Songklanakarin J. Sci. Technol.
36 (4), 425-432, Jul. - Aug. 2014

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Xylitol does not directly affect adiponectin production and adipogenesis in 3T3-L1 cells

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Received 1 October 2013; Accepted 1 May 2014

Abstract

Xylitol is widely used as a low-calorie sweetener in various kinds of food products, including diabetic foods. Adiponectin, secreted by adipocytes, plays a key role in carbohydrate and lipid metabolism. Low levels of plasma adiponectin are associated with cardiovascular disease and type II diabetes. The aims of this study were to determine effects of xylitol on the adipogenesis of pre-adipocytes, adiponectin synthesis and secretion. To assess adipogenesis, pre-adipocyte 3T3-L1 cells were treated with xylitol during cell differentiation and fat droplets in the mature adipocytes were stained with oil red O. Adiponectin levels were determined by Western blot in both culture media and mature adipocytes treated with xylitol. There were no significant differences in the levels of adipogenesis, adiponectin synthesis and secretion in the xylitol-treated 3T3-L1 cells compared with the untreated control cells. This suggests that xylitol does not have a direct effect on adipogenesis or on adiponectin synthesis and secretion.

Keywords: 3T3-L1 cells, adiponectin, adipogenesis, xylitol, adipocyte

1. Introduction

Obesity, especially visceral fat obesity, is presently considered to be a severe public health problem, associated with metabolic syndrome, atherosclerosis, and cardiovascular disease (Akagiri *et al.*, 2008; Despres *et al.*, 2008). It is characterized by an increase in body fat mass which is a result of increases in either adipocyte number (hyperplasia) or adipocyte size (hypertrophy), caused by triglyceride accumulation within the cells (Drolet *et al.*, 2008; Jo *et al.*, 2009). Causes of obesity include environmental factors, especially increases in dietary intake. It has been reported that the development of obesity in rats fed with a high-fat diet involves both hypertrophy and hyperplasia of adipocytes (Li *et al.*, 2002; Marques *et al.*, 2000).

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Xylitol is a five-carbon sugar alcohol with hydrogenated monosaccharide properties. It is used as a low-calorie sweetener or food additive in various kinds of food products such as chewing gum, candies, soft drinks, energy drinks, dental care products and medications (Llop *et al.*, 2010; Ly *et al.*, 2006). In addition, xylitol is also used as a sweetener in diabetes diets for glycemic control because it provides low energy and has little effect on plasma glucose (Islam, 2011; Islam and Indrajit, 2012). Xylitol is slowly absorbed via the intestine by passive diffusion and is mainly metabolized in the liver (Dills, 1989), where it is metabolized to xylose-5-phosphate. This metabolite is an activator of the pentose phosphate pathway, which in turn stimulates fatty acid synthesis and increases in glucose-6-phosphate (Mourrieras *et al.*, 1997; Woods and Krebs, 1973). In contrast, xylitol has also been reported to reduce lipogenic and gluconeogenic enzymes such as glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme in the liver. Moreover, xylitol increases G6PD and malic enzyme in epididymal fat tissue (Ellwood *et al.*, 1999). In addition, rats fed with a high-fat diet

mixed with xylitol have decreased visceral fat mass, plasma insulin and lipid levels, but increased levels of peroxisome proliferator-activated receptor γ (PPARγ) and adiponectin mRNA, compared to rats fed with a high-fat diet alone (Amo *et al.*, 2011).

Differentiation of pre-adipocytes to mature adipocyte involves stimulations of ERK1/2, PI3K/Akt, and PPARγ pathway (Chuang *et al.*, 2007). Therefore, PPARγ is an important transcription factor that induces the expression of the adipocyte specific genes required for adipocyte differentiation (adipogenesis) (Porras *et al.*, 2002). Adiponectin is an adipokine, a peptide hormone synthesized from mature adipocytes in adipose tissue, produced as a monomer and assembled into low molecular weight (LMW) trimers, medium molecular weight (MMW) hexamers, and high molecular weight (HMW) oligomers of 12–18 monomeric units (Pajvani *et al.*, 2003). HMW adiponectin is biologically active, with lower levels associated with diabetes (Waki *et al.*, 2003). In addition, plasma adiponectin levels also decrease in obesity and cardiovascular diseases (Hotta *et al.*, 2000; Kawano and Arora, 2009; Pilz *et al.*, 2006). Adiponectin mediates its effects by binding to the adiponectin receptor found in skeletal muscle and liver. After the binding, the adenosine monophosphate-activated protein kinase pathway is activated (Yamauchi *et al.*, 2002), leading to an increase of β-oxidation of fatty acids and translocation of GLUT4 to the plasma membrane. Consequently, gluconeogenesis and hepatic glucose output are reduced (Kurth-Kraczek *et al.*, 1999). Furthermore, adiponectin helps reduce fat accumulation in macrophages and foam cells associated with atherosclerosis, by reducing oxidized LDL uptake and increasing HDL-mediated cholesterol efflux (Fu *et al.*, 2005; Tian *et al.*, 2009).

