

# The Influence of Hyperglycemia on Liver Triglyceride Deposition in Partially Pancreatectomized Rats



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## Key words

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## ABSTRACT

Nonalcoholic fatty liver disease and diabetes always coexist. The relationship of fatty liver and hyperglycemia is not clear. We studied the influence of hyperglycemia on triglyceride (TG) accumulation in the liver and explored its possible mechanisms. SD rats were divided into three groups: Group A (sham operation control), Group B (partially pancreatectomized rats), and Group C (partially pancreatectomized rats treated with insulin). At 4 weeks after surgery, pancreatic weights and liver TG contents were measured. Serum biochemical parameters were determined, and oral glucose tolerance tests (OGTT) were performed. The gene expression of sterol regulatory element-binding protein 1c (SREBP-1c), carbohydrate regulatory element-binding protein (ChREBP), fatty acid synthase (FAS), carnitine palmitoyltransferase 1 (CPT-1), and fibroblast growth factor 21 (FGF21) was determined by real-time PCR. Compared with Group A, postprandial glucose increased significantly; the concentrations of insulin and C-peptides, pancreatic weights and serum FGF21 levels were decreased, liver TG was increased significantly in Group B, and insulin treatment improved these changes. Compared with Group A, the gene expressions of FGF21, CPT-1 and FAS in the liver were decreased in Group B (all  $p < 0.05$ ). Compared with Group B, the gene expressions of FGF21, FAS, ChREBP, SREBP-1c and CPT-1 in the liver in Group C were all increased significantly ( $p < 0.05$ , respectively). Hyperglycemia induced by partial pancreatectomy could lead to increased liver TG. Insulin treatment could decrease glucose levels and improve fatty liver, and genes related to lipid metabolism may play a role in this process.

## ABBREVIATIONS

|          |   |
|----------|---|
| NAFLD    | Nonalcoholic fatty liver disease                |
| T2DM     | Type 2 diabetes                                 |
| T1D      | Type 1 diabetes                                 |
| DNL      | de novo lipogenesis                             |
| SREBP-1c | Sterol regulatory element-binding protein 1c    |
| ChREBP   | Carbohydrate regulatory element-binding protein |
| TG       | Triglyceride                                    |

|        |                                  |
|--------|----------------------------------|
| FA     | Fatty acid                       |
| FAS    | Fatty acid synthase              |
| L-CPTI | Carnitine palmitoyltransferase I |
| FGF21  | Fibroblast growth factor         |
| SPF    | Specific pathogen-free           |
| OGTTs  | Oral Glucose tolerance tests     |
| AMS    | Amylase                          |
| ALT    | Alanine aminotransferase         |
| AST    | Aspartate aminotransaminase      |

## Introduction

Nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2DM) always coexist [1, 2], and this phenomenon can also be seen in some type 1 diabetes (T1D) [3, 4]. The relationships of NAFLD and hyperglycemia are not clear.

Obesity is a risk factor for developing diabetes [5] and is closely associated with the prevalence of NAFLD [6]. When obesity develops, insulin resistance may play a key role in linking hepatic fat to the incidence of T2DM [7]. From this point of view, NAFLD may play a role in the development of diabetes.

It has been reported that the overall NAFLD prevalence in patients with T1D has increased [4], that most type 1 diabetic patients are nonobese, and that  $\beta$  cell dysfunction is the major pathophysiological change in T1D [8]. This suggests that for NAFLD in diabetes, in addition to obesity, there are other factors related to fatty liver development.

Hepatic lipids may be derived from dietary intake, esterification of plasma free fatty acids (FAs) or hepatic *de novo* lipogenesis (DNL); hepatic DNL is increased in individuals with NAFLD [9], and DNL is regulated mainly by two key transcription factors, sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate regulatory element-binding protein (ChREBP) [10, 11]. Enzymes of TG synthesis, such as fatty acids synthase (FAS), which catalyzes the last step in the FA biosynthetic pathway, are transcriptionally regulated by ChREBP and SREBP-1c. Carnitine palmitoyltransferase I (L-CPTI) is the rate-limiting enzyme of FA  $\beta$ -oxidation [10–12], since FA entry into the mitochondria through CPT-1. Fibroblast growth factor (FGF21) is mainly synthesized in the liver, it also synthesized from the pancreas, adipose tissue, and skeletal muscle. FGF21 plays an important role in regulating hepatic oxidation of FA, suppressing caloric burden, reducing *de novo* lipogenesis, and increasing fat oxidation in the liver [13, 14]. We wanted to know whether hyperglycemia plays a causible role in the development of NAFLD. With studies in humans, there are many factors that affect research results, and biopsy is a gold standard to diagnose fatty liver; however, it is not convenient to address this question in humans, so we used a hyperglycemia animal model to study the relationship. Several toxins, including streptozotocin and alloxan, are usually used to induce hyperglycemia in rats, but these chemicals are toxic to the liver and may be metabolized in and affect the liver. Therefore, we used partial pancreatectomy to decrease insulin production to induce hyperglycemia and observed fat accumulation in the liver. Then, we measured serum FGF21 levels and the gene expression of SREBP-1c, ChREBP, FAS, CPT-1, and FGF21. We hope this study will be helpful to guide the clinical experience.

