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1 Anti-Obesity Potential of Rare Sugar D-psicose by Regulating Lipid Metabolism in

2 Rats

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Abstract

D-psicose is a new generation sugar substitute with low calorie value while still offering desirable sweetness. This objective of this study was to investigate the anti-obesity potential of D-psicose and possible mechanism using Wistar rats as animal model. Animals were divided into five groups and supplemented with diets containing 5% of different carbohydrates including glucose, fructose, cellulose, D-psicose and control diet for 4 weeks, respectively. After sacrifice, blood lipid profile, tissue morphology and related genes participated in lipid metabolism were analyzed. Results indicated supplementation of D-psicose lead to minimum fat accumulation in rats compared with other carbohydrates. The blood lipid profile and anti-oxidative activity of the rat was also improved. D-psicose can regulate lipid metabolism by increasing lipid metabolism related enzymes such as SDH in serum and liver, and HL in the liver. D-psicose can prevent fat accumulation by suppressing the expression of lipogenesis related gene ACC α , hepatic fatty acid uptake gene including FAS and SREBP-1c gene, while stimulating the expression for fatty acid oxidation related gene including AMPK2 α , HSL and PPAR α . In conclusion, D-psicose can be a healthy alternative to traditional sweetener.

Keywords: D-psicose; Body weight; Lipid profile; Lipid metabolism; Antioxidant

1.Introduction

Overweight and obesity has been a serious health problem all over the world. According to WHO (World Health Organization), the global obese population has increased nearly three times since 1975. In 2016, 1.9 billion adults were overweight, accounting for more than one quarter of world population ¹. Overweight and obesity were proved to cause complications such as heart disease, cardiovascular disease, high blood pressure, type 2 diabetes and even cancers ^{2,3}. Studies have confirmed that increased intake of food and beverage rich in calories such as sugar and fat is one of the major reason that cause obesity ⁴. Over the past several decades, researchers had developed sugar substitutes to replace nature sugar in foods and beverages. But there was constant controversy over using synthetic sugar substitutes sweeteners such as aspartame, saccharine and sucralose. Studies reported these synthetic sweeteners may increase body weight and interfere physiological process ⁵. Hence there is a growing interest in developing and evaluating nature sugar substitutes such as sugar alcohols and rare sugars with significantly lower calorie, but still offer comparable sweetness. D-psicose is a kind of rare sugar with 70% the sweetness of sucrose but only 0.3 % of calorie ⁶. D-psicose, also known as D-allulose and D-ribo-2-hexulose, is a C-3 epimer of D-fructose. It occurs in small quantity in some fruits and some foods that has been processed. D-psicose can be produced by enzymatic transformation from D-fructose using D-psicose 3-epimerase ⁷. It has been listed as GRAS (Generally Regarded as

Safe) by the U.S. Food and Drug Administration ⁸. D-psicose has gained tremendous interest in recent years for its significant health promoting potentials ⁸. Several studies had reported the anti-obesity effect of D-psicose. More importantly, it cannot be metabolized in the body ⁹. D-psicose cannot be absorbed in the small intestine and do not ferment in large intestine ¹⁰. Furthermore, D-psicose can help to normalize body weight and prevent obesity. For example, rats/mice fed with D-psicose had lower body weight, reduced visceral fat mass, reduced food intake and decreased lipogenesis, lower blood insulin and glucose level compared to rats/mice with normal diet ¹¹⁻¹⁴. Han ¹⁵ found that D-psicose can regulate lipid metabolism disorder in mice induced by high fat diet. The potential mechanism was regulation of genes participated in lipid metabolism such as fatty acid synthase and fatty acid β -oxidation. Nagata ¹⁴ reported consumption of D-psicose could increase energy expenditure of rats in the day and fat oxidization at night by altering lipid metabolism through the regulation of related genes. Studies have reported D-psicose can decrease final body weight in obese rats by regulating major genes such as *Fos*, *Mmp3*, *Fgf21*, and *Abcd2* in lipid metabolism and inflammatory responses ¹⁶. Rats supplemented with both high fat diet and D-psicose showed less fat pad mass compared with control group. It was caused by the potential of D-psicose to regulate lipid metabolism and gene expression related to fecal lipids. D-psicose was reported to enhance 24h energy expenditure in Sprague-Dawley rats and therefore contribute to weight management. D-psicose can reduce visceral fat in diet induced obesity rats¹³. Luminal D-psicose may stimulate

83 Glucagon-like peptide 1 (GLP-1), a gastrointestinal hormone that is responsible for
84 body weight regulation ¹⁷. Until now, there are only a limited number of studies
85 reported the underlying mechanism behind these phenomena.
86 In the present study, we examined the effect of four different dietary carbohydrates
87 including glucose, fructose, D-psicose and cellulose on lipid metabolism of rats
88 supplemented with normal diet. We compared the effect of these carbohydrates on the
89 body weight, lipid profile, antioxidant level and expression of lipid metabolism
90 related genes in rat liver. Since liver played a key role in lipid metabolism, we further
91 investigated the effect of D-psicose on some key genes in lipid metabolism that has
92 not been reported before. It may provide a more comprehensive insight regarding how
93 D-psicose exhibit its anti-obesity potential.

