Curcumin attenuates insulin resistance and hepatic lipid accumulation in a rat model of intrauterine growth restriction through insulin signaling pathway and SREBP

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Key words: intrauterine growth restriction, insulin resistance, lipid metabolism, curcumin, rat

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Abbreviations used: Actb, β-actin.; Fasn, fatty acid synthase; FASN, fatty acid synthase; GSK3α/β, glycogen synthesis kinase-3 α/β; HDL-C, high-density lipoprotein cholesterol; HL, hepatic lipase; HOMA-IR, homeostasis model assessment index; HSL, hormone sensitive lipase; IR, insulin resistance; IRS-1, insulin receptor substrate 1; IUGR, intrauterine growth restriction; LDL-C, low-density lipoprotein cholesterol; LPL, lipoprotein lipase; NAFLD, non-alcoholic fatty liver disease; NEFA, non-esterified fatty acid; PI3K, PI3 Kinase p85; PPARα, peroxisome proliferator-activated receptor α; Ppara, peroxisome proliferator-activated receptor α; PK, pyruvate kinase; Srebf1, sterol regulatory element binding proteins 1c; SREBP1, sterol regulatory element binding proteins 1; TC, total cholesterol; TG, triglyceride; TL, total lipase.

Abstract

The objective of present study was to investigate the effect of curcumin on insulin resistance and hepatic lipid accumulation in intrauterine growth restriction (IUGR). Rats with a normal birth weight (NBW) or IUGR were fed basic diets (NBW and IUGR groups) or basic diets supplemented with curcumin (NBW-C and IUGR-C groups) from 6 to 12 weeks. Rats with IUGR showed higher levels of glucose and homeostasis model assessment index (HOMA-IR) ($P<0.05$) than in NBW group. The livers of IUGR rats exhibited higher ($P<0.05$) concentration of triglyceride and lower ($P<0.05$) activities of lipolysis enzymes compared with the normal rats. In response to dietary curcumin supplementation, concentrations of serum insulin, glucose and HOMA-IR, pyruvate, triglyceride, total cholesterol and non-esterified fatty acid in the liver were decreased ($P<0.05$). The concentrations of glycogen and activities of lipolysis enzymes in the liver were increased ($P<0.05$) in the IUGR-C group than in the IUGR group. These results were associated with lower ($P<0.05$) phosphorylated insulin receptor substrate 1 (IRS-1), Akt, glycogen synthase kinase 3β (GSK3β) and expressions of sterol regulatory element binding proteins 1 (SREBP1)
and fatty acid synthase (FASN); decreased expressions for Cd36, Srebf1 and Fasn; increased (P<0.05) expression of peroxisome proliferator-activated receptor α (PPARα) and expressions for Ppara and hormone sensitive lipase (HSL) in the liver of IUGR-C rats than the IUGR rats. Maternal malnutrition caused insulin resistance and lipid accumulation in the liver. Curcumin supplementation prevented insulin resistance by regulating insulin signaling pathways and attenuated hepatic lipid accumulation.

Introduction

Intrauterine growth restriction (IUGR) is a serious problem in human beings and mammals, which affects approximately 5-10% of human infants worldwide and 15-20% of newborn animals (1; 2). It has been observed that at least 13.7 million infants were born with low birth weight each year (3). IUGR leads to high morbidity and mortality, low feed utilization, and permanent adverse effects on postnatal life. David Barker et al. (4) originally stated the “early” or “fetal” origins of adult disease hypothesis. The hypothesis described that environmental factors, particularly poor nutrition, act in early life to program the risks for the early onset of metabolic diseases in adult life and premature death. A significant relationship was observed between children born with IUGR and the development of a variety of adult diseases, including hyperinsulinemia dyslipidemia and nonalcoholic fatty liver disease (NAFLD) (5; 6).

Liver is a major organ for lipid metabolism and insulin target organ, often influenced by IUGR during pregnancy (7; 8). The liver also plays a critical role in maintaining blood glucose homeostasis by controlling hepatic glucose production. IUGR has been linked to glucose intolerant and insulin resistance (IR) (9). Previous studies suggested that IUGR not only closely linked with lipid dysfunction but also related to fatty liver disease (10).

Curcumin is a naturally occurring phenolic compound, which is widely used in food, beverage, medicine...
and so forth \(^{(11)}\). Curcumin \((C_{21}H_{20}O_{6})\), first described in 1910 by Lampe and Milobedeska, is the most active ingredient of turmeric and makes up 2-5\% of this spice \(^{(12)}\). The beneficial effects of curcumin on IR have been widely researched in animal models \(^{(13)}\). Curcumin treatment also has been illustrated to attenuate IR by decreasing insulin receptor substrate 1 phosphorylation in the muscle of Wistar rats fed with high fructose \(^{(14)}\). In addition, they found that curcumin attenuated hyperinsulinemia and the homeostasis model assessment index (HOMA-IR). However, the related researches about curcumin on insulin resistance in IUGR are very limited.

