms in participants with angina pectoris [16]. The Dan-Lou prescription, which is a transformation of the GLXBBX decoction, inhibits foam cell formation induced by ox-LDL via the TLR4/NF-κB and PPARγ signaling pathways [17]. Although the lipid-lowering effect of GLXBBX has previously been proposed, the molecular mechanism has not been clarified.

In the present study, we attempted to depict the underlying mechanism by which GLXBBX improves hyperlipidemia induced by P407 based on the interaction between lipid-lowering and LPL activity. After verification, we found that GLXBBX alleviated hyperlipidemia induced by P407 by activating LPL activity, and GLXBBX inhibited early hepatic steatosis by antagonizing the mRNA overexpression of SCD1, blocking hepatic TG synthesis. In conclusion, we provided evidence that GLXBBX treatment effectively attenuates hyperlipidemia by activating LPL activity.

### Materials and methods

#### Reagents and chemicals

P407 and d-glucose were purchased from Beijing Solarbio Technology Co., Ltd. (Beijing, China). TRNzol Universal total RNA extraction reagent and RNase/DNase-free ddH₂O were purchased from Tiangen Biochemical Technology Co., Ltd. (Beijing, China). TB Green Premix Ex Taq II was purchased from Takara Biomedical Technology Co., Ltd. and Trans DNA Marker II and DNase I were purchased from TransGen Biotech (Beijing, China). The Hematoxylin and Eosin (H&E) staining solution was purchased from Nanjing Jiancheng Bioengineering Institute. The Oil Red O staining solution was purchased from Servicebio Co., Ltd. (Wuhan, China).

#### Composition and preparation of the GLXBBX decoction

The composition of GLXBBX decoction is listed in Table 1. GLXBBX herbal slices (1320 g) were obtained from Anguo Shenbao Pharmaceutical Co., Ltd., Baoding, China and the proportions of the components in the GLXBBX formula were maintained. Rice wine was purchased from Shaoxing Nuerhong Brewing Co., Ltd., and the content was 15.5% alcohol/volume. The herbal mixture was crushed and soaked with 3500 ml of rice wine overnight, and pure water was added to the medicinal herbs, which were decocted on a slow fire, and the extract was filtered twice. The resulting

### Table 1 The composition of GLXBBX decoction

| Pharmaceutical          | Pinyin   | Composition  |
|-------------------------|----------|--------------|
| Trichosanthes Gualou    | 37.50%   |
| Allium macrostemon Xiebai | 25%     |
| Pinellia ternata Banxia | 37.50%   |
| Rice wine Hua Huangji    | 15.5% vol|
solution was concentrated to obtain exactly 1320 ml, which was cooled to room temperature and stored at −20°C after cooling for later use. The extract was rewarmed to 37°C before use.

Animals
Male Wistar rats (6–8 weeks old) were purchased from Beijing Weitong Lihua Animal Laboratory Technology Co., Ltd. (Beijing, China). They were housed in an animal room at a consistent temperature (23 ± 2°C) and humidity and maintained on a 12-h light and dark cycle. Rats were divided randomly into two groups: one group received the GLXBBX decoction (5 g/kg) (GLXBBX group; n=11) and the other group (n=14) received an equal volume of pure water. One week later, the pure water group was randomized into two groups: the P407 group (n=8) and the control group (n=6). Both the P407 group and the GLXBBX decoction group were given bolus intraperitoneal (i.p.) injections of P407 (0.5 g/kg) to establish the hyperlipidemia model [18], and the control group received 0.9% saline (n=6), twice a week for 4 weeks. P407 was dissolved in cold saline (0.9%) and kept overnight at 4°C for complete dissolution according to the cold method.

All rats were killed on the last day at the same time. After anesthesia with sodium pentobarbital (40 mg/kg, i.p.), blood was drawn from the abdominal aorta, anticoagulated using heparin sodium, and plasma was separated centrifugally. All experimental protocols were approved by the Animal Ethics Committee of the Institute of Environmental Medicine and Occupational Medicine, Institute of Military Medicine, Academy of Military Sciences (IAUC No. AMMS-04-2020-053).

Measurement of plasma lipid profiles, liver tissue lipid content, and the liver/body ratio
The intact liver was removed after the rats were sacrificed, and the liver/body weight ratio was calculated to evaluate the amount of fat deposited in the liver. The TG and TC levels in the plasma were measured using a fully automatic bio-analysis machine (Mindray BS 180, China). TG and TC levels in liver tissue homogenates were determined using an ELISA kit (Nanjing Jiancheng, China).