As mentioned above, the earlier study in rats fed with high-fat diet reveals that xylitol has effects on fat accumulation and expression of PPARγ and adiponectin mRNA synthesized from adipocytes. Therefore, the aims of this study were to assess if xylitol directly affects adipogenesis of pre-adipocyte 3T3-L1 cells and adiponectin production by mature adipocytes.

2. Methods and Materials

2.1 Chemical reagents

Mouse 3T3-L1 pre-adipocytes (American type culture collection, Manessas, VA) were kindly provided by Associate Professor Sarawut Jitrapakdee (Biochemistry Department, Faculty of Sciences, Mahidol University, Thailand). Dulbecco modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Gibthai, Thailand). 3-isobutyl-1-methylxanthine (IBMX), Dexamethasone, Oil Red O, xylitol, insulin, cycloheximide, Troglitazone and GW9662 were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Antibodies used included mouse anti-adiponectin, mouse monoclonal antibody (Chemicon International, Inc., Temecula, CA), anti-β-actin, mouse monoclonal antibody (Santa Cruz cruz biotechnology, California, USA), mouse anti-PPARg (E-8; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and goat anti-mouse HRP (Bio-Rad Laboratories, Hercules, CA).

2.2 Cell cytotoxicity

MTT (Thiazolyl Blue Tetrazolium Bromide) assay is a colorimetric assay, as described by Mosmann T, (Mosmann, 1983) and Bagchi *et al.*, 1998, (Bagchi *et al.*, 1998), used to study cell viability after xylitol treatments. The principle of MTT assay is based on the activity of cellular enzymes that metabolize the tetrazolium dye, MTT, to its purple insoluble formazan product, giving a color intensity that reflects cell viability. About 10,000 pre-adipocyte 3T3-L1 cells were cultured in a 96-well plate at 37ºC, 5% CO_2 overnight to allow cell attachment. Xylitol was then added in triplicates and cells were further incubated for 48 hours. After that, 20 µL of fresh MTT solution (5mg/mL) was added and incubated at 37ºC for 4 hours to allow MTT to be metabolized. All media were removed; formazan product was resuspended in 200 µL of dimethyl sulfoxide (DMSO), and the optical density (OD) was read at 570 nm against a background wavelength at 630 nm. Optical density reflects viable cell quantity. The data were represented as percentage of viable cells compared to control (cells treated with PBS), using the following equation; [OD value of sample / OD value of control] x 100.

2.3 Adipogenesis assay by Oil Red O staining

3T3-L1 differentiation was studied using the adipogenesis assay kit (Chemicon International, Inc., Temecula, CA). Pre-adipocytes 3T3-L1 cells were cultured in 24-well plates at 60,000 cells per well under different conditions; DMEM containing 10% FBS alone (no inducer/negative control), DMEM with IBMX and dexamethasone (inducers/control), and DMEM with IBMX, dexamethasone and various xylitol concentration (0.5, 5, 50 mM), 10 µM troglitazone (PPARγ agonist, an adipogenesis activator) or 5 µM GW9662 (PPARγ antagonist, adipogenesis inhibitor) for two days. Cells were then incubated in the same medium, except with insulin substituted for IBMX and dexamethasone for two days. Cells were then incubated with DMEM/10%FBS with or without various xylitol concentrations, troglitazone, or GW9662 for three days. At day 7 of differentiation, cells were stained with Oil Red O, and dye was extracted. Extracted dye was transferred to a 96-well plate and absorbance of extracted Oil Red O was measured in a plate reader at 490 nm.

2.4 Xylitol treatment on 3T3-L1 adipocytes

Pre-adipocytes 3T3-L1 cells were cultured in DMEM containing 25 mM glucose (DMEM/High glucose) supplemented with 10% FBS, 100U/mL penicillin and 100 µg/mL streptomycin at 37ºC in 5%CO_2. To induce differentiation, cells

were grown to confluence in 6-well tissue culture plates and stimulated after two-day post-confluence with the induction medium (0.5 mM IBMX, 250 nM dexamethasone, and 5 μg/mL insulin in DMEM/10% FBS) for 48 hours. The induction medium was then removed and replaced with 3T3-L1 adipocyte medium (5 μg/mL insulin in DMEM/10% FBS). The 3T3-L1 adipocyte medium was removed and replaced every two days. On day 6 after induction, when 3T3-L1 adipocytes had attained maturity, adipocyte medium was removed and replaced with serum-free medium (DMEM/low glucose) containing increasing concentrations of xylitol (0, 0.1, 0.25, 0.5, 5, 50 mM) for 24 or 48 hours. On day 7 or 8 after induction, the medium was aspirated and replaced with serum-free medium (DMEM/high glucose) containing 10 μg/mL cycloheximide (CHX) for eight hours to stop new protein synthesis (Miller *et al.*, 2008). After that, medium was collected and cells were harvested in PBS. Cell lysate was prepared for adiponectin and PPARg analysis by sonication, and analyzed for total protein. Equivalent amounts of total protein (10 mg for adiponectin and 25 μg for PPARg) were loaded for SDS-PAGE and Western blot analysis, as described below.