Accordingly, we hypothesized that dietary supplementation of curcumin has a protective effect on IUGR insulin resistance by modulating the insulin signaling pathway. Furthermore, we took advantage of a maternal malnutrition rat model, which is an identified model for human IUGR study, and determined the hepatic lipid content and protein expressions related to fatty acid synthesis and lipid oxidation, to investigate whether these changes of IUGR-induced hyperlipidemia were alleviated after curcumin administration.

**Materials and methods**

*Curcumin preparation*

The curcumin used in this study was kindly provided by the Kehu Bio-technology Research Center (Guangzhou, China). The content of curcumin was 98\% measured by using high performance liquid chromatography (HPLC).

*Animal experiment design*

The experimental design and procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University following the requirements of the Regulations for the Administration of Affairs Concerning Experimental Animals of China (SYXX(Su)2017-0007). The feed
restiction method was used for maternal rats during pregnancy lead to induction of IUGR mode according to a previous study\(^\text{15}\). First-time-pregnant Sprague Dawley rats (Nanjing Qinglongshan Experiment Animal Center, Nanjing, China) were housed in a facility at a constant temperature and humidity. The light regimen was adjusted 12 h light-12h dark cycle. At day 10 of gestation, rats were provided either a diet of standard laboratory diet (LabDiet 5001) (n=12) \textit{ad libitum} or a 50% feed-restricted (n=12) diet determined by the quantification of normal feed intake in \textit{ad libitum}-fed rats. Dam gave birth normally, and birth weights of offspring were recorded on day 1 of postnatal life and divided into normal or IUGR groups. Rats were limited to 10 per litter to normalize rearing (average 11.83 and 12.1 rats per litter in normal and IUGR groups, respectively). During the 21 day of lactation period, each litter of rats from the normal or IUGR group were nursed by their own dams. During the lactation, all dams were free to feed. At 3 weeks of age, offspring in each litter were weaned and housed individually until they were 6 weeks old for observing the early growth of rats. At 6 weeks of age, 24 female rats with nearly equal body weights (within each group) were allocated to the NBW (normal birth weight), NBW-C (normal birth weight with supplementation), IUGR, and IUGR-C (IUGR with curcumin supplementation) groups (1 rat per litter, n=6/group), respectively. The rats were allowed water and a standard granulated diet (AIN-93G diet) \textit{ad libitum}. During the entire experimental period, rats in the NBW-C and IUGR-C groups were fed a standard diet supplemented with 400 mg curcumin/kg. Curcumin was added to the feed before it was made into pellets. The light regimen was a 12 h light-12 h dark cycle and the temperature was maintained at 22 ± 2°C. At 12 weeks of age, all rats were fasted overnight, and blood was collected via cardiac puncture after anesthesia. Serum was obtained from the blood via centrifugation for 15 min at 3000 g at 4°C. Liver tissue (the same area for each sample) was removed after death and snap-frozen in liquid nitrogen and then stored at -80°C for further analysis.
Serum biochemistry parameters

Concentrations of total cholesterol (TC) and triglyceride (TG) in serum and liver were measured according to previous studies(16;17). Concentrations of very low-density lipoprotein (VLDL) in serum and liver were determined by ELISA kit from Shanghai YILI Biological Technology Co., Ltd. Concentrations of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glycogen, pyruvate, non-esterified fatty acid (NEFA) and activities of hepatic pyruvate kinase (PK), hepatic lipase (HL), lipoprotein lipase (LPL) were determined using colorimetric kits (Nanjing Jiancheng Institute of Bioengineering) with a spectrophotometer, according to the manufacturer’s instructions. Total lipase (TL) activity was defined as equal to HL and LPL activities. The concentrations of curcumin in serum and liver of rats were determined by HPLC-MS/MS system according to the previous study(18).