94 2.Experimental

95 2.1 Material

96 D-psicose with the purity of 98% was prepared by our own lab by biotransformation
97 method as described before ¹⁸. Bovine serum albumin (BSA) was obtained from
98 Sangon Biotech (Shanghai, China). Coomassie brilliant blue G-250, glucose, ethanol,
99 sulfuric acid, casein, L-leucine, corn starch, sucrose, cellulose, choline chloride,
100 formaldehyde and sodium chloride were all analytical grade and purchased from
101 Sinopharm Chemical Reagent (Shanghai, China). Total cholesterol (TC), triglyceride,
102 free fatty acid (FFA), low density lipoprotein-cholesterol (LDL-C), superoxide
103 dismutase (SOD), succinate dehydrogenase (SDH), hepatic lipase (HL), catalase

(CAT), glycogen assay kit, glucose assay kit, rat insulin ELISA kit, and RNA storage solution were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Diethyl pyrocarbonate (DEPC) water, animal total RNA isolation kit, design and synthesis of primer were obtained from Shanghai Generay Biotech Co., Ltd (Shanghai, China). RT-PCR kit and SYBR fluorescent dye were purchased from TaKaRa Clontech (Dalian, China).

2.2 Animals, diets, and experimental procedure

Animal experiments were approved by Jiangnan University Institutional Animal Care and Use Committee (JU20140430-1) and conducted in compliance with the *Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities at Jiangnan University*. Thirty male Wistar rats (3-week-old) were obtained from Shanghai Slac Laboratory Animal, Co Ltd (Shanghai, China). Upon arrival, the rats were kept in animal room with controlled temperature of 23 ± 2 °C, humidity of $60 \pm 5\%$, 12h day/night cycle. The rats had free access to food and water. After acclimation for 1 week, rats were randomly divided into 5 groups and administered with diets only differ in dietary carbohydrate type. The diets were prepared in reference to AIN-76A diets with 5% of different carbohydrate in each group^{11, 19, 20}. During the whole experiment period, the body weight of the rats was measured every other day. The food intake was also recorded. After 4 weeks, the rats were sacrificed by carbon dioxide asphyxiation. Cardiac blood was collected, centrifuged at 1300 g for 10 min (4°C). Serum on the top layer was collected and stored in a -80 °C freezer.

125 Liver, heart, soleus, abdominal fat, epididymal fat were harvested and weighed
126 immediately. The liver was then cut into three pieces: one piece was stored in RNA
127 storage solution, one was immediately frozen with liquid nitrogen and stored in a -80
128 °C freezer. Another piece was fixed with 4% paraformaldehyde for histopathological
129 analysis. The abdominal fat was also fixed with 4% paraformaldehyde for
130 histopathological analysis.

131 2.3 Blood lipid profile analysis

132 Total cholesterol, triglyceride, low-density lipoprotein cholesterol and free fatty acid
133 in the serum were determined with analytical kit following advised protocols.

134 2.4 Histopathological analysis

135 Paraformaldehyde fixed hepatic and epididymal fat were processed for dehydration,
136 paraffin embedding, sectioning and Hematoxylin and Eosin staining according to
137 method described before ²¹. The tissue slides were observed under a Nikon Eclipse Ti
138 microscope (Nikon Corporation, Tokyo, Japan) for hepatic cell morphology.

139 2.5 Biological analysis

140 Hepatic sample preparation: a small piece of hepatic sample (about 100 mg) was
141 thawed. The tissue was then placed in a precooled blender. Then 9 times of
142 physiological saline by weight of the sample was added. The tissue was homogenized
143 at full speed every 30 s for a total of 2 min. The hepatic homogenate was then
144 centrifuged at 3000 g for 10min. The supernatant was collected. The protein content
145 in hepatic tissue was determined by Bradford method ²².

146 The catalase (CAT) and superoxide dismutase (SOD) in serum were determined with
147 commercial kits (Nanjing Jiancheng Bioengineering Institute). The CAT, SOD,
148 Succinate dehydrogenase (SDH) and Hepatic lipase (HL) in hepatic tissue were also
149 determined with commercial kit.

