Anti-Obesity Effect of Galacto-Oligosaccharides in Obese Rats

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Research

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Abstract

Background: Galacto-oligosaccharides (GOS) is a commonly used as a prebiotic with a variety of metabolic benefits. Whether GOS plays a protective role in obesity is still unknown. Here we demonstrated that GOS possesses an anti-obesity activity by promoting adipose tissue browning and thermogenesis.

Results: Our results showed that GOS effectively slow weight gain of diet-induced obese (DIO) rats without affecting energy intake. GOS significantly suppressed the hypertrophy and hyperplasia of white adipose tissue (WAT), as well as markedly lessened the ratio of fat pad to fat body. Consistently, GOS significantly improved serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels, which indicated an appropriate weight loss activity of GOS. Interestingly, GOS also significantly increased the expression levels of browning proteins (UCP1, PPARγ, PGC1α and PRMD16) both in the WAT and brown adipose tissue (BAT). We further found that GOS markedly increased the expression levels of LXRα, PPARα, LDLR and CYP7A1 proteins in the liver of obese rats.

Conclusions: Taken together, we concluded that GOS inhibits obesity by accelerating the browning of white fat cells and the thermogenesis of brown fat cells, moreover GOS improves host lipid homeostasis by promoting cholesterol catabolism.

Background

In today's society, obesity is a chronic, complex disease characterized by excessive accumulation of fat and with an increasing occurrence rate [1]. For example, more than 340 million people aged between 5 and 19 were diagnosed as overweight or obese in 2016 [2]. And obesity is associated with chronic diseases such as hypertension, type 2 diabetes, cardiovascular disease and various types of cancers [3] {Ulian, 2018 #19;Ulian, 2018 #1301}. So far the major therapeutic treatments for obesity consist of lifestyle changes (making healthy dietary choices and increasing exercise), bariatric surgery and medications [4]. Bariatric surgery is helpful for morbidly obese patients to significantly reduce weight and to improve obesity-induced cardiovascular disease and type 2 diabetes [5]. However, weight loss surgery is expensive and has numerous risks and side effects. And the relapse occurred in about one-third of surgical patients within five years after bariatric surgery [6]. The FDA-approved anti-obesity drugs mechanisms of action mainly include suppression of appetite and calorie intake [7], increased energy expenditure [8], and reduced fat absorption [9]. As the most widely used diet pills, central anti-appetite drugs have been demonstrated to be difficult to avoid, and have various side effects on the human body due to the multi-factor pathogenesis and the complex neurohormonal regulation system [10, 11]. Hormone drugs have been banned due to their large side effects and strong dependence [12]. Therefore, the development of new strategies for fighting against obesity and its complications has become urgent needs.

Adipose tissue usually makes up 20-28% of the total weight of healthy people, and can make up 80% of the body weight of obese patients [13]. Human adipose tissue mainly includes white adipose tissue (WAT) and brown adipose tissue (BAT). As the body's main storage area for energy, WAT contain very few mitochondria. Meanwhile, WAT is also the largest endocrine organ in the human body. WAT secretes various cytokines to regulate a series of physiological processes such as metabolism, appetite, angiogenesis, immunity and cardiovascular system [14]. In contrast, the cytoplasm of BAT is rich in mitochondria, and uncoupling protein 1 (UCP1) are concentrated in the inner membrane of these mitochondria [15]. UCP1 is the main effector of adaptive (non-shivering) thermogenesis in brown fat cells. In adults, beige adipose tissue is another type of adipose tissue between the white and brown adipose tissue [16, 17]. Beige fat cells are found mainly in subcutaneous fat and are derived from white fat cells in a process known as "Browning of white fat cells" [18]. Beige fat cells have basic metabolic functions similar to those of white fat cells, and under chronic cold exposure or beta-adrenergic stimulation, they can be transformed into thermogenic fat cells with a comparable high level of UCP1 expression as the brown fat tissue, and display metabolic activity [19, 20]. UCP1 in BAT is critical in regulating overall energy balance, which makes it a potential therapeutic target for many metabolic diseases, such as obesity and diabetes [21]. In fact, BAT activity has been shown to decrease in obese patients [16]. Thus, unraveling the mechanism of BAT activation is the key for the development of treatments for obesity.

Prebiotics are foods (usually high-fiber foods) used as food for the human microbiome [22]. As a prebiotic, Galacto-oligosaccharides (GOS) are formed by the linkage of 2-10 galactose monomers via glycosidic bonds with a relative molecular weight of about 300-2000Da [23]. GOS are safest oligosaccharides, a functional food additive with natural properties [24]. It is an important prebiotic for obtaining wide recognition and optimal therapeutic effect [25]. It has been widely used in infant formula, fermented milk and other foods [26]. GOS is naturally present in breast milk and is present in trace amounts in animal milk. Among all types of functional oligosaccharides, it is the only non-synthetic oligosaccharide derived from animal milk [27]. In recent years, due to its own physical and chemical properties and numerous physiological functions [28], more and more research has been done on GOS. Previous studies have shown that GOS can improve lipid metabolism in mice by regulating short-chain fatty acids [29, 30]. At the same time, GOS is also one of the hot points in the academic circle, but most of the studies are aimed at improving intestinal flora and promoting the growth of beneficial bacteria [31]. However, whether GOS has the activity of weight loss remains unclear.

In this study, we induced an obese sprague-dawley (SD) rat model through an 8-week high-fat diet, given GOS intervention for 8 weeks, dissecting, and measuring various indicators in plasma. Differential gene expression (DGE) analysis of liver and epididymal fat showed that there are differential genes in lipid metabolism and thermogenic pathway. Western Blotting were performed on related genes, and it was found that GOS can promote the browning of epididymis and the formation of brown fat. It promotes heat production and promotes cholesterol breakdown to regulate lipid metabolism. These results indicate that GOS can be used as a functional food to prevent and treat obesity.

Methods

Material

Orlistat was purchased from Zhongshan Wanhan Pharmaceutical Co., Ltd. (Guangzhou, Guangdong, China). GOS were supplied by New Francisco (Yunfu City) Biotechnology CO., Ltd. (Yunfu, Guangdong, China). The reagent information used in the experiment is provided in the supplementary material (Supplementary Tab. S1).

Experimental Animals and Treatment

Eighty male SD rats (200±20g, eight weeks old) purchased from the Guangdong Meddical Laboratory Animal Center (Guangzhou, China) were fed under the specific pathogen free environment of the experimental animal center with a temperature of 24±2℃, a relative humidity of 50%-60%, and a light rhythm of 12 hours. After ten days of adaptive feeding with normal feed (NF), they were randomly divided into two groups, ten of them were fed with normal feed as the blank group, and the remaining rats continued to be fed high-fat diet (HFD). The composition of HFD was basic feed 54%, lard 15%, sucrose 15%, milk powder 4%, peanut 3%, egg yolk powder 5%, salt 2%, sesame oil 1%, $CaHPO_4$ 0.6%, and mountain flour 0.4%. After two weeks of feeding, each rat was weighed, and twenty rats with obesity resistance were eliminated. After eight weeks, average weight of the high-fat model group is greater than 20% of the average weight of the blank group, which means that the model of obese rats was successful. Fifty DIO rats were randomly divided into five groups (n=10): HFD group (Model group) intragastrically 0.9% sodium chloride solution; GOS high-dose group (GOS-H, 1200mg/kg•day), GOS medium dose group (GOS-M, 600mg/kg•day), GOS low dose group (GOS-L, 300mg/kg•day) [32], Orlistat group (7mg/kg•day, positive drug control group) [33], for eight weeks, respectively. These rats were continuously fed HFD.