Oral glucose tolerance tests
Rats were fasted overnight for 12–14 h with free access to water, and glucose was administered orally at 2.5 g/kg of body weight. Blood glucose was measured from the tail using an automatic glucometer (OneTouch UltraEasy, U.S.A.) at baseline (0), 15, 30, and 120 min after administration.

Analysis of serum glucose, alanine aminotransferase, and aspartate aminotransferase
Plasma glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were measured using a biochemical analyzer (Mindray BS 180, China), and the AST/ALT ratio was calculated.

Plasma LPL activity
LPL activity was determined using an LPL activity kit (Mrybio, China) according to the manufacturer’s instructions.

Histopathological observation
Liver tissue was fixed in 4% paraformaldehyde. After 2 days, the tissue was dehydrated using ethanol concentrations (from 70 to 100%) and subsequently embedded in paraffin. The tissue was sliced into 5-μm-sections and dried overnight for staining. The tissue sections were then stained with H&E and Toluidine Blue after deparaffinization and rehydration. Each section was dehydrated with ethanol, cleared with xylene, and mounted on slides using a mounting medium. Lipid deposition in liver cells was assessed by Oil Red O staining. The liver tissue sections were stained with 0.5% Oil Red O solution for 1 h and then differentiated in a 60% propylene glycol solution for 5 min. The slides were observed and measured in five high-power fields using a light microscope (Leica, Germany) at 40× magnification. At least five sections of four mice from each group were randomly selected and analyzed.

Quantitative real-time polymerase chain reaction
Liver tissue was obtained, flash frozen, and stored at −80°C until processing. Total RNA was isolated using the TRIzol reagent. RNA was reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, U.S.A.). The mRNA expression levels of FAS, ACC, and SCD1 were evaluated by quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis using the StepOne Real-Time PCR System (ABI, U.S.A.).
Table 2 Primer sequences used in the present study

| Primer name | Primer sequence (5′-3′) |
|-------------|-------------------------|
| LPL         | Forward: AGCCCTAGTCGCCCTTTCTCCTG  
              | Reverse: GCCTTGCTGCGGGTTTTCTTCATTC |
| Fas         | Forward: TTGATGAAGAGGGACCATAAAGA  
              | Reverse: TGGGAACAAGGCATTAGGGT |
| Acc         | Forward: CCGTGAAGGGCTACCTCTAATG  
              | Reverse: ATCACAACCCAAGAACCACCC |
| Scd1        | Forward: CCTCATCATTGCCAACACCAT  
              | Reverse: GCACAAGCAGCCAACCCAC |
| Gapdh       | Forward: TGCCACTCAGAAGACTGTGG  
              | Reverse: TTCAGCTCTGGGATGACCTT |

The primer sequences are listed in Table 2. Target mRNA expression in each sample was normalized to that of the housekeeping gene, GAPDH. The $2^{-\Delta\Delta C_t}$ method was used to calculate relative mRNA expression levels.

Western blotting assay

Whole liver tissue lysates were generated using urea lysis buffer and run on 10% SDS/PAGE gel. Proteins were transferred on to PVDF membranes (Millipore, U.S.A.), and incubated with antibodies against FAS (1:1000, Abcam), ACC (1:1000, Abcam), SCD1 (1:1000, Abcam), respectively, followed by incubation with corresponding secondary antibodies conjugated to horseradish peroxidase. The β-Actin (1:1000, Beyotime Biotechnology) Western blotting was performed for each membrane as a loading control. Immunoreactive proteins were visualized using ECL reagents (Millipore, U.S.A.).

Statistical analysis

All results are presented as the mean ± SD. Multigroup comparisons were performed using one-way analysis of variance followed by Dunnett’s test. GraphPad Prism software was used to perform the statistical analysis (version 7.0; GraphPad Software, Inc., San Diego, CA, U.S.A.). Differences were considered statistically significant at $P < 0.05$.

Results

GLXBBX treatment ameliorates hyperlipidemia induced by P407

As previously described, P407, a highly efficient inhibitor of LPL, has been extensively used to induce hyperlipidemia in rodents and other experimental animals [19,20]. To investigate the potential role of GLXBBX decoction on lipid lowering, we first estimated its phenotype in the plasma and liver of rats. The carcass weight was similar in all groups ($P > 0.05$). Both the plasma TG and TC levels were clearly higher in the group that was treated with P407 compared with their control littermates (Figure 1A,B). Furthermore, an over-accumulation of TG was observed in the liver of the P407 group (Figure 1C), with a significant elevation in the liver/body weight ratio ($P < 0.05$) (Figure 1E) and a tendency toward elevated TC levels (Figure 1D).