Gene expression assays

Total RNA from the liver samples stored at -80°C was isolated using the Trizol reagent (Invitrogen). The determination of RNA content, mRNA quantification and real-time PCR (Applied Biosystems) were performed according to previously described methods(9). The primer sequences for the target and housekeeping genes (Cd36, peroxisome proliferator-activated receptor α (Ppara), sterol regulatory element binding proteins 1c (Srebf1), fatty acid synthase (Fasn), hormone sensitive lipase (HSL) and β-actin (Actb) used for real-time PCR are listed in Table 1. Gapdh was also used as a control gene to normalise the expression of target genes. Briefly, a reaction system of 20 μL was composed of 0.4 μL of forward primers, 0.4 μL of reverse primers, 0.4 μL of ROX Reference Dye, 10 μL of SYBR Premix Ex Taq (TaKaRa Biotechnology Co. Ltd), 6.8 μL of double-distilled water and 2 μL of complementary DNA. The 2⁻ΔΔCt method was used to calculate relative levels of mRNA expression after normalisation with housekeeping genes (19). The values for the NBW group were used for calibration.
**Protein analysis**

Primary antibodies against: insulin receptor substrate 1 (IRS-1; 1:1 000; Cell Signaling Technology); Glycogen synthesis kinase-3 α/β (GSK3α/β; 1:1 000; Cell Signaling Technology); Akt/Protein kinase B (Akt; 1:1 000; Cell Signaling Technology); PI3 Kinase p85 (PI3K; 1:1 000; Cell Signaling Technology); Sterol regulatory element binding proteins 1 (SREBP1; 1:1 000; Affinity); Fatty acid synthase (FASN; 1:1 000; Cell Signaling Technology); Peroxisome proliferator-activated receptor α (PPARα; 1:500; Affinity); phosphorylated IRS-1Ser302 (pIRS-1Ser302; 1:1 000; Cell Signaling Technology); phosphorylated GSK3αSer21/βSer9 (pGSK3αSer21/βSer9; 1:1 000; Cell Signaling Technology); phosphorylated AktSer473 (pAktSer473; 1:1 000; Cell Signaling Technology); phosphorylated PI3KTyre458 (pPI3KTyre458; 1:1000; Cell Signaling Technology) and antibodies against β-actin (1:1 000; Cell Signaling Technology). The total protein and cytomembrane protein of the liver were extracted using assay kits according to the manufacturer’s instructions (Beyotime, Jiangsu, China). The protein content of the sample was measured using the BCA Protein Assay Kit (Beyotime, Jiangsu, China). For western blotting analyses, 40 μg of protein from each sample were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis. After electrophoresis, proteins were separated and transferred to polyvinylidene difluoride membranes. The membranes were blocked with blocking buffer (5% non-fat dry milk) for 12 h at 4°C. Then, the membranes were probed with appropriate primary and secondary antibodies (horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse immunoglobulin G; Cell Signaling Technology 1:5 000 dilution in 1 × TBS with 0.1% Tween 20). The blots were detected using enhanced chemiluminescence reagents (ECL-Kit, Beyotime, JiangSu, China) followed by autoradiography. Photographs of the membranes were taken using the Luminescent Image Analyzer LAS-4000 system (FujiFilm Co.) and quantified by Gel-Pro Analyzer 4.0 software (Media Cybernetics). Results were
corrected for total protein.

**Statistical analysis**

Differences between groups were analyzed using a two-way ANOVA. The classification variables were birth weight (NBW+NBW-C × IUGR+IUGR-C), diet (NBW+IUGR × NBW-C+IUGR-C), and the interaction between birth weight and diet (NBW × NBW-C × IUGR × IUGR-C). A Tukey’s post hoc analysis was used to determine the differences between the four groups when a statistically significant birth weight × diet interaction was observed. SPSS 17.0 (SPSS, Inc., Chicago, IL) was used for these analyses. A probability level of *P* < 0.05 was considered statistically significant, and *P* < 0.01 was considered very significant. Data were presented as mean ± standard deviation.

**Results**

**Serum hormone levels**

IUGR rats exhibited higher concentrations of serum insulin (*P*<0.05), glucose (*P*<0.05) and HOMA-IR (*P*<0.05) compared with NBW rats. Curcumin supplementation reduced (*P*<0.01) the concentrations of serum insulin, glucose and HOMA-IR in IUGR rats. In addition, a significant effect of birth weight × dietary interaction (*P*<0.05) for concentrations of serum insulin, glucose and HOMA-IR were observed. There were no significant differences in the concentrations of serum insulin, glucose and HOMA-IR between NBW and NBW-C groups rats (*P*>0.05) (Table 2).

**Serum biochemistry and hepatic lipid metabolic parameters**

The concentrations of TC and TG in the serum of IUGR rats were significantly lower (*P*<0.05) than the NBW rats. Dietary curcumin supplementation significantly decreased (*P*<0.05) the concentration of HDL-C and increased (*P*<0.05) the concentration of LDL-C in the serum of NBW and IUGR rats. In addition, a birth weight × dietary interaction effect (*P*<0.05) was observed for concentrations of TG and
HDL-C in the serum. The concentrations of TC, TG and NEFA in the liver of IUGR rats were significantly higher ($P<0.05$) than the NBW rats. In the IUGR-C group, the concentrations of TC, TG and NEFA in the liver of rats were lower ($P<0.05$) than the IUGR group. A birth weight × dietary interaction effect was observed in the concentrations of TC and TG in the liver of rats. The NBW-C group had lower ($P<0.05$) concentrations of VLDL and NEFA in the liver than in the NBW group rats (Table 3).