Determination of Food Intake and Weigh Gain

The weekly body weight changes and daily food intake of rats were measured. All rats can eat and drink freely. The rats were fasted for 12 hours, then anesthetized after eight weeks of intervention. The liver, perirenal fat, epididymal fat, subcutaneous fat and BAT were quickly taken and weighed, the blood sample were taken from the abdominal aorta. The fat/body ratio and fat pad were calculated.

Serum, Liver and Fecal Biochemical Analysis

The level of TC, TG, HDL-C, LDL-C, glucose (GLU) in serum, TC, TG, HDL-C, LDL-C in liver, and TC, TG in fecal were measured using kits obtained from Feiya Biotechnology, Inc. (Jiangsu, China). The rats fasted for 12 hours and drank water freely. Determination of fasting blood glucose: after sterilizing the tails with alcohol, cut off the tails by 0.1cm, collect blood, and measure fasting blood glucose with a blood glucose meter, which is 0 min blood glucose value. Glucose solution (1.5g/kg) was injected into the rats according to their body weight, and the blood glucose values at 15min, 30min, 60min, 90min, and 120min were recorded.

Determination of TC in faeces: put the faeces in a vacuum dryer for 30 min, pound it, weigh 0.1 g of faeces in a 5 ml centrifuge tube, add 0.9 ml of ether, shake it for 15 min, let it rest for 10 min at room temperature, then absorb the upper layer of liquid and determine the TC content according to the instructions of the kit.

Determination of TG in faeces: Weigh 0.25g of crushed feces and place it in a 10ml centrifuge tube, add 3ml of 95% ethanol, mix well, and then extract in a water bath (60℃, 30min), after stratification, add 3ml of 95% ethanol, extract three times, and place the extract in another 10ml centrifuge tube at 80℃ in a water bath. After evaporation, add 10 ml of 60% acetic acid, dissolve with repeated shaking, and determine the TG content according to the kit instructions.

Histopathological Examination

The epididymis, perirenal, subcutaneous adipose tissue and liver were fixed in 4% paraformaldehyde solution for 24 hours, dehydrated by gradient ethanol, embedded in paraffin, cut into 3-5μm thick sections, stained with hematoxylin and eosin (HE), observed under a microscope with 200 times magnification.

Gene Sequencing Method

The liver and the epididymal fat RNA in the NF (Control), HF (Model) and GOS (GOS-H) groups were extracted and processed by the mRNA enrichment method (the mRNA enriched with polyA with oligodT). Then the DNA probe is used to digest the DNA/RNA hybrid chain, and then the DNA probe is removed with DNaseI, and RNA is obtained after purification. Then use buffer to fragment RNA, reverse transcription RNA, and synthesize double-stranded DNA. Fill in the ends of the obtained double-stranded DNA, reduce the phosphorylation of the 5'end, and then connect a bubbling linker with a protruding "T" at the 3'end to perform PCR amplification of the product. The PCR product was circularized with a bridge primer to obtain a single-stranded circular DNA library [34]. Finally, it was sequenced on the computer. After sequencing, the NOISeq method and cluster analysis of differentially expressed genes were used to screen differentially expressed genes (Supplementary Fig. S1).

Western Blotting

Strong RIPA lysis buffer containing protease and phosphatase inhibitors was used to extract total protein from the liver and adipose tissue of SD rats. The protein sample was diluted with loading buffer and denatured at 98℃ for 5 minutes. The concentration of the protein sample in sodium dodecyl sulfate-polyacrylamide gel electrophoresis is 10-15% (v/v) and the separated protein is then transferred to a polyvinylidene flfluoride (PVDF) membrane (microporous Corp . Billerica, Mom, USA). The PVDF membrane was incubated in the diluted primary antibody at 4℃ overnight, and then incubated with goat anti-rabbit secondary antibody (Beijing Biosynthetic Biotechnology Co., Ltd.) for 2 hours after blocked with 5% skimmed milk powder. Use Image J 1.48V software to perform gray analysis on Western blots.

Statistical Analysis

In this subject, SPSS20.0 software was used for data processing, and means±SEM was used for experimental results. A single factor was used to compare the mean of multiple groups of samples, and p value less than 0.05 indicated statistical significance. GraphPad Prism5.0 was used for plotting.

Results

GOS Ameliorates Obesity In DIO Rats

During the administration, the average daily food intake of SD rats was recorded, and the results showed that GOS had no appetite inhibition (Fig. 1a and Table S2). After GOS and orlistat treatment, the weight gain trends were slower than that of the Model group, among which GOS-H had the best effect (Fig. 1b-c). These results indicated that GOS can decelerate the trend of weight gain without affecting appetite in DIO rats. After weighing the fat mass (fat pad) that was anatomically obtained, the fat/ body ratio of the rat was calculated. The results showed that GOS significantly reduced the fat content and the fat/body ratio caused by HFD (Fig. 1d-e and Table S3).

GOS Improves Serum and Liver Lipid Metabolism In DIO Rats

The effects of GOS on serum and liver lipid metabolism levels (TC, TG, LDL-C and HDL-C) were determined. Compared with the Model group, GOS significantly increased serum HDL-C level and decreased TC, TG and LDL-C levels (Fig. 2a-d and Table S4), although the GOS-H was slightly inferior to the orlistat. Similarly the levels of TC, TG and LDL-C in liver were obviously lower, while the levels of HDL-C were markedly increased after GOS treatment (Fig. 2e-h and Table S5), indicating that GOS can improve dyslipidemia induced by HFD in rats.

GOS PromotesFecal LipidExcretion

The contents of TC and TG in the feces of DIO rats were examined, the results are presented in Fig. 2i-j and Table S6. The TC and TG levels after orlistat treatment were obviously higher than those of the Model group, due to the fact that orlistat is a lipase inhibitor, which can inhibit the absorption of fat in the body. The contents of TC and TG in GOS group were significantly higher than Model group. The results showed that GOS can improve obesity symptoms by promoting the excretion of TC and TG.

GOS Improves Serum Glucose Level In DIO Rats

Blood glucose is the main source of normal energy supply in the body. Glucose tolerance is the body's ability to regulate blood glucose concentration. As shown in Fig. 2k, serum glucose levels were measured. Blood glucose were decreased after GOS treatment, but only GOS-H showed significant difference,which indicates that GOS had a certain hypoglycemic effect. In order to observe whether the glucose tolerance of rats was abnormal after the administration of GOS, a glucose tolerance test was conducted to observe the body's sensitivity to glucose level. The results are shown in Fig. 2l-m, indicated that GOS-H reduced glucose tolerance in obese rats.

GOS Reduces Lipogenesis In the Liver and Inhibits Adipocyte Hypertrophy

The DIO rats were dissected, the liver was removed, photographed and the overall morphology was observed, as shown in Fig. 3a. The liver of the Model group was hypertrophy, the surface was not smooth and the color was white. In contrast, the livers in the Control group were bright red in color, small in size, soft in texture and with tissue elasticity. Interestingly, the livers of GOS groups were bright red in color, soft in texture, and smooth in leaf. In addition, liver tissues of DIO rats were sectioned and stained, as shown in Fig. 3b. The hepatocytes in the Control group were orderly and uniform in size, there was no inflammatory cell infiltration. However, liver cells in Model group were deformed and had uneven size and more fat drop accumulation. After GOS administration, the fat droplets were significantly reduced.