After treatment with GLXBBX decoction, rats showed a 24% reduction in the liver/body weight ratio ($P < 0.05$) (Figure 1E) and a 40% reduction in the plasma TG level ($P < 0.05$) (Figure 1A). Both TG and TC levels in the livers of the GLXBBX-treated rats were 28 and 32% lower, respectively, while there were no obvious changes in TG and TC levels in the liver tissue.

GLXBBX decoction provides protection against hepatotoxicity induced by P407

The liver plays an important role in lipid metabolism. The initial step in hepatic metabolism of lipoproteins is their transfer through 100–200 nm pores (fenestrations) in the liver sinusoidal endothelial cells prior to receptor-mediated uptake [21]. H&E staining of the liver showed that the control group had normal hepatic lobules, hepatic cords orderly arranged around the central vein, and clearly visible hepatic sinuses (Figure 2D upper). Conversely, normal hepatocytes were almost invisible in the liver tissue of the P407-treated rats, with enlarged hepatocytes, disordered hepatic cords, numerous fat vacuoles, and lightly stained cytoplasm. Oil Red O staining showed that there was an accumulation of lipid droplets in the P407 group, and GLXBBX treatment significantly decreased P407-induced hepatic fat deposition (Figure 2D bottom).
Figure 1. GLXBBX decoction modulates plasma and hepatic-rich lipid levels induced by P407
(A, B) Plasma TGs (A) and TC (B). (C, D) Hepatic TGs (C) and TC (D). (E) Liver wet weight to carcass body weight ratio in the control, model, and GLXBBX-treated rats. (F) Carcass body weights for the different groups. *P<0.05; **P<0.01; ***P<0.001.

For the GLXBBX-treated rats, hepatic lipid accumulation was significantly reduced. To further understand the effect of P407 on the liver and the GLXBBX decoction-associated lipid-lowering expression pattern, we assessed liver function tests. The increased serum concentrations of AST and ALT in those treated with P407 were down-regulated by GLXBBX. Compared with the P407-treated rats, the AST and ALT levels in the GLXBBX-treated rats decreased sharply by 32 and 33%, respectively (P<0.05) (Figure 2A, B). The AST/ALT ratio, which is considered an alternative marker of hepatic steatosis, was significantly increased with P407 (P<0.05) (Figure 2C).

P407-induced impaired glucose tolerance and high random blood glucose levels
Although fasting blood glucose levels were normal in all treatment groups, the model rats with P407-induced hyperlipidemia had more impaired glucose tolerance compared with the controls. The model rats’ ability to process
Figure 2. GLXBBX decoction improves hepatic function and reduces hepatic lipid accumulation

(A,B) Plasma liver enzymes, AST (A) and ALT (B). (C) AST/ALT ratios for the different groups. (D) H&E staining of hepatic tissue (D, top) and Oil Red O staining of hepatic tissue (D, bottom). *P<0.05.

GLXBBX decreases the expression of SCD1

Sterol regulatory element binding protein (SREBP1c), a key transcription factor for lipid synthesis, activates downstream target genes such as FAS, ACC, SCD1, and other key enzymes involved in lipid synthesis, which promote TG synthesis and lipid deposition [22]. The expression of FAS, ACC, and SCD1 is dysregulated in the livers of rats with fatty liver and obesity [23]. Therefore, the mRNA expression of these key enzymes was investigated using RT-qPCR, and the results are shown in Figure 4 and Supplementary Data. The mRNA expression of FAS, ACC, and SCD1 in the model rats increased to varying degrees, though the expression of FAS and SCD1 in the model rats significantly differed from that of the controls.

SCD1 is involved in the final step of TG synthesis in the liver. The mRNA expression of SCD1 in GLXBBX-treated rats was significantly lower than that in the P407 group (P<0.05) (Figure 4C). Furthermore, Western blot also confirmed that the protein expression level of SCD1 in the GLXBBX group was remarkably decreased than that in the P407 group (Figure 4D).
Figure 3. P407-induced hyperlipidemia accompanied by high random blood glucose levels and impaired glucose tolerance
(A) Random blood glucose levels for the different groups. (B) Fasting blood glucose levels for the different groups. (C,D) Time course of blood glucose levels after 2.5 g/kg glucose gavage and area under the curve (AUC) in the control and P407 groups (C) and the P407 and GLXBBX groups (D) are shown. *P<0.05; **P<0.01.

