Original Article

Effects of xylitol on metabolic parameters and visceral fat accumulation

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Xylitol is widely used as a sweetener in foods and medications. Xylitol ingestion causes a small blood glucose rise, and it is commonly used as an alternative to high-energy supplements in diabetics. In previous studies, a xylitol metabolite, xylulose-5-phosphate, was shown to activate carbohydrate response element binding protein, and to promote lipogenic enzyme gene transcription *in vitro*; however, the effects of xylitol *in vivo* are not understood. Here we investigated the effects of dietary xylitol on lipid metabolism and visceral fat accumulation in rats fed a high-fat diet. Sprague-Dawley rats were fed a high-fat diet containing 0 g (control), 1.0 g/100 kcal (X1) or 2.0 g/100 kcal (X2) of xylitol. After the 8-week feeding period, visceral fat mass and plasma insulin and lipid concentrations were significantly lower in xylitol-fed rats than those in high-fat diet rats. Gene expression levels of ChREBP and lipogenic enzymes were higher, whereas the expression of sterol regulatory-element binding protein 1c was lower and fatty acid oxidation-related genes were significantly higher in the liver of xylitol-fed rats as compared with high-fat diet rats. In conclusion, intake of xylitol may be beneficial in preventing the development of obesity and metabolic abnormalities in rats with diet-induced obesity.

Key Words: xylitol, visceral fat, lipid metabolism, lower insulin level, high fat diet

V isceral fat obesity has become a severe problem in the world and is a major risk factor for many diseases, such as metabolic syndrome, atherosclerosis, cardiovascular disease, stroke, and even cancer.[1–3] Recently, it has been established that obesity is caused by various environmental factors, including dietary energy content and nutrient composition.[4,5] A high-fat diet induces obesity, hyperglycemia, dyslipidemia and hyperinsulinemia, leading to insulin resistance and type 2 diabetes.[6,7] In rodents, such overnutrition as high-fat feeding activates sterol regulatory-element binding protein 1c (SREBP-1c) in the liver, and the increased accumulation of fat promotes metabolic syndrome.[8,9] In addition, SREBP-1c plays a key role in the mechanism of high-fat-induced obesity and insulin resistance in the liver.[10] A high-fat diet leads to the increased expression of fatty acid oxidation-related genes including peroxisome proliferators-activated receptor (PPAR) α in the liver to allow adaptation to the high intake of fat.[8] However, an impaired ability to increase fat oxidation in response to a high-fat diet leads to the development of obesity and insulin resistance.[11]

Xylitol is a five-carbon sugar alcohol with an energy value of 3 kcal/g. Significant quantities of xylitol have been detected in a wide variety of plants, including fruits and vegetables such as plums, strawberries, raspberries, and cauliflower.[12] It is used widely as a low-calorie sweetener in medications, dental care products, chewing gums, and candies. Xylitol is absorbed from the small intestine by passive diffusion and is mostly metabolized in the liver.[13] The ingestion of xylitol causes a smaller rise in plasma glucose and insulin concentrations than does the ingestion of glucose in healthy men and diabetics.[14,15] Hence, it has been used in patients with diabetes mellitus as an energy source in place of other carbohydrates. In the liver, xylitol is phosphorylated and metabolized to xylulose 5-phosphate (Xu5P), an intermediate of the nonoxidative branch of the pentose phosphate pathway.[16–18] The xylitol metabolite Xu5P specifically activates both nuclear transport and the DNA-binding activities of carbohydrate response element binding protein (ChREBP) through the activation of protein phosphatase 2A (PP2A) *in vitro*.[19,20] ChREBP is a transcription factor that activates lipogenic enzyme genes, such as acetyl coenzyme A carboxylase (ACC) and fatty acid synthase (FAS), and that stimulates lipogenesis in the liver.[21,22] Enhanced hepatic lipogenesis result in steatosis and obesity.[23,24] If xylitol stimulates lipogenesis, it might induce steatosis and obesity, leading to metabolic syndrome. In addition, the long-term effects of xylitol intake on lipid metabolism are not fully understood.

In the present study, we investigated the effects of dietary xylitol on visceral fat accumulation and lipid metabolism in rats fed a high-fat diet.

Materials and Methods

Animals. Sprague-Dawley (SD) rats were purchased from the Japan SLC (Hamamatsu, Japan) and used in all experiments. The rats were individually caged in the facility under a 12-h light/dark cycle and constant temperature (23 ± 2°C). Prior to the initiation of our study, the rats were fed a standard rodent diet (MF; Oriental Yeast, Tokyo, Japan) and water ad libitum. This study was approved by the Tokushima University Animal Use Committee, and the rats were maintained according to guidelines of Tokushima University for care of laboratory animals.