150 2.6 Real-Time PCR Analysis

151 Hepatic tissue (about 100mg) was mixed with 1mL TRIzol reagent and crushed in
152 liquid nitrogen with a mortar and pestle. Total RNA in each sample was extracted
153 following method described before²³. The RNA was then converted into
154 single-stranded cDNA using reverse transcription. The fluorescent quantification of
155 genes in lipid metabolism were carried out using SYBR Premix Ex Taq II in a
156 Bio-Rad CFX96 Real-Time PCR Detection system (Hercules, CA). The primers used
157 in this study were: GADPH F: 5'-ATGGCCTTCCGTGTTCTACCC-3', R:
158 5'-GCCTGCTTCACCTTCTTGATG-3', AMPK α 2 F:
159 5'-CAGAGCAAACCATACGACA-3', R: 5'-GCATTCACCTTCCATTC-3',
160 SREBP-1c F: 5'-CTGCTTGGCTCTTCTCTTT-3', R:
161 5'-CTTGTTTGCGATGTCTCC-3', PPAR α F: 5'-ACGGTGTGTATGAAGCCA-3',
162 R: 5'-GAACTCTCGGGTGATGAAG-3', ACC F:
163 5'-ATGTTGGGAGTTGTGTGTG-3', R: 5'-AGAAGTGTGTGAGCAGGAAG-3',
164 HSL F: 5'-CCTCTGCTTCTCCCTCTC-3', R: 5'-AAAATGGTCCTCCGTCTC-3',
165 FAS F: 5'-GGAGTTGAAGAGGAGCGT-3', R:
166 5'-ACGGTTGACAGCAAAATG-3'. The copy number of each transcript was

calculated with GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) as internal control using the $2^{-\Delta\Delta C_t}$ method ²⁴.

2.7 Statistic analysis

All data are presented as mean \pm SD. ANOVA was used to compare the differences among more than two groups followed by Tukey's Honestly Significant Difference Test. A $p < 0.05$ was considered statistical significant.

3 Results and Discussion

3.1 Food intake, body weight and tissue weight

The rats were administered with diets containing 5% carbohydrates for 4 weeks to determine the effect of different carbohydrates on weight increase. As shown in Figure 1, there was a steady increase in body weight for rats in all groups. As shown in Table 2, the final body weight of rats in D-psicose group was lower than other four groups. It was 13.4% less than control group. The increase of body weight in rats supplemented with D-psicose was significantly lower than other four groups ($p < 0.05$). It was 20.8% lower than the control group. The result was in agreement with earlier published studies ^{13, 16}. The daily food intake in D-psicose group was higher than control group. The daily food intake in cellulose group was higher than other groups. This might lead to the high body weight increase secondary to control group. The excessive calorie was stored in the body in the form of white adipose including abdominal adipose, epididymal fat and mesentery fat ²⁵. As shown in Table 1, the

abdominal fat and epididymal fat in D-psicose group were significantly lower than other groups ($p < 0.05$). They were 53.8% and 49.3% lower than that of control group. The weight of liver in D-psicose group was lower than control group, glucose group and fructose group. This may indicate that D-psicose can prevent fat from accumulating in the liver. Short term intake of D-psicose lead to least weight gain ($p < 0.05$) compared with other carbohydrates. This might due to the fact that D-psicose had relatively low energy value compared with other carbohydrates. Another possible reason is that D-psicose is absorbed in the small intestine without metabolism and then excreted in urine or feces, no calorie was generated^{10, 13}. All these results suggested that D-psicose can prevent significant body weight increase compared with other dietary carbohydrates such as corn starch, glucose, fructose and cellulose.

3.2 Blood lipid profile

We then examined the effect of different dietary carbohydrates on blood lipid profile including triglyceride, free fatty acid (FFA) and low-density lipoprotein cholesterol (LDL-C). The level of TG, FFA and LDL-C in blood are important indicators for lipid metabolism disorder²⁶. As seen in Table 2, after 4 weeks, the triglyceride in D-psicose group was clearly lower than other groups. It was 41.5% lower than the TG content in control group ($p < 0.05$). The TC level in D-psicose group was lower than control and cellulose group but higher than glucose and fructose group. The free fatty acid (FFA) in the blood is associated with lipid and sugar metabolism. FFA content in

D-psicose group was 51.1% less than control group ($p<0.05$). Low density lipoprotein cholesterol (LDL-C) was also known as ‘bad cholesterol’. Increased LDL-C in the blood may increase the risk of cardiovascular disease ²⁷. The LDL-C levels in fructose group, D-psicose group and cellulose group was lower than control group ($p<0.05$). The LDL-C in D-psicose group was 34.5% lower than control. In conclusion, supplementation of D-psicose may contribute to blood lipid profile improvement.

3.3 Effect of D-psicose on antioxidant level in serum and hepatic tissue

Body antioxidant level is one important indicator of obesity. High carbohydrate diet may increase oxidative stress in rats and induce lipid metabolism disorder ²⁸. Therefore, we examined the antioxidant capacities of liver and serum after supplemented with different dietary carbohydrates. Two enzymes, superoxidative dismutase (SOD) and catalase (CAT) were analyzed. These enzymes can remove free radicals inside the body and decrease the damage to liver caused by increased fat accumulation. The effect of different dietary carbohydrates on antioxidant activity in serum and hepatic tissue was shown in Table 3. The serum and hepatic level of CAT in D-psicose treated group were significantly ($p<0.05$) higher than other groups. They were 139.1% and 165.7% higher than control group. The serum and hepatic level of SOD in D-psicose group were also higher than other groups. In conclusion, D-psicose can improve the antioxidant capacity of the body through the increase of CAT activity.