Figure 4. Expression of key enzymes related to TG synthesis and decomposition modulated by GLXBBX decoction
(A–C) The mRNA levels of TG synthesis in hepatic tissue of the control, P407, and GLXBBX-treated rats. (D) The protein levels of TG synthesis in hepatic tissue of the control, P407, and GLXBBX-treated rats. (E) The mRNA levels of LPL in brown adipose tissue of the control, P407, and GLXBBX-treated rats. (F) Plasma LPL activity of the different groups are shown. *P<0.05; **P<0.01.
Table 3 Comparison of plasma LPL activity in different groups (± s)

| Group          | Plasma LPL activity |
|----------------|---------------------|
| Control group  | 0.2 ± 0.06          |
| P407 group     | 0.14 ± 0.02 \(^1\) |
| GLXBBX group   | 0.19 ± 0.03 \(^2\)  |

Compared with control group.
\(^1\)P < 0.01; compared with P407 group.
\(^2\)P < 0.05.

GLXBBX decoction enhances LPL activity

LPL is a regulatory enzyme involved in the hydrolysis of TGs in both chylomicrons and very LDL particles and in the conversion of high-density lipoproteins. The mRNA expression level of LPL of rats in the P407 group decreased compared with control group, while the treatment of GLXBBX could restore the drop in LPL expression level caused by P407 (Figure 4E). The LPL activity in the plasma of the P407-treated rats sharply decreased 33% (P < 0.01) compared with the controls. Furthermore, the LPL activity in the GLXBBX-treated rats was considerably higher than those in the P407 group (P < 0.05) (Figure 4F and Table 3).

Discussion

LPL deficiency has been reported to contribute to hypertriglyceridemia in previous studies [6], and the rat model induced by P407 showed decreased LPL expression and activity and increased plasma TG levels [8]. This study explored whether the oral administration of GLXBBX could improve hyperlipidemia induced by the i.p. injection of P407, thereby protecting cardiovascular function. Previous studies of GLXBBX have established a basis for the clinical application of GLXBBX in cardiovascular diseases accompanied by hyperlipidemia, which has promoted further investigation into the efficacy of GLXBBX in the treatment of hyperlipidemia.

The liver plays an important role in lipid metabolism. The initial step in the hepatic metabolism of lipoproteins is their transfer through 100–200 nm pores (fenestrations) in the liver sinusoidal endothelial cells prior to receptor-mediated uptake [21]. P407 is a ubiquitous manmade surfactant and non-ionic detergent composed of repeating polyoxyethylene and polyoxypropylene units and is chemically a polyether-based compound [24]. P407 is taken up by the liver, resulting in dramatic defenestration of liver sinusoidal endothelial cells [25]. It has been suggested that the mechanism of P407-induced hypertriglyceridemia involves elevated TG levels resulting from the inhibition of LPL [20], and P407-induced hypercholesterolemia involves the indirect stimulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and decreased LDL receptor expression in the liver [26,27]. Dramatic fenestration has been caused by P407 in isolated liver sinusoidal endothelial cells. Based on this model, we found that hyperlipidemia was accompanied by liver steatosis and abnormal glucose metabolism. We demonstrated that GLXBBX treatment inhibits hyperlipidemia and ameliorates P407-induced hepatic damage, and we further investigated the mechanism by which GLXBBX could improve hypertriglyceridemia. As shown in the present study, the clinical condition of rats can be improved by GLXBBX through a significant reduction in plasma TG levels, though with no resulting change in plasma TC. Gualou Xiebai decoction is a classic prescription in Chinese medicine used for the treatment of cardiac conditions, but also serves many other functions, including antioxidation, anti-apoptosis, and anti-inflammation [28]. Decoctions containing Banxia have also been used for hyperlipidemia treatment. The Banxia Baizhu Tianma decoction is made up of six Chinese herbal medicines, including Banxia. Research on rats has shown that the Banxia Baizhu Tianma decoction can reduce serum TC, TG, LDL cholesterol, apolipoprotein B, superoxide dismutase, and malondialdehyde in rats with hyperlipidemia [29]. Moreover, Jiang et al. found that the Banxia Baizhu Tianma decoction has a significant effect on body weight and TC in mice with obesity-related hypertension, reducing lipid deposition in the roots of arteries, protecting endothelial cells, reducing apoptosis, decreasing the migration rate caused by oxidized LDLs, and maintaining cell morphology [30].