HE staining was performed on epididymis (Fig. 3c), perirenal (Fig. 3d) and subcutaneous adipose tissue (Fig. 3e). The adipocytes in the Control group had normal structure, compact arrangement, uniform size, large number and small cell volume, while the adipocytes in the Model group were not uniform in size. After GOS administration, the size of fat cells significantly decreased and the hypertrophy were improved. All data indicated that GOS can relieve fatty liver, inhibit the growth and accumulation of fat cells in WAT, so as to play an anti-obesity role.

DGE In Liver and Adipose Tissue

To explore the mechanism of GOS weight loss and lipid-lowering, genes differentially expressed in the liver and epididymal fat were identified. The differentially expressed genes were mapped by differential gene analysis as shown in Fig. 4a and Fig. 5a, respectively. The significantly up-regulated and down-regulated genes in liver and epididymal fat were counted. Compared with Model, GOS up-regulated 300 genes and down-regulated 266 genes in liver. Similarly, GOS up-regulated 120 genes and down-regulated 428 genes in adipose tissue. The above results showed that feeding on different diets (normal diet and high-fat diet), and the administration of GOS can regulate gene transcription levels in DIO rats. The results of the Gene Ontology (GO) annotation analysis in liver and epididymal fat are shown in Fig. 4b and Fig. 5b, respectively. In the liver, the impacted biological processes mainly involved cell process, cell development process, biological regulation, metabolic process and multicellular tissue process. The cell composition was mainly associated with cell membrane and organelle. The molecular function mainly covered binding function and catalytic function (Fig. 4b). Furthermore, the results of GO annotation analysis in the epididymal fat were the same as those in the liver (Fig. 5b).

In order to further understand the connections between genes in organisms, pathway analysis was conducted, the cooperative effects of different genes were deeply explored to complete certain functions. The Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation analysis in liver and epididymal fat are shown in Fig. 4c and Fig. 5c, respectively. It can be known that the KEGG pathway classification in liver and epididymal fat mainly involved energy metabolism, bile acid metabolism, amino acid metabolism, lipid metabolism, sterol and steroid metabolism. In addition, enriched bubble map analysis was performed on the KEGG pathway analysis of differential genes in liver and epididymal fat, and the results are presented in Fig. 4d and Fig. 5d, respectively. The results suggested that the differential genes

in liver were concentrated on metabolism-related pathways, including ether-lipid metabolism, lipid metabolism, mitogen-activated protein kinase (MAPK) signaling pathway, metabolism of cytochrome P450 to heterologous organisms, biliary secretion, and tyrosine metabolism (Fig. 4d). These results indicated that GOS was associated with lipid metabolism pathways in the liver. Therefore, the lipid metabolism-related proteins in the liver were selected for lipid lowering mechanism study.

In addition, the results in epididymal fat suggested that the differential genes were mainly related to thermogenesis, including MAPK, peroxisome proliferators-activated receptors (PPAR), phosphatidylinositol 3 kinase/protein kinase B (PI3K/AKT) signaling pathway, cholesterol metabolism, fatty acid metabolism, and arachidonic acid metabolism (Fig. 5d). These results indicated that GOS was associated with the browning and lipid metabolism of white fat in epididymal fat. Therefore, proteins related to the browning and lipid metabolism of white fat were selected for anti-obesity mechanism study.

GOS Promotes Epididymal Fat Browning

Based on the results of gene sequencing, genes related to lipid metabolism and energy metabolism were verified (Fig. 6a), including browning marker genes and thermogenic genes, namely, UCP1, peroxisome proliferative proliferator-activated receptor γ (PPARγ), peroxlsome proliferator-activated receptor-γ coactlvator-*1α* (PGC1α), zinc lipoprotein 16 (PR domain-containing 16, PRDM16) and activating transcription factor2 (ATF2). The results indicated that protein levels of UCP1, PPARγ, PGC1α and PRDM16 were significantly increased after GOS treatment (Fig. 6b-e). Protein levels of ATF2 in GOS-H and GOS-M group was markedly higher than those in the Model group (Fig. 6f). According to these results, we can preliminarily infer that GOS may promote the browning of epididymal fat cells in DIO rats.

GOS Promotes Brown Fat Formation

BAT loses weight by increasing the amount of energy consumed by nontremor heat production. In order to investigate the changes of BAT in DIO rats after GOS intervention, we detected the expression of thermogenic protein in BAT (Fig. 7a). The results were consistent with the expression of these proteins in epididymal fat. Compared with HFD alone, the protein expression levels of UCP1, PRDM16 and PPARγ in the brown fat of DIO rats treated with GOS were obviously increased (Fig. 7b,c,e). In addition, the high and medium dose GOS significantly increased PGC1α protein expression (Fig. 7d). On the premise of suppressing weight gain and promoting the increase of brown fat cells, the results indicated that GOS can promote the formation of BAT and thermogenesis.

GOS Regulates Cholesterol Catabolism In Liver andEpididymal Fat

Cholesterol metabolic-related proteins, cholesterol 7α-hydroxylase (CYP7A1), low density lipoprotein receptor (LDLR), liver X receptor α (LXRα), peroxisome proliferative proliferator-activated receptor *α* (PPARα), sterol-responsive element binding protein-2 (SREBP2) in liver, and CYP7A1, LDLR, PPARα, LXRα in epididymal fat were detected, as shown in Fig. 8a and Fig. 9a, respectively. The results suggested that

the protein expression levels of CYP7A1 and PPARα in the liver of DIO rats treated with GOS were significantly increased compared with that given only HFD (Fig. 8b,e).

Compared with the Model group, the expression level of LDLR and LXRα protein were significantly increased after GOS-H and GOS-M treatment (Fig. 8c,d). GOS, especially the medium and low dose, reversed the upregulated expression of SREBP2 induced by HFD (Fig. 8f). Moreover, the results in epididymal fat were consistent with the expression of these protein in liver. Protein expression levels of CYP7A1, LDLR, LXRα, PPARα in epididymal fat were markedly higher in DIO rats treated with GOS than in those using HFD intervention (Fig. 9b-e). These results suggested that GOS can promote the decomposition of cholesterol in liver and epididymal fat by regulating the expression of proteins involved in cholesterol catabolism to relieve obesity and its related metabolic diseases.