2.5 Analysis of adiponectin and PPARg by Western blot

For analysis of adiponectin and PPARγ, cell lysate was incubated with sample buffer [20 mM Tris-HCl, pH 6.8, 4% (w/v) SDS, 16% (v/v) glycerol, and 0.004% (w/v) bromophenol blue] containing 10% Dithiothreitol (DTT) as reducing agent for 5 min at 100ºC, to change oligomeric forms to monomeric forms, and resolved on 12.5% (w/v) SDS-polyacrylamide gels. For analysis of adiponectin oligomers, medium samples were incubated for 10 min at room temperature in non-reducing sample buffer without DTT and resolved on 5% (w/v) SDS-polyacrylamide gels. After electrophoresis, proteins were transferred to nitrocellulose membrane and incubated overnight with mouse anti-mouse adiponectin anti-body (1:5,000), mouse anti-mouse PPARγ antibody (1:100) or mouse anti-mouse actin antibody (1:1,000) followed by incubation for 1.5 hours with the goat anti-mouse HRP (1:5,000). Target protein was detected by chemiluminescence (Roche Diagnostics) and protein band densities were semi-quantified, as arbitrary units (a.u.), using ImageJ software. Adiponectin and PPARγ band densities were normalized with cellular actin. Three independent experiments were performed, each in triplicate.

2.6 Statistical analysis

The results are expressed as means±SD or means± SEM for triplicate of three independent experiments. Mann-Whitney tests were used to estimate differences between groups for adipogenesis assay. For adiponectin analysis, all treatments were compared by one-way ANOVA using Tukey's posthoc test to identify individual differences.

3. Results

3.1 Cell cytotoxicity detected by MTT assay

First, xylitol was tested to determine if it had cytotoxicity to 3T3-L1 cells by using MTT assay, a colorimetric assay measuring the quantity of viable cells. The results showed that viability of cells treated with xylitol at the range of 0.05 and 100 mM was higher than 80%, i.e. xylitol caused less than 20% cytotoxic effect (Figure 1). Therefore, all xylitol concentrations chosen in this study (0.1, 0.25, 0.5, 5, and 50 mM) caused minimal cytotoxic effects on the cells.

3.2 No effect of xylitol on adipogenesis of 3T3-L1

Xylitol was then tested for its effects on adipogenesis. Pre-adipocyte 3T3-L1 cells (Figure 2-A1) were treated with the differentiation inducers to create mature adipocytes (see methods and materials). Adipogenesis or mature adipocytes were examined by determining fat droplets within cells using oil red O staining (Figure 2-A2). Fat droplet accumulation correlates with increased absorbance of oil red O at 490 nm. Negative control, 3T3-L1 pre-adipocytes cultured in medium without the differentiation inducers, showed absorbance of 0.162±0.023, while control cells, cultured with the inducers, showed absorbance of 0.236±0.041, reflecting higher accumulations of fat droplets in the control than in the negative control. In addition, troglitazone and GW9662, which are a PPARγ activator and inhibitor, respectively, were tested. It was found that absorbance of troglitazone-treated cells was 0.270±0.045, while that of GW9662-treated cells was 0.133±0.028. These results showed that GW9662 and troglitazone significantly decreased (p=0.0002) and increased (p=0.049) 3T3-L1 adipogenesis compared to control, respec-

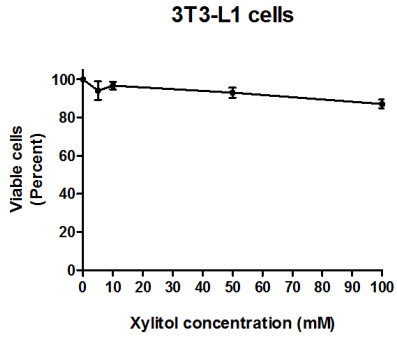

Figure 1. Effect of xylitol on viability of 3T3-L1 cells determined by MTT assay. Pre-adipocyte 3T3-L1 cells were cultured in the presence of xylitol (0.1, 0.25, 0.5, 5, 50 mM) for 48 hours. Cell viability was assessed using the MTT assay. The data is represented as percentage of viable cells compared to control using the following equation: [MTT OD value of sample/MTT OD value of control (cell treated with PBS)] x 100.