**Hepatic glycogen and metabolic parameters**

IUGR rats exhibited lower ($P<0.05$) concentration of glycogen and higher ($P<0.05$) concentration of pyruvate in the liver compared with the NBW rats. Dietary curcumin supplementation significantly increased the concentration of glycogen and decreased the concentration of pyruvate and activity of PK in the liver of IUGR group ($P<0.05$). Dietary curcumin supplementation, the activity of PK and the concentration of glycogen were significantly lower in the liver of NBW-C group than the NBW group ($P<0.05$). In addition, a birth weight × dietary interaction effect ($P<0.05$) was observed for concentrations of pyruvate (Table 4).

**Hepatic lipolysis enzymes**

The IUGR group showed lower activities of LPL ($P<0.01$), HL ($P<0.05$) and TL ($P<0.05$) in the liver of rats than the NBW group rats. The activities of hepatic LPL ($P<0.01$), HL ($P<0.05$) and TL ($P<0.01$) were higher ($P<0.05$) in the IUGR-C group rats than in the IUGR group rats. Increased ($P<0.05$) activities of hepatic LPL, HL and TL were observed in the NBW-C group rats (Table 5).

**Hepatic protein expression**

The IUGR rats exhibited higher phosphorylated IRS-1 ($P>0.05$) (Figure 1A), Akt ($P<0.05$) (Figure 1C) and glycogen synthase kinase 3β (GSK3β) ($P<0.05$) (Figure 2) levels, and lower ($P>0.05$) phosphorylated GSK3α and PI3K levels (Figure 1B) in the liver than the NBW rats. Dietary curcumin supplementation
significantly decreased ($P<0.05$) the phosphorylated levels of IRS, Akt and GSK3β, and had a tendency to increase ($P>0.05$) the phosphorylated levels of GSK3α and PI3K in the liver of the IUGR-C group. A birth weight × dietary interaction effect ($P<0.05$) was observed for the phosphorylated levels of IRS, GSK3β and PI3K. The protein expressions of SREBP1 (Figure 3A) and FASN (Figure 3B) were significantly increased ($P<0.05$) in the liver of IUGR rats compared with the NBW rats. The protein expressions of SREBP1 and FASN in the liver of IUGR-C group were lower ($P<0.05$) than in the IUGR rats. The IUGR rats also showed lower ($P<0.05$) protein expression of PPARα in the liver than the NBW group rats (Figure 3C). Dietary curcumin supplementation significantly increased ($P<0.05$) the protein expression of PPARα in the IUGR-C group rats. Dietary curcumin supplementation had no significant effects ($P>0.05$) on the protein expressions of SREBP1, FASN and PPARα in the liver of NBW rats. Additionally, a birth weight × dietary interaction effect ($P<0.01$) was noted for expressions of SREBP1, FASN and PPARα.

**Gene expression**

In the liver of the IUGR rats, the mRNA expression levels for *Cd36*, *Srebf1* and *Fasn* were higher ($P<0.05$) and the mRNA expression levels for *Ppara* and *HSL* were lower ($P<0.05$) than the NBW rats. Dietary curcumin supplementation significantly increased ($P<0.05$) the mRNA expression levels for *Ppara* and *HSL*, decreased ($P<0.05$) the mRNA expression levels for *Cd36*, *Srebf1* and *Fasn* in the liver of IUGR-C group. A birth weight × dietary interaction effect was noted for the mRNA expressions for *Cd36*, *Ppara*, *Srebf1* and *HSL* in the liver of rats ($P<0.01$) (Figure 4).

**Discussion**

IUGR impairs liver metabolism during the early period in piglets and induces insulin resistance in rats. Previous studies of Magee et al. found that the IUGR male rats increased hepatic fatty synthase and...
triglyceride contents. Mina et al. (15) found that IUGR female adult had higher percent of body fat. Our previous study also found that curcumin was beneficial in preventing IUGR-induced inflammation, oxidative damage and injury in the liver of IUGR rats (21). Nevertheless, the studies on insulin resistance and the relationship between glycogen and lipid metabolism in IUGR female rats are very limited. Therefore, we chose IUGR female rats as our research animal model and investigated the effect of dietary curcumin supplementation on insulin levels, hepatic lipid and glycogen metabolism of female rats.