Discussion

Obesity is one of the main symptoms of metabolic syndrome WAT is largely distributed in visceral and subcutaneous locations [35], mainly including subcutaneous fat, perirenal fat and epididymal fat. The subcutaneous adipose tissue accounts for most of the total white adipose tissue in human [14]. Brown fat cells are rich in mitochondria, in which UCP1 is transported to all parts of the body in the form of heat energy through the capillaries in brown fat by decoupling during oxidative respiration. As a result, the efficiency of fatty acid oxidation and decomposition is increased, thus increasing the body's heat production [36]. Activation of UCP1 also requires an auxiliary activator, including PGC1α, PRDM16 and PPARγ[37]. PRDM16 promotes the differentiation of $Myf5^{+/-}$ adipocytes into brown adipocytes, promotes the expression of PGC1α and UCP1 in WAT, and activates the interaction between PGC1α and PPAR [38], thus promoting the browing process of adipose tissue. PGC1α is expressed in fat cells and muscle and regulates the expression of related proteins during thermogenesis. PGC1α also binds to PPARγ and PPARα to form a transcription complex, which then binds to the PPAR response element in the UCP1 promoter to activate UCP1. Phosphorylated ATF2 binds to the promoter region of PGC1α, thereby enhancing the expression of PGC1α [39]. LDLR is a multifunctional egg white, which is synthesized mainly in the liver and can bind to 65-70% LDL in plasma to reduce LDL content. SREBP2 is a key factor in the regulation of cholesterol synthesis. CYP7A1 is the most important regulatory factor in the decomposition of cholesterol, which can catalyze the decomposition of cholesterol to produce bile acids. Studies have shown that the activity of CYP7A1 is regulated by a variety of factors, such as PPAR and LXR, which can activate the expression of CYP7A1. CYP7A1 is also regulated by the feedback effect of cholesterol and bile acid in the body [40]. LXR is a transcription factor activated by nuclear receptor superfamily ligand and mainly expressed in the liver, which regulates cholesterol metabolism in vivo by stimulating the expression of CYP7A1 [41]. Studies have shown that PPAR can promote the synthesis of CYP7A1. PPAR also promotes the expression of LXR, thus increasing bile acid synthesis [42].

In this study, it was found that GOS could significantly reduce weight gain in obese rats induced by HFD, improve lipid levels in obese rats, improve liver function injury and alleviate lipid accumulation in the liver. In addition, GOS has a certain hypoglycemic function, GOS-H can improve glucose tolerance in obese

rats. GOS was found to be related to biliary secretion and lipid metabolism in liver and associated with thermogenesis, MAPK, PPAR signaling pathways and cholesterol metabolism in adipose tissue by gene differential spectrum. Therefore, the results of this study indicated that GOS can be used as functional foods to prevent and treat obesity.

Conclusions

To sum up, GOS can lose weight without affecting appetite, improve blood lipid of obese SD rats, relieve fat accumulation in liver, and inhibit fat cell hypertrophy. Genetic differential spectrum analysis showed that GOS was associated with lipid metabolism in liver and heat production and lipid metabolism in adipose tissue. In addition, GOS promoted browning and brown fat formation of epididymal fat, as well as regulating of lipid metabolism of liver and epididymal fat.

Abbreviations

GOS: Galacto-oligosaccharides; DIO: Diet-induced obese; WAT: White adipose tissue; TC: Total cholesterol;TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol;GLU: Glucose; BAT: Brown adipose tissue; UCP1: Uncoupling protein 1; SD: Sprague-dawley; NF: Normal feed; HFD: High-fat diet; HE: Hematoxylin and eosin; PVDF: Polyvinylidene flfluoride: DGE: Differential gene expression; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; MAPK: Mitogen-activated protein kinase; PPAR: Peroxisome proliferators-activated receptors; PI3K/AKT: Phosphatidylinositol 3 kinase/protein kinase B; PPARγ: Peroxisome proliferative proliferator-activated receptor γ; PPARα: Peroxisome proliferative proliferator-activated receptor *α;* PGC1α: Peroxlsome proliferator-activated receptor-γ coactlvator-*1α*; PRDM16: Zinc lipoprotein 16 (PR domain-containing 16, PRDM16);ATF2: Activating transcription factor2; LDLR: Low density lipoprotein receptor; LXRα: Liver X Receptor α; LDL: Low density lipoprotein; SREBP2: Sterol-responsive element binding protein-2;CYP7A1: Cholesterol 7α-hydroxylase.

Declarations

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Authors' contributions

Conceptualization, S.K., H.N., and Z.S.; formal analysis, S.K., X.H.; funding acquisition, Z.S.; methodology, S.K., H.C., and Z.S.; project administration, H.N. and Z.S.; software, H.C and Q.C.; supervision, Z.S.; validation, Q.C., and H.N.; writing—original draft, S.K., and X.H.; writing—review and editing, S.K., H.N., and Z.S.

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Availability of data and materials

The datasets analysed in the current study are available from the corresponding author on request.

Ethics approval and consent to participate

All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of Guangdong Pharmaceutical University (Guangzhou, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interest.

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Figures

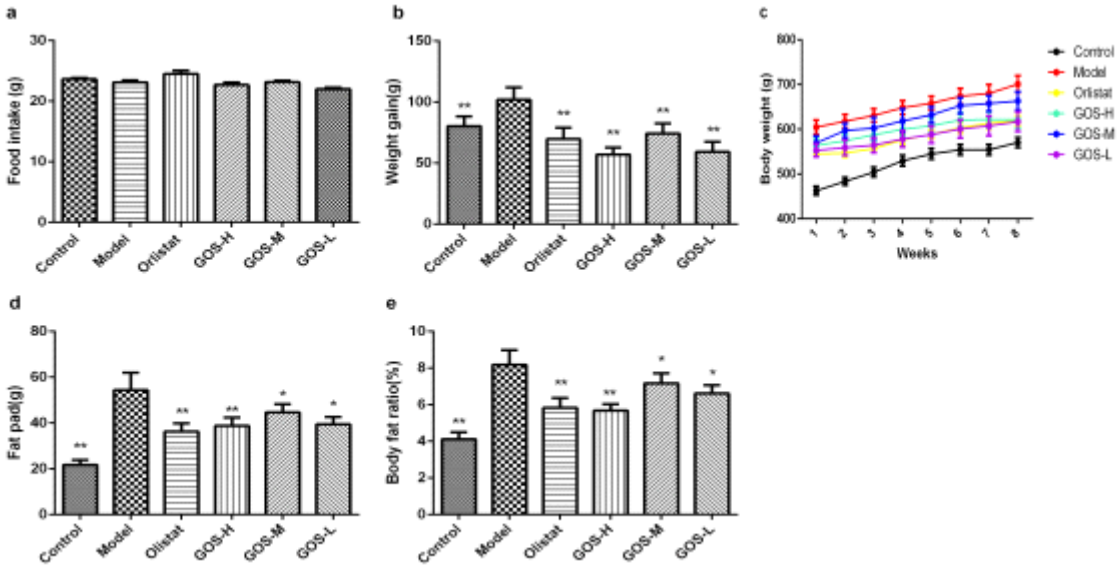

Figure 1

The main index of GOS. Changes in the food intake, body weight gain, body weight, fat pad and body fat ratio after 8 weeks of treatment. The data are presented as the means ± SEM (n = 10). * $p < 0.05$; ** $p < 0.01$ vs. the Model group.

