British Journal of Nutrition (2020), 124, 1241–1250 doi:10.1017/S0007114520002408

Effects of dietary carbohydrate sources on lipid metabolism and SUMOylation modification in the liver tissues of yellow catfish

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(Submitted 18 May 2020 – Final revision received 18 June 2020 – Accepted 23 June 2020 – First published online 30 June 2020)

Abstract

Dysregulation in hepatic lipid synthesis by excess dietary carbohydrate intake is often relevant with the occurrence of fatty liver; therefore, the thorough understanding of the regulation of lipid deposition and metabolism seems crucial to search for potential regulatory targets. In the present study, we examined TAG accumulation, lipid metabolism-related gene expression, the enzyme activities of lipogenesis-related enzymes, the protein levels of transcription factors or genes involving lipogenesis in the livers of yellow catfish fed five dietary carbohydrate sources, such as glucose, maize starch, sucrose, potato starch and dextrin, respectively. Generally speaking, compared with other carbohydrate sources, dietary glucose promoted TAG accumulation, up-regulated lipogenic enzyme activities and gene expressions, and down-regulated mRNA expression of genes involved in lipolysis and small ubiquitin-related modifier (SUMO) modification pathways. Further studies found that sterol regulatory element binding protein 1 (SREBP1), a key transcriptional factor relevant to lipogenic regulation, was modified by SUMO1. Mutational analyses found two important sites for SUMOylation modification (K254R and K264R) in SREBP1. Mutant SREBP lacking lysine 264 up-regulated the transactivation capacity on an SREBP-responsive promoter. Glucose reduced the SUMOylation level of SREBP1 and promoted the protein expression of SREBP1 and its target gene stearoyl-CoA desaturase 1 (SCD1), indicating that SUMOylation of SREBP1 mediated glucose-induced hepatic lipid metabolism. Our study elucidated the molecular mechanism of dietary glucose increasing hepatic lipid deposition and found that the SREBP-dependent transactivation was regulated by SUMO1 modification, which served as a new target for the transcriptional programmes governing lipid metabolism.

Key words: ***Pelteobagrus fulvidraco*: Glucose: Liver tissues: SUMOylation: Lipid metabolism**

Dietary carbohydrate sources range from simple monosaccharides to disaccharides to complex polysaccharides. Among these carbohydrate sources, dietary disaccharides and polysaccharides are broken down to monosaccharides before being transported into the intestinal epithelial cells, and delivered to the liver via the portal blood[1]. In the livers, they were metabolised to provide substrates for the biosynthesis of fatty acids and TAG[2,3]. Increasing evidences have shown that the biosynthesis of hepatic fatty acids from carbohydrates contributes to excessive lipid deposition and the development of fatty liver diseases, and the increased intake of dietary carbohydrate is the reason of these diseases[1,4]. These have attracted wide attentions in unravelling the regulation of lipogenic genes in response to dietary carbohydrate signals. The biosynthesis of fatty acids and TAG involves many enzymes, such as NADPH-generating enzymes, fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1), acetyl-CoA carboxylase (ACC) and lipoprotein lipase (LPL), to induce *de novo* lipogenesis for the storage of energy source[4,5]. On the other hand, several transcription factors, such as carbohydrate-responsive element binding protein (ChREBP), sterol regulatory elements binding protein 1 (SREBP1), PPAR and liver X receptor (LXR), are important candidates for the induction of lipogenesis[5–7]. They modulate the transcription of many genes relevant to fatty acid metabolism and accordingly play

Abbreviations: 6PGD, 6-phosphogluconate dehydrogenase; ACC, acetyl-CoA carboxylase; B2M, beta-2-microglobulin; bHLH-zip, basic Helix-Loop-Helix-zipper domain; C/EBP, CCAAT/enhancer binding protein; ChREBP, carbohydrate-responsive element binding protein; ELFA, translation elongation factor; FAS, fatty acid synthase; G6PD, glucose 6-phosphate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ICDH, isocitrate dehydrogenase; LPL, lipoprotein lipase; LXR, liver X receptor; ME, malic enzyme; *n*-3 PfaA, PUFA synthase PfaA; PIAS1, protein inhibitor of activated STAT1; SAE1, SUMO-activating enzyme subunit 1; SAP130-C, mSin3A-associated proteins 130 C-terminus; SCD1, stearoyl-CoA desaturase 1; SENP, sentrin-specific protease; SIRT1, NAD-dependent deacetylase sirtuin-1; SRE-1, sterol regulatory element 1; SREBP1, sterol regulatory elements binding protein 1; SUMO, small ubiquitin-related modifier; TBP, TATA-box-binding protein; TUBA, tubulin alpha chain; UBA2, SUMO-activating enzyme subunit 2; UBC9, ubiquitin-conjugating enzyme 9.

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key roles in lipid homoeostasis[8]. At present, the impacts of dietary carbohydrate on the control of these enzymes and genes are not well understood[5,9]. A thorough understanding into the mechanisms of carbohydrate affecting gene expression will help build the nutritional preventive pathways for diseases involving the metabolic dysfunction in the livers, such as the metabolic syndrome and fatty liver diseases[4].

Genes and proteins involved in lipid metabolism are acutely controlled at the transcriptional and post-translational levels. Among the post-translational modification, small ubiquitin-related modifier (SUMO) modification acts as an important mechanism affecting the cellular localisation, activities and stabilities of the target proteins[10,11]. Four SUMO isoforms, SUMO1, SUMO2, SUMO3 and SUMO4, have been detected in mammals, but only SUMO1, SUMO2 and SUMO3 can be conjugated to their target proteins. SUMO-activating enzyme subunit 1 (SAE1) and SAE1 subunits activate SUMO proteins[12]. Activated SUMO proteins are transferred to ubiquitin-conjugating enzyme 9 (UBC9), the E2-conjugating enzyme, and they are conjugated to specific lysine residues in the target proteins[13]. The Protein Inhibitor of Activated STAT (PIAS) family work as the E3 ligases, which contribute to the SUMOylation substrate specificity and efficiency[12,14]. SUMOylation is a highly dynamic process, which can be revised by SUMO-specific proteases (SENP)[15]. At present, several transcription factors involved in lipid metabolism were reported for SUMOylation modification, including PPARγ[16] and SREBP[17]. However, the effect and the underlying mechanism of their SUMOylation modifications on lipid metabolism are largely unknown.

Here, we explored the influences of dietary carbohydrate sources on lipid metabolism in the liver tissues of yellow catfish; by using its primary hepatocytes, the mechanism of glucose incubation up-regulating lipogenesis and lipid deposition was also investigated. Our findings provide evidences that SREBP1 is an important regulator of lipid metabolism and that its SUMOylation modification acts as a molecular switch to influence gene expression in response to dietary glucose signals.

Materials and methods

Our study is divided into two parts, an *in vivo* study and an *in vitro* study. The experimental protocols were approved by the Committee of Huazhong Agricultural University on the Ethics of Laboratory Animal Experiments.

Expt 1: in vivo *study*

Experimental procedures. The experimental protocols for fish culture and management have been described in our parallel study[18]. Briefly, yellow catfish from a local fish farm (Wuhan, China) were subjected to a 2-week acclimation in the indoor tanks. Then, 450 uniform-sized fish (4·68 (SE 0·02 g)) were stocked in fifteen tanks (300 litres in water volume), thirty fish for each tank. Five diets were formulated to contain 25 % of carbohydrates, based on our published studies[19]. Dietary carbohydrate sources consisted of glucose, maize starch, sucrose, potato starch and dextrin, and they were all purchased from Aladdin company. Each diet was assigned to three tanks, and the feeding was similar to our recent study[20]. The feeding experiment continued for 10 weeks. Then, prior to sampling, all fish were fasted for 24 h. They were euthanised, dissected on ice to obtain the liver tissues for the analysis of TAG and glycogen contents, histology, enzymatic activities, mRNA and protein expression assays and RNA sequencing.