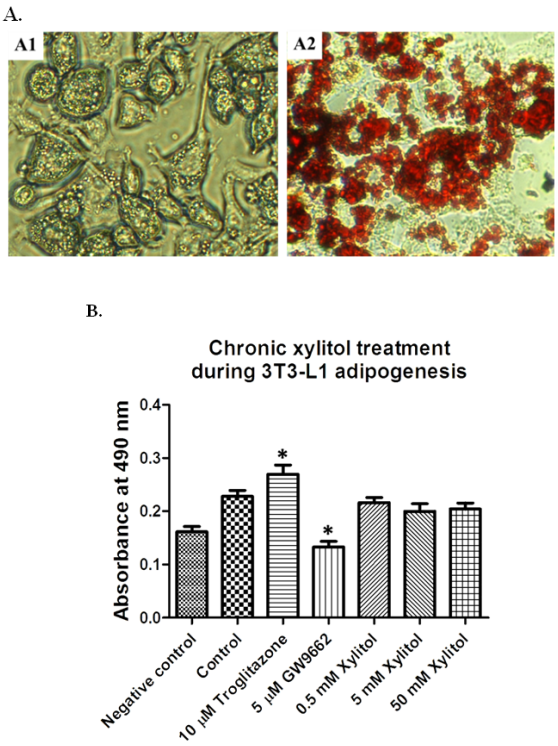

Figure 2. Effects of xylitol on adipogenesis of 3T3-L1 cells. Cells were plated in 24-well plates and incubated in 10% FBS/DMEM without differentiation inducers (negative control), with the inducer (control) or with the inducer and treatments. The treatments included xylitol (0.5, 5 and 50 mM), 5 mM GW9662, and 10 mM troglitazone. Cells were stained with Oil Red O and absorbance of extracted dye was measured at 490 nm. A: Representative images of unstained (A1) and oil Red O-stained (A2) mature adipocytes. B: Absorbances of oil Red O in adipogenesis assay are shown as mean±SD (n=6-9). *p<0.05 vs Control. [Control vs troglitazone; p=0.049, Control vs GW9662; p= 0.0002]

tively. For xylitol-treated cells, the absorbances were 0.216±0.026, 0.200±0.037 and 0.204±0.033 for 0.5, 5, and 50 mM xylitol treatments, respectively. Xylitol slightly decreased the absorbance, compared to the control. However, there was no significant difference in absorbance among these xylitol-treated cells and control (p>0.05) (Figure 2-B).

3.3 Xylitol does not directly affect adiponectin synthesis and secretion

In addition, xylitol was examined for its effects on adiponectin production. Pre-adipocyte 3T3-L1 cells were grown and differentiated to mature adipocytes, and then treated with various xylitol concentrations (0, 0.1, 0.25, 0.5, 5 and 50 mM) for 24 and 48 hours. Cell lysates and culture media were collected for analysis of adiponectin in intracellular and secreted pools, respectively, by using Western blot technique. For cell lysates, adiponectin and β-actin band densities were assessed (Figure 3-A) and calculated as adiponectin/β-actin ratio. Fold changes in adiponection/β-actin ratio induced by 24 and 48 hours xylitol treatment compared to control are shown in Figure 3-B respectively. The results show that 24 and 48 hour xylitol treatments did not significantly affect the intracellular pool of adiponectin, hence its production (p>0.05).

For the secreted pool of adiponectin, the culture media were analyzed after 48 hours of xylitol treatments and additional incubation for eight hours with CHX, to block new adiponectin synthesis. Adipocytes can secrete different forms of adiponectin; HMW, MMW, and LMW, which are approximately 270-300 kDa, 180 kDa, and 90 kDa, respectively (Figure 4-A). To assess different forms of adiponectin, secreted adiponectin from xylitol-treated cells was analyzed by SDS-PAGE without reducing agent in the sample buffer and immunoblot. Band densities of HMW, MMW, and LMW adiponectin were assessed and fold changes of each adiponectin forms secreted by xylitol-treated 3T3-L1 cells, compared to control cells, are shown in Figure 4-B. After 48-hour xylitol treatments, the secreted levels of LMW form showed a slight increase, while HMW form showed a slight decrease, compared to the control. However, the sum of LMW, MMW and HMW forms secreted by xylitol-treated

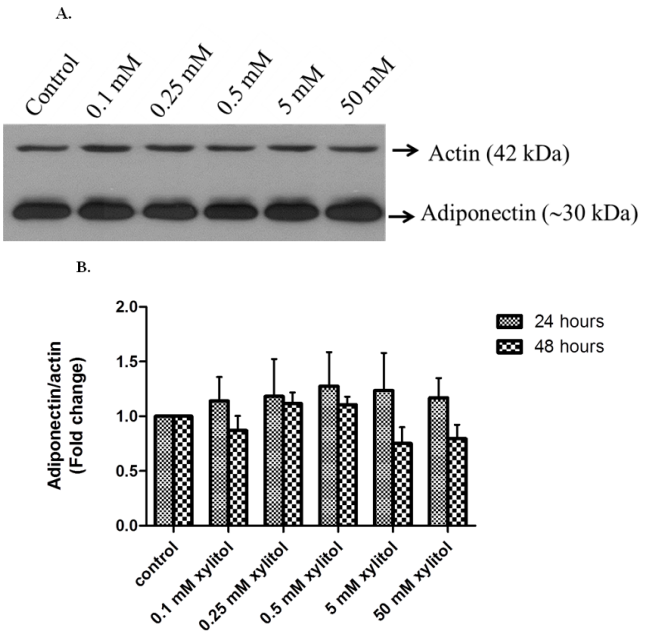

Figure 3. Effects of xylitol on adiponectin synthesis in mature adipocytes. Mature 3T3-L1 cells were treated without (control) or with xylitol (0.1, 0.25, 0.5, 5 and 50 mM) for 24 and 48 hours. Adiponectin mass in cell lysates were determined by SDS-PAGE and Western blot. A: Immunoblot shows adiponectin and β-actin bands at 30 and 42 kDa, respectively. B: Adiponectin band densities were normalized with β-actin. The data are presented as fold changes of adiponectin/actin, compared with the control. These results are shown as mean±SEM from three experiments.