In the present study, IUGR increased serum HOMA-IR, insulin and glucose levels, which were considered as markers of IR. The results were consistent with the previous study on insulin sensitivity index, which first formally reported the sequence of insulin resistance in short children with IUGR (22). IR is related to a postreceptor defect in the intracellular insulin signaling pathway, leading to the failure of insulin to reduce the level of glucose and improve hepatic glycogen synthesis (23). Insulin regulation effect on glucose metabolism are mediated by insulin receptor substrate 1 (IRS-1), which activates PI3 kinase p85 (PI3K) and Akt. The phosphorylation of Akt leads to inaction of glycogen synthesis kinase-3 (GSK3) and thus enhancing the glycogen synthesis (24). Our results showed that IUGR induced rats increased levels of phosphorylated IRS-1 and Akt. It has been demonstrated that the increased serine phosphorylation of IRS-1 plays an important role in the pathogenesis of insulin resistance (25). The activation of Akt induced the significant phosphorylation level of GSK3β in IUGR, not GSK3α in our study. GSK3α and GSK3β are two functional isoforms of GSK3, which originate from different genes, but share 97% amino acid homology. GSK3β is the primary kinase in regulating glycogen synthesis in muscle which has a positive effect on glycogen deposition as compared with GSK3α (26; 27). The different effects of GSK3α and 3β on liver are not well documented. Our research implied that the causes of IR in IUGR might be related to the failure of Akt to suppress GSK3β. Nachimuthu et al. (14) reported that curcumin
treatment attenuated the insulin resistance by decreasing IRS-1 phosphorylation in rats and alleviated hyperinsulinemia and HOMA-IR levels. In the present study, the concentrations of curcumin in serum and liver were accumulated in NBW and IUGR rats after treated with curcumin. We found that dietary curcumin supplementation decreased the levels of serum insulin, glucose and HOMA-IR in IUGR rats. Furthermore, results also showed that the levels of IRS-1 serine phosphorylation, Akt and GSK3β phosphorylation were lower in IUGR rats when supplemented with dietary curcumin. Pyruvate kinase (PK) acts as a key glycolytic enzyme that catalyses the final step of glycolysis and generates pyruvate in the metabolic process. In our present study, the activity of pyruvate kinase (PK) and concentration of pyruvate were decreased and concentration of hepatic glycogen was increased in liver of IUGR rats supplemented with curcumin. These results implied that curcumin could attenuate insulin resistance, inhibit hepatic glycolysis and improve hepatic glycogen deposition through regulating IRS-1/Akt/GSK3 pathway and decreasing glycolytic enzyme activity.

Triglycerides, the most common non-toxic form of fatty acid, are the main lipids stored in the liver of NAFLD patients (28). In the present study, IUGR rats significantly increased the liver concentration of triglyceride and total cholesterol and decreased the serum concentration of triglyceride. These results indicated that more triglyceride was exported to liver in IUGR rats. Some previous studies revealed that inhibiting triglyceride synthesis was helpful for the treatment of hepatic steatosis (29). In NAFLD patients, non-esterified fatty acid (NEFA) provides most of lipid content for hepatic triglyceride synthesis (30). In the present experiment, we observed that hepatic NEFA concentration was increased in IUGR rats. It is well known that accumulation of triglyceride and increase of NEFA in liver is due to imbalance between hepatic lipids acquisition and removal (32). Notably, the activities of enzymes related to lipolysis in liver were decreased in IUGR rats. Lipoprotein lipase (LPL) catalyzes the hydrolysis of triacylglycerol in
circulating chylomicrons, thus supplying NEFAs for tissue utilization \(^{(31)}\). Hepatic lipase (HL) plays a critical role in hydrolyzing triglycerides and phospholipids in the blood. In our experiment, the decreased activities of LPL and HL in IUGR rats might be the main reasons for inefficient NEFA utilization and excessive triglyceride accumulation in liver. More importantly, after dietary curcumin supplementation, the activities of LPL, HL and TL in IUGR rats were increased and triglyceride accumulation was obviously attenuated to a normal level. Moreover, curcumin significantly decreased the concentrations of hepatic NEFA in IUGR rats. The findings were agreement with the previous results reported by Asai et al. \(^{(32)}\) and Jang et al. \(^{(33)}\), reported that curcumin was beneficial to inhibit fatty acids synthases and attenuate hepatic triglyceride accumulation in high-fat-fed rats. These results suggested that IUGR might have a high risk of hepatic lipid metabolic disorder and dietary curcumin supplementation could alleviate the accumulation of triglyceride in liver.