Expt 2: in vitro *study*

Part 1. Isolation and culture of primary hepatocytes from yellow catfish. The hepatocytes were isolated from yellow catfish and cultured according to our previous studies[20]. Three glucose concentrations, control (5·44 mM), 15 mM (15·60 mM) and 30 mM (29·84 mM), were used to incubate these hepatocytes at 28°C. Sampling occurred at the 48-h incubation for the following analyses: TAG content, enzymatic activity, quantitative PCR and immunoblotting. In addition, immunoprecipitation was conducted to analyse the mechanism of glucose influencing the SUMOylation of SREBP1. Each treatment was performed in triplicate.

Part 2. Hela cell culture and treatment. Here, we performed the two independent experiments. The first experiment was conducted to explore the SREBP1's SUMOylation. Hela cells were transfected with SREBP1-Flag, SUMO1-6 × His and UBC9-6 × His plasmids by Lipofectamine 2000. The second experiment was conducted to explore the SUMOylation sites of SREBP1. Hela cells were transfected with SUMO1-6 × His, UBC9-6 × His and the mutations of SREBP1-Flag. The protocols for transfection followed the manufacturer's instructions. After 48 h, the Hela cells were collected for the analysis of Western blot and immunoprecipitation.

Sample analysis. *RNA sequencing and bioinformatics.* In this study, the liver tissues from fish fed glucose (monosaccharide) and maize starch (polysaccharide) were selected for transcriptome analysis because the liver of fish fed the two groups of diets showed significantly different TAG contents. RNA library sequencing was conducted according to our recent publications[20,21]. Six RNA libraries from two treatments were built and read on the Illumina HiSeq 4000 platform by the Novogene company. Differentially expressed genes were sieved by the DEseq software, and the parameters were designated below: P value < 0·05 and the absolute value of $\log_2$ ratio > 1 (Anders & Huber, 2010). We performed Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis to identify these enriched metabolic pathways. Furthermore, fifteen candidate genes were arbitrarily selected for real-time quantitative PCR validation. Primers are showed in online Supplementary Table S1. We have chosen a set of eight housekeeping genes (*β-actin*, ribosomal protein L7 (*rpl7*), tubulin alpha chain (*tuba*), beta-2-microglobulin (*b2m*), TATA-box-binding protein (*tbp*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), *18S rRNA* and translation elongation factor (*elfa*)) from the literature so as to analyse their expression stabilities. The $2^{-\Delta\Delta Ct}$ method was performed to calculate the fold variations in their relative expression[22].

Histological and histochemical observation. Haematoxylin–eosin and Oil Red O stainings were conducted based on our recent publication[18].

Cell viability, TAG, glucose, glycogen contents and enzymatic activities assays. Cell viability was measured according to our recent publication[23]. The contents of TAG, glucose and glycogen were measured by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The activities of lipogenic enzymes (glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), malic enzyme (ME), isocitrate dehydrogenase (ICDH) and FAS) were measured as previously described[24]. Soluble protein concentrations were detected with the Bradford Protein Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

mRNA expression analysis (quantitative PCR). Quantitative PCR assays were performed according to Yang *et al.*[23]. The primers are given in online Supplementary Table S1. Eight housekeeping genes (*β-actin, rpl7, tuba, b2m, tbp, gapdh, 18S rRNA* and *elfa*) were selected to test their transcription stability. The relative gene expression was analysed with the $2^{-\Delta\Delta Ct}$ method when normalising to the geometric mean of the best combination of two genes, based on the geNorm[23].

Plasmid construction. We constructed SUMO1-6 × His, UBC9-6 × His and SREBP1-Flag plasmids based on the methods described previously[25]. Mutants with the K145R, K254R, K264R, K392R and K419R mutations were obtained from wild-type SREBP1 after the site-directed mutagenesis. All the primers are listed in online Supplementary Table S1.

Western blot analysis. Western blot analysis was performed according to our recent publication[18]. Antibodies against SREBP1 (ab28481; Abcam), SUMO1 (ab32058; Abcam), SCD1 (ab19862; Abcam), LXRα (ab41902; Abcam), GAPDH (10494-1-AP; Proteintech Group) and IgG (Alexa Fluor® 647, ab150079; Abcam) were used to measure the expression of the corresponding proteins. We visualised the protein bands using the Vilber Fusion FX6 Spectra imaging system (Vilber Lourmat) and quantified them by the software Image-Pro Plus 6.0.

Immunoprecipitation. We used immunoprecipitation to analyse the SUMOylation level of SREBP1, based on Lee *et al.*[26]. At first, we prepared for whole-cell lysates with modified radioimmunoprecipitation buffer containing 1 % SDS, 1 μM phenylmethylsulfonyl fluoride, 10 mM N-ethylmaleimide and 0·1 % protease inhibitor cocktail. We performed the immunoprecipitation by using the antibody indicated above. The immunoprecipitated complexes were then subjected to Western blot analysis. IgG was used as the loading control.

Statistical analysis. All the results are presented as means and their standard errors. Before the statistical analysis, all data were tested for normality of distribution using the Kolmogorov–Smirnov test. Data were evaluated using one-way ANOVA. Duncan's multiple range tests were used to compare the differences among more than three groups, and Student's *t* test was used to analyse the differences between two groups. The analysis was carried out using SPSS 19.0 for Windows (SPSS), and differences were considered statistically significant at $P < 0{\cdot}05$.

Results

Expt 1: in vivo *study*

Histology, histochemistry, glycogen and TAG contents. Yellow catfish fed with maize starch, sucrose and potato starch had normal histological structures, and the hepatocytes possessed the round nucleus with obvious nucleolus. In contrast, yellow catfish fed with dietary glucose and dextrin resulted in the vacuolisation in hepatocytes (Fig. 1(A)–(E)) and had more lipid droplets (Fig. 1(F)–(J)). These observations were confirmed by the quantitative analysis for vacuoles in the haematoxylin–eosin and lipid droplets in Oil Red O staining (Fig. 1(K) and (L)). Hepatic glycogen content was the highest for yellow catfish fed the glucose, and the lowest for yellow catfish fed the sucrose (Fig. 1(M)). TAG contents from the glucose and dextrin groups were higher than those in other three groups (Fig. 1(N)).

Enzyme activities and gene expression. Dietary carbohydrate sources significantly influenced enzymatic activities and gene expression. Generally speaking, activities of G6PD, ME and ICDH were higher in dietary glucose group than other groups. 6PGD activities were higher in dietary glucose, maize starch and potato starch groups than those in the sucrose and dextrin groups. FAS activity for fish fed the potato starch was higher than those from other groups (Fig. 2(A)).

The *fas* mRNA abundances were the highest for yellow catfish fed glucose and dextrin and lowest for fish fed the maize starch. The mRNA expression of *pparγ* was the highest for yellow catfish fed the sucrose and maize starch and the lowest for yellow catfish fed glucose and potato starch. Among three lipolytic genes (*accb*, *cpt1* and *pparα*), *accb* mRNA abundances were the highest for yellow catfish fed the dextrin and lowest for yellow catfish fed glucose and maize starch (Fig. 2(B)). *Cpt1* mRNA levels were higher in fish fed dextrin than those for fish fed glucose, maize starch and potato starch. *Pparα* mRNA levels were the highest for yellow catfish fed the dextrin and lowest for yellow catfish fed the glucose.