The metabolic pathways and primary metabolic sites of TGs are different from those of TC metabolism. LPL, an enzyme that is secreted by adipocytes and myocytes, acts as an intravascular TG hydrolase by converting TGs into fatty acids for utilization by vital tissues once it reaches its site of action in the capillary lumen. To investigate the efficacy of GLXBBX from a histological perspective, macroscopic and microscopic scores were obtained. GLXBBX was found to have a significant intervention effect, through the reduction in tissue damage and improvement in liver steatosis. GLXBBX decoction has been found to down-regulate TG levels by increasing LPL activity. Previous studies
[5] have established that glycosylphosphatidyl inositol-anchored high-density lipoprotein-binding protein 1 (GPI-HBP1) is needed for the transportation of LPL into the capillaries, lipoprotein margination, and the preservation of LPL structure and activity. Deficiency in either LPL or GPIHBP1 impairs TG hydrolysis, resulting in severe hypertriglyceridemia. LPL activity in the tissues is regulated by angiopoietin-like proteins [31]. Angiopoietin-like proteins inactivate LPL by converting LPL homodimers into monomers, rendering them highly susceptible to spontaneous unfolding and the loss of enzymatic activity. Future research is needed that focuses on the properties of LPL and explores the mechanism by which GLXBBX regulates LPL activity. GPIHBP1 and angiopoietin-like proteins are good potential points of penetration.

Although there was a trend of hepatic lipid down-regulation in the GLXBBX decoction group, this finding was not statistically significant, which might have been due to the small sample size. The antihyperlipidemic effects of the GLXBBX decoction were confirmed by analyzing the mRNA expression of lipogenesis-related genes, revealing that GLXBBX reduced the mRNA expression of SCD1 and FAS. Therefore, the antihyperlipidemic effects of GLXBBX could be attributed to the suppression of these lipogenic genes. Interestingly, it was reported that Ganoderma lucidum exhibited anti-obesity activity by down-regulating FAS, SCD1, and SREBP1c, and also displayed antihyperglycemic and antihyperinsulinemic activities by enhancing the activation of AMP-activated protein kinase (AMPK), ACC, insulin receptor, insulin-receptor substrate, and protein kinase B. AMPK activation attenuates the transcription and translation of lipogenic genes such as FAS and SCD1 and increases ACC protein expression [32]. This finding is consistent with our results. It is worth noting that the GLXBBX decoction treats hyperlipidemia induced by P407 by inhibiting the synthesis of TGs via the AMPK/SREBP1c/SCD1 signaling pathway.

Previous evidence [33] has shown that systemic LPL deficiency causes lipid accumulation in tissues (liver and skeletal muscle), which ultimately results in impaired glucose tolerance. Consistent with this finding, Li et al. [34] found that the glucose tolerance of LPL gene knockout heterozygous mice was impaired. P407-induced hyperlipidemia was accompanied by high random blood glucose levels and impaired glucose tolerance. However, GLXBBX had no effect on improving blood glucose levels. Therefore, the impaired glucose tolerance induced by P407 was not only due to the inhibition of LPL activity, but also failed to be reversed by the GLXBBX decoction.

ALT and the AST/ALT ratio have been associated with non-alcoholic fatty liver disease and insulin resistance [35]. Our results revealed that the plasma AST and AST/ALT ratio of rats with P407-induced hyperlipidemia significantly improved. Moreover, GLXBBX treatment reduced both plasma AST levels and the AST/ALT ratio.

In the present study, it is important to mention that the model rats exhibited a strong stress response during the course of the experiment. Specifically, after all the rats were sacrificed, a strong stress response was observed in the organs of the model rats, with two rats showing severe edema of the kidney. In contrast, both the control rats and GLXBBX-treated rats were in good condition. Unfortunately, we were unable to examine this phenomenon in depth.