## Materials and Methods

### Animal model

Specific pathogen-free (SPF) male SD rats (6–8 weeks of age; weighing 200–300 g) were purchased from the Experimental Animal Center of ShanXi Medical University (TaiYuan, ShanXi, China) and adapted to the environment for 1 week before the study. All rats were housed in SPF colony cages (4 rats in each cage) with a 12-hour light/dark cycle in a temperature-controlled environment. The rats were randomly divided into three groups according to the

random number table method. Group A: sham-operated controls ( $n = 10$ ); group B: partially pancreatectomized rats ( $n = 10$ ); and group C: partially pancreatectomized rats treated with insulin ( $n = 10$ ). All rats were fed a normal chow diet (66.5% carbohydrate, 10.2% fat, 23.3% protein). The animals had free access to food and drinking water. All animal care and experimental procedures were performed in accordance with the guidelines of the Animal Care Committee of ShanXi Medical University of China. Partial pancreatectomy was used to induce hyperglycemia in the rats, and each experiment was performed in our laboratory. Rats were anesthetized with 0.5% pentobarbital (5 mg/100 g body weight) (Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. Shanghai, China) by an i.p. injection before the operation. Under sterile conditions, the abdominal cavities of the rats in Groups A, B and C were opened, through a midline abdominal incision, partial pancreatectomy (90%) were performed in Group B and C rats, according to the reference [15]. The pancreatic tissue was removed by gentle abrasion. All major blood vessels were left intact, only the pancreatic tissue between the common bile duct extending to the first loop of the duodenum was preserved. The rats in Group A underwent a sham pancreatectomy, the tail and body of the pancreatic tissue were disengaged from the mesentery and gently manipulated before being repositioned to the abdominal cavity. After partial pancreatectomy was performed, insulin glargine ( $0.1 \text{ U} \cdot \text{kg}^{-1}$ , Sanofi-Aventis) was injected i.p. into Group C rats (once-daily). After surgery, the rats were put into single cages, and wound care was applied. Body weights were measured at the beginning and after 4 weeks.

### Oral Glucose tolerance tests (OGTTs)

Four weeks after surgery, an OGTT was performed by glucose gavage (5 g glucose/kg body wt) after an overnight fast. The rat tail was pierced by a needle, and a drop of venous blood was taken to measure blood glucose. Another 300  $\mu\text{l}$  of tail vein blood was collected and centrifuged at 2000 rpm for 15 minutes to obtain serum for insulin and C-peptide measurement. Blood glucose, insulin, and C-peptide were monitored after glucose gavage for 0 h, 0.5 h, 1 h, and 2 h, and blood glucose was monitored using a glucometer (Roche Diagnostics, Switzerland). Insulin and C-peptide concentrations were determined using a rat enzyme-linked immunosorbent assay kit (RD system) using a rat standard. The area under the curve for the OGTT was calculated as previously described [16].

### Biochemical analysis

At 4 weeks after surgery, under anesthesia, blood samples were collected from the abdominal aorta and centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum concentrations of triglycerides (TG), amylase (AMS), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined using a microplate (according to the manufacturer's protocol, Nanjing Jiancheng Corp, Nanjing, China). Serum concentrations of FGF21 were determined using a rat FGF21 enzyme-linked immunosorbent assay kit (RD system).

### Triglyceride contents in the liver

The livers were isolated from rats under anesthesia and washed rapidly three times with ice-cold isotonic saline. The tissue was in-

cised into several pieces on a filter paper and immediately preserved between aluminum foil at  $-70^{\circ}\text{C}$ . When measuring, after liver tissue was thawed, one hundred milligram of tissue was weighed, and triglycerides were extracted from the tissues and measured according to the manufacturer's protocol (Triglycerides Assay Kit, Nanjing Jiancheng Corp, Nanjing, China). For each sample, two different parts of the tissue were measured, and the average value of the two measurements was used.