Dietary carbohydrate sources significantly influenced the mRNA abundances of SUMOylation-related genes (Fig. 3). Among five dietary carbohydrate sources, mRNA expression of *sumo2* and *sumo3* was relatively lower for yellow catfish fed the glucose than other four dietary carbohydrate sources. *Sumo1* mRNA expression was lower in yellow catfish fed the glucose and maize starch than those fed potato starch and dextrin. mRNA levels of SUMO-activating enzyme subunit 2 (*uba2)*, protein inhibitor of activated STAT1 (*pias1)*, *senp2* and *senp3* were lower for yellow catfish fed the glucose than those for yellow catfish fed the dextrin. *ubc9* mRNA levels were higher for fish fed potato starch and dextrin than those for fish fed glucose, maize starch and sucrose. *sae1* and *senp1* mRNA expression showed no significant differences among five treatments.

Transcriptomic analysis of the liver of yellow catfish fed the diets containing glucose and maize starch. Since hepatic TAG content possessed significant differences in fish fed the glucose and maize starch, we performed RNA-seq to examine

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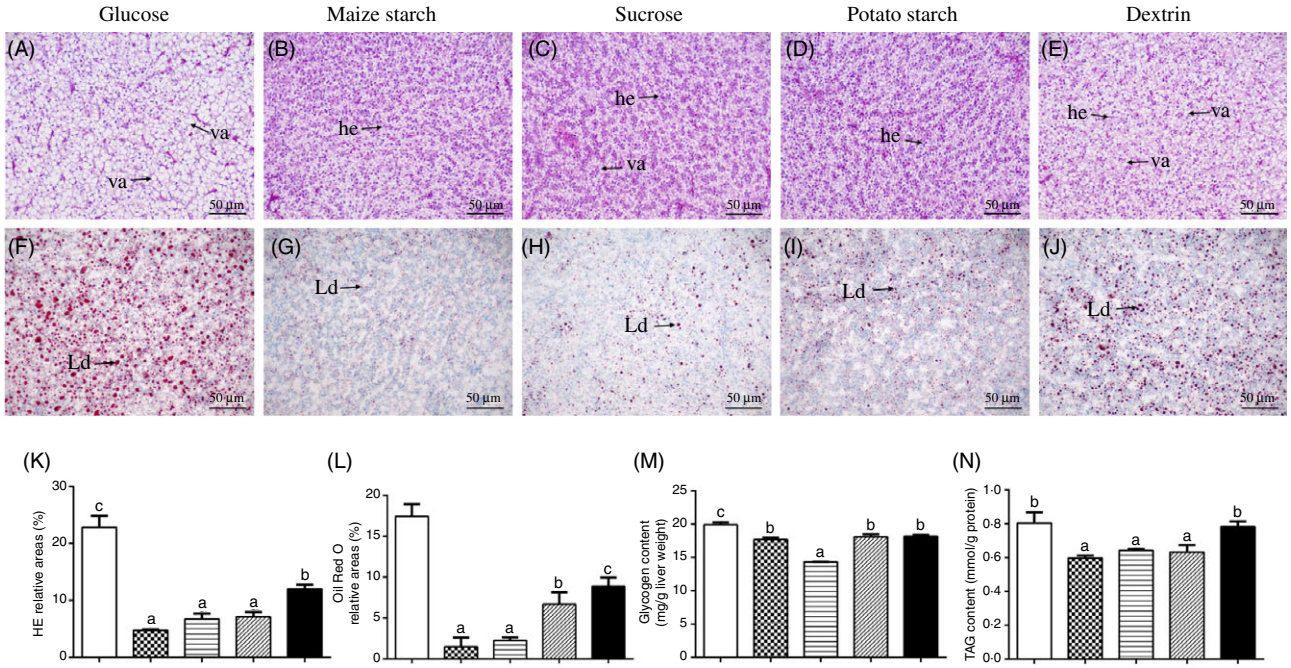

Fig. 1. Effect of dietary carbohydrate sources on histology, histochemistry, glycogen and TAG content in the liver tissues of yellow catfish. Liver histology (haematoxylin–eosin; H&E) (A–E) and histochemistry (Oil Red O staining) (original magnification 200×, bars 50 μm) (F–J). (K) Relative areas for hepatic vacuoles in H&E staining; (L) relative areas for lipid droplets in Oil Red O staining; (M) glycogen content; (N) TAG content. Values are means with their standard errors (for analysing glycogen/TAG content, *n* 3 replicate tanks and six fish were sampled for each tank; for analysing H&E and Oil Red O staining, *n* 3 replicate tanks and three fish were sampled for each tank). [a,b,c] Unlike letters indicate significant differences among the five groups ($P < 0{\cdot}05$). he, Hepatocytes; va, vacuoles; Ld, lipid droplet. ▭, Glucose; ▩, maize starch; ▭, sucrose; ▨, potato starch; ■, dextrin.

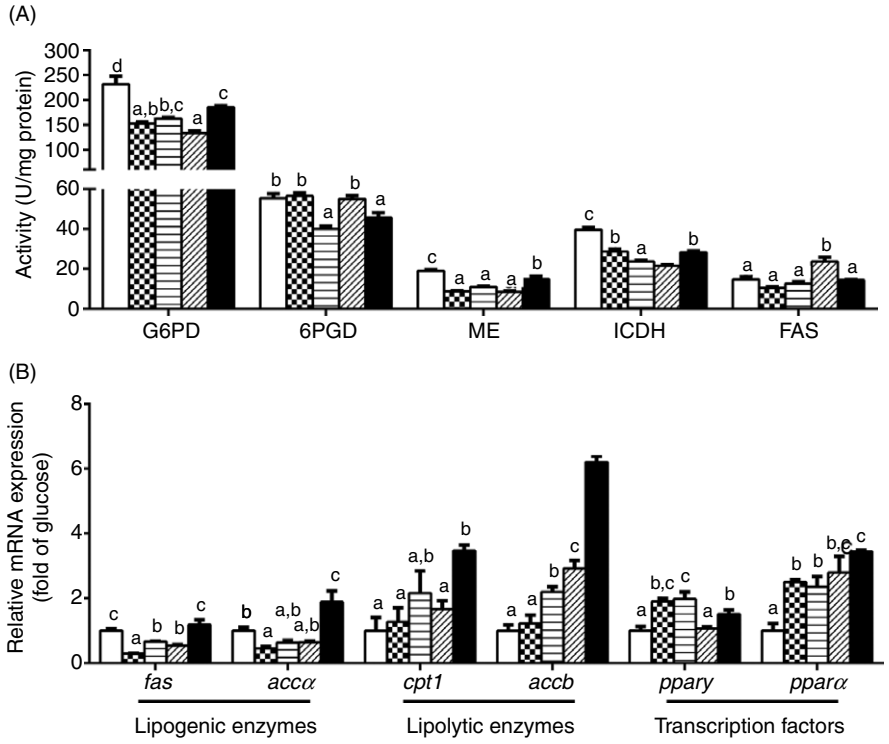

Fig. 2. Effect of dietary carbohydrate sources on lipid metabolism in the liver tissues of yellow catfish. (A) Activities of lipogenic enzymes; (B) mRNA expression of genes. Values are means with their standard errors, *n* 3 replicates of six fish. [a,b,c,d] Unlike letters indicate significant differences among the five groups ($P < 0{\cdot}05$). *acc*, Acetyl-CoA carboxylase; *cpt 1*, carnitine palmitoyltransferase 1; FAS, fatty acid synthase; G6PD, glucose 6-phosphate dehydrogenase; ICDH, isocitrate dehydrogenase; ME, malic enzyme; 6PGD, 6-phosphogluconate dehydrogenase. (A, B) ▭, Glucose; ▩, maize starch; ▭, sucrose; ▨, potato starch; ■, dextrin.