3.4 D-psicose on lipid metabolism related enzymes

In this part, we examined the effect of D-psicose on succinate dehydrogenase (SDH) and hepatic lipase (HL) in serum and liver. Succinate dehydrogenase is an enzyme complex located in the mitochondrial membrane. It played an important role in citric acid cycle²⁹. The SDH content reflects the speed of citric acid cycle, which is essential in lipid, protein and carbohydrate metabolism. The SDH and HL level in Wistar rats can be seen in Table 4. The SDH level in D-psicose group were significantly ($p<0.05$) higher than other groups for both liver and serum samples. This may suggest D-psicose can stimulate the metabolism of lipid, protein and carbohydrate, which will ultimately lead to the decrease of fat accumulation in the body and decreased body weight. The major function of HL is to hydrolyze triglycerides and phospholipids of lipoprotein³⁰. As shown in Table 5, the D-psicose group had the highest HL content. It was 27.1% higher than control group. This result may suggest that D-psicose can increase lipid metabolism and maintain the cholesterol level inside the cell.

3.5 Effect of D-psicose on hepatic and adipose tissue morphology

Liver is the main location for lipid metabolism, abnormality in liver morphology may indicate hepatic disorder³¹. As shown in Figure 2d, the morphology of hepatic lobule in D-psicose group was clear, and the hepatic cells were evenly distributed. There was no obvious abnormality observed. But for glucose group (Figure 2b) and fructose group (Figure 2c), there was no obvious borders between different cells. There were more

fat deposits in samples from other four carbohydrates than D-psicose. For control group (Figure 2a) and cellulose group (Figure 2e), the hepatic morphology was better than glucose group and fructose group but still not as good as D-psicose group. Inflammatory infiltrate and fat vacuolation could be observed in these samples. From this experiment, we may conclude that D-psicose could reduce the accumulation of fat in the liver. The morphology of epididymal adipocyte was shown in Figure 3. As shown in Figure 3, the adipocytes in D-psicose group was significantly smaller than other groups, which indicated less fat deposition. When there is an energy surplus in the body, the energy will accumulate as fat in adipocyte and cause hypertrophy³². This result was in accordance with the analysis result of body weight. The rats in this group had lowest body weight and body fat content. Meanwhile, there was no pathological change for hepatic tissue in this group. For normal carbohydrate groups, the accumulation of lipids and fat mass may trigger the release of cytokines and chemokines, thus initiating inflammation response and cause the dysfunction of hepatic and adipose tissue ³³. These results may suggest D-psicose could modulate the lipid metabolism disorder, control body weight and body fat. To further elucidate the potential mechanism for the regulation of lipid metabolism, we then analyzed the expression of lipid metabolism related genes in hepatic tissue.

3.6 Effect of D-psicose on mRNA levels of lipid metabolism-related genes

In order to elucidate the underlying mechanism for the anti-obesity activity of D-psicose, we analyzed lipid metabolism related genes in rats fed with different carbohydrates.

3.6.1 ACC α

Acetyl-CoA carboxylase²³ is a carboxyl transferase that catalyze the carboxylation of acetyl-CoA to malonyl-CoA. The main function of ACC is to provide substrate for the synthesis of free fatty acid. There are two subunits of ACC: ACC α and ACC β . ACC α mainly existed in cell cytoplasm of lipogenic tissue such as liver, mammary gland and adipose tissue. It is the main regulatory enzyme that catalyzes the synthesis of long chain fatty acid. As shown in Figure 4a, the expression of ACC α was significantly ($p < 0.05$) lower than other groups. The expression of ACC α was significantly inhibited by D-psicose. This result was in agreement with previous published study where mice were supplemented with high fat diet containing 5% D-psicose¹⁵.

3.6.2 FAS

Fatty acid synthase (FAS) is the enzyme that catalyzes the synthesis of fatty acids. The activity of FAS had a direct impact on the synthesis of fatty acid in the body. It played a very important role in lipid metabolism. As the FAS activity increases, extra free fatty acid can form fat, thus resulting in the accumulation of fat in the body. It was reported when blood TG increase, the FAS expression also increase. With the

improvement of FAS expression, TG will also accumulate and lead to obesity. As

shown in Figure 4b, the D-psicose group had lowest ($p<0.05$) FAS level. The mRNA expression of FAS was significantly inhibited by supplementing D-psicose.

3.6.3 HSL

In addition to enzymes participated in lipogenesis, we also determined the effect of D-psicose on enzymes participated in lipolysis. Hormone sensitive lipase (HSL) is one major lipase that catalyze the breakdown of triglyceride, diglyceride and monoglyceride into free fatty acid and glycerol³⁴. Its substrates can also be cholesterol and steroids. As the mRNA expression of HSL gene increase, the accumulation of triglyceride in adipose tissue will decrease. As shown in Figure 4c, the HSL level in D-psicose group was higher than other groups. This may indicate supplementation of D-psicose can increase the breakdown of fatty acid in hepatic tissue.