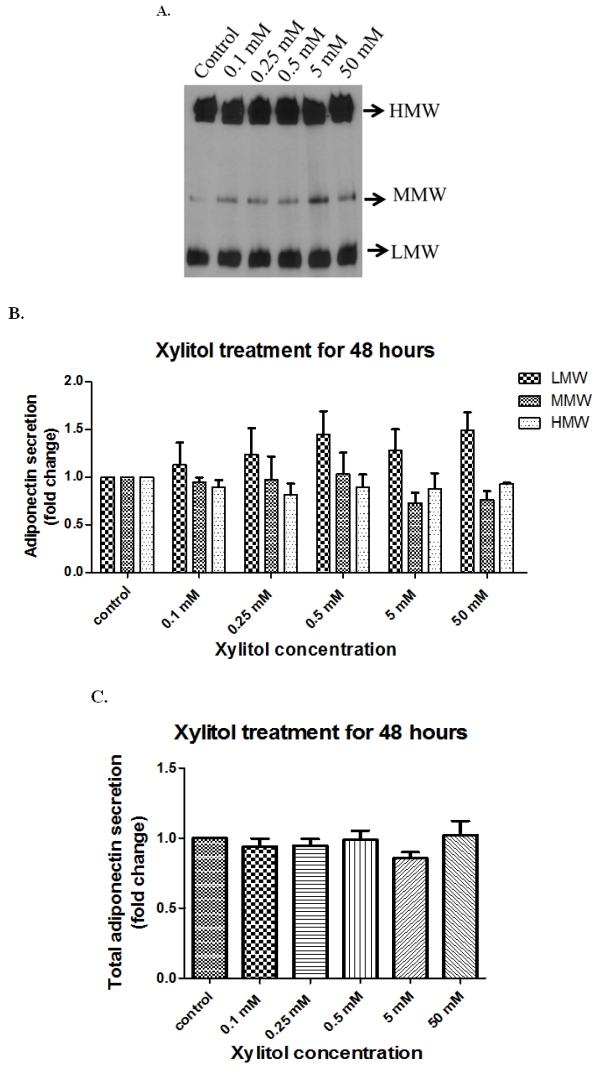

Figure 4. Effects of xylitol on adiponectin secretion. Mature 3T3-L1 cells were treated without (control) or with xylitol (0.1, 0.25, 0.5, 5 and 50 mM) for 48 hours. Secreted adiponectin in the culture medium was determined by SDS-PAGE without reducing agent and Western blot. A: Immunoblot shows HMW, MMW and LMW forms of adiponectin. B: Adiponectin band densities were determined and the amounts of HMW, MMW and LMW forms of adiponectin are presented as fold changes, compared to control. C: Total adiponectin was the sum of all band densities of three adiponectin forms. Data were normalized and presented as fold changes, compared to control. These results are shown as mean±SEM from three experiments.

cells were not significantly different from that secreted by control cells (p>0.05). Fold changes of the sum of secreted adiponectin are presented in Figure 4-C. Mature adipocytes treated with xylitol (0.1, 0.25, 0.5, 5 and 50 mM) secreted similar amounts of adiponectin to control cells. This result indicates that xylitol did not affect the adiponectin secretion from 3T3-L1 adipocytes.

3.4 Xylitol does not affect PPARg synthesis

PPARγ has been shown to have an important role in adipogenesis because its activation and inhibition cause adipogenesis stimulation and suppression, respectively. To examine effects of xylitol on PPARγ synthesis in mature adipocytes, differentiated 3T3-L1 adipocytes were treated with various xylitol concentrations (0, 5, and 50 mM) for 48 hours. Cell lysates were collected to determine protein levels of PPARγ by Western blot technique. The results were presented as the PPARγ/actin ratios compared to control (Figure 5-A, 5-B). It was shown that xylitol had no significant effect on the PPARg/actin ratios (p>0.05).