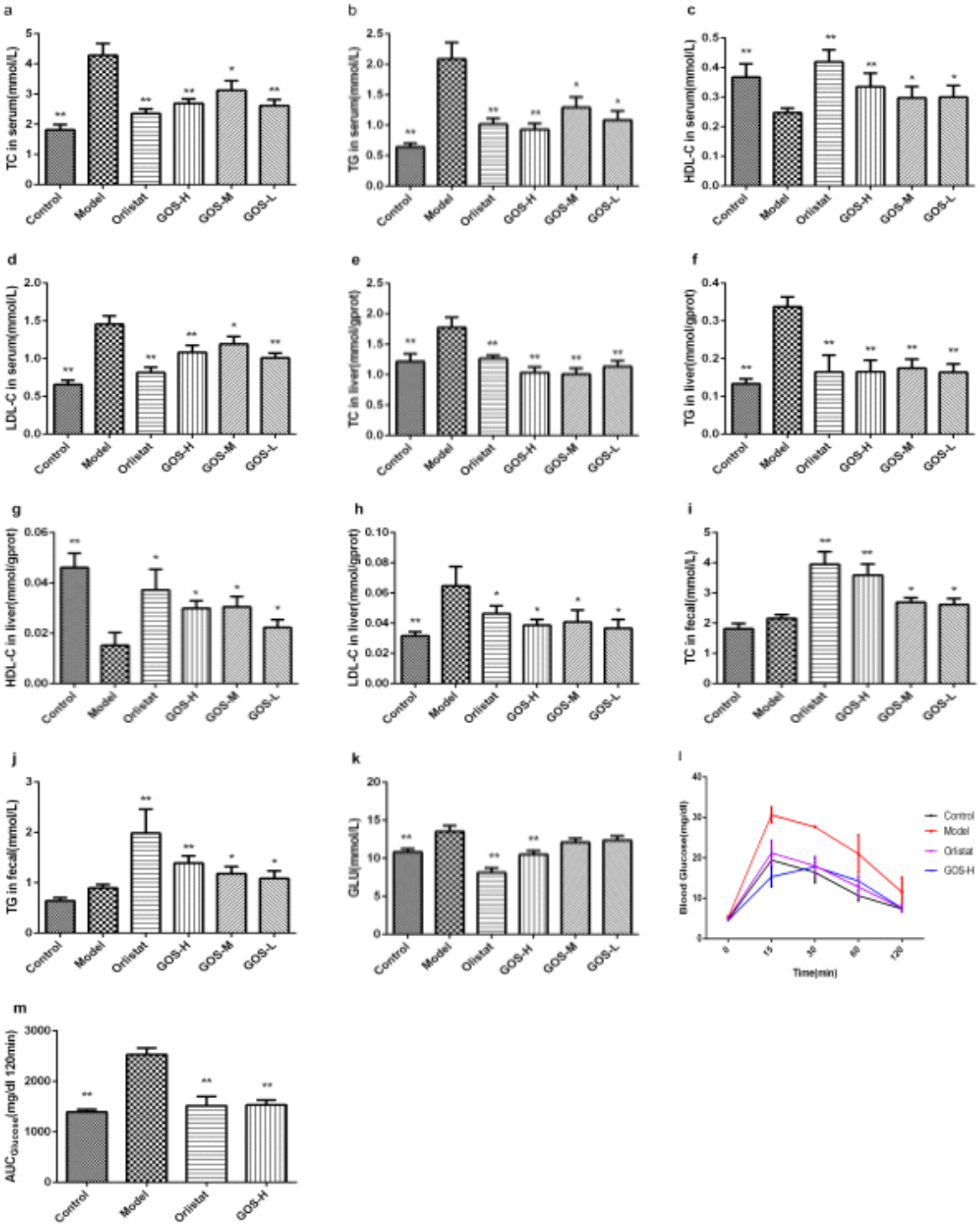

Figure 2

The effect of GOS on serum,liver and fecal lipid levels in HFD rats after 8 weeks of treatment. The data are presented as the means ± SEM (n = 10). * $p < 0.05$; ** $p < 0.01$ vs. the Model group.

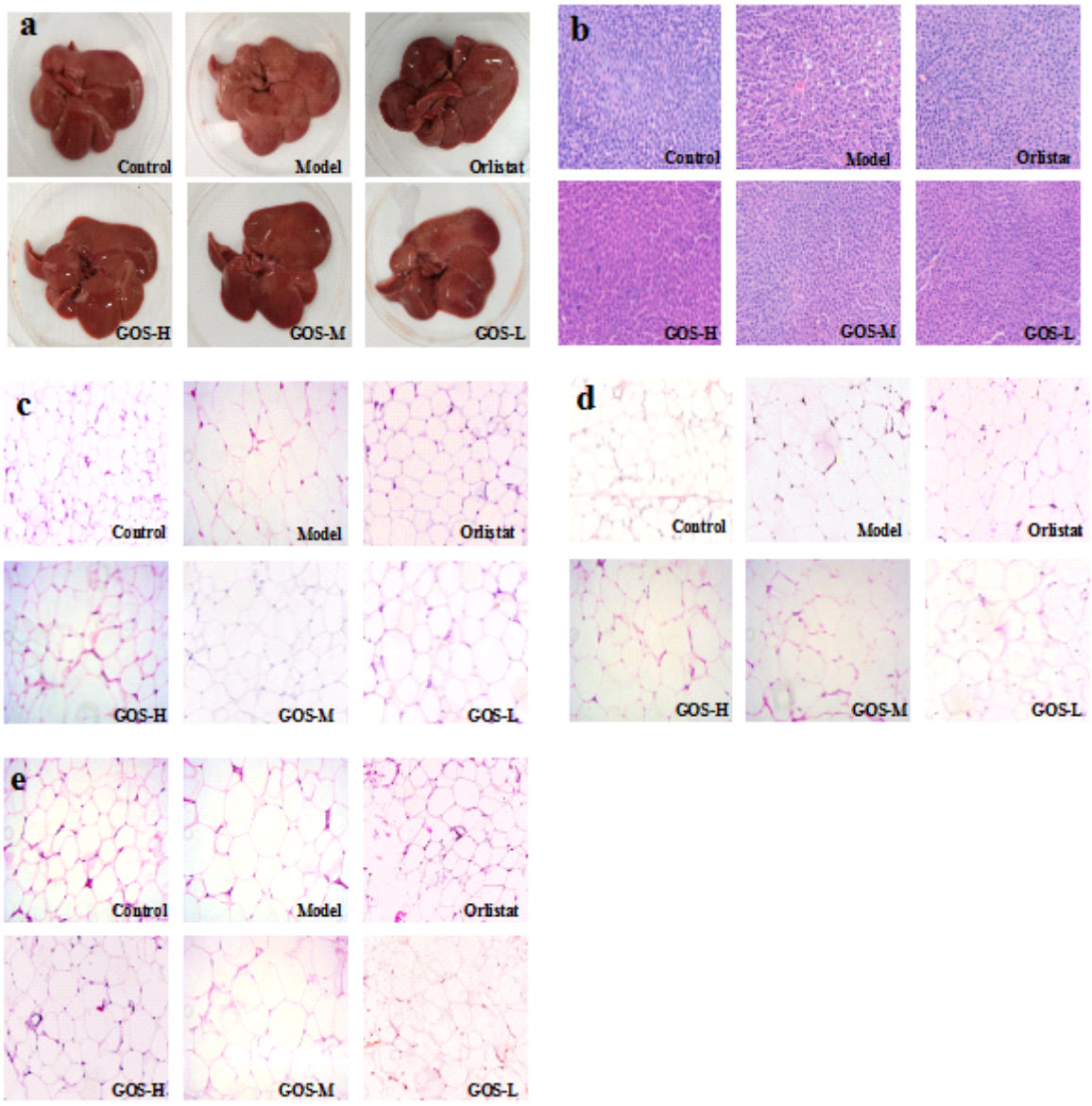

Figure 3

The whole liver (a), slices of liver (b), Epididymal fat (c), perirenal fat (d) and subcutaneous fat (e) from different groups of rats (200×) after 8 weeks of treatment. Tissue sections were stained with HE.