Diet and experimental design of long-term xylitol feeding test. Eighteen male, 9-week-old SD rats (weight 290–310 g) were used in a long-term xylitol feeding test. After acclimation for 2 weeks, the rats were divided into 3 groups (6 rats per group) and subjected to the following studies. The rats were fed one of three high-fat-based diets containing different amounts of xylitol: 0 g (HFD, control), 1.0 g/100 kcal (X1) or 2.0 g/100 kcal (X2). The exact composition of each diet is shown in Table 1. Xylitol was

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Table 1. Composition of experimental diets

Composition	Diet		
	HFD	X1	X2
	g/kg diet		
Milk casein	222.5	222.5	222.5
L-Cystine	2.5	2.5	2.5
Lard	150.0	150.0	150.0
Soybean oil	50.0	50.0	50.0
Cornstarch	312.3	267.3	222.3
α-Cornstarch	50.0	50.0	50.0
Sucrose	100.0	100.0	100.0
Xylitol	0.0	45.0	90.0
Vitamin mixture†	12.5	12.5	12.5
Mineral mixture†	50.0	50.0	50.0
Fiber (cellulose)	45.0	45.0	45.0
Choline bitartrate	3.2	3.2	3.2
tert-butylhydroquinone	0.01	0.01	0.01
Vitamin E acetate	2.0	2.0	2.0

HFD, high-fat diet; X1, high-fat diet containing xylitol at 1.0 g/100 kcal; X2, high-fat diet containing xylitol at 2.0 g/100 kcal. † AIN-93M[25].

substituted with starch, because sucrose contents have greater effects on serum lipid levels, postprandial glucose level, and body fat mass than starch.[26–28] To eliminate impact of differences in energy states on body fat mass, isocaloric pair-feeding was performed through the feeding period. Food intake was monitored daily, and body weight was recorded weekly throughout the feeding period. After 8 week, blood samples were collected from the tail vein for the determination of plasma glucose and insulin levels after 12 h of food deprivation. The rats were then anesthetized with diethylether and blood was withdrawn from the jugular vein for the determination of all other measurements. After the rats were killed by exsanguination, organs including the liver, visceral fat and soleus muscle were collected, weighted and stored at −80°C until analysis.

Plasma glucose, insulin and lipids measurements. Plasma glucose levels were measured by the glucose dehydrogenase method using an Accu-Chek blood glucose meter (Roche Diagnostics, Mainz-Hechtsheim, Germany). Plasma insulin levels were measured by ELISA (Morinaga, Yokohama, Japan). Plasma triglyceride, total cholesterol and non-esterified fatty acids (NEFA) concentrations were measured by enzymatic methods using Triglyceride E, Cholesterol E and NEFA C-tests (Wako, Osaka, Japan), respectively.

Hepatic lipids concentration. Hepatic lipids were extracted from 1.0 g of liver with chloroform/methanol (2:1 v/v), according to the method of Folch *et al*.[29] Triglyceride and cholesterol concentrations were determined in the extracted samples by commercial kits (Triglyceride E and Cholesterol E-tests).

RNA preparation and quantitative RT-PCR. Total RNA was isolated from frozen liver and mesenteric adipose tissue samples with TRIzol Regent (Invitrogen, Carlsbad, CA) and RNeasy kit (QIAGEN, Tokyo, Japan), respectively. First-standard cDNAs were synthesized with M-MLV reverse transcriptase (Invitrogen) and oligo-dT primer. We performed real-time PCR by using the primers described in Table 2, and SYBR green dye (SYBR Premix Ex Taq; TAKARA BIO, Shiga, Japan) in a LightCycler real-time PCR system (Roche Diagnostics), according to the manufacturer's instructions. The relative amounts of mRNA were calculated with β-actin mRNA as the invariant control. The ratio for the data from the HFD group was set arbitrarily at 1.

Primary culture of hepatocytes. Hepatocytes were isolated from male SD rats aged 6–10 weeks using the collagenase perfusion method.[30] The cells were cultured in glucose-free Dulbecco's

modified Eagle's medium (Invitrogen) supplemented with 1 nmol/l of insulin (Sigma, St. Louis, MO), 1 nmol/l of dexamethasone (Sigma), 10% (v/v) fetal bovine serum (Invitrogen), and 1% (v/v) penicillin-streptomycin (Sigma). After 6 h of attachment, the medium was removed and changed to fresh medium. After an overnight culture, cells were stimulated with 5 mmol/l xylitol for 16 h, after which total RNA was extracted (*n* = 3 in each experiment) and cDNA synthesis and real-time PCR analysis was performed.