4. Discussion

Xylitol is widely used as a sweetener and is involved in lipid and carbohydrate metabolism. Understanding the effect of xylitol on adipogenesis is important because both adipocyte hyperplasia and hypertrophy can contribute to the onset of obesity, which is a risk factor of metabolic syndrome, insulin resistance, and cardiovascular disease. Adiponectin is also another important factor associated with diabetes and cardiovascular events. Plasma adiponectin is reduced in obesity, insulin resistance, type II diabetes and cardiovascular disease (Fasshauer *et al.*, 2004; Hotta *et al.*, 2000; Otsuka *et al.*, 2006; Pilz *et al.*, 2006). Therefore, the effects of xylitol

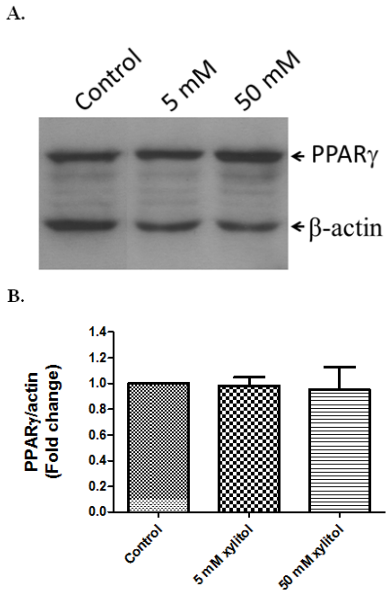

Figure 5. Effects of xylitol on PPARg gene expression in mature adipocytes. Mature 3T3-L1 cells were treated without (control) or with xylitol (5 and 50 mM) for 48 hours. The amounts of PPAR protein in mature adipocytes were determined by Western blot. A: Immunoblot shows PPARγ and β-actin bands at 57 and 42 kDa, respectively. B: PPARγ band density was normalized with β-actin and presented as fold changes of PPARγ/actin ratio. These results are shown as mean±SEM.

on adipogenesis and adiponectin production were assessed in the present study. In this study, xylitol was tested using an in-vitro strategy for its effects using chronic treatment on 3T3-L1 adipogenesis and acute treatment of differentiated adipocytes for adiponectin production.

In comparison with control, there was higher absorbance of oil red O extracted from 10 μM troglitazone-treated cells and lower absorbance of dye extracted from 5 μM GW9662-treated cells. This validates the use of our adipogenesis assay system to effectively examine in xylitol-treated cells. Unexpectedly, we found that xylitol did not significantly affect adipogenesis of 3T3-L1 cells (p >0.05) compared with the control. In addition, pre-adipocyte 3T3-L1 cells treated with xylitol tended to differentiate to mature adipocytes at a lower rate than the control. This suggests that xylitol does not promote and cause problems with adipocyte hyperplasia or hypertrophy in adipose tissue. This is consistent with the findings by Amo *et al.* (2011). They reported that rats fed with a high-fat diet mixed with xylitol for 8 weeks (long term) had decreased visceral fat mass compared with rats fed with a high-fat diet alone as a control.

In addition, it has been reported that rats fed with a high-fat diet mixed with xylitol had decreased plasma insulin and lipid levels, but increased PPARγ and adiponectin mRNA levels (Amo *et al.*, 2011). PPARγ, which is mainly expressed in adipose tissue, triggers adipocyte differentiation and up-regulates adiponectin gene expression (Hamm *et al.*, 1999; Just, 2010). Our study found that adiponectin within xylitol-treated cells, and the culture media was not significantly different from the control. In addition, PPARγ in xylitol-treated cells was not changed, compared with the control. It is possible that the increase of adiponectin mRNA levels in rats fed with xylitol is not a direct effect of xylitol on adipocytes. It is likely that there are other contributing factors such as decreased fat accumulation in adipose tissue and suppressed plasma insulin (Amo *et al.*, 2011). High plasma insulin might be an important factor since it is related to high lipid accumulation within adipocytes in adipose tissues. However, there is no reported evidence of high plasma insulin in xylitol-fed rats (Amo *et al.*, 2011; Ellwood *et al.*, 1999) because xylitol intake attenuated the increase in plasma insulin induced by high-fat diet. In the previous report, it has been found that lipogenic and gluconeogenic enzymes were increased in epididymal fat tissue of xylitol-fed rats (Ellwood *et al.*, 1999), but no data has shown an increase of lipid accumulation in adipose tissue (Amo *et al.*, 2011). Further studies are needed to investigate these potential mechanisms.

Xylitol concentrations chosen for these cultured cell treatments were similar to the xylitol treatments in HepG2 cells, hepatocytes (Mourrieras *et al.*, 1997; Woods and Krebs, 1973) and 3T3-L1 cells (Wang *et al.*, 1999). The xylitol concentrations used in the present study were responsible for less than 20% toxicity as measured by MTT assay. Xylitol is mainly metabolized in liver cells, but a small amount is taken up into adipose tissue (<5%) (Quadflieg and Brand, 1976). This may be the cause for the non-significant change in the amount of adiponectin in all xylitol-treated cells compared with the control. However, CCAAT/Enhancer-binding protein-∝ (C/EBP∝) gene expression decreases in 10 mM xylitol treated 3T3-L1 cells (Wang *et al.*, 1999). Thus, the down-regulating effects of high xylitol concentration on the expression of C/EBP∝ mRNA suggest that xylitol is taken up, metabolized and affects gene expression in 3T3-L1 adipocytes. Therefore, 0.5-50 mM xylitol concentrations used in the present study should have been taken up and metabolized by the 3T3-L1 cells.