## Histopathology of the liver

At the end of the experiment, under anesthesia, the total preserved pancreas was removed and weighed in all three groups. Livers ( $n = 5$ ) were selected from each group randomly according to the random number table method, rapidly rinsed with PBS and immersed in 10% formaldehyde (Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. Shanghai, China). Specimens were fixed in 10% formaldehyde for 2–3 days, embedded in paraffin, serially sectioned ( $4\ \mu\text{m}$ ) and stained with hematoxylin and eosin (HE) to assess liver morphology. Oil Red O staining was performed in the liver according to a reference [17]. Images were analyzed using Image-Pro Plus software (Media Cybernetics, Rockville, MD, USA).

## Quantitative real-time PCR analysis

Total RNA was extracted from the liver ( $n = 3$ ), randomly selected from each group according to the random number table method, and reverse transcription was performed with 460 ng of total RNA in a total volume of  $10\ \mu\text{l}$  under conditions of  $37^{\circ}\text{C}$  for 15 minutes,  $85^{\circ}\text{C}$  for 5 seconds, and  $4^{\circ}\text{C}$  for 5 minutes. cDNA was synthesized using the Reverse Transcriptase kit according to the manufacturer's protocol (PrimeScript RT Master Mix, RR036A, Takara, Biomedical Technology Co., Ltd.), and the cDNA product was then amplified by real-time PCR in a total volume of  $20\ \mu\text{l}$  according to the manufacturer's protocol (SYBR premix Ex Taq II, RR820A, Takara,

Biomedical Technology Co., Ltd) using gene-specific primers (► **Table 1**) on an ABI 7300/7500 real-time PCR instrument (Applied Biosystems, Carlsbad, CA, USA). To  $2\ \mu\text{l}$  of cDNA,  $0.8\ \mu\text{l}$  of primers for the gene of interest and  $0.8\ \mu\text{l}$  of primers for the reference gene were added, and the reaction conditions were 40 cycles of  $95^{\circ}\text{C}$  for 30 seconds,  $95^{\circ}\text{C}$  for 5 seconds, and  $55\text{--}60^{\circ}\text{C}$  for 34 seconds. Relative mRNA expression levels were calculated using the  $\Delta\Delta\text{Cq}$  method and normalized to  $\beta$ -actin mRNA levels. Individual samples were assayed in triplicate, and the average quantification cycle (Cq) was calculated for the gene of interest and the reference gene. Based on the difference between both Cq values, the comparison was calculated. All primers were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. (Shanghai, China). The primer efficiencies were 99.98–101.01%.

## Statistical analyses

Values for normal distributions are presented as the mean  $\pm$  standard deviation (SD). Statistical analysis was performed with the Statistics Package for Social Science 19 (SPSS 19). The average difference in parameters was analyzed using two-way ANOVA and individual comparisons with Fisher's LSD test. Statistical significance was assumed at  $p < 0.05$ .

## Results

### Biometric parameters

After 4 weeks of surgery, the body weights of the three groups were not significantly different ( $p > 0.05$ ), and pancreatic weight in Group B was significantly decreased compared to that in Group A ( $p < 0.05$ ), there were no significant differences between groups B and C (► **Table 2**).

► **Table 1** Primer used for qPCR.

| Gene            | Accession number | Nucleotide sequence (from 5' to 3') | Amplicon (bp) |
|-----------------|------------------|-------------------------------------|---------------|
| <i>SREBP-1c</i> | NM-001276708.1   | F: ACAAGATTGTGGAGCTCAAG             | 72            |
|                 |                  | R: TGGCAAGACAGCAGATTTA              |               |
| <i>FAS</i>      | XM-031354836.1   | F: GGATGTCAACAAGCCCAAGTA            | 101           |
|                 |                  | R: TTACAGAGGAGAAGGCCACAA            |               |
| <i>CPT-1</i>    | XM-031389083.1   | F: GGAGAGAATTCATCCACTT              | 92            |
|                 |                  | R: ATGGCTTGCTCAAGTGCTT              |               |
| <i>ChREBP</i>   | FN432819.1       | F: AATCCCAGCCCTACACC                | 60            |
|                 |                  | R: CTGGGAGGAGCCAATGTG               |               |
| <i>FGF21</i>    | XM_032893629.1   | F: CACACCCAGTCCAGAAAG               | 77            |
|                 |                  | R: GGCTTGACACCCAGGATT               |               |
| $\beta$ -actin  | XM-032887061.1   | F: AAGTCCCTCACCTCCAAAAG             | 96            |
|                 |                  | R: AAGCAATGCTGTACCTTCCC             |               |

*SREBP-1c*: Sterol regulatory element binding protein-1c; *FAS*: Fatty acid synthase; *ChREBP*: Carbohydrate responsive element-binding protein; *CPT-1*: Carnitine palmitoyl transferase-1; *FGF21*: Fibroblast growth factor 21.