3.6.4 AMPK α 2

AMP-activated protein kinase (AMPK) is an enzyme that stimulates the fatty acid oxidation and ketogenesis³⁵. It also played an important role in inhibiting cholesterol and triglyceride synthesis. AMPK is made up of three subunits: α , β and γ . The major AMPK expressed in the liver is AMPK α 2. As shown in Figure 4d, the mRNA expression of AMPK α 2 in D-psicose group was significantly ($p<0.05$) higher than fructose group and glucose group. It was higher than control group and cellulose group although there was no statistical significance. The result may indicate the

309 dietary supplementation of D-psicose can suppress lipid synthesis by activating

310 AMPK pathway.

311 3.6.5 PPAR α

312 Peroxisome proliferator activated receptors (PPARs) are nuclear transcription factors

313 activated by specific ligands that can induce the expression of lipid metabolism

314 related genes ^{36, 37}. There are three types of PPAR, including PPAR α , PPAR β and

315 PPAR γ . The major function of PPAR α is to regulate genes involved in fatty acid

316 metabolism. Activation of PPAR α can induce mitochondrial fatty acid oxidation and

317 improve hepatic steatosis ^{38,39}. Expression of AMPK α 2 will lead to fat accumulation

318 in the liver ⁴⁰. As shown in figure 4e, the mRNA expression of PPAR α in D-psicose

319 group was significantly ($p < 0.05$) higher than other groups. This result may indicate

320 supplementation of D-psicose can activate the expression of PPAR α gene, hence

321 increase the fatty acid oxidation in the liver.

322 3.6.6 SREBP-1c

323 Sterol regulatory element binding proteins (SREBPs) are a group of lipid synthetic

324 transcription factors existed in endoplasmic reticulum and nuclear envelop. The main

325 function of SREBP is to regulate lipogenesis and maintain homeostasis ⁴¹. SREBPs

326 can activate at least 30 genes that was associated with lipid metabolism pathway

327 including FAS ⁴². There are three types of SREBPs, i.e., SREBP-1a SREBPs-1c and

328 SREBPs-2. Among which, SREBP-1 was mainly expressed in liver, skeletal muscle,

329 adipose tissue and spleen ⁴³. SREBP-1c is the major form of SREBP-1 in the liver. As

shown in Figure 4f, the expression of SREBP-1c was significantly ($p<0.05$) induced by fructose. This may suggest fructose could increase the SREBP-1c, which will increase the production of free fatty acid. In the meantime, D-psicose group was shown to suppress the expression of SREBP-1c, leading to a reduced triglyceride content in the liver which would result in less fat accumulation ³¹. In summary, D-psicose can increase the mRNA expression of AMPK - an important enzyme in modulating glucose and fat metabolism⁴⁴. When AMPK phosphorylates SREBP-1c, fatty acid synthesis was inhibited ³⁵. The expression of fatty acid synthesis related genes including ACC α and FAS were significantly decreased ($p<0.05$). In the meantime, supplementation of D-psicose can suppress the expression of PPAR α and lead to increased β -oxidation. In addition, activation of AMPK α 2 can induce the expression of HSL, which contributes to lipolysis. Taken together, D-psicose contribute to least body weight increase by inhibiting expression of fatty acid synthesis related genes and increasing expression lipolysis related genes. Several recent studies also reported D-psicose could prevent lipid metabolism by regulating target genes. Kim found D-psicose can up-regulate β -oxidation related genes including *Abcd2*, CPT1 and HSL in C57BL/6J-*ob/ob* mice ¹⁶. However, Han reported D-psicose may decrease body weight by decreasing the mRNA expression of CD36, ApoB48, FATP4 in small intestine, while downregulating the fatty acid synthase and β -oxidation in the liver¹⁵. In addition to the regulation of lipid metabolism, Kim et al. also reported that the ability of D-psicose to change

inflammatory response networks might contribute to its anti-obesity potential. Genes related to obesity induced inflammation such as TNF- α (tumor necrosis factor-alpha) and IL-6 (interleukin-6) were suppressed by supplementation D-psicose¹⁶. More study needed to be carried out before identifying the specific mechanism of its anti-obesity potential. Further studies should focus on the elucidating of D-psicose anti-obesity potential from a comprehensive perspective.

4 Conclusion

In this study, we evaluated the effect of D-psicose on rat body weight gain, lipid profile and lipid metabolism related activities compared with other four carbohydrates including glucose, fructose and cellulose. Supplementation of D-psicose can inhibit fat accumulation in rats caused by high carbohydrate intake. Rats supplemented with D-psicose had minimum body weight increase, least epididymal fat and adipose cell size. They also had better blood lipid profile and antioxidant capacity. The fat accumulation was slowed down by activation of genes involved in fatty acid synthesis including AMPK2 α , HSL and PPAR α . At the same time, D-psicose can suppress the expression of genes participated in fatty acid synthesis including ACC α , FAS, SREBP-1c. D-psicose is a healthy low-calorie sweetener that can provide enough sweetness but do not cause lipid metabolism disorder or significant weight increase. These results may contribute to the justification of D-psicose utilization in foods as natural sweetener.

Conflicts of interest

There are no conflicts of interest to declare.