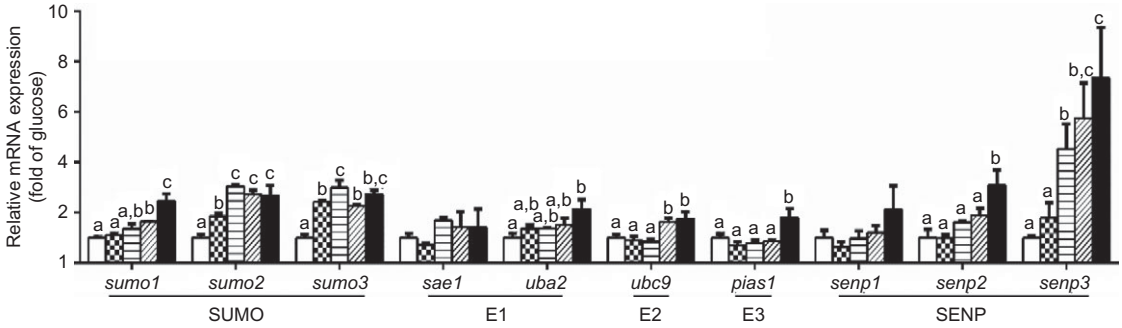

Fig. 3. Effect of dietary carbohydrate sources on mRNA expression of SUMOylation-related genes in the liver tissues of yellow catfish. Values are means with their standard errors, *n* 3 replicates of six fish. [a,b,c] Unlike letters indicate significant differences among the five groups ($P < 0{\cdot}05$). *pias1*, Protein inhibitor of activated STAT1; *sae1*, SUMO-activating enzyme subunit 1; *senp*, sentrin-specific protease; *sumo*, small ubiquitin-related modifier; *uba2*, SUMO-activating enzyme subunit 2; *ubc9*, ubiquitin-conjugating enzyme 9. ▭, Glucose; ▩, maize starch; ▭, sucrose; ▨, potato starch; ■, dextrin.

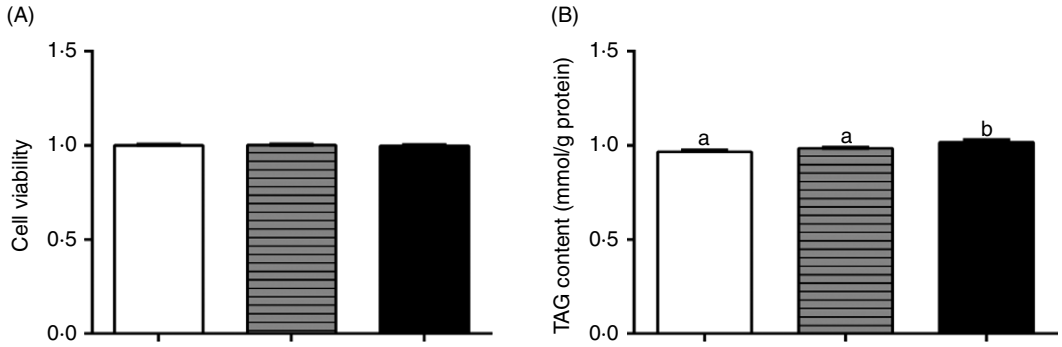

Fig. 4. Effects of 48-h glucose incubation on cell viability and TAG content in primary hepatocytes from yellow catfish. (A) Cell viability, (B) TAG content. Values are means with their standard errors, *n* 3 (replicates of three biological experiments). [a,b] Unlike letters indicate significant differences among the three groups ($P < 0{\cdot}05$). (A, B) ▭, Control; ▩, 15 mM; ■, 30 mM.

the molecular mechanisms of TAG changes in fish fed the two carbohydrate sources. The RNA-seq data were uploaded to the Sequence Read Archive and could be accessed on https://www.ncbi.nlm.nih.gov/sra/PRJNA624795 (accession number: PRJNA624795). Three hundred sixteen unigenes were identified as the differentially expressed genes, including 178 up- and 138 down-regulated genes (online Supplementary Fig. S1). The GO (online Supplementary Fig. S2) and Kyoto Encyclopedia of Genes and Genomes (online Supplementary Fig. S3) database were utilised to perform the pathway analysis. We validated our RNA-seq data by our quantitative PCR, which indicated that RNA-seq data were reliable (online Supplementary Tables S2 and S3 and Fig. S4). All these results indicated that, compared with dietary maize starch, dietary glucose impacted the hepatic physiological function. The Kyoto Encyclopedia of Genes and Genomes found that dietary glucose significantly up-regulated the expression of genes related to lipogenesis (*scd1*, *elovl6*), down-regulated the expression of gene related to fatty acid transport (*lpl*) and adipocytokine signalling pathway (*socs3*); dietary glucose significantly influenced the expression of genes related to citrate cycle (up-regulated: *suclg2*; down-regulated: *mdh1*) (online Supplementary Fig. S5). These results confirmed that dietary glucose activated lipogenesis and promoted the TAG accumulation.

Expt 2: in vitro *study*

Cell viability and TAG content. Glucose incubation did not adversely affect cell viability (Fig. 4(A)). TAG content in 30 mM glucose group was significantly higher than those in the control and 15 mM glucose (Fig. 4(B)).

Enzyme activities and gene expression. Activities of lipogenic enzymes (G6PD, 6PGD and ME) increased with increasing glucose concentration (Fig. 5(A)). ICDH activity was higher in 30 mM glucose than those in the control and 15 mM glucose. FAS activity showed no marked difference among the three groups.

The mRNA levels of *chrebp*, *srebp1* and *fas* were higher for 15 mM glucose than those of other two groups (Fig. 5(B)). The mRNA levels of *pparγ*, *lxrα* and *accα* were significantly higher in 15 and 30 mM glucose than those in the control. The mRNA levels of *scd1* and *lpl* showed no obvious discrepancies among the three groups.

Protein expression of liver X receptor α, sterol regulatory elements binding protein 1 and stearoyl-CoA desaturase 1. The SREBP1 protein expression was the highest for 30 mM glucose and showed no significant differences between other

https://doi.org/10.1017/S0007114520002408 Published online by Cambridge University Press

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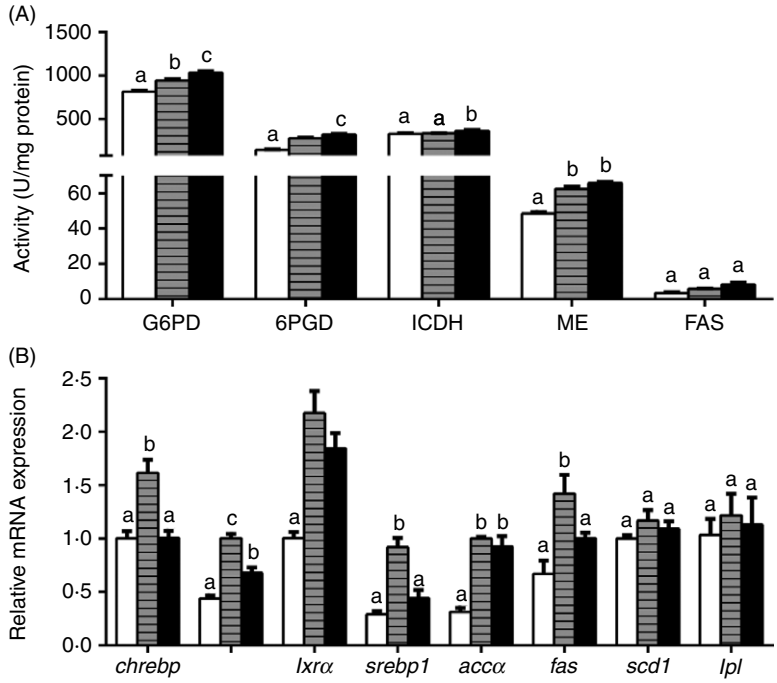