► **Table 2** Biometric parameters and serum biochemical analysis.

| Group                             | A (n=10)     | B (n=10)     | C (n=10)                |
|-----------------------------------|--------------|--------------|-------------------------|
| Body weight (g)                   | 323.97±60.59 | 324.09±44.25 | 356.86±107.35           |
| Pancreatic weight (g)             | 1.10±0.21    | 0.14±0.20*   | 0.26±0.19               |
| Pancreatic weight/body weight (%) | 0.34±0.08    | 0.04±0.06*   | 0.08±0.06               |
| Serum AST (U/l)                   | 36.75±23.44  | 38.37±20.04  | 31.34±23.04             |
| Serum ALT (U/l)                   | 17.96±10.84  | 15.81±7.47   | 14.40±11.15             |
| Serum GGT (U/l)                   | 19.17±3.63   | 18.84±4.55   | 16.49±7.00              |
| Serum AMS (U/μl)                  | 18.13±18.70  | 19.73±21.38  | 21.91±15.43             |
| Serum TG (mmol/l)                 | 0.37±0.13    | 0.39±0.14    | 0.32±0.14               |
| Serum FGF21 (ng/l)                | 865.5±88.8   | 728.5±65.5*  | 819.6±42.5 <sup>#</sup> |
| Liver TG (mol/g)                  | 19.03±4.89   | 28.66±12.90* | 20.61±3.84 <sup>#</sup> |

Group A: Sham operation group; Group B: Partially pancreatectomy group; Group C: Partially pancreatectomized rats treated with insulin glargine; TG: Triglycerides; Alb: Albumin; ALT: Alanine aminotransferase; AST: Aspartate aminotransaminase; GGT: Transglutaminase; AMS: Aspartate aminotransaminase; FGF21: Fibroblast growth factor 21. \* $p < 0.05$  versus group A; <sup>#</sup> $p < 0.05$  versus group B.

### Serum biochemical analysis and liver TG measurement

After 4 weeks, the levels of serum ALT, AST, GGT, AMS and TG were not significantly different among the three groups ( $p > 0.05$ ). Compared with Group A, serum FGF21 concentrations in Group B were decreased significantly ( $p < 0.05$ ), and after insulin glargine treatment, FGF21 was increased significantly in Group C ( $p < 0.05$ ). Compared with Group A, the liver TG contents in Group B were increased significantly ( $p < 0.05$ ), and compared with Group B, the liver TG contents in Group C were decreased significantly ( $p < 0.05$ ) (► **Table 2**).

### OGTTs, insulin and C-peptide concentrations

After 4 weeks, compared with Group A, fasting glucose in Group B was not changed significantly ( $p > 0.05$ ), however, after 0.5 h, 1 h and 2 h, postprandial glucose increased significantly (all  $p < 0.05$ ), and after insulin glargine treatment in Group C, 0.5 h, 1 h, and 2 h postprandial glucose improved to some extent. Compared with Group A, fasting insulin levels were not obviously changed, 0.5 h and 1 h postprandial insulin decreased significantly (all  $p < 0.05$ ), and 2 h postprandial insulin levels decreased slightly ( $p > 0.05$ ) in Group B. Compared with Group A, fasting C-peptide levels were not obviously changed, 0.5 h and 1 h postprandial C-peptides decreased significantly (all  $p < 0.05$ ), and 2 h postprandial C-peptide levels decreased slightly ( $p > 0.05$ ) in Group B, while after insulin glargine treatment in group C, 0.5 h, 1 h, and 2 h postprandial C-peptide levels changed slightly compared with Group B. Compared with Group A, the areas under the OGTT curves increased significantly ( $p < 0.05$ ), but the areas under the C-peptide curves decreased significantly ( $p < 0.05$ ) in Group B. Compared with Group B, after insulin glargine treatment, the areas under the OGTT curves in Group C decreased ( $p < 0.05$ ), and the areas under the C-peptide curves increased slightly ( $p > 0.05$ ) (► **Fig. 1**).