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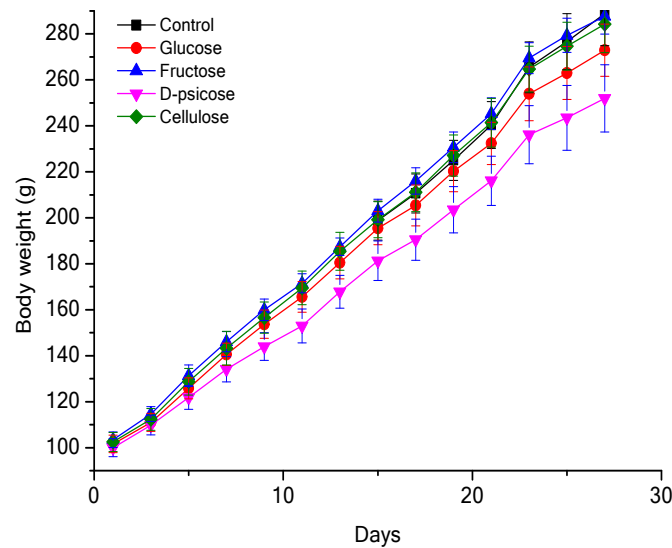


Figure 1. Effect of different carbohydrates on rat body weight. Rats were divided into five groups and followed AIN-76A diet containing 5% (w/w) of different carbohydrates (control/corn starch, glucose, fructose, D-psicose and cellulose). The body weight was measured every other day for a total of 28 days.

Table 1. Effects of dietary carbohydrates on the body weight and organ weight of rats in different groups.

Groups	Control	Glucose	Fructose	D-psicose	Cellulose
Initial weight (g)	100±6	101±5	103±4	100±7	102±5
Final weight (g)	291±16 ^a	272±14 ^{ab}	288±12 ^a	252±8 ^b	284±15 ^a
Weight gain (g)	192±12 ^a	171±12 ^b	184±10 ^{ab}	152±3 ^c	181±10 ^{ab}
Food intake (g/day)	25.7±3.1 ^b	28.2±2.4 ^b	28.3±4.4 ^b	29.1±1.7 ^b	34.1±2.7 ^a
Organ weight					
Liver (g)	10.3±0.9 ^a	8.8±0.6 ^b	9.5±0.4 ^{ab}	9.1±0.4 ^b	9.0±0.5 ^b
Heart (mg)	958±67	940±87	1058±82	946±50	1060±101
Soleus (mg)	110±17	136±27	118±13	106±15	104±15
Abdominal fat (g)	2.6±0.3 ^a	2.2±0.5 ^a	2.7±0.9 ^a	1.2±0.3 ^b	2.5±0.4 ^a
Epididymal fat (g)	6.7±0.4 ^a	5.2±1.0 ^a	6.3±0.7 ^a	3.4±0.3 ^b	6.5±1.3 ^a

All data are expressed as mean \pm SE (n=6). Data with different letters indicate statistical difference (p<0.05).

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Table 2. Effects of dietary carbohydrates on rat blood lipids.

	Control	Glucose	Fructose	D-psicose	Cellulose
TG (mmol/L)	1.42 \pm 0.48 ^a	1.36 \pm 0.23 ^{ab}	0.94 \pm 0.27 ^{ab}	0.83 \pm 0.11 ^b	1.20 \pm 0.31 ^{ab}
TC (mmol/L)	3.46 \pm 0.90	2.92 \pm 0.77	2.41 \pm 0.69	3.31 \pm 0.76	3.54 \pm 0.68
FFA (mmol/L)	0.94 \pm 0.21 ^a	0.60 \pm 0.06 ^b	0.58 \pm 0.07 ^b	0.42 \pm 0.04 ^b	0.94 \pm 0.07 ^a
LDL-C (mmol/L)	0.58 \pm 0.10 ^a	0.53 \pm 0.07 ^{ab}	0.39 \pm 0.10 ^{bc}	0.38 \pm 0.05 ^c	0.27 \pm 0.04 ^c

All data are expressed as mean \pm SE (n=6). Data with different letters indicate statistical difference (p<0.05). TG, triglyceride; TC, total cholesterol; FFA, free fatty acid; LDL-C, low density lipoprotein-cholesterol.

Table 3. Serum and hepatic level of SOD and CAT.

	Control	Glucose	Fructose	D-psicose	Cellulose
<i>Serum</i>					
SOD (U/mL)	326.3 \pm 23.2 ^{ab}	322.6 \pm 15.4 ^{ab}	296.3 \pm 24.4 ^b	352.2 \pm 16.6 ^a	335.4 \pm 18.2 ^{ab}
CAT (U/mL)	6.03 \pm 0.47 ^c	6.33 \pm 0.62 ^{bc}	6.47 \pm 0.63 ^{bc}	14.42 \pm 0.70 ^a	7.84 \pm 0.72 ^b
<i>Liver</i>					
SOD (U/mg prot)	323.2 \pm 35.1	348.3 \pm 37.7	335.4 \pm 35.8	383.3 \pm 39.9	326.5 \pm 31.1
CAT (U/mg prot)	10.31 \pm 1.23 ^b	10.69 \pm 1.54 ^b	11.62 \pm 1.71 ^b	27.39 \pm 1.49 ^a	13.58 \pm 1.30 ^b

SOD, superoxide dismutase; CAT, catalase. All data are expressed as mean \pm SE (n=6). Data with different superscript letters indicate statistical difference (p<0.05).