Effects of xylitol on postprandial glucose and insulin levels in rats. Male SD rats aged 10–13 weeks were used in an oral sucrose tolerance test. Before 4 days of the oral study, rats underwent a catheterization to insert catheter into the left femoral vein as described previously.[31] After an overnight fast (18–20 h), sucrose (1 g/kg body weight), either alone or with xylitol (0.25 g/kg body weight), was orally administered to rats. Mannitol, a type of sugar alcohol, was used as a reference against xylitol. Blood samples were taken from the femoral vein of unanesthetized rats at 0, 30, 60 and 120 min after administration.

Statistical analysis. Values are expressed as means ± SEM. Significance of the differences was determined by analysis of variance (ANOVA) or the Kruskal-Wallis test, followed by the post-hoc test. Differences with a value of *p*<0.05 were considered significant. Statistical tests were performed with Excel-Toukei 2006 (SSRI, Tokyo, Japan).

Results

Xylitol supplimentation suppresses visceral fat accumulation induced by HFD. During the 8-week feeding period, the energy intake was similar in all groups (Table 3). Diarrhea was not found in any rats throughout the study. After the experimental period, body weight did not differ among the groups; however, the accumulation of visceral fat was significantly smaller in the xylitol-fed (X1 and X2) groups than in the control (HFD) group (*p*<0.05; Table 3). In particular, the relative weight of mesenteric fat was significantly lower in the xylitol-fed (X2) group than in the HFD group by 23.2% (*p*<0.05; Table 3). In addition, the relative weight of epididymal fat was significantly lower in the xylitol-fed (X1 and X2) groups than in the HFD group by 15.5% and 17.0%, respectively (*p*<0.05; Table 3). The relative weights of soleus muscle and liver, and the hepatic triglyceride and cholesterol concentrations were not affected by dietary interventions (Table 3).

Xylitol supplementation suppresses the increased insulinemia and lipidemia induced by HFD. The plasma glucose and non-esterified fatty acid (NEFA) concentrations did not differ among the three groups (Fig. 1). The plasma insulin and triglyceride concentrations were significantly lower in the xylitol-fed (X2) group than in the HFD group by 29.3% and 54.5%, respectively (*p*<0.05; Fig. 1). The plasma total cholesterol concentrations in the xylitol-fed (X1 and X2) groups were significantly reduced as compared with the HFD group (*p*<0.05; Fig. 1).

Adipose gene expression in xylitol long-term feeding test. As shown in Table 3, the visceral fat mass, especially mesenteric and epididymal fat mass, was significantly lower in the xylitol-fed (X1 and X2) groups than in the HFD group. Therefore, we examined the expression of lipid metabolism-related genes in the mesenteric adipose tissue by real-time RT-PCR. The mRNA levels of PPARγ, a key regulator of adipocyte differentiation, and the insulin-sensitizing hormone adiponectin were significantly upregulated in the xylitol-fed (X2) group as compared with the HFD group (*p*<0.05; Fig. 2). Moreover, the mRNA levels of hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), the lipolytic enzymes in adipocytes, were significantly elevated in the xylitol-fed (X2) group as compared with the HFD group (*p*<0.05; Fig. 2).

Hepatic gene expression in xylitol long-term feeding test. To investigate the molecular mechanisms underlying the