Fig. 5. Effects of 48-h glucose incubation on enzymatic activities and gene expression in primary hepatocytes from yellow catfish. (A) Enzymatic activities, (B) gene expression. G6PD, glucose 6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; ICDH, isocitrate dehydrogenase; ME, malic enzyme; FAS, fatty acid synthase; *chrebp*, carbohydrate-responsive element binding protein; *lxr*, liver X receptor; *srebp1*, sterol regulatory elements binding protein 1; *accα*, acetyl-CoA carboxylase α; *scd1*, stearoyl-CoA desaturase 1; *lpl*, lipoprotein lipase. Values are means with their standard errors, *n* 3 (replicates of three biological experiments). [a,b,c] Unlike letters indicate significant differences among the three groups ($P < 0{\cdot}05$). (A, B) ▭, Control; ▤, 15 mM; ■, 30 mM.

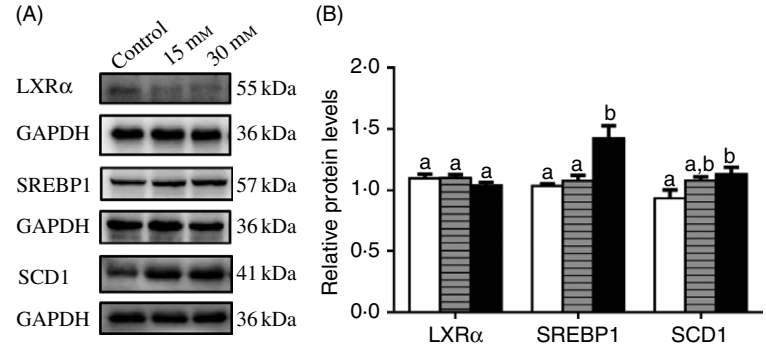

Fig. 6. Effects of 48-h glucose incubation on protein levels of liver X receptor α (LXRα), sterol regulatory elements binding protein 1 (SREBP1) and stearoyl-CoA desaturase 1 (SCD1) in primary hepatocytes from yellow catfish. (A) Western blot analysis of LXRα, SREBP1 and SCD1 expression. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. (B) Protein levels of LXRα, SREBP1 and SCD1. Values are means with their standard errors, *n* 3 (replicates of three biological experiments). [a,b] Unlike letters indicate significant differences among the three groups ($P < 0{\cdot}05$). (B) ▭, Control; ▤, 15 mM; ■, 30 mM.

two groups (Fig. 6). The protein levels of SCD1 increased with glucose concentration. The protein levels of LXRα showed no significant differences among three groups.

Small ubiquitin-related modifier 1 modification and the SUMOylation sites of sterol regulatory elements binding protein 1. Studies indicated that the transcription factors that were conjugated with SUMO proteins often inhibited transcription[27]. Thus, we analysed whether the potential SUMO-targeted lysines in SREBP1 could be SUMOylated. At first, to test whether SREBP1 was a target for SUMOylation modification, we analysed whether SREBP1 could be modified by SUMO1. SREBP1 and SUMO1 coexpression produced a shifted band whose size corresponded to the estimated size of SUMOylated SREBP1, and immunoblotting analysis of the immunoprecipitated complex with SREBP1 (anti-Flag) against SUMO1 (anti-6 × His) confirmed the SUMOylated SREBP1 (Fig. 7(A)), indicating that SREBP1 was a target for SUMOylation. Then, SUMOplot 2.0 software was used to analyse the putative SUMOylation site(s) of SREBP1 protein sequence, and five potential SUMOylation sites were mapped at Lys145, Lys254, Lys264, Lys392 and Lys419 in yellow catfish SREBP1 (Fig. 7(B)). The Clustal-W multiple alignment algorithm indicated that four potential SUMOylation sites (K254, K264, K392 and K419) were conserved (online Supplementary Fig. S6). Domains were analysed by online CDD tool at NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and the SMART programme (http://smart.embl-heidelberg.de/). The *P. fulvidraco* SREBP1 consisted of three

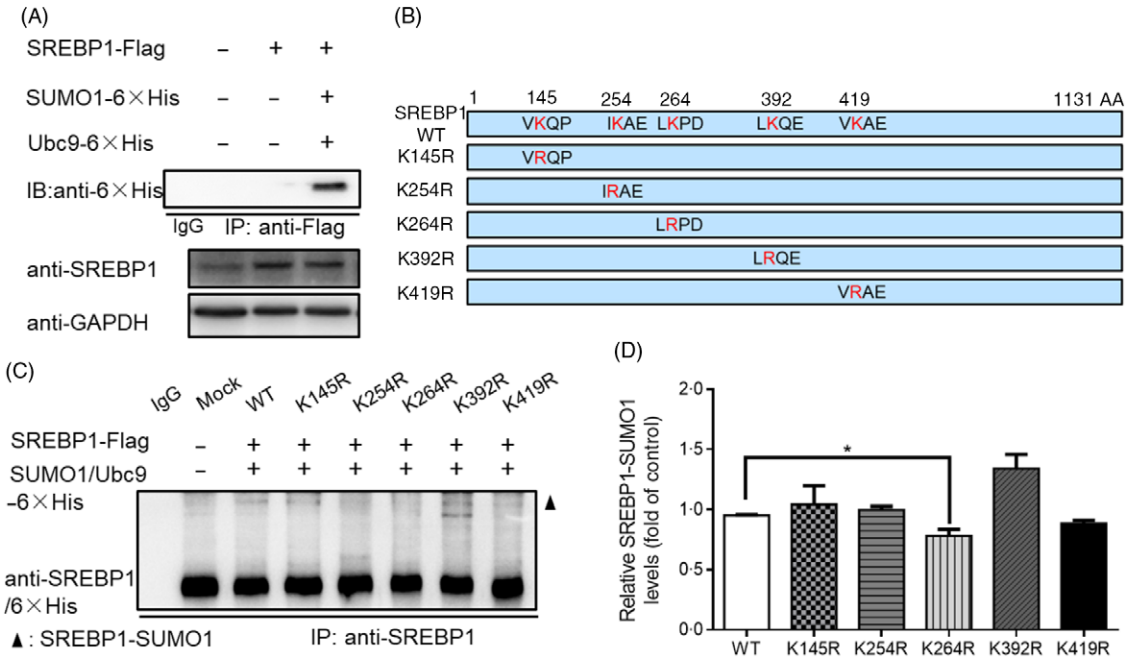

Fig. 7. Small ubiquitin-related modifier 1 (SUMO1) modification and the SUMOylation sites of sterol regulatory elements binding protein 1 (SREBP1). (A) Schematic representation of wild-type (WT) SREBP1 protein and the indicated mutant proteins. AA, amino acid; (B) HeLa cells were transfected with SREBP1-Flag, SUMO1-6 × His and Ubc9-6 × His. Immunoprecipitation (IP) of SREBP1-Flag was probed for SUMOylation using an anti-Flag antibody. The levels of SREBP1 protein in total cell lysates are also shown; (C) HeLa cells were transfected with SUMO1/Ubc9-6 × His and either WT SREBP1-Flag or the K145R, K254R, K264R, K392R or K419R mutant of SREBP1-Flag. Immunoprecipitation of SREBP1-Flag was probed for SUMOylation using an anti-SREBP1 antibody. (D) SUMOylation levels of WT SREBP1-Flag or the K145R, K254R, K264R, K392R or K419R mutant of SREBP1-Flag. Values are means with their standard errors, *n* 3 (replicates of three biological experiments). * Differences are significant between the two groups ($P < 0{\cdot}05$).

domains, such as mSin3A-associated proteins 130 C-terminus (SAP130-C), PUFA synthase PfaA (*n*-3 PfaA) domain and basic Helix-Loop-Helix-zipper domain (bHLH-zip), and these domains included four potential SUMOylation sites, such as K145, K254, K264 and K392 (online Supplementary Fig. S7).