Table 4. Serum and hepatic level of SDH and HL.

	Control	Glucose	Fructose	D-psicose	Cellulose
<i>Serum</i>					
SDH (U/mL)	21.33 \pm 3.21 ^b	21.67 \pm 4.73 ^b	19.67 \pm 4.93 ^b	35.00 \pm 5.00 ^a	22 \pm 4.00 ^b
<i>Liver</i>					
SDH (U/mg prot)	2.72 \pm 0.80 ^b	3.15 \pm 1.11 ^{ab}	2.96 \pm 1.16 ^{ab}	5.42 \pm 0.94 ^a	3.59 \pm 0.84 ^{ab}
HL (U/mg prot)	1.33 \pm 0.28	1.21 \pm 0.34	1.44 \pm 0.33	1.69 \pm 0.37	1.37 \pm 0.29

SDH, succinate dehydrogenase; HL, hepatic lipase. All data are expressed as mean \pm SE (n=6). Data with different superscript letters indicate statistical difference (p <0.05).

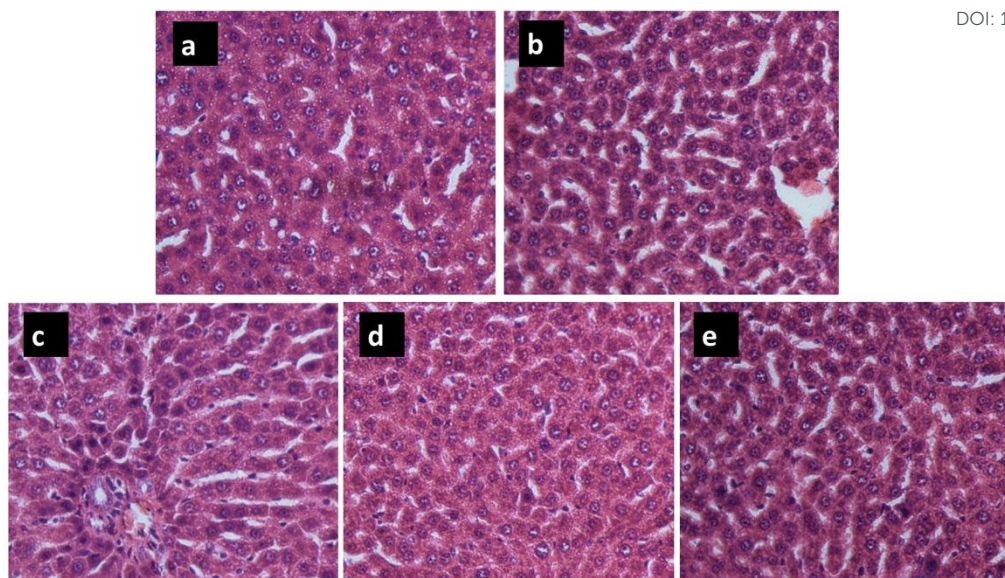


Figure 2. The effect of D-psicose on hepatic tissue morphology of rats supplemented with different carbohydrates (by HE stain $\times 400$). (a) control group, (b) glucose group, (c) fructose group, (d) D-psicose group, (e) cellulose group. Representative image from each group was used.