Insulin regulates the lipogenesis through the activation of sterol regulatory element-binding proteins-1 (SREBP-1) \(^{(34)}\). Liver plays a vital role in the regulation of gene expression for lipid metabolism, including SREBP-1c and peroxisome proliferator-activated receptor \(\alpha\) (PPAR\(\alpha\)) \(^{(35)}\). SREBP-1c is one of the isoforms of SREBPs and firstly enhances transcription of genes associated with biosynthesis of fatty acid and triglyceride. Fatty acids synthase (FASN) is well known to catalyze the last step in the biosynthesis of fatty acid, directly activated by SREBPs and involved in triglyceride synthesis. Overexpression of SREBP-1c led to the increase of triglyceride content by more than 4-fold and expression of FASN to be increased by 3.9-fold in the liver \(^{(36)}\). Emerging evidence has suggested that CD36 mediated fatty acid uptake and increased CD36 expression resulted in the dyslipidemia and hepatic triglyceride storage \(^{(37)}\). We found that the transcription levels of Cd36, Srebf1 and Fasn were increased in IUGR rats. The protein expressions of SREBP1 and FASN in the liver of IUGR rats also were increased.
Previous study indicated that IUGR could easily cause hepatic triglyceride accumulation by increasing the levels of SREBP and FASN proteins during early life (10), which were similar to our results. PPARα promotes β-oxidation of fatty acids which is mainly expressed in the liver. Hormone-sensitive lipase (HSL) is believed to be a key enzyme for improving the decomposition of triglycerides (38) and inhibited by insulin (39). Our results showed that the transcription levels of HSL and the protein and mRNA expressions of PPARα were decreased in the liver of IUGR rats. The present study suggested that IUGR inhibited the hepatic β-oxidation of fatty acids by regulating HSL and PPARα. In mice administrated with a methionine choline-deficient diet, PPARα-deficiency provoked more severe steatosis, whereas PPARα activation enhanced hepatic lipid turnover (40). Furthermore, activation of PPARα obviously reduced adiposity in mice fed with a high-fat diet (41). These results implied that IUGR could improve lipid accumulation and inhibit lipolysis in the liver which might be related to the alterations of the expression of SREBP1 and target genes. Interestingly, curcumin had a potential ability of attenuating hepatic lipid deposition in IUGR rats. In the present study, dietary curcumin supplementation significantly decreased the protein expressions of SREBP1, FASN and increased the protein expression of PPARα in the liver of IUGR rats. Curcumin also down-regulated the transcription levels of Cld36, Srebfl and Fasn, and up-regulated the transcription levels of Ppara and HSL in IUGR rats. Previous studies indicated that curcumin could suppress the gene expression of Fasn (42) and activate protein expression of PPARα (43). According to the results of our present study, we could confirm that curcumin could promote lipolysis and fatty-acid oxidation in the liver of IUGR rats.

In conclusion, our present data suggested that IUGR rats exhibited a high risk of insulin resistance and hepatic lipid accumulation in the liver. Curcumin efficiently attenuated insulin resistance of IUGR rats, which might contribute to reduce insulin level in serum and regulate the insulin signaling pathways in
liver. Curcumin also alleviated hepatic lipid accumulation of IUGR rats by regulating SREBPs target genes and lipid metabolism related genes in liver, which might contribute to the inhibition of lipogenesis and promotion of lipolysis. Our findings may be helpful in finding a new nutritional strategy for the prevention or treatment of IUGR in humans in future.

Acknowledgments

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Conflict of Interest

None conflicts of interest existed in the manuscript.

Authorship

The authors’ responsibilities were as follows—YN designed the research, conducted the research, analysed the data, wrote and revised the manuscript; HA revised the manuscript; JTH and CW designed the research; TC, LLZ, XZ and JFZ conducted the research; TW had primary responsibility for final content. All authors read and approved the final manuscript. None of the authors declared a conflict of interest.
References


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Figure legends

**FIGURE 1** Abundance of phosphorylated proteins in liver of normal birth weight rats (NBW), NBW rats supplemented with curcumin (NBW-C), intrauterine growth restriction (IUGR) rats (IUGR) and IUGR rats supplemented with curcumin (IUGR-C). (A) IRS-1; (B) PI3K; (C) Akt.

Values are means ± SD; n = 6/group. Data were analyzed by using two-way ANOVA and Tukey’s *post hoc* testing, where appropriate.

a,b,c Mean values within a row with unlike superscript letters were significantly different (P < 0.05).

Results were corrected for total protein.

B, birth weight; D, diet; B × D is the interaction between the corresponding parameters.