Conclusion

In summary, our experimental results showed that GLXBBX treatment significantly regulated hyperlipidemia-like symptoms and ameliorated dysfunctional hepatic lipid metabolism in rats with P407-induced hyperlipidemia. The latent mechanisms of action may involve suppressing the mRNA expression of SCD1 and fortifying LPL activity to reduce TG levels. The present experimental findings confirm the therapeutic effect of GLXBBX and provide a scientific rationale for prescribing this herbal formula for lipid-lowering treatment. Further work must be conducted to explore the mechanism by which GLXBBX reduces LPL activity.

Data Availability

All supporting data are included in the main article. There are no links or online datasets.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This work was supported by the Basic Research Projects [grant numbers BWS16J009, HZ18C01].

Author Contribution

Yonghe Hu and Xuewei Chen conceived the idea for the present study. Mingzhu Luo, Rong Fan, Ping Li, and Wenbin Chu collected and analyzed the data. Xiaoming Wang and Junyu Lu drafted the paper. Xuewei Chen and Rong Fan revised the final paper.
Ethics Approval
All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978). All experimental protocols were approved by the Animal Ethics Committee of the Institute of Environmental Medicine and Occupational Medicine, Institute of Military Medicine, Academy of Military Sciences (IAUC No. AMMS-04-2020-053).

Abbreviations
ACC, acetyl-CoA carboxylase; ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; AST, aspartate aminotransferase; FAS, fatty acid synthase; GLXBXX, Gualou Xiebai Banxia; GPHBP1, glycosylphosphatidyl inositol-anchored high-density lipoprotein-binding protein 1; H&E, Hematoxylin and Eosin; i.p., intraperitoneal; LDL, low-density lipoprotein; LPL, lipoprotein lipase; ox-LDL, oxidation low lipoprotein; P407, Poloxamer 407; PPAR, peroxisome proliferator-activated receptor; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RT-qPCR, real-time quantitative polymerase chain reaction; SCD1, stearoyl-CoA desaturase; SREBP1c, sterol regulatory element binding protein; TC, total cholesterol; TG, triglyceride; TLR4, Toll-like receptor 4.