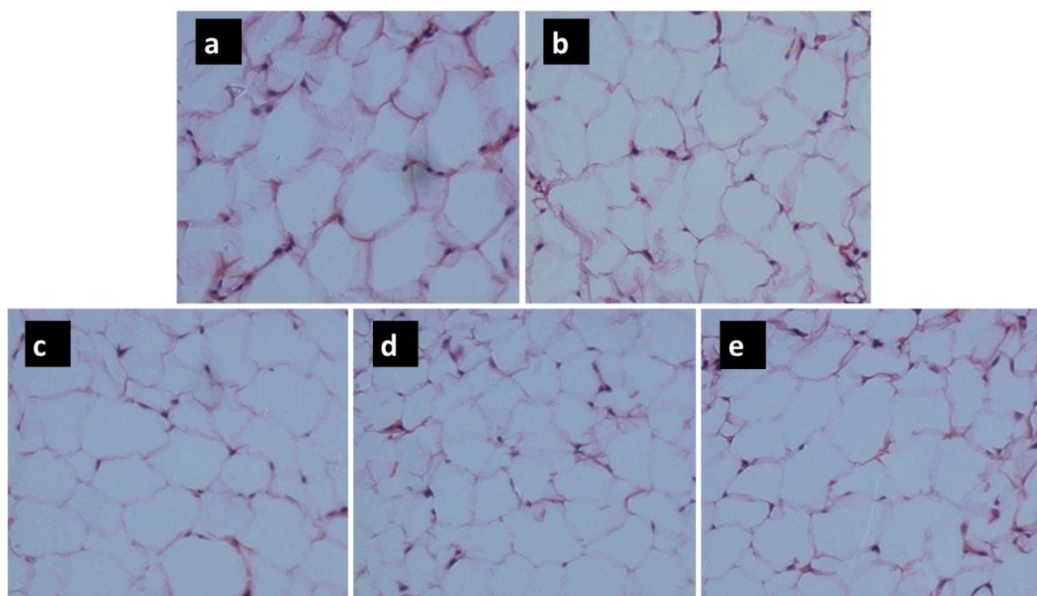


Figure 3. The effect of D-psicose on adipose tissue morphology of rats supplemented with different carbohydrates (by HE stain $\times 400$). (a) control group, (b) glucose group, (c) fructose group, (d) D-psicose group, (e) cellulose group. Representative image from each group was used.

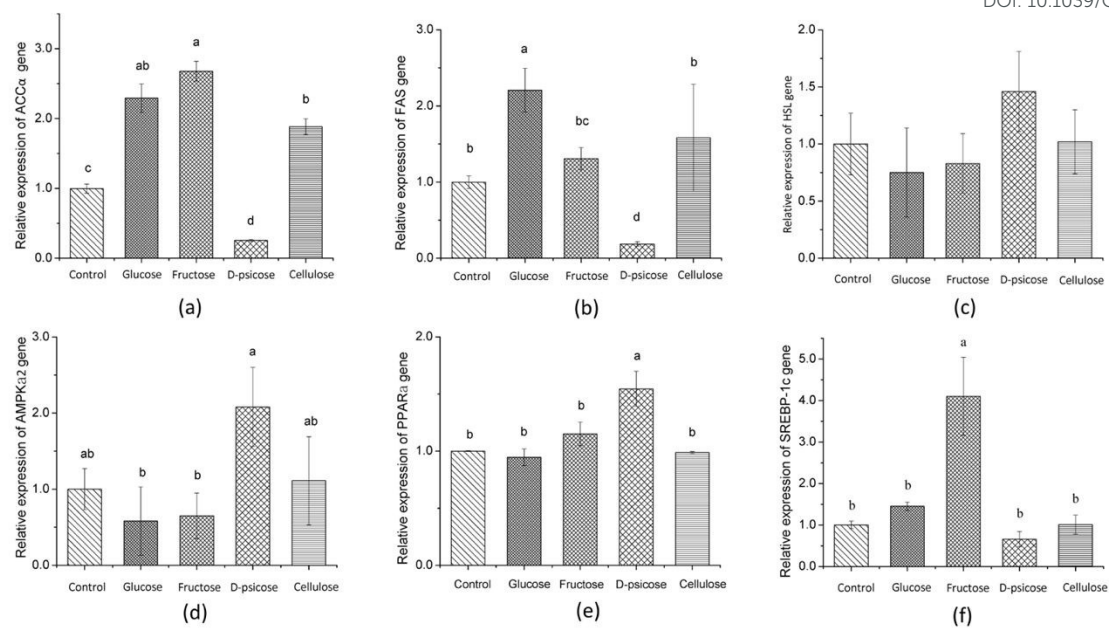
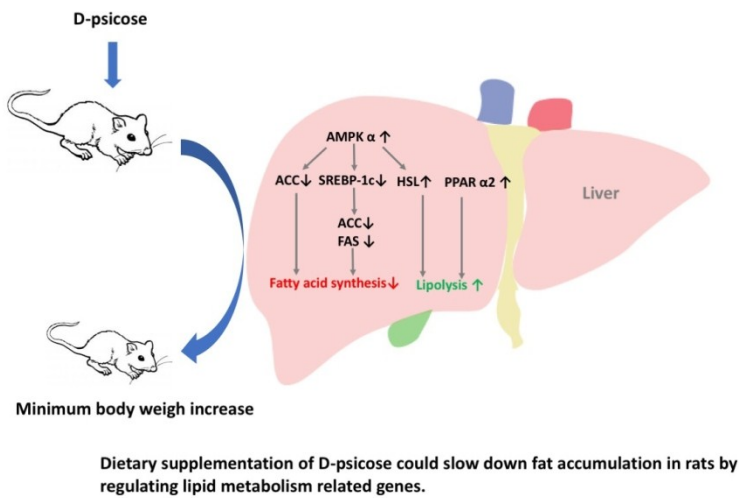


Figure 4. Effect of different sugars on RNA expression of enzymes participated in lipid synthesis and metabolism. (a) ACC α , (b) FAS, (c) HSL, (d) AMPK α 2, (e) PPAR α , (f) SREBP-1c. All values are means \pm SE (n=6). Different letters indicate statistical difference (P<0.05). ACC α : acethyl-CoA carboxylase; FAS, fatty acid synthase; HSL, hormone sensitive lipase; AMPK, AMP-activated protein kinase; PPAR, peroxisome proliferator activated receptors; SREBP, sterol regulatory element binding proteins.



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