### Morphological changes in the liver

Compared with Group A, HE staining and Oil Red O staining showed that lipid droplets were increased significantly in Group B, and compared with Group B, after insulin glargine treatment, lipid droplets in Group C decreased significantly (► **Fig. 2**).

### Correlations between the liver TG contents and serum FGF21 levels

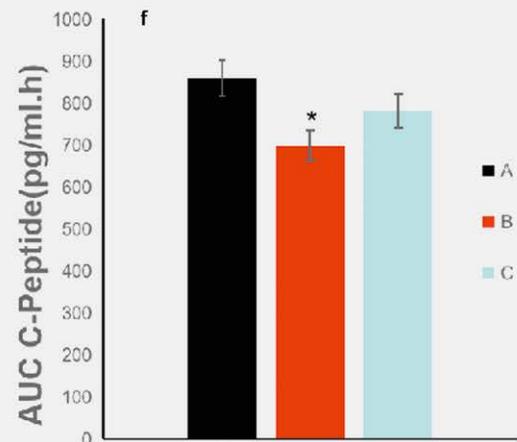
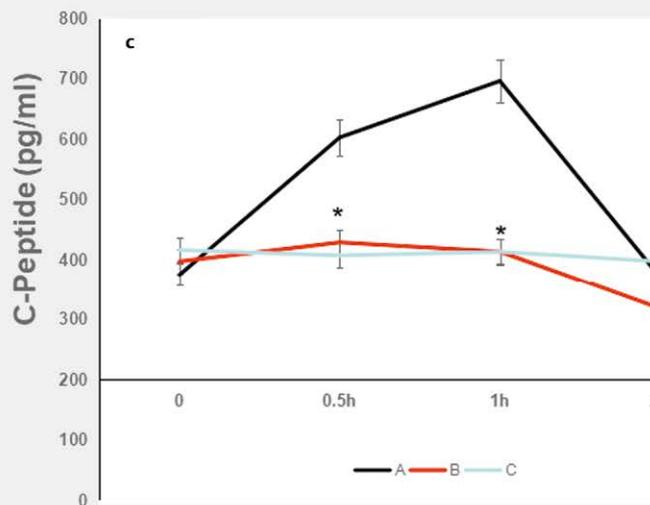
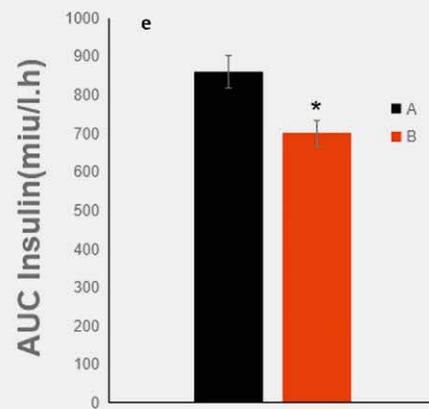
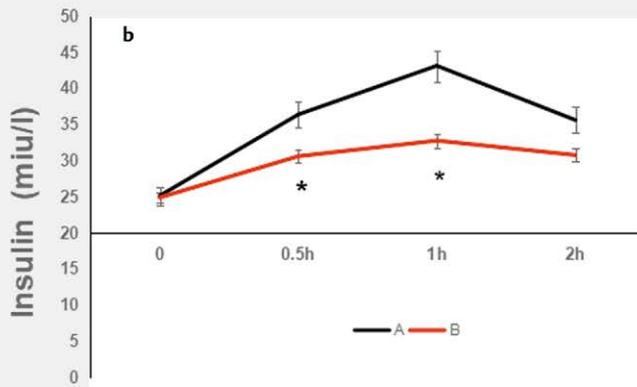
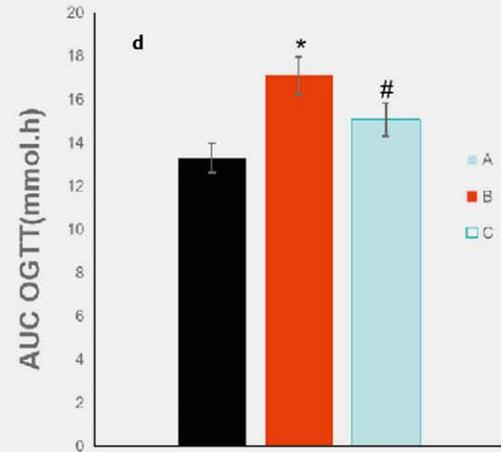
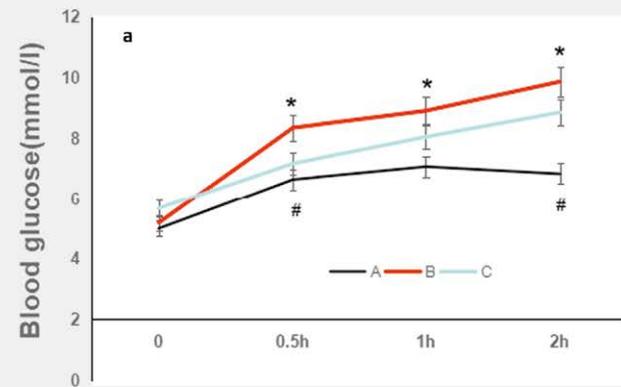
Correlation analysis revealed a significant negative correlation between the liver TG contents and serum FGF21 levels after 4 weeks of surgery ( $r = -0.75$ ,  $p < 0.01$ ) (► **Fig. 3**).