Each of these five lysine residues of SUMOylation sites of SREBP1 was then replaced by arginine, and they were analysed for SUMOylation modification. The mutation at K254R and K264R alleviated the SUMO1 modification of SREBP1, whereas the K145R, K392R and K419R SREBP1 mutants still exhibited SUMO conjugates similar to those in wild-type SREBP1 (Fig. 7(C)**)**. According to the greyscale value, we confirmed that the SUMOylation site of SREBP1 was Lys264 (Fig. 7(D)).

Glucose mediated the SUMOylation of sterol regulatory elements binding protein 1. Immunoblotting analysis of the immunoprecipitated complex with SREBP1 against SUMO1 found that 30 mM glucose reduced the SUMOylation levels of SREBP1 (Fig. 8(A) and (B)). In contrast, 30 mM glucose incubation increased the protein levels of SREBP1 (Fig. 8(C) and (D)).

Discussion

A perfect understanding of the regulatory mechanism of lipid metabolism will be essential in both physiology and physiopathology of vertebrates[7]. In the present study, we found that dietary glucose increased lipid deposition by up-regulating fatty acid biosynthesis and lipogenesis, and down-regulating lipolysis in the livers. Similarly, Morral *et al.*[4] found that glucose disposal activated the transcription of key genes in lipogenic pathways, accompanied by down-regulation of many genes involved in fatty acid oxidation. Here, high-throughput sequencing of liver tissues from dietary glucose and dietary maize starch further confirmed these. Accordingly, the enhanced lipogenesis and inhibited lipolysis increased hepatic TAG accumulation yellow catfish.

To decipher the mechanism for dietary glucose inducing lipid accumulation in yellow catfish, several key transcriptional factors were analysed. ChREBP, SREBP1, PPARγ and LXRα are key transcriptional factors and they transcriptionally regulate many key enzymes (such as ACC, FAS and SCD1) of *de novo* fatty acid and TAG synthesis[7,28–30]. We found that glucose incubation up-regulated the mRNA levels of *chrebp*, *srebp1*, *pparγ* and *lxrα*, similar to many other studies[31–34]. In the present study, glucose-induced up-regulation of mRNA expression of these transcriptional factors was paralleled by an increase in gene transcription of these enzymes, indicating that the activation of these transcriptional factors induced the expression of lipogenic genes, in agreement with several reports[5,32,35]. We also found that dietary glucose addition tended to reduce mRNA expression of three SUMO proteins (*sumo1*, *sumo2* and *sumo3*); mRNA levels of *uba2* and *pias1* were lower for yellow catfish fed the glucose than those fed the dextrin, and *ubc9* mRNA levels were lower for yellow catfish fed the glucose than those fed potato starch. SUMO1, SUMO2 and SUMO3 are SUMO proteins, and they can be linked to target proteins mediated by several enzymes, including SUMO activating enzymes (E1), conjugating

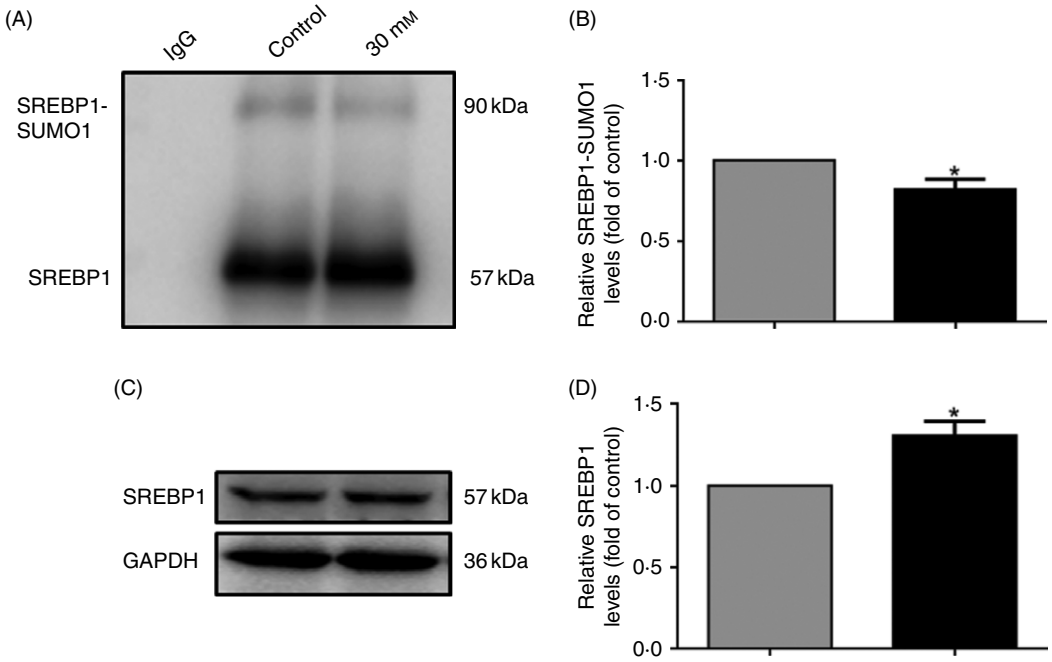

Fig. 8. Forty-eight-hour glucose incubation changed the SUMOylation levels of sterol regulatory elements binding protein 1 (SREBP1) and influenced the protein expression of SREBP1 in primary hepatocytes from yellow catfish. (A) Immunoprecipitation of SREBP1 was probed for SUMOylation using an anti-SREBP1 antibody. (B) SUMOylation levels of SREBP1. (C) Levels of SREBP1 protein in total cell lysates are also shown. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. (D) Protein expression of SREBP1. Values are means with their standard errors, *n* 3 (replicates of three biological experiments). * Differences are significant between the two groups ($P < 0{\cdot}05$). (B, D) ▭, Control; ■, 30 mM.

enzyme (E2, UBC9) and ligases (E3)[12,13,15]. PIAS were initially considered to be inhibitors of the STAT family of transcription factors[36] and possessed the activity of SUMO E3 ligase[37]. Here, glucose-induced down-regulation of these genes meant that dietary glucose tended to reduce mRNA expression of genes associated with SUMO modification. SUMO1 and SUMO2/3 act as cellular stress proteins in response to high glucose, and they mediates various translational modifications of many signalling proteins. Through the SUMOylation suppression, we speculate that high glucose influenced many signalling pathways. Similarly, Zhang *et al.*[38] reported that the mRNA expression of hepatic PIAS1 and PIAS3 was inversely linked with those of lipogenic genes in mouse models with diet-induced obesity. Our recent study indicated that dietary carbohydrate levels affected their mRNA expression in a dose-dependent manner[23]. In contrast, Huang *et al.*[39] pointed out that high glucose up-regulated the expression of SUMO1 and SUMO2/3. We did not know the reason for these differences. However, the carbohydrate-induced variations of their expression will influence lipid metabolism, as suggested by other studies[26,38,39].