Table 2. Sequence of oligonucleotide primers for quantitative RT-PCR analysis

Gene name	Size (*bp*)	Accession No.	Primer sequence
PPARγ	147	AF156665	F: 5'-GGAAACTTGTGCAAGGTTGGA-3' R: 5'-CAGGCTCTACTTTGATCGCA-3'
adiponectin	140	NM_144744	F: 5'-GGAAACTTGTGCAAGGTTGGA-3' R: 5'-GGTCACCCTTAGGACCAAGA-3'
HSL	229	X51415	F: 5'-AGAGCCATCAGACAGCCCCGAGAT-3' R: 5'-TGACGAGTAGAGGGGCATGTGGAG-3'
ATGL	148	NM_001108509	F: 5'-GAGATGTGCAAACAGGGCTA-3' R: 5'-CAGTCCTCTCCTCAGTCACG-3'
SREBP-1c	190	AF286470	F: 5'-GGAGCCATGGATTGCACATTT-3' R: 5'-TCCTTCCGAAGGTCTCTCCTC-3'
ChREBP	113	AB074517	F: 5'-CAGCTTCTCGACTTGGACTG-3' R: 5'-TTGCCAACATAAGCGTCTTC-3'
ACC	233	J03803	F: 5'-CCAGTCTACATCCGCTTGGCTGAG-3' R: 5'-AGTCGCCAGTAGAAGAAGGTGCGG-3'
FAS	104	M76767	F: 5'-TGGGCCCATCTTCTTAGCC-3' R: 5'-GGAACAGCGCAGTACCGTAGA-3'
PPARα	112	M88529	F: 5'-TGTATGAAGCCATCTTCACG-3' R: 5'-GGCATTGAACTTCATAGCGA-3'
ACO	114	J02752	F: 5'-ATGGCAGTCCGGAGAATACCC-3' R: 5'-CCTCATAACGCTGGCTTCGAGT-3'
UCP2	107	NM_019354	F: 5'-TCTCCCAATGTTGCCCGAAA-3' R: 5'-GGGAGGTCGTCTGTCATGAG-3'
PGC-1α	109	AY237127	F: 5'-TGTTCGATGTGTCGCCTTGT-3' R: 5'-GAACGAGAGCGCATCCTTTG-3'
CYP7A1	127	NM_012942	F: 5'-CACCTTTGACGACATGGAGAAG-3' R: 5'-TGCTTTCATTGCTTCAGGACTC-3'
ABCG5	111	NM_053754	F: 5'-TGTGTTACTGGACTCTGGGC-3' R: 5'-CAAGCAGCACAAGTGTCAGA-3'
β-actin	171	NM_031144	F: 5'-GTCCCAGTATGCCTCTGGTCGTAC-3' R: 5'-CCACGCTCGGTCAGGATCTTCATG-3'

F, forward; R, reverse; PPAR, peroxisome proliferator-activated receptor; HSL, hormone sensitive lipase; ATGL, adipose trigyceride lipase; SREBP-1c, sterol regulatory-element binding protein 1c; ChREBP, carbohydrate response-element binding protein; ACC, acetyl coenzyme A carboxylase; FAS, fatty acid synthase; ACO, acyl coenzyme A oxidase; UCP2, uncoupling protein 2; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator 1α; CYP7A1, cholesterol 7α hydroxylase; ABCG5, ATP-binding cassette subfamily G member 5.

Table 3. Energy intake, body and organ weights, and hepatic lipid concentrations in rats fed three different diets for 8 week

|	HFD	X1	X2
Energy intake, *kcal/d*	95.5 ± 1.1	94.1 ± 1.2	93.5 ± 1.2
Body weight, *g*	543.2 ± 10.2	525.1 ± 9.6	540.4 ± 9.9
Visceral fat, *g/kg body weight*	96.7 ± 3.8	84.2 ± 5.3*	81.7 ± 4.2*
Mesenteric fat	27.5 ± 1.3	25.4 ± 1.3	21.1 ± 1.0*
Epididymal fat	30.2 ± 2.1	25.6 ± 1.5*	25.1 ± 1.9*
Retroperitoneal fat	39.0 ± 1.7	33.2 ± 3.9	35.5 ± 2.1
Soleus muscle, *g/kg body weight*	0.64 ± 0.02	0.68 ± 0.02	0.66 ± 0.04
Liver, *g/kg body weight*	29.7 ± 1.1	29.6 ± 1.1	28.4 ± 1.2
Liver triglyceride, *μmol/γ tissue*	21.1 ± 4.3	27.3 ± 5.4	22.6 ± 5.6
Liver cholesterol, *μmol/γ tissue*	3.7 ± 0.6	4.5 ± 0.6	3.9 ± 0.7

Values are mean ± SEM, *n* = 6. *$p < 0.05$ vs HFD group.