### Correlations between the liver TG contents and plasma blood glucose levels

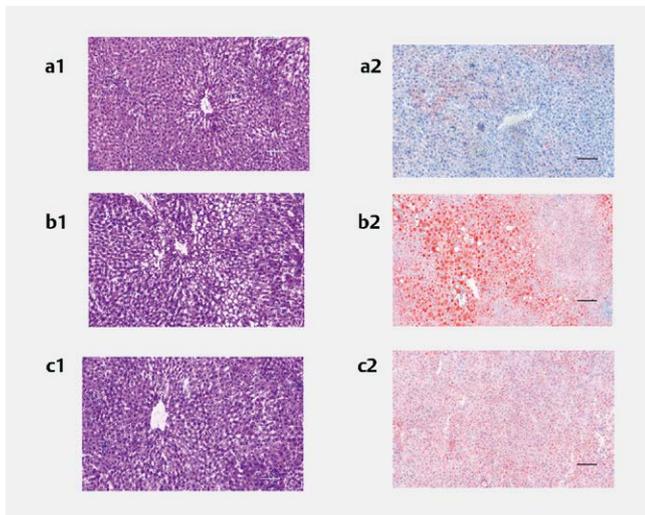
Correlation analysis revealed a significant positive correlation between the liver TG contents and the areas under the OGTT curves after 4 weeks of surgery ( $r = 0.543$ ,  $p < 0.05$ ) (► **Fig. 3**).

### Gene expression of SREBP-1c, ChREBP, FAS, CPT-1, and FGF21

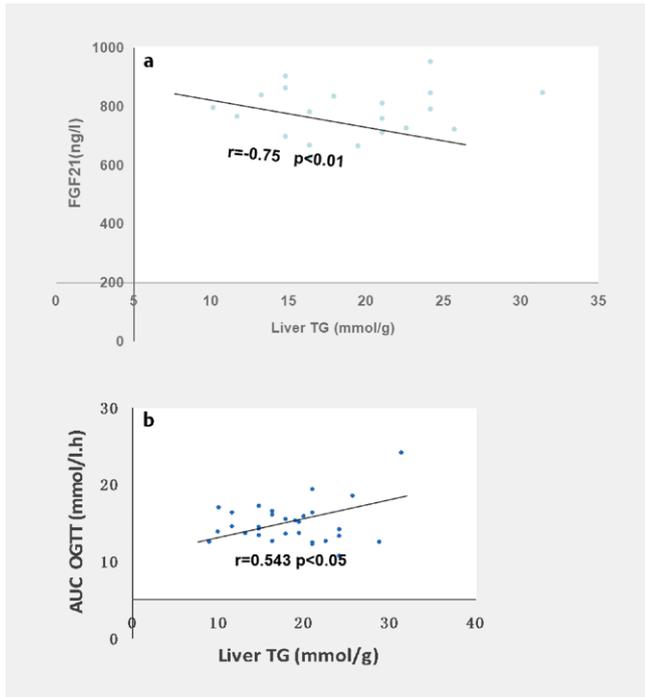
Compared with Group A, the mRNA expression of SREBP-1c and ChREBP changed slightly (all  $p > 0.05$ ), FAS mRNA expression decreased by about 55%, the mRNA expression of CPT-1 decreased by about 45% and FGF21 mRNA expression decreased by approximately 20% in Group B, respectively (all  $p < 0.05$ ). Compared with Group B, after insulin treatment in Group C, ChREBP mRNA expression was increased by about 57%, SREBP-1c mRNA expression was increased by about 53%, FAS mRNA expression was increased by about 80%, CPT-1 mRNA expression increased by about 24% (all  $p < 0.05$ ), and FGF21 mRNA expression increased by about 1.36-fold significantly ( $p < 0.01$ ), respectively (► **Fig. 4**).