References
1. Bays, H.E., Jones, P.H., Brown, W.V. and Jacobson, T.A. (2014) National Lipid Association Annual Summary of Clinical Lipidology 2015. J. Clin. Lipidol. 8, S1–S36, https://doi.org/10.1016/j.jacl.2014.10.002
2. Bozkurt, B., Aguilar, D., Deswal, A., Dunbar, S.B., Francis, G.S., Horwich, T. et al. (2016) Contributory risk and management of comorbidities of hypertension, obesity, diabetes mellitus, hyperlipidemia, and metabolic syndrome in chronic heart failure: a scientific statement from the American Heart Association. Circulation 134, e535–e578, https://doi.org/10.1161/CIR.0000000000000450
3. Miller, M., Stone, N.J., Ballantyne, C., Criqui, M.H., Ginsberg, H.N. et al. (2011) Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 123, 2292–2333, https://doi.org/10.1161/CIR.0b013e3182160726
4. Mendoza-Camal, E.C., Barajas-Olmos, F., García-Ortiz, H., Cicerón-Arellano, I., Martínez-Hernández, A., Córdova, E.J. et al. (2020) Metabolic syndrome in indigenous communities in Mexico: a descriptive and cross-sectional study. BMC Public Health 20, 339, https://doi.org/10.1186/s12889-020-0378-5
5. Young, S.G., Feng, L.G., Beigneux, A.P., Allan, C.M., He, C., Jiang, H. et al. (2019) GPHBP1 and lipoprotein lipase, partners in plasma triglyceride metabolism. Cell Metab. 30, 51–65, https://doi.org/10.1016/j.cmet.2019.05.023
6. Gehrisch, S. (1999) Common mutations of the lipoprotein lipase gene and their clinical significance. Curr. Atheroscler. Rep. 1, 70–78, https://doi.org/10.1007/s11883-999-0052-4
7. Dallinga-Thie, G.M., Kroon, J., Borén, J. and Chapman, M.J. (2016) Triglyceride-rich lipoproteins and remnants: targets for therapy? Curr. Cardiol. Rep. 18, 67, https://doi.org/10.1007/s10558-016-0745-6
8. Johnston, T.P. (2004) The P-407-induced murine model of dose-controlled hyperlipidemia and atherosclerosis: a review of findings to date. J. Cardiovasc. Pharmacol. 43, 595–600, https://doi.org/10.1177/0022351404430505
9. Gabor, R.D., El-Sherbeni, A.A., Ben-Eliyahu, M., El-Kadi, A.O. and Brocks, D.R. (2017) Pharmacokinetics of metformin in the rat: assessment of the effect of hyperlipidemia and evidence for its metabolism to guanylurea. Can. J. Physiol. Pharmacol. 95, 530–538, https://doi.org/10.1139/cjpp-2016-0329
10. Jensen-Urstad, A.P. and Semenkovich, C.F. (2012) Fatty acid synthase and liver triglyceride metabolism: housekeeper or messenger? Front. Physiol. 3, 747–753, https://doi.org/10.3389/fphys.2012.00747
11. Brown, J.M., Chung, S., Sawyer, J.K., Degirolamo, C., Alger, H.M., Nguyen, T. et al. (2008) Inhibition of stearoyl-coenzyme A desaturase 1 dissociates insulin resistance and obesity from atherosclerosis. Circulation 118, 1467–1475, https://doi.org/10.1161/CIRCULATIONAHA.108.793182
12. Imai, N. and Cohen, D.E. (2018) Trimming the fat: acetyl-CoA carboxylase inhibition for the management of NAFLD. Hepatology 68, 2062–2065, https://doi.org/10.1002/hep.30206
13. Chen, M., Li, M., Ou, L., Kuang, R., Chen, Y., Li, T. et al. (2018) Effectiveness and safety of Chinese herbal medicine formula Gualou Xiebai Banxia (GLXBXX) decoction for the treatment of stable angina pectoris: protocol for a systematic review. Medicine (Baltimore) 97, e11680, https://doi.org/10.1097/MD.000000000001680
14. Zhang, Y.Y., Zhao, Z.D., Kong, P.Y., Gao, L., Yu, Y.N., Liu, J. et al. (2020) A comparative pharmacogenomic analysis of three classic TCM prescriptions for coronary heart disease based on molecular network modeling. Acta Pharmacol. Sin. 41, 735–744, https://doi.org/10.1038/s41401-019-0352-3
15. Lu, X.Y., Xu, H., Zhao, T. and Li, G. (2018) Study of serum metabonomics and formula-pattern correspondence in coronary heart disease patients diagnosed as pheigm or blood stasis pattern modeling based on ultra performance liquid chromatography mass spectrometry. Chin. J. Integr. Med. 24, 905–911, https://doi.org/10.1007/s11655-018-2564-7
16. Liu, W., Xiong, X., Yang, X., Chu, F. and Liu, H. (2016) The effect of chinese herbal medicine Gualouxiebaibanxia decoction for the treatment of angina pectoris: a systematic review. Evid. Based Complement. Alternat. Med. 2016, 8565907, https://doi.org/10.1155/2016/8565907
17. Gao, L.N., Zhou, X., Lu, Y.R., Li, K., Gao, S., Yu, C.Q. et al. (2018) Dan-Lou prescription inhibits foam cell formation induced by ox-LDL via the TLR4/NF-kB and PPAR signaling pathways. Front. Physiol. 9, 590, https://doi.org/10.3389/fphys.2018.00590