effects of xylitol on hepatic lipid metabolism *in vivo*, we examined the expression levels of lipid metabolism-related genes in the liver by real-time RT-PCR. The mRNA levels of ChREBP, and the lipogenic enzymes ACC and FAS were higher in the xylitol-fed (X1 and X2) groups than in the HFD group ($p < 0.05$; Fig. 3A). On the other hand, the mRNA level of another lipogenic transcription factor SREBP-1c, which regulated principally by insulin,[32] was significantly lower in the xylitol-fed (X2) group than in the HFD group ($p < 0.05$; Fig. 3A). The mRNA levels of the transcription factors PPARα and PPARγ coactivator 1α (PGC-1α) which regulate fatty acid oxidation were significantly higher in the xylitol-fed (X2) group than in the HFD group ($p < 0.05$; Fig. 3B). The mRNA levels of acyl-coenzyme A oxidase (ACO) and un-coupling protein 2 (UCP2), downstream target genes of PPARα were significantly higher in the xylitol-fed (X1 and X2) groups than in the HFD group ($p < 0.05$; Fig. 3B). Additionally, the expres-
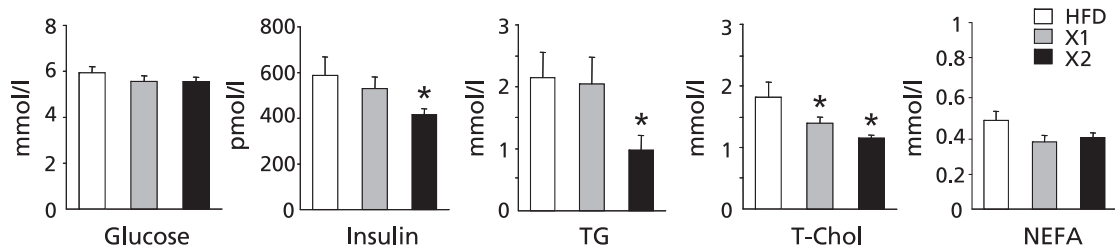
Fig. 1. Plasma glucose, insulin, and lipids levels in rats fed three different diets for 8 week. Values are mean ± SEM (n = 6 for each group). *$p<0.05$ vs HFD group. TG, triglyceride; T-Chol, total cholesterol; NEFA, non-esterified fatty acid.
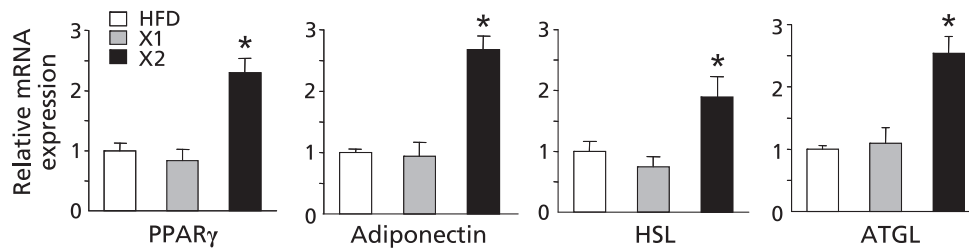
Fig. 2. Adipose gene expression in rats fed three different diets for 8 week. mRNA levels of genes in the mesenteric fat tissues were determined by quantitative RT-PCR analysis. Values are mean ± SEM (n = 5 for each group). *$p<0.05$ vs HFD group. The ratio for the data from the HFD group was set arbitrarily at 1. PPARγ, peroxisome proliferator-activated receptor γ; HSL, hormone sensitive lipase; ATGL, adipose triglyceride lipase.

sion levels of genes involved in bile acid synthesis and cholesterol metabolism such as cholesterol 7α hydroxylase (CYP7A1) and ATP-binding cassette, subfamily G, member 5 (ABCG5) increased in the xylitol-fed (X2) group compared with HFD group ($p<0.05$; Fig. 3C).

Effect of xylitol on gene expression in rat primary hepatocytes. Xylitol is metabolized predominantly in the liver,[13] so we next investigated the direct effect of xylitol on lipid metabolism-related gene expression using rat primary hepatocytes. Xylitol induced lipogenic gene expressions including ChREBP and FAS ($p<0.05$; Fig. 4A), as well as previously reported.[19,33] Moreover, xylitol also induced fatty acid oxidation-related gene expressions including PPARα and ACO as observed in long-term feeding test ($p = 0.057$ and $p<0.05$; Fig. 4B). However, the direct effect of xylitol on PPARα and ACO seems to be not so potent. In addition, xylitol stimulation did not change the expression level of SREBP-1c differently from the result *in vivo* (Fig. 4A). These results indicated that xylitol could also have the indirect effect on hepatic gene expression.

Xylitol suppresses postprandial rises in blood glucose and insulin in rats. In the long-term xylitol feeding test, fasting plasma insulin level was reduced in the xylitol-fed groups than in the HFD group (Fig. 1). One potential factor that regulates lipogenic gene expression is insulin. Thus, we investigated whether xylitol suppress the postprandial rises in blood glucose and insulin by performing the oral co-administration with sucrose to rats as described previously.[34,35] The plasma glucose levels were significantly reduced by the coadministration of xylitol at 30 min after the sucrose administration ($p<0.05$; Fig. 5A). In addition, the co-administration of xylitol tended to decrease the plasma insulin levels (Fig. 5B). There were no significant differences in the plasma glucose and insulin levels between the sucrose alone and the coadministration of mannitol (Fig. 5 A and B). These data showed that xylitol could suppress postprandial rise in glucose and insulin levels.