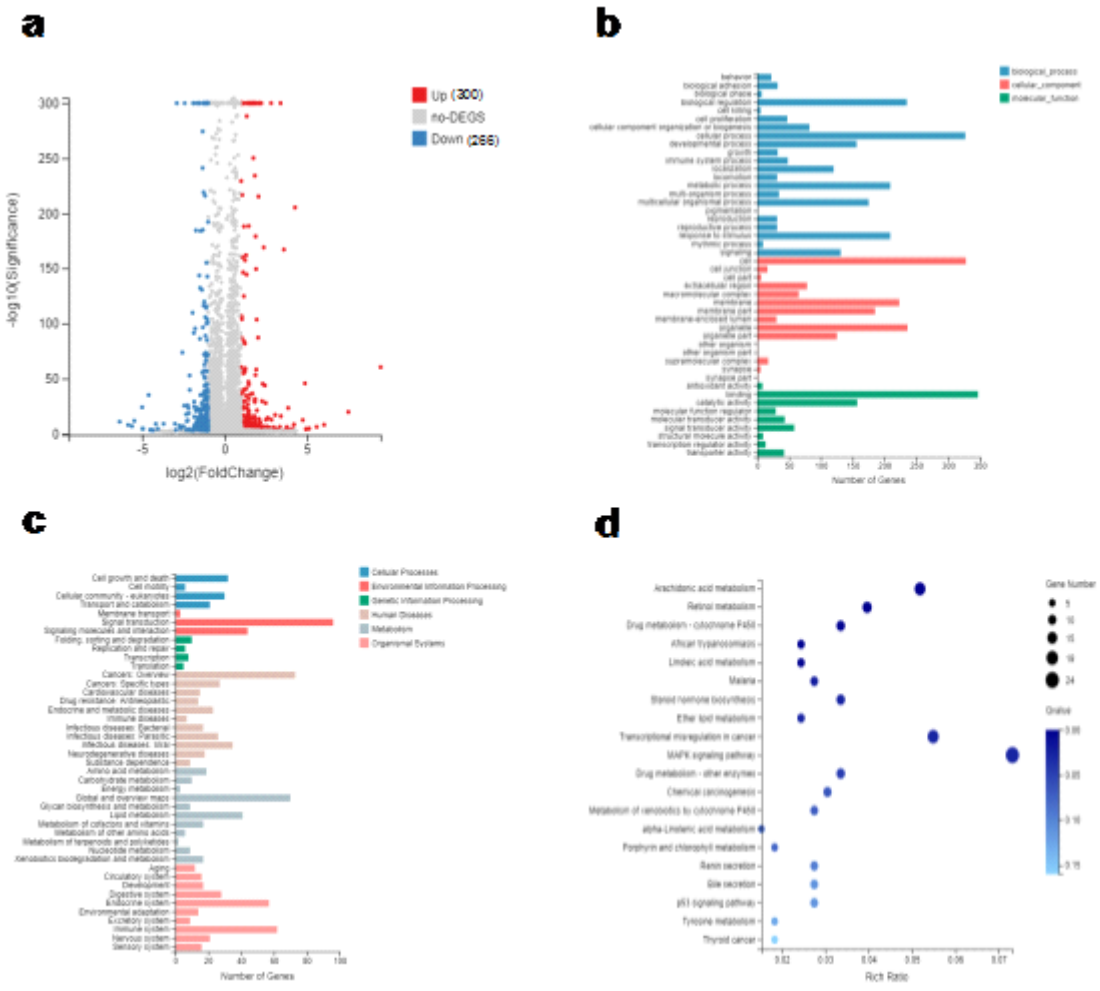

Figure 4

Differential gene expression analysis between Model and GOS group in the liver. (a) Scatter Map of Expressed Genes,(b) Gene Ontology Functional Annotation Classification Chart, (c) KEGG Pathway Classification Statistical Map, (d) KEGG Pathway Enrichment Bubble Map.

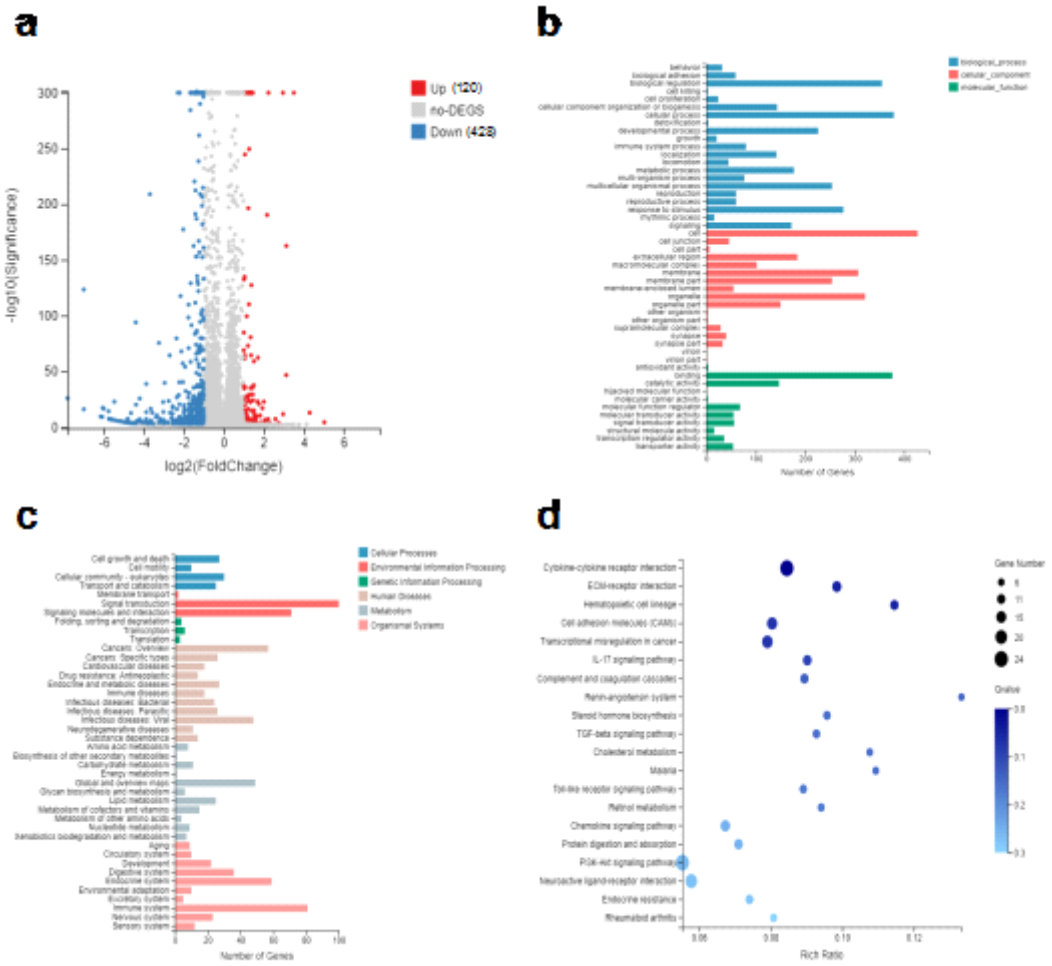

Figure 5

Differential gene expression analysis between Model and GOS group in the epididymal fat. (a) Scatter Map of Expressed Genes,(b) Gene Ontology Functional Annotation Classification Chart,(c) KEGG Pathway Classification Statistical Map,(d) KEGG Pathway Enrichment Bubble Map.