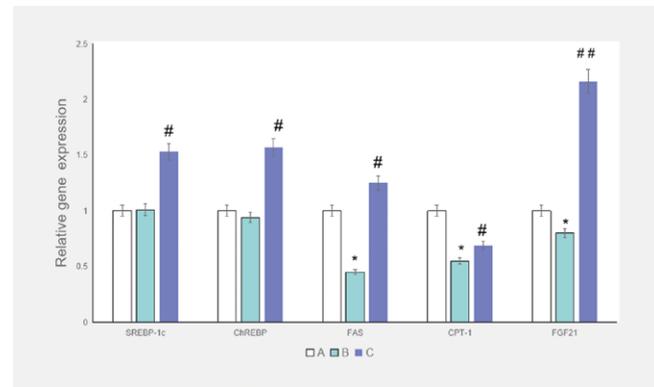
► **Fig. 1** **a:** Oral Glucose tolerance tests (OGTTs) in the three groups. black line, Group A; red line, Group B; light blue line, Group C. \* $p < 0.05$  versus Group A; # $p < 0.05$  versus Group B. **b:** Concentrations of insulin during OGTT in groups A and B. black line, Group A; red line, Group B. \* $p < 0.05$  versus Group A. **c:** Concentrations of C-peptide during OGTT in the three groups. black line, Group A; red line, Group B; light blue line, Group C. \* $p < 0.05$  versus Group A. **d:** The areas under the curve (AUC) for the OGTT in the three groups. black square, Group A; red square, Group B; light blue square, Group C. \* $p < 0.05$  versus Group A. # $p < 0.05$  versus Group B. **e:** The areas under the curve (AUC) for concentrations of insulin during OGTT in groups A and B. black square, Group A; red square, Group B. \* $p < 0.05$  versus Group A. **f:** The areas under the curve (AUC) for concentrations of C-peptide during OGTT in the three groups. black square, Group A; red square, Group B; light blue square, Group C. \* $p < 0.05$  versus Group A. Group A, sham operation control; Group B, partial pancreatectomy; Group C, partially pancreatectomized rats treated with insulin glargine.



► **Fig. 2** Liver tissues were stained with HE and Oil Red O after 4 weeks in the three groups. **a1**, Group A (HE × 100); **a2**, Group A (Oil Red O staining × 100); **b1**, Group B (HE × 100); **b2**, Group B (Oil Red O staining × 100); **c1**, Group C (HE × 100); **c2**, Group C (Oil Red O staining × 100). Oil Red O stains the fat and neutral fat, and red dots indicate lipid droplets accumulated in the liver. Scale bars: 100 μm. HE: Hematoxylin and eosin; (Images were randomly chosen from 5 rats in the three groups, n = 5) (The data are representative of 5 rats in the three groups, n = 5). Group A, sham operation control; Group B, partial pancreatectomy; Group C, partially pancreatectomized rats treated with insulin glargine.



► **Fig. 3** **a**: Correlations between the liver TG contents and serum FGF21 levels. **b**: Correlations between the liver TG contents and the areas under the curve (AUC) for the OGTT.



► **Fig. 4** Genes of SREBP-1c, ChREBP, FAS, FGF21, CPT-1 mRNA expression in the three groups (n = 3). White squares, Group A (sham operation); light blue squares, Group B (partial pancreatectomy); sky blue squares, Group C (partially pancreatectomized rats treated with insulin glargine). \*p < 0.05 versus Group A; #p < 0.05 versus Group B; ##p < 0.01 versus Group B.

## Discussion

The prevalence of NAFLD and diabetes is increasing worldwide [18]. The relationship between diabetes and NAFLD is controversial. We want to know whether hyperglycemia could lead to fatty liver and explore its mechanisms. Therefore, we constructed a hyperglycemic rat model by partial pancreatectomy.