In conclusion, 0.5-50 mM xylitol treatments did not directly affect adiponectin synthesis and secretion of differentiated adipocytes, contrasting with previous reports, which showed an increase in adiponectin mRNA in rats fed with xylitol. In addition, xylitol did not promote the differentiation of pre-adipocytes to mature adipocytes. Therefore, xylitol intake may be useful in food supplements for obesity, diabetes, and metabolic disorders because it did not affect adipocyte hyperplasia and visceral fat accumulations.

Acknowledgements

We thank Dr. Stefano O. Casalotti for critical reading of the manuscript. This work was financially supported by Thammasat University and National Research Council of Thailand (NRCT). The authors declare no conflicts of interest.

Abbreviations

LMW; low molecular weight trimers,
MMW; medium molecular weight or hexamers
HMW; high molecular weight

References

Akagiri, S., Naito, Y., Ichikawa, H., Mizushima, K., Takagi, T., Handa, O., Kokura, S., Yoshikawa, T. 2008. A mouse model of metabolic syndrome; Increase in visceral adipose tissue precedes the development of fatty liver and insulin resistance in high-fat diet-fed male KK/Ta mice. Journal of Clinical Biochemistry and Nutrition. 42, 150-157.

Amo, K., Arai, H., Uebanso, T., Fukaya, M., Koganei, M., Sasaki, H., Yamamoto, H., Taketani, Y., Takeda, E. 2011. Effects of xylitol on metabolic parameters and visceral fat accumulation. Journal of Clinical Biochemistry and Nutrition. 49, 1-7.

Bagchi, D., Kuszynski, C., Balmoori, J., Bagchi, M., Stohs, S.J. 1998. Hydrogen peroxide-induced modulation of intracellular oxidized states in cultured macrophage J774A.1 and neuroactive PC-12 cells, and protection by a novel grape seed proanthocyanidin extract. Phytotherapy Research. 12, 568-571.

Chuang, C.C., Yang, R.S., Tsai, K.S., Ho, F.M., Liu, S.H. 2007. Hyperglycemia enhances adipogenic induction of lipid accumulation: involvement of extracellular signal-regulated protein kinase 1/2, phosphoinositide 3-kinase/Akt, and peroxisome proliferator-activated receptor gamma signaling. Endocrinology. 148, 4267-4275.

Despres, J.P., Lemieux, I., Bergeron, J., Pibarot, P., Mathieu, P., Larose, E., Rodes-Cabau, J., Bertrand, O.F., Poirier, P. 2008. Abdominal obesity and the metabolic syndrome: Contribution to global cardiometabolic risk. Arteriosclerosis Thrombosis and Vascular Biology. 28, 1039-1049.

Dills, W.L., Jr., 1989. Sugar alcohols as bulk sweeteners. Annual Review of Nutrition. 9, 161-186.

Drolet, R., Richard, C., Sniderman, A.D., Mailloux, J., Fortier, M., Huot, C., Rheaume, C., Tchernof, A. 2008. Hypertrophy and hyperplasia of abdominal adipose tissues in women. International Journal of Obesity. 32, 283-291.

Ellwood, K.C., Bhathena, S.J., Johannessen, J.N., Bryant, M.A., O'Donnell, M.W. 1999. Biomarkers used to assess the effect of dietary xylitol or sorbitol in the rat. Nutrition Research. 19, 1637-1648.

Fasshauer, M., Paschke, R., Stumvoll, M. 2004. Adiponectin, obesity, and cardiovascular disease. Biochimie. 86, 779-784.

Fu, Y., Luo, N., Klein, R.L., Garvey, W.T. 2005. Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. Journal of Lipid Research. 46, 1369-1379.

Hamm, J.K., el Jack, A.K., Pilch, P.F., Farmer, S.R. 1999. Role of PPAR gamma in regulating adipocyte differentiation and insulin-responsive glucose uptake. Annals of the New York Academy Sciences. 892, 134-145.

Hotta, K., Funahashi, T., Arita, Y., Takahashi, M., Matsuda, M., Okamoto, Y., Iwahashi, H., Kuriyama, H., Ouchi, N., Maeda, K., Nishida, M., Kihara, S., Sakai, N., Nakajima, T., Hasegawa, K., Muraguchi, M., Ohmoto, Y., Nakamura, T., Yamashita, S., Hanafusa, T., Matsuzawa, Y. 2000. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thrombosis and Vascular Biology. 20, 1595-1599.

Islam, M.S. 2011. Effects of Xylitol as a Sugar Substitute on Diabetes-Related Parameters in Nondiabetic Rats. Journal of Medicinal Food. 14, 505-511.

Islam, M.S., Indrajit, M. 2012. Effects of Xylitol on Blood Glucose, Glucose Tolerance, Serum Insulin and Lipid Profile in a Type 2 Diabetes Model of Rats. Annals of Nutrition and Metabolism. 61, 57-64.