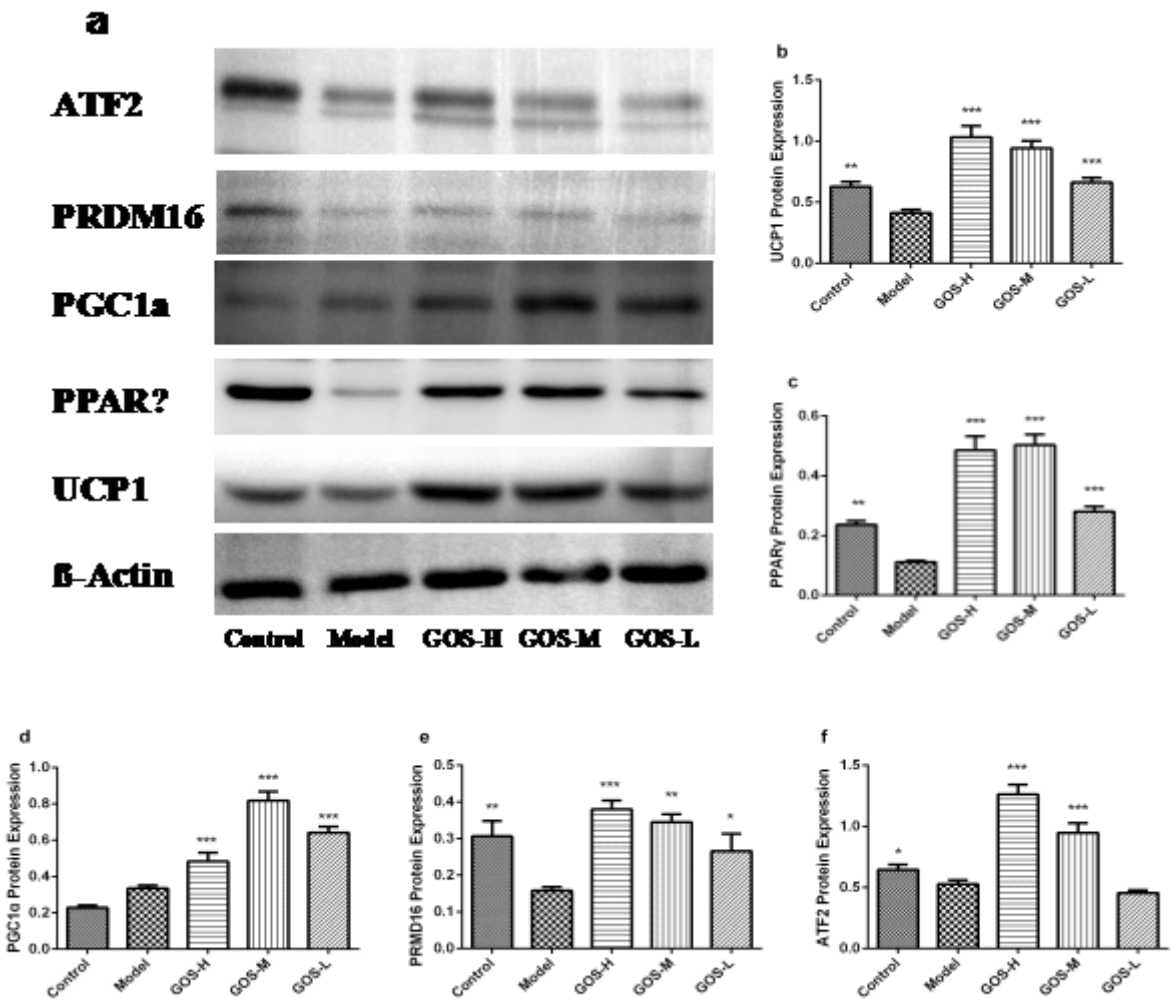

Figure 6

The protein expression in the epididymal fat (n=5, means ± SEM). (b) UCP1; (c) PPARγ; (d) PGC1α; (e) PRMD16; (f) ATF2. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. the Model group.

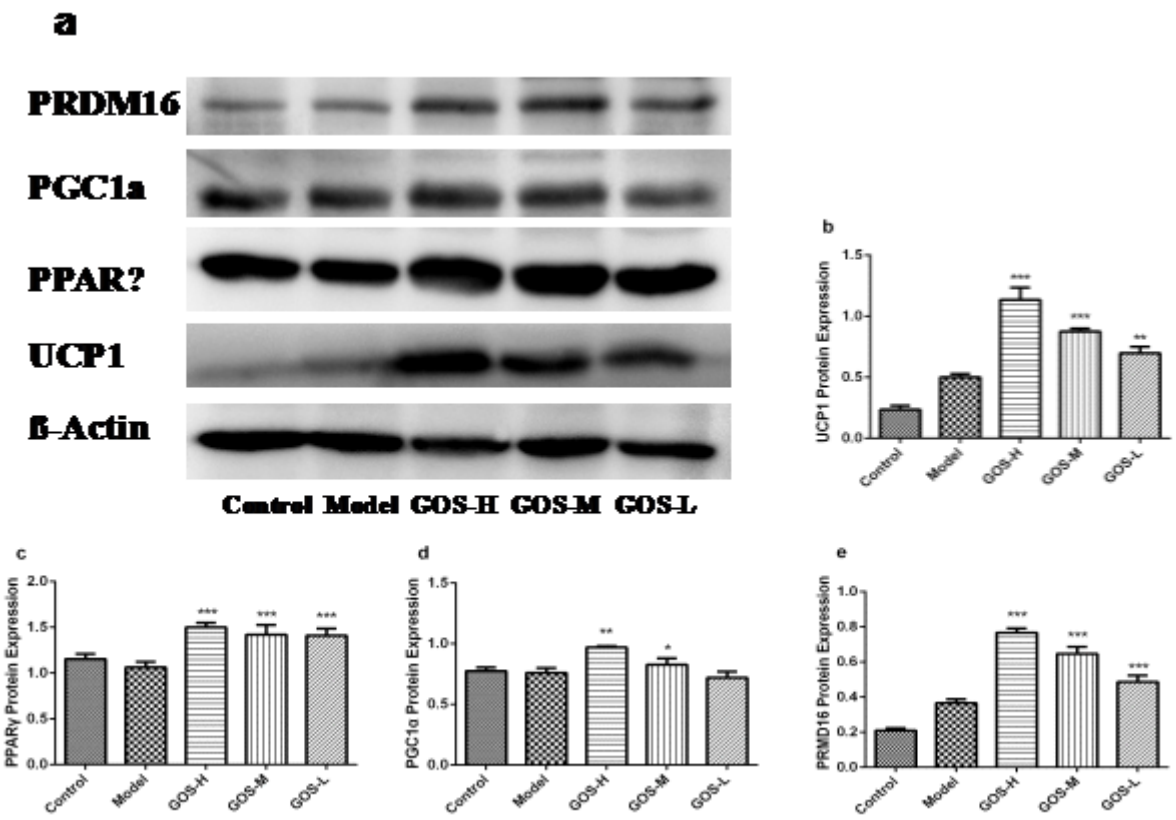

Figure 7

The protein expression in the BAT (n=5, means ± SEM). (b) UCP1; (c) PPARγ; (d) PGC1α; (e) PRMD16. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. the Model group.