IRS-1, insulin receptor substrate-1; pIRS-1Ser302, phosphorylated IRS-1Ser302; PI3K, PI3 kinase p85; pPI3KTyr458, phosphorylated PI3 kinase p85Tyr458.
FIGURE 2 Abundance of phosphorylated glycogen synthesis kinase-3 \( \alpha/\beta \) (GSK3\( \alpha/\beta \)) in liver of normal birth weight rats (NBW), NBW rats supplemented with curcumin (NBW-C), intrauterine growth restriction (IUGR) rats and IUGR rats supplemented with curcumin (IUGR-C).

Values are means ± SD; \( n = 6 \)/group. Data were analyzed by using two-way ANOVA and Tukey’s post hoc testing, where appropriate.
Mean values within a row with unlike superscript letters were significantly different \((P < 0.05)\).

Results were corrected for total protein.

B, birth weight; D, diet; \(B \times D\) is the interaction between the corresponding parameters.

GSK3\(\alpha\), glycogen synthase kinase 3\(\alpha\); pGSK3\(\alpha^{\text{Ser21}}\), phosphorylated glycogen synthase kinase 3\(\alpha^{\text{Ser21}}\); GSK3\(\beta\), glycogen synthase kinase 3\(\beta\); pGSK3\(\beta^{\text{Ser9}}\), phosphorylated glycogen synthase kinase 3\(\beta^{\text{Ser9}}\).

**FIGURE 3** Abundance of proteins in liver of normal birth weight rats (NBW), NBW rats supplemented with curcumin (NBW-C), intrauterine growth restriction (IUGR) rats and IUGR rats supplemented with curcumin (IUGR-C). (A) SREBP1; (B) FASN; (C) PPAR\(\alpha\).

Values are means \(\pm\) SD; \(n = 6\)/group. Data were analyzed by using two-way ANOVA and Tukey’s post hoc testing, where appropriate.

Mean values within a row with unlike superscript letters were significantly different \((P < 0.05)\).

Results were corrected for total protein.

B, birth weight; D, diet; \(B \times D\) is the interaction between the corresponding parameters.

FASN, fatty acid synthase; PPAR\(\alpha\), peroxisome proliferator-activated receptor \(\alpha\); SREBP1, sterol regulatory element binding proteins 1.
FIGURE 4 Effect of curcumin on the hepatic gene expressions of rats with intrauterine growth restriction (IUGR).

Values are means ± SD; n = 6/group. Data were analyzed by using two-way ANOVA and Tukey’s post hoc testing, where appropriate.

a,b,c Mean values within a row with unlike superscript letters were significantly different (P < 0.05).

B, birth weight; D, diet; B × D is the interaction between the corresponding parameters.

NBW, normal birth weight; NBW-C, normal birth weight rats supplemented with curcumin (NBW-C); IUGR, intrauterine growth restriction; IUGR-C, IUGR rats supplemented with curcumin.

Fasn, fatty acid synthase; HSL, hormone sensitive lipase; Ppara, peroxisome proliferator-activated receptor α; Srebf1, sterol regulatory element binding proteins 1c.
TABLE 1 Primers

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</tbody>
</table>

1Ppara, peroxisome proliferator-activated receptor α; Srebfl, sterol regulatory element binding proteins 1c; Fasn, fatty acid synthase; HSL, hormone sensitive lipase; Actb, β-actin.

TABLE 2 Effect of curcumin on the serum concentrations of insulin, glucose and HOMA-IR of rats with intrauterine growth restriction (IUGR)

<table>
<thead>
<tr>
<th>Items</th>
<th>Experiment groups</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBW</td>
<td>NBW-C</td>
</tr>
<tr>
<td>Insulin mU/L</td>
<td>8.46±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.85±0.66&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose mmol/L</td>
<td>3.60±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.79±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.43±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.38±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means ± SD; n = 6/group. Data were analyzed by using two-way ANOVA and Tukey’s post hoc testing, where appropriate.

<sup>a,b,c</sup>Mean values within a row with unlike superscript letters were significantly (P < 0.05).
NBW, normal birth weight rats; NBW-C, normal birth weight rats fed diets supplemented with 400 mg/kg curcumin; IUGR-C, IUGR fed diets supplemented with 400 mg/kg curcumin; B, birth weight; D, dietary curcumin supplementation; B × D, interaction between the corresponding parameters; I, IUGR. HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (μU/mL)]/22.5; HOMA-IR, homeostasis model of assessment for insulin resistance index.