On the other hand, SENP desumoylase family catalyses the de-conjugation of SUMO from their substrate. Our study indicated that *senp2* and *senp3* mRNA levels were lower for yellow catfish fed the glucose than those fed the dextrin. In contrast, Jung *et al.*[40] found that the expression of Senp2, but not Senp1, was triggered by chronic high-glucose stimulation in insulin-producing cells. Moreover, Jung *et al.*[40] pointed out that the induction of different SENP needed different stimulations according to cell types. In response to glucose, the expression of SENP2 was predominant in the cytoplasm than in the nucleus[40]. Obviously, the physiological importance of the *senp* in SUMOylation needs to be clarified, and further studies were needed to elucidate the molecular mechanism of the *senp* transcriptional repression by glucose.

SREBP1 is a key transcription factor which regulates lipogenesis. Studies have shown that the SUMOylation modification of transcription factors often inhibits transcription[27]. In this work, *P. fulvidraco* SREBP1 possessed three distinct domains, including the SAP130-C domain, the *n*-3 PfaA domain and the bHLH-zip domain. The SAP130-C domain interacts with the mSin3A-histone deacetylase complex and appears to function with acetylation[41]. The *n*-3 PfaA domain reveals to be involved in polyketide-like biosynthetic mechanisms of PUFA biosynthesis[42]. The bHLH-zip domain found in SREBP1 and other similar proteins. SREBP1 is a member of bHLH-zip transcription factor family that recognises sterol regulatory element 1 (SRE-1) and acts as a transcriptional activator required for lipid homoeostasis[43]. Thus, next, we explored whether the lysine residue in SREBP1 could be SUMOylated. Here, we found that SREBP1 was a target for SUMOylation and that the SUMOylation site of SREBP1 was Lys264. Since Lys264 mapped at the SAP130-C domain of SREBP1, and studies indicated that NAD-dependent deacetylase sirtuin-1 (SIRT1) deacetylates the SREBP1[44]. It suggests that sumoylation and acetylation work together to regulate the activity of SREBP1. Thus, further work should be clarified to explore the mechanism of two posttranslational modifications modulating SREBP1 activity. Lee *et al.*[26] found that the SUMO E3 ligase sumoylated SREBP1c at Lys98, suppressing the lipogenic programme in response to fasting. SUMOylation plays versatile functions in transcriptional

regulation. It influences the localisation of transcription factors and other post-translational modifications[13,45–47]. More studies are needed to investigate the effects of SUMO1 modification on the subnuclear localisation of SREBP1. Studies pointed out that SUMO attachment sites have been shown to be critical functional components of transcriptional inhibitory domains, and transient SUMO attachment may mediate transcriptional repression[48]. Moreover, our studies found that 30 mM glucose reduced the SUMOylation levels of SREBP1, but increased the protein levels of SREBP1. These results indicated that glucose-mediated SUMOylation of SREBP1 might regulate *de novo* lipogenesis resulting in TAG accumulation. Similarly, Hirano *et al.*[17] found that residues Lys123 and Lys418 of SREBP1a acted as potential SUMO1 acceptor sites, and SUMO1 can negatively regulate the transactivation function of SREBP. Other studies also indicated that SUMO modification inhibited the transcriptional activities of target nuclear receptors[27,48,49]. Lee *et al.*[26] found SREBP1c sumoylation might repress hepatic lipogenic pathways. In the present study, since glucose incubation increased SUMO1 expression, it is reasonable to speculate that glucose-induced reduction of SREBP1 SUMOylation was attributable to the up-regulation of SUMO1 expression. Although our studies suggested the regulatory functions of SUMOylation modification in CCAAT/enhancer binding protein (C/EBP) proteins, the underlying mechanism is not yet clear. Further definition of the relevant mechanism will provide insights into the roles of SUMOylation in regulating SREBP1 function.

Conclusion

In summary, our data elucidated the molecular mechanism of dietary glucose increasing lipid deposition and up-regulating lipogenesis. The present study is the first one to elucidate the SUMO1 modification of SREBP1 and SREBP1 SUMOylation at Lys264. High glucose incubation decreased the SUMOylation level of SREBP1 and enhanced the SREBP1 expression in primary hepatocytes, indicating that glucose-mediated SUMOylation of SREBP1 affected its transcriptional activity and protein expression, which further regulated its target genes involving lipogenesis.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (NSFC, 31572605) and National Key R&D Program of China (2018YFD0900400).

X.-Y. T. and S.-B. Y. designed the experiment; S.-B. Y. conducted the feeding and analytical experiment with the skillful help of T. Z., L.-X. W. and Y.-C. X.; S.-B. Y. analysed the data and X.-Y. T. provided some critical suggestions for the analysis of the data; S.-B. Y. drafted the manuscript and X.-Y. T. revised the manuscript; all of the authors read and approved the manuscript.

No conflict of interest is declared.

Supplementary material

For supplementary materials referred to in this article, please visit https://doi.org/10.1017/S0007114520002408

References

1. Neuschwander-Tetri BA (2013) Carbohydrate intake and nonalcoholic fatty liver disease. *Curr Opin Clin Nutr Metab Care* **16**, 446–452.
2. Iizuka K, Bruick RK, Liang G, *et al.* (2004) Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proc Natl Acad Sci U S A* **101**, 7281–7286.
3. Uyeda K & Repa JJ (2006) Carbohydrate response element binding protein, ChREBP, a transcriptional factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metab* **4**, 107–110.
4. Morral N, Edenberg HJ, Witting SR, *et al.* (2007) Effects of glucose metabolism on the regulation of genes of fatty acid synthesis and triglyceride secretion in the liver. *J Lipid Res* **48**, 1499–1510.
5. Foretz M, Pacot C, Dugail I, *et al.* (1999) ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Mol Cell Biol* **19**, 3760–3768.
6. Yamashita H, Takenoshita M, Sakurai M, *et al.* (2001) A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver. *Proc Natl Acad Sci U S A* **98**, 9116–9121.
7. Postic C, Dentin R, Denechaud PD, *et al.* (2007) ChREBP, a transcriptional regulator of glucose and lipid metabolism. *Annu Rev Nutr* **27**, 179–192.
8. Brown MS & Goldstein JL (1997) The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* **89**, 331–340.
9. Kim JB, Sarraf P, Wright M, *et al.* (1998) Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J Clin Invest* **101**, 1–9.
10. Flotho A & Melchior F (2013) SUMOylation: a regulatory protein modification in health and disease. *Annu Rev Biochem* **82**, 357–385.
11. Guo C & Henley JM (2014) Wrestling with stress: roles of protein SUMOylation and deSUMOylation in cell stress response. *IUBMB Life* **66**, 71–77.
12. Johnson ES, Schwienhorst I, Dohmen RJ, *et al.* (1997) The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. *EMBO J* **16**, 5509–5519.
13. Desterro JMP, Rodriguez MS & Hay RT (1998) SUMO-1 modification of IkBα inhibits NF-kB activation. *Mol Cell* **2**, 233–239.
14. Rytinki MM, Kaikkonen S, Pehkonen P, *et al.* (2009) PIAS proteins: pleiotropic interactors associated with SUMO. *Cell Mol Life Sci* **66**, 3029–3041.
15. Hay RT (2005) SUMO: a history of modification. *Mol Cell* **18**, 1–12.
16. Pascual G, Fong AL, Ogawa S, *et al.* (2005) A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature* **437**, 759–763.
17. Hirano Y, Murata S, Tanaka K, *et al.* (2003) Sterol regulatory element-binding proteins are negatively regulated through SUMO-1 modification independent of the ubiquitin/26 S proteasome pathway. *J Biol Chem* **278**, 16809–16819.
18. Zhao T, Yang SB, Chen GH, *et al.* (2020) Dietary glucose increases glucose absorption and lipid deposition via SGLT1/2 signaling and acetylated ChREBP in the intestine and isolated intestinal epithelial cells of yellow catfish. *J Nutr* **150**, 1790–1798.
19. Ye WJ, Tan XY, Chen YD, *et al.* (2009) Effects of dietary protein to carbohydrate ratios on growth and body composition of juvenile yellow catfish, *Pelteobagrus fulvidraco* (*Siluriformes*, *Bagridae*, *Pelteobagrus*). *Aquacult Res* **40**, 1410–1418.