Jo, J., Gavrilova, O., Pack, S., Jou, W., Mullen, S., Sumner, A.E., Cushman, S.W., Periwal, V. 2009. Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth. Plos Computational Biology. 5.

Just, W. 2010. Focus on ... the role of PPAR gamma in adipogenesis Introduction. Febs Letters. 584, 3241-3241.

Kawano, J., Arora, R. 2009. The role of adiponectin in obesity, diabetes, and cardiovascular disease. Journal of the Cardiometabolic Syndrome. 4, 44-49.

Kurth-Kraczek, E.J., Hirshman, M.F., Goodyear, L.J., Winder, W.W. 1999. 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. Diabetes. 48, 1667-1671.

Li, J., Yu, X., Pan, W., Unger, R.H. 2002. Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity. American Journal of Physiology - Endocrinology and Metabolism. 282, E1334-1341.

Llop, M.R., Jimeno, F.G., Acien, R.M., Dalmau, L.J.B. 2010. Effects of xylitol chewing gum on salivary flow rate, ph, buffering capacity and presence of *Streptococcus mutans* in saliva. European Journal of Paediatric Dentistry. 11, 9-14.

Ly, K.A., Milgrorn, P., Rothen, M. 2006. Xylitol, sweeteners, and dental caries. Pediatric Dentistry. 28, 154-163.

Marques, B.G., Hausman, D.B., Latimer, A.M., Kras, K.M., Grossman, B.M., Martin, R.J. 2000. Insulin-like growth factor I mediates high-fat diet-induced adipogenesis in Osborne-Mendel rats. American Journal of Physiology - Regulatory, Integrative and Comparative. 278, R654-662.

Miller, J.R., Siripurkpong, P., Hawes, J., Majdalawieh, A., Ro, H.S., McLeod, R.S. 2008. The trans-10, cis-12 isomer of conjugated linoleic acid decreases adiponectin assembly by PPARgamma-dependent and PPAR gamma-independent mechanisms. Journal of Lipid Research. 49, 550-562.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 65, 55-63.

Mourrieras, F., Foufelle, F., Foretz, M., Morin, J., Bouche, S., Ferre, P. 1997. Induction of fatty acid synthase and S14 gene expression by glucose, xylitol and dihydroxyacetone in cultured rat hepatocytes is closely correlated with glucose 6-phosphate concentrations. Biochemical Journal. 326 (Pt 2), 345-349.

Otsuka, F., Sugiyama, S., Kojima, S., Maruyoshi, H., Funahashi, T., Matsui, K., Sakamoto, T., Yoshimura, M., Kimura, K., Umemura, S., Ogawa, H. 2006. Plasma adiponectin levels are associated with coronary lesion complexity in men with coronary artery disease. Journal of the American College of Cardiology. 48, 1155-1162.

Pajvani, U.B., Du, X., Combs, T.P., Berg, A.H., Rajala, M.W., Schulthess, T., Engel, J., Brownlee, M., Scherer, P.E. 2003. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. Journal of Biological Chemistry. 278, 9073-9085.

Pilz, S., Maerz, W., Weihrauch, G., Sargsyan, K., Almer, G., Nauck, M., Boehm, B.O., Winkelmann, B.R., Mangge, H. 2006. Adiponectin serum concentrations in men with coronary artery disease: the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study. Clinica Chimica Acta. 364, 251-255.

Porras, A., Valladares, A., Alvarez, A.M., Roncero, C., Benito, M. 2002. Differential role of PPAR gamma in the regulation of UCP-1 and adipogenesis by TNF-alpha in brown adipocytes. Febs Letters. 520, 58-62.

Quadflieg, K.H., Brand, K. 1976. Comparison of xylitol and glucose metabolism in nonhepatic rat tissues. Z Ernahrungswiss. 15, 345-354.

Tian, L., Luo, N., Klein, R.L., Chung, B.H., Garvey, W.T., Fu, Y. 2009. Adiponectin reduces lipid accumulation in macrophage foam cells. Atherosclerosis. 202, 152-161.

Waki, H., Yamauchi, T., Kamon, J., Ito, Y., Uchida, S., Kita, S., Hara, K., Hada, Y., Vasseur, F., Froguel, P., Kimura, S., Nagai, R., Kadowaki, T. 2003. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. Journal of Biological Chemistry. 278, 40352-40363.

Wang, Y., Lee-Kwon, W., Martindale, J.L., Adams, L., Heller, P., Egan, J.M., Bernier, M. 1999. Modulation of CCAAT/enhancer-binding protein-alpha gene expression by metabolic signals in rodent adipocytes. Endocrinology. 140, 2938-2947.

Woods, H.F., Krebs, H.A. 1973. Xylitol metabolism in the isolated perfused rat liver. Biochemical Journal. 134, 437-443.

Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., Yamashita, S., Noda, M., Kita, S., Ueki, K., Eto, K., Akanuma, Y., Froguel, P., Foufelle, F., Ferre, P., Carling, D., Kimura, S., Nagai, R., Kahn, B.B., Kadowaki, T. 2002. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature Medicine. 8, 1288-1295.