### TABLE 3 Effect of curcumin on serum and liver lipid metabolic measurements of rats with intrauterine growth restriction (IUGR)

<table>
<thead>
<tr>
<th>Items</th>
<th>Experiment groups</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBW</td>
<td>NBW-C</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC mmol/L</td>
<td>2.52±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.48±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG mmol/L</td>
<td>0.52±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-C mmol/L</td>
<td>0.53±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-C mmol/L</td>
<td>1.32±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VLDL mmol/L</td>
<td>11.41±1.51</td>
<td>10.95±0.81</td>
</tr>
<tr>
<td>Curcumin ng/mL</td>
<td>—</td>
<td>1.79±0.29</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC mmol/g prot</td>
<td>1.48±0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.97±0.66&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG mmol/g prot</td>
<td>1.81±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.55±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NEFA μmol/g prot</td>
<td>294.44±12.63</td>
<td>269.52±18.43</td>
</tr>
</tbody>
</table>
Values are means ± SD; n = 6/group. Data were analyzed by using two-way ANOVA and Tukey’s post hoc testing, where appropriate.

Mean values within a row with unlike superscript letters were significantly (P < 0.05). — means not determined.

NBW, normal birth weight rats; NBW-C, normal birth weight rats fed diets supplemented with 400 mg/kg curcumin; IUGR-C, IUGR fed diets supplemented with 400 mg/kg curcumin; B, birth weight; D, dietary curcumin supplementation; B × D, interaction between the corresponding parameters; I, IUGR.

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acid; VLDL, very low-density lipoprotein.

**TABLE 4** Effect of curcumin on hepatic glycogen and enzymes of rats with intrauterine growth restriction (IUGR)

<table>
<thead>
<tr>
<th>Items</th>
<th>Experiment groups</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen mg/g</td>
<td>NBW</td>
<td>NBW-C</td>
</tr>
<tr>
<td>prot</td>
<td>5.36±0.98^a</td>
<td>3.56±0.47^c</td>
</tr>
<tr>
<td>Pyruvate µmol/g</td>
<td>13.21±0.84^b</td>
<td>13.82±1.03^b</td>
</tr>
</tbody>
</table>
Pyruvate kinase
U/g prot

14.77±0.76\textsuperscript{a}  11.23±1.87\textsuperscript{c}  13.00±0.97\textsuperscript{b}  8.78±1.31\textsuperscript{d}  <0.01  <0.01  0.53

\textsuperscript{1}Values are means ± SD; n = 6/group. Data were analyzed by using two-way ANOVA and Tukey’s post hoc testing, where appropriate.

\textsuperscript{a,b,c,d}Mean values within a row with unlike superscript letters were significantly (P < 0.05).

NBW, normal birth weight rats; NBW-C, normal birth weight rats fed diets supplemented with 400 mg/kg curcumin; IUGR-C, IUGR fed diets supplemented with 400 mg/kg curcumin; B, birth weight; D, dietary curcumin supplementation; B × D, interaction between the corresponding parameters; I, IUGR.

\textbf{TABLE 5} Effect of curcumin on hepatic lipolysis enzymes of rats with intrauterine growth restriction (IUGR)\textsuperscript{1}

\begin{tabular}{lcccccc}
\hline
\textbf{Items} & \textbf{Experiment groups} & \textbf{P-value} \\
 & NBW & NBW-C & IUGR & IUGR-C & B & D & B×D \\
\hline
LPL U/mg prot & 0.97±0.03\textsuperscript{b} & 1.26±0.21\textsuperscript{a} & 0.88±0.07\textsuperscript{c} & 0.99±0.10\textsuperscript{b} & <0.01 & <0.01 & 0.09 \\
HL U/mg prot & 1.05±0.14\textsuperscript{b} & 1.18±0.17\textsuperscript{a} & 0.92±0.13\textsuperscript{c} & 1.06±0.10\textsuperscript{b} & 0.04 & 0.03 & 0.93 \\
TL U/mg prot & 1.92±0.20\textsuperscript{c} & 2.43±0.34\textsuperscript{a} & 1.89±0.11\textsuperscript{d} & 2.10±0.10\textsuperscript{b} & 0.05 & <0.01 & 0.09 \\
\hline
\end{tabular}

\textsuperscript{1}Values are means ± SD; n = 6/group. Data were analyzed by using two-way ANOVA and Tukey’s post hoc testing, where appropriate.

\textsuperscript{a,b,c,d}Mean values within a row with unlike superscript letters were significantly (P < 0.05).

NBW, normal birth weight rats; NBW-C, normal birth weight rats fed diets supplemented with 400 mg/kg curcumin; IUGR-C, IUGR fed diets supplemented with 400 mg/kg curcumin; B, birth weight; D, dietary curcumin supplementation; B × D, interaction between the corresponding parameters; I, IUGR.
weight; D, dietary curcumin supplementation; B × D, interaction between the corresponding parameters; I, IUGR.

LPL, lipoprotein lipase; HL, hepatic lipase; TL, total lipase, equal to hepatic lipase and lipoprotein lipase activities.