20. Zhao T, Wu K, Hogstrand C, *et al.* (2020) Lipophagy mediated carbohydrate-induced changes of lipid metabolism via oxidative stress, endoplasmic reticulum (ER) stress and ChREBP/PPARγ pathways. *Cell Mol Life Sci* **77**, 1987–2003.
21. Pan YX, Zhuo MQ, Li DD, *et al.* (2019) SREBP-1 and LXRα pathways mediated Cu-induced hepatic lipid metabolism in zebrafish *Danio rerio*. *Chemosphere* **215**, 370–379.
22. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**, e45.
23. Yang SB, Tan XY, Zhang DG, *et al.* (2018) Identification of 10 SUMOylation-related genes from yellow catfish *Pelteobagrus fulvidraco*, and their transcriptional responses to carbohydrate addition *in vivo* and *in vitro*. *Front Physiol* **9**, 1544.
24. Wu K, Luo Z, Hogstrand C, *et al.* (2018) Zn stimulates the phospholipids biosynthesis via the pathways of oxidative and endoplasmic reticulum stress in the intestine of freshwater teleost yellow catfish. *Environ Sci Technol* **52**, 9206–9214.
25. Xu YH, Tan XY, Xu YC, *et al.* (2019) Novel insights for SREBP-1 as a key transcription factor in regulating lipogenesis in a freshwater teleost, grass carp *Ctenopharyngodon idella*. *Br J Nutr* **122**, 1201–1211.
26. Lee GY, Jang H, Lee JH, *et al.* (2014) PIASy-mediated sumoylation of SREBP1c regulates hepatic lipid metabolism upon fasting signaling. *Mol Cell Biol* **34**, 926–938.
27. Gill G (2005) Something about SUMO inhibits transcription. *Curr Opin Genet Dev* **15**, 536–541.
28. Joseph SB, Laffitte BA, Patel PH, *et al.* (2002) Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. *J Biol Chem* **277**, 11019–11025.
29. Kalaany NY & Mangelsdorf DJ (2006) LXRs and FXR the yin and yang of cholesterol and fat metabolism. *Annu Rev Physiol* **68**, 159–191.
30. Yao D, Luo J, He Q, *et al.* (2017) SCD1 alters long-chain fatty acid (LCFA) composition and its expression is directly regulated by SREBP-1 and PPARγ1 in dairy goat mammary cells. *J Cell Physiol* **232**, 635–649.
31. Kawaguchi T, Takenoshita M, Kabashima T, *et al.* (2001) Glucose and cAMP regulate the L-type pyruvate kinase gene by phosphorylation/dephosphorylation of the carbohydrate response element binding protein. *Proc Natl Acad Sci U S A* **98**, 13710–13715.
32. Sandberg MB, Fridriksson J, Madsen L, *et al.* (2005) Glucose-induced lipogenesis in pancreatic *β*-cells is dependent on SREBP-1. *Mol Cell Endocrinol* **240**, 94–106.
33. Anthonisen EH, Berven L, Holm S, *et al.* (2010) Nuclear receptor liver X receptor is O-GlcNAc-modified in response to glucose. *J Biol Chem* **285**, 1607–1615.
34. Hasty AH, Shimano H, Yahagi N, *et al.* (2000) Sterol regulatory element-binding protein-1 is regulated by glucose at the transcriptional level. *J Biol Chem* **275**, 31069–31077.
35. Mitro N, Mak PA, Vargas L, *et al.* (2007) The nuclear receptor LXR is a glucose sensor. *Nature* **445**, 219–223.
36. Liu B, Liao J, Rao X, *et al.* (1998) Inhibition of Stat1-mediated gene activation by PIAS1. *Proc Natl Acad Sci U S A* **95**, 10626–10631.
37. Hochstrasser M (2001) SP-ring for SUMO: new functions bloom for a ubiquitin-like protein. *Cell* **107**, 5–8.
38. Zhang Y, Gan Z, Huang P, *et al.* (2012) A role for protein inhibitor of activated STAT1 (PIAS1) in lipogenic regulation through SUMOylation-independent suppression of liver X receptors. *J Biol Chem* **287**, 37973–37985.
39. Huang W, Xu L, Zhou X, *et al.* (2013) High glucose induces activation of NF-κB inflammatory signaling through IκBα sumoylation in rat mesangial cells. *Biochem Biophys Res Commun* **438**, 568–574.
40. Jung HS, Kang YM, Park HS, *et al.* (2016) Senp2 expression was induced by chronic glucose stimulation in INS1 cells, and it was required for the associated induction of Ccnd1 and Mafa. *Islets* **8**, 207–216.
41. Fleischer TC, Yun UJ & Ayer DE (2003) Identification and characterization of three new components of the mSin3A corepressor complex. *Mol Cell Biol* **23**, 3456–3467.
42. Allen EE & Bartlett DH (2002) Structure and regulation of the *n*-3 polyunsaturated fatty acid synthase genes from the deep-sea bacterium *Photobacterium profundum* strain SS9. *Microbiology* **148**, 1903–1913.
43. Parraga A, Bellsolell L, Ferre-DAmare AR, *et al.* (1998) Co-crystal structure of sterol regulatory element binding protein 1a at 2.3 A resolution. *Structure* **15**, 661–672.
44. Ponugoti B, Kim DH, Xiao Z, *et al.* (2010) SIRT1 deacetylates and inhibits SREBP-1c activity in regulation of hepatic lipid metabolism. *J Biol Chem* **285**, 33959–33970.
45. Muller S, Matunis MJ & Dejean A (1998) Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. *EMBO J* **17**, 61–70.
46. Prudden J, Pebernard S, Raffa G, *et al.* (2007) SUMO-targeted ubiquitin ligases in genome stability. *EMBO J* **26**, 4089–4101.
47. Yang WH, Heaton JH, Brevig H, *et al.* (2009) SUMOylation inhibits SF-1 activity by reducing CDK7-mediated serine 203 phosphorylation. *Mol Cell Biol* **29**, 613–625.
48. Girdwood DW, Tatham MH & Hay RT (2004) SUMO and transcriptional regulation. *Semin Cell Dev Biol* **15**, 201–210.
49. Sentis S, Le Romancer M, Bianchin C, *et al.* (2005) Sumoylation of the estrogen receptor *α* hinger region regulates its transcriptional activity. *Mol Endocrinol* **19**, 2671–2684.

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