Discussion

In the present study, we demonstrated that long-term intake of xylitol suppressed the accumulation of visceral fat and the increase in plasma insulin and lipids concentrations in rats fed a high-fat diet. Intake of xylitol stimulated the expression of fatty acid oxidation genes in the liver, and lipid degradation and adiponectin genes in the adipose tissue. Furthermore, in oral sucrose tolerance test, we found for the first time that xylitol ingestion lowered postprandial hyperglycemia. The dose of xylitol in this study is the non-effective dose in causing diarrhea, and within the limits of orally administered physiological amounts (1–4 g/kg body weight daily) of xylitol on human and rats identified previously.[36,37]

The adipose tissue of xylitol-fed rats showed significantly higher levels of mRNAs encoding PPARγ, adiponectin, HSL and ATGL. These data indicates a miniaturization of adipocytes and lipolysis were caused in the adipose tissue, that they could contribute to lowering fat mass in xylitol-fed rats. PPARγ is mainly expressed in adipose tissue, which triggers adipocyte differentiation,[38] and it up-regulates the expression level of adiponectin in small adipocytes.[39] Adiponectin is an important modulator of insulin sensitivity and activates PPARα, thereby stimulating fatty acid oxidation in the liver.[40] In addition, PPARγ agonists can stimulate lipolysis by increasing the expression levels of lipolysis-related enzymes, including HSL and ATGL in adipose tissue.[41] Therefore, these observations suggest that the elevation in PPARγ expression in the adipose tissue of rats fed a high-fat diet with xylitol might contribute to the suppression of visceral fat accumulation and increased level of adiponectin mRNA that occurs concomitantly with the provision of fatty acid as an energy source to the liver. At present, however, it is uncertain how dietary xylitol promotes the expression of PPARγ in adipose tissue. Because xylitol is mainly metabolized in the liver[16–18] and little is taken up into adipose tissue,[42,43] the effects of xylitol on adipose tissue may be not a direct effect. Additional studies are needed to investigate the underlying mechanism.