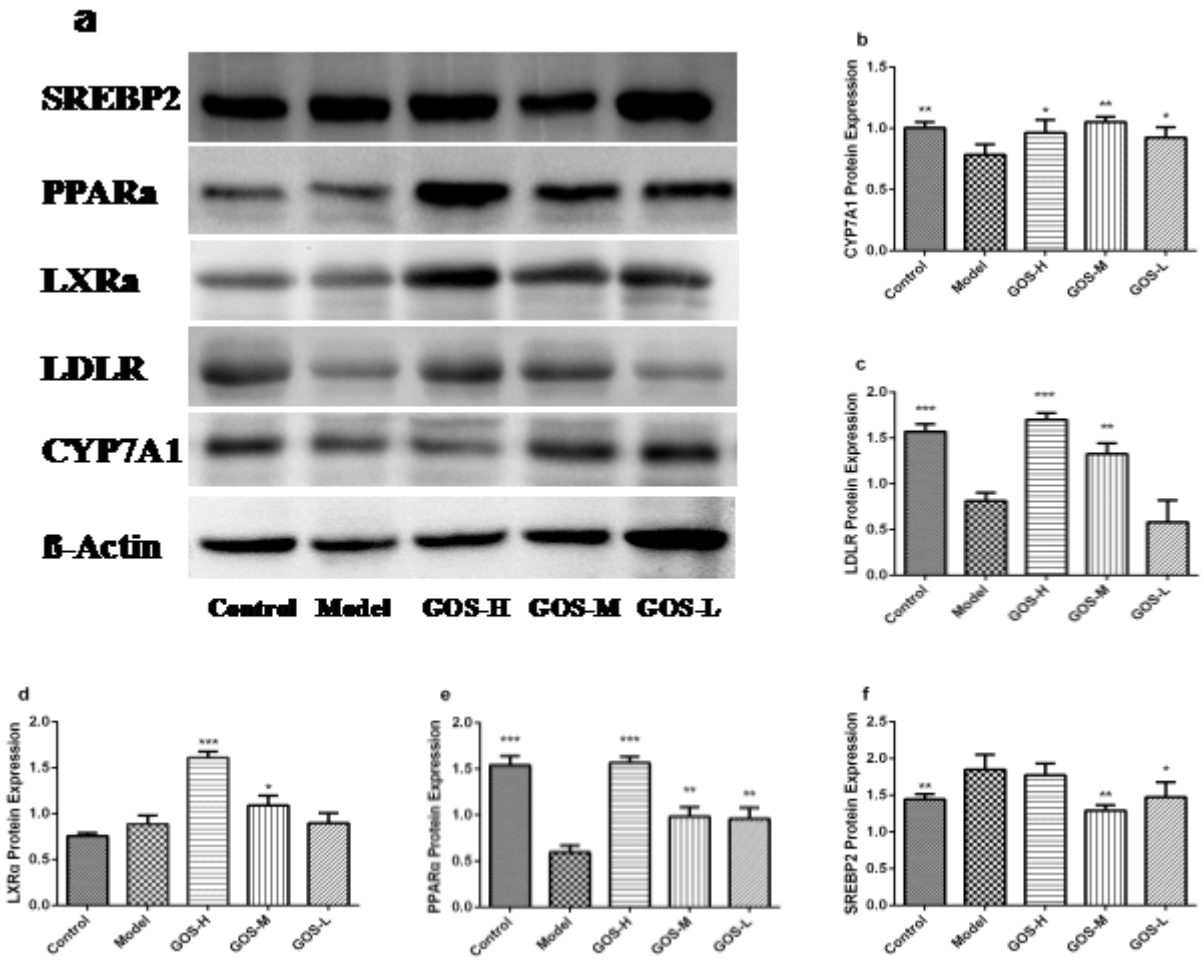

Figure 8

The protein expression in the liver (n=5, means ± SEM). (b) CYP7A1; (c) LDLR; (d) LXRα; (e) PPARα; (f) SREBP2. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. the Model group.

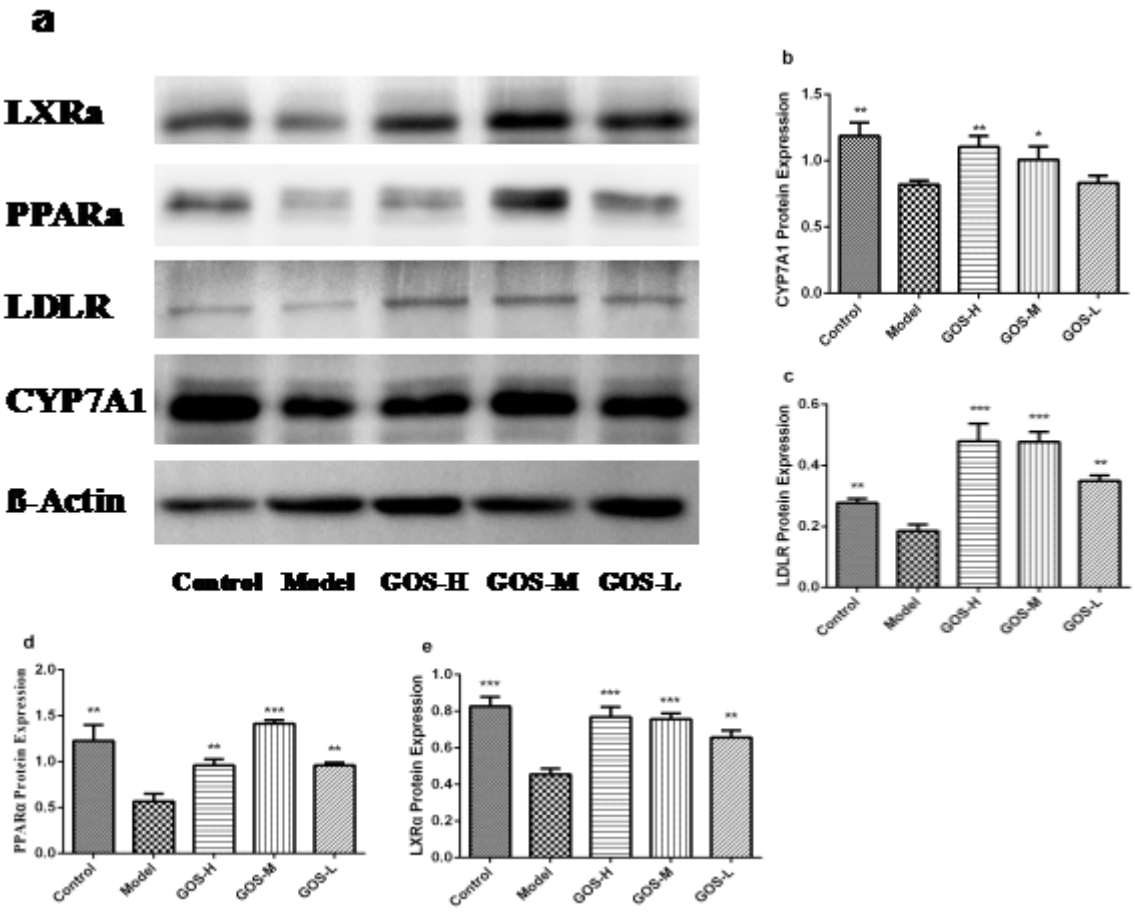

Figure 9

The protein expression in the epididymal fat (n=5, means ± SEM). (b) CYP7A1; (c) LDLR; (d) LXRα; (e) PPARα. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. the Model group.

Supplementary Files

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- SupplementaryMaterial.docx