Research Article

Metabolic response of soy pinitol on lipid-lowering, antioxidant and hepatoprotective action in hamsters fed-high fat and high cholesterol diet

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This study was performed to investigate the lipid-lowering, antioxidant, and hepato-protective effects of pinitol in dose-dependent manners in hamsters fed-high fat and high cholesterol (HFHC) diet. Pinitol supplementation (0.05%, P-I and 0.1% pinitol, P-II) with an HFHC diet (10% coconut oil plus 0.2% cholesterol) for 10 wks significantly lowered the white adipose tissue weights