In the liver, two main transcription factors regulate the gene
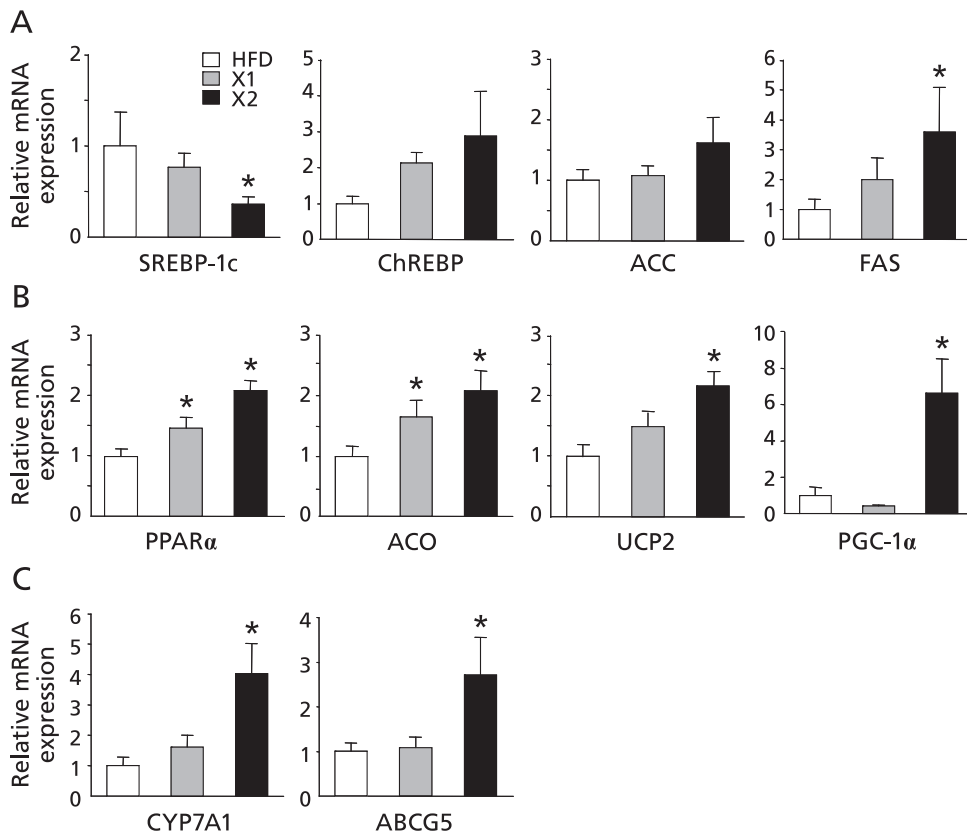
Fig. 3. Hepatic gene expression in rats fed three different diets for 8 week. mRNA levels of genes related to (A) lipogenesis, (B) fatty acid oxidation, and (C) cholesterol metabolism in the liver were determined by quantitative RT-PCR analysis. Values are mean ± SEM (n = 6 for each group). *$p<0.05$ vs HFD group. The ratio for the data from the HFD group was set arbitrarily at 1. SREBP-1c, sterol regulatory-element binding protein 1c; ChREBP, carbohydrate response-element binding protein; ACC, acetyl coenzyme A carboxylase; FAS, fatty acid synthase; PPARα, peroxisome proliferator-activated receptor α; ACO, acyl coenzyme A oxidase; UCP2, uncoupling protein 2; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator 1α; CYP7A1, cholesterol 7α hydroxylase; ABCG5, ATP-binding cassette subfamily G member 5.
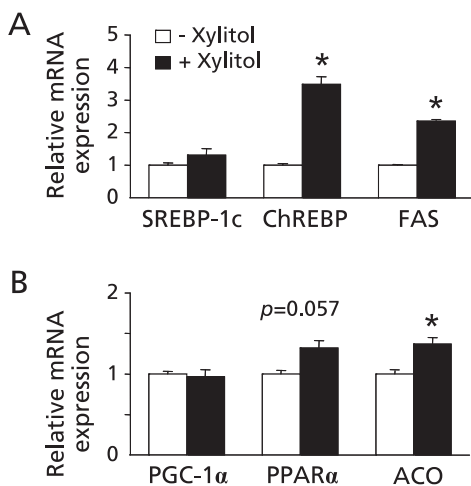
Fig. 4. Effects of xylitol on gene expression in rat primary hepatocytes. Xylitol stimulation was performed in rat primary hepatocytes, and mRNA levels of genes related to (A) lipogenesis and (B) fatty acid oxidation were determined by quantitative RT-PCR analysis. Values are mean ± SEM, n = 3. *$p<0.05$ vs control (- xylitol) group.

expression of lipogenic enzymes: one is SREBP-1c, whose expression can be up-regulated by insulin;[32] the other is ChREBP, which can be activated by the xylitol metabolite Xu5P through the activation of PP2A, independently of insulin.[21,22] Although it has been reported that SREBP-1c and ChREBP synergistically regulate lipogenesis,[44] the liver of xylitol-fed rats showed the increase in the expression of ChREBP and the reduction of SREBP-1c in the long-term xylitol feeding test, and caused the suppression of visceral fat accumulation, resulting from not trending toward an increase in lipid synthesis. When we tried to confirm the direct effect of xylitol on above-mentioned genes expression related to hepatic lipid metabolism by using rat primary hepatocytes, xylitol induced the gene expression of ChREBP, but did not affect SREBP-1c. In oral sucrose tolerance test, xylitol supplementation exhibited a suppression of postprandial hyper-glycemia. Therefore, the suppression of plasma insulin levels in xylitol-fed rats of the long-term test could be attributed to the suppression of postprandial hyperglycemia. The smaller demand for insulin secretion in xylitol-fed rats can contribute to lower the level of plasma insulin and the hepatic lipid metabolism-related gene expression. Because SREBP-1c is regulated mainly by insulin, a low level of SREBP-1c mRNA expression *in vivo* study might be caused by the suppression of insulin secretion in xylitol-fed rats.