We found that compared with Group A, after partial pancreatectomy for 4 weeks, insulin secretion (postprandial 0.5 h, 1 h and 2 h) during OGTT was decreased, and the glucose levels (postprandial 0.5 h, 1 h and 2 h) were increased. This result suggested that we successfully constructed a hyperglycemia model. We measured TG contents in the liver and found that compared with those in Group A, TG contents in the liver were increased significantly in Group B. After insulin treatment in Group C, glucose levels were decreased, and TG contents in the liver were also decreased significantly. These results were further confirmed by Oil Red O and HE staining, which demonstrated that lipid droplets were significantly increased in the group B, and insulin treatment decreased liver TG contents significantly. Correlation analysis showed liver TG positively related to the areas under the OGTT curves. This result suggested that hyperglycemia plays a very important role in lipid accumulation in the liver. Chon et al. [19] reported that subjects with T2DM had a higher prevalence of severe NAFLD than those with normal glucose, and T2DM worsened the course of NAFLD, doubling the risk of disease progression [20]. In an in vitro study, dose-dependent lipid accumulation was induced by glucose in HepG2 cells [21]. A high glucose diet increased fat content in the liver [22, 23], and low diet glucose levels decreased liver fat [24]. These findings are in accordance with our results to some extent.

FGF21 is a metabolic hormone, in mice, the role of hepatic FGF21 has been shown to be involved in the regulation of mitochondrial fatty acid oxidation and ketone body production, and FGF21 also regulates whole body fat oxidation and energy expenditure [14]. In our study, after partial pancreatectomy, compared with Group A, serum FGF21 concentrations decreased in Group B, and after insulin treatment, FGF21 increased in Group C. Correlation analysis suggested liver TG contents negatively related to serum FGF21 levels. Fletch-

er et al. [25] found compared with wild type mice, liver TG contents were increased in FGF21-Knockout mice. Our finding is in accordance with their results. It suggested FGF21 decrease plays an important role in the liver TG accumulation.

It is usually believed that insulin activates SREBP-1c, which transcriptionally activates genes involved in FA synthesis, whereas glucose activates ChREBP, which activates both glycolysis and FA synthesis. These overlapping, but distinct actions ensure that the liver synthesizes FAs only when insulin and carbohydrates are both present. Hepatic ChREBP deficiency resulted in reduced mRNA levels and protein levels of SREBP-1c [26]. SREBP-1c and ChREBP are coordinated, and they have a synergistic relationship [27, 28]. In our study, after partial pancreatectomy, insulin secretion was decreased, and in response to decreased insulin secretion, glucose levels were increased. We also found that compared with Group A, the gene expression of SREBP-1c and ChREBP slightly changed, and its downstream gene FAS mRNA expression decreased significantly in Group B, after insulin treatment, SREBP-1c, ChREBP and FAS mRNA expression were all increased in Group C. When insulin level decreased, which may have led to the decreased gene expression of SREBP-1c and its downstream gene FAS. An increase in glucose may induce an increase in ChREBP gene expression. Because SREBP-1c and ChREBP are coordinated, the effect of decreased insulin levels and high glucose levels may eventually result in slight changes in the gene expression of SREBP-1c and ChREBP.

Compared with Group A, FGF21 mRNA expression was decreased, CPT-1 mRNA expression in the liver was also decreased in Group B, after insulin treatment in Group C, they both increased significantly. FGF21 plays an important role in regulating hepatic oxidation of FA, FGF21 treatment reversed NAFLD [29], and the FGF21 decrease may be related to low insulin levels [30, 31]. Miotto et al. reported that the ablation of insulin resulted in reductions in mitochondrial oxidative capacity [32]. In our study, after partial pancreatectomy for 4 weeks, FGF21 gene expression in the liver was decreased, it is in accordance with serum FGF21 levels.

Compared with Group B, after insulin treatment in Group C, the mRNA expression of ChREBP, SREBP-1c, FAS, CPT-1, and FGF21 was all increased significantly, which suggested insulin treatment activated SREBP-1c and led to the increased mRNA expression of SREBP-1c and its downstream FAS. When SREBP-1c and ChREBP are coordinated it led to an increase of ChREBP mRNA expression. Also, the mRNA expressions of FGF21 and CPT-1 were all increased. Eventually, when FA oxidation exceeded FA synthesis, which led to decreased liver TG accumulation.

In conclusion, we found that after partial pancreatectomy, insulin secretion was decreased, glucose levels increased, and liver TG was increased. Insulin treatment decreased glucose levels and improved fatty liver. Genes related to FA synthesis and oxidation may play a role in this process. Our findings suggest antihyperglycemic treatment could improve NAFLD, and our results are meaningful to guide the prevention and treatment of NAFLD in clinical experience.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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