18. Mahmoud, M.O., Aboud, H.M., Hassan, A.H., Ali, A.A. and Johnston, T.P. (2017) Transdermal delivery of atorvastatin calcium from novel nanovesicular systems using polyethylene glycol fatty acid esters: ameliorated effect without liver toxicity in poloxamer 407-induced hyperlipidemic rats. J. Control. Release 254, 10–22, https://doi.org/10.1016/j.jconrel.2017.03.039
19 Wasan, K.M., Subramanian, R., Kwong, M., Goldberg, I.J., Wright, T. and Johnston, T.P. (2003) Poloxamer 407-mediated alterations in the activities of enzymes regulating lipid metabolism in rats. J. Pharm. Pharm. Sci. 6, 189–197
20 Johnston, T.P. and Palmer, W.K. (1993) Mechanism of poloxamer 407-induced hypertriglyceridemia in the rat. Biochem. Pharmacol. 46, 1037–1042, https://doi.org/10.1016/0006-2952(93)90668-M
21 Cogger, V.C., Hilmer, S.N., Sullivan, D., Muller, M., Fraser, R. and Le Couteur, D.G. (2006) Hyperlipidemia and surfactants: the liver sieve is a link. Atherosclerosis 189, 273–281, https://doi.org/10.1016/j.atherosclerosis.2005.12.025
22 Asrih, M., Montessuit, C., Philippe, J. and Jornayvaz, F.R. (2015) Free fatty acids impair FGF21 action in HepG2 cells. Cell. Physiol. Biochem. 37, 1767–1778, https://doi.org/10.1159/000438540
23 Serviddio, G., Bellanti, F. and Vendemiale, G. (2013) Free radical biology for medicine: learning from nonalcoholic fatty liver disease. Free Radic. Biol. Med. 65, 952–968, https://doi.org/10.1016/j.freeradbiomed.2013.08.174
24 Dumortier, G., Grossiord, J.L., Agnely, F. and Chaumeil, J.C. (2006) A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Pharm. Res. 23, 2709–2728, https://doi.org/10.1007/s11095-006-9104-4
25 Warren, A., Benseler, V., Cogger, V.C., Bertolino, P. and Le Couteur, D.G. (2015) The impact of poloxamer 407 on the ultrastructure of the liver and evidence for clearance by extensive endothelial and kupffer cell endocytosis. Toxicol. Pathol. 39, 390–397, https://doi.org/10.1177/0197346615594212
26 Leon, C., Wasan, K.M., Sachs-Barrable, K. and Johnston, T.P. (2006) Acute P-407 administration to mice causes hypercholesterolemia by inducing cholesterolenes and down-regulating low-density lipoprotein receptor expression. Pharm. Res. 23, 1597–1607, https://doi.org/10.1007/s11095-006-0276-8
27 Palmer, W.K., Emeson, E.E. and Johnston, T.P. (1997) The poloxamer 407-induced hyperlipidemic atherogenic animal model. Med. Sci. Sports Exerc. 29, 1416–1421, https://doi.org/10.1097/00005768-199711000-00005
28 Yan, L.L., Zhang, W.Y., Wei, X.Y., Han, L., Pan, C.S., Yu, Y. et al. (2018) Gualou Xiebai decoction, a traditional Chinese medicine, prevents cardiac reperfusion injury of hyperlipidemia rat via energy modulation. Front. Physiol. 9, 296, https://doi.org/10.3389/fphys.2018.00296
29 Cai, H., Guo, Y., Zhao, Z., Chen, Y., Zhao, S. and Chen, B. (2018) Banxia Baizhu Tianma decoction for hyperlipidemia: protocol for a systematic review and meta-analysis. Medicine (Baltimore) 97, e13067, https://doi.org/10.1097/MD.0000000000013067
30 Jiang, Y.H., Zhang, P., Tao, Y., Liu, Y., Cao, G., Zhou, L. et al. (2021) Banxia Baizhu Tianma decoction attenuates obesity-related hypertension. J. Ethnopharmacol. 266, 113453, https://doi.org/10.1016/j.jep.2020.113453
31 Kristensen, K.K., Leth-Espensen, K.Z., Mertens, H.D.T., Birrane, G., Meiyyappan, M., Olivcrona, G. et al. (2020) Unfolding of monomeric lipoprotein lipase by ANGPTL4: Insight into the regulation of plasma triglyceride metabolism. Proc. Natl. Acad. Sci. U.S.A. 117, 4337–4346, https://doi.org/10.1073/pnas.1920202117
32 Lee, H.A., Cho, J.H., Afinanisa, Q., An, G.H., Han, J.G., Kang, H.J. et al. (2020) Ganoderma lucidum extract reduces insulin resistance by enhancing AMPK activation in high-fat diet-induced obese mice. Nutrients 12, 3338, https://doi.org/10.3390/nu12113338
33 Holzl, B., Iglseder, B., Sandhofer, A., Malaimare, L., Lang, J., Paulweber, B. et al. (2002) Insulin sensitivity is impaired in heterozygous carriers of lipoprotein lipase deficiency. Diabetologia 45, 378–384, https://doi.org/10.1007/s00125-001-0771-8
34 Li, Y.X., Han, T.T., Liu, Y., Zheng, S., Zhang, Y., Liu, W. et al. (2015) Insulin resistance caused by lipotoxicity is related to oxidative stress and endoplasmic reticulum stress in LPL gene knockout heterozygous mice. Atherosclerosis 239, 276–282, https://doi.org/10.1016/j.atherosclerosis.2015.01.020
35 Labayen, I., Ruiz, J.R., Ortega, F.B., Davis, C.L., Rodriguez, G., Gonzalez-Gross, M. et al. (2015) Liver enzymes and clustering cardiometabolic risk factors in European adolescents: the HELENA study. Pediatr. Obes. 10, 361–370, https://doi.org/10.1111/jpo.273