In the current *in vivo* study, the liver of xylitol-fed rats showed a significant increase in mRNAs levels of the genes encoding PPARα, PGC-1α, ACO and UCP2, which are related to fatty acid
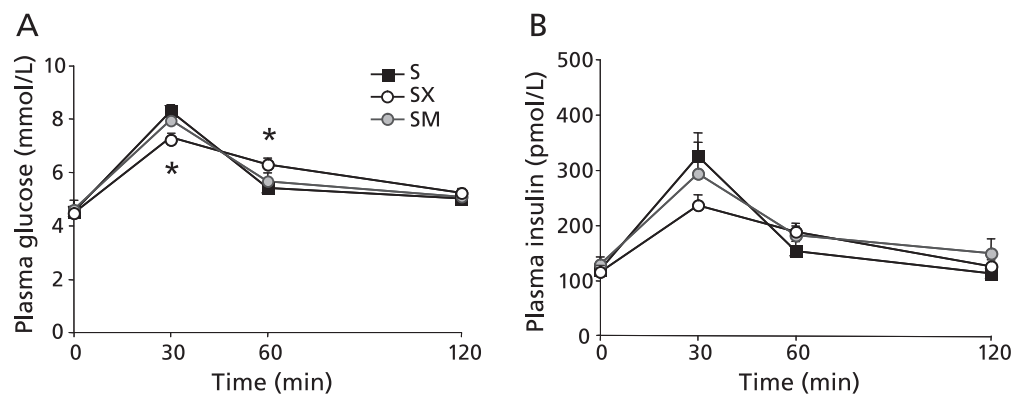
Fig. 5. Effect of oral sucrose administration with or without xylitol on plasma glucose and insulin levels. After fasting for 18–20 h, the rats were orally administrated sucrose (1 g/kg body weight) either alone or with xylitol or mannitol (0.25 g/kg body weight). Blood samples were taken at 0, 30, 60 and 120 min after administration. (A, B) Time-dependent curve for plasma levels of (A) glucose and (B) insulin. Values are mean ± SEM, n = 4–7. *$p < 0.05$ vs S group. S, sucrose (1 g/kg body weight); SX, sucrose (1 g/kg body weight) with xylitol (0.25 g/kg body weight); SM, sucrose (1 g/kg body weight) with mannitol (0.25 g/kg body weight).

oxidation.[45,46] Activation of PPARα not only suppresses adipocyte hypertrophy in adipose tissue but also stimulates fatty acid oxidation in the liver.[47] In addition, the rate of fatty acid oxidation in the liver is a major determinant of plasma triglyceride levels.[48] The increased fatty acid oxidation-related gene expression may also contribute to the suppression of visceral fat accumulation in xylitol-fed rats. However, it is likely that the direct effect of xylitol on the induction of PPARα and oxidation-related enzyme is not much powerful as shown Fig. 4B. The other candidate for the regulator of fatty acid oxidation can be suspected insulin. The attenuation of hyperinsulinemia enhances fat oxidation rates, and assist in preventing obesity and insulin resistance.[49,50] And, insulin negative-regulator of fatty acid oxidation-related gene expression including PPARα.[51,52] Therefore, it is possible that hepatic oxidation-related gene expression in xylitol-fed rats could be affected by insulin. Taken together, hepatic lipid metabolism in xylitol-fed rats might be affected predominantly by the suppression of insulin, and contribute to lower visceral fat accumulation. Because xylitol intake attenuated the increase in plasma insulin induced by HFD and postprandial hyperglycemia, it may be possible that intake of xylitol prevents the onset or progression of type 2 diabetes. However, further studies using model animals of diabetes are needed.

Conclusion

To date, xylitol is used widely in foods and medications, but its metabolite has been reported to activate ChREBP, up-regulating the gene transcription of lipogenic enzymes *in vitro*. This discrepancy suggests caution in the use of xylitol for the patients with obesity, type 2 diabetes, and other metabolic disorders. Our study has demonstrated that the intake of xylitol *in vivo* did not cause problems with lipogenesis, because of the suppression of high-fat induced-visceral fat accumulation. In addition, xylitol may have some beneficial effects such as lower postprandial hyperglycemia. These preferable effects suggest that xylitol intake

may be useful to control or prevent humans from obesity, diabetes, and other metabolic disorders.

Conflict of interest

No potential conflicts of interest were disclosed.

Abbreviations

ABCG5	ATP-binding cassette subfamily G member 5
ACC	acetyl coenzyme A carboxylase
ACO	acyl coenzyme A oxidase
ATGL	adipose trigyceride lipase
ChREBP	carbohydrate response-element binding protein
CYP7A1	cholesterol 7α hydroxylase
FAS	fatty acid synthase
HFD	high-fat diet
HSL	hormone sensitive lipase
PGC-1α	peroxisome proliferator-activated receptor-gamma coactivator 1α
PPAR	peroxisome proliferator-activated receptor
SREBP-1c	sterol regulatory-element binding protein 1c
UCP2	uncoupling protein 2
X1	high-fat diet containing xylitol at 1.0 g/100 kcal
X2	high-fat diet containing xylitol at 2.0 g/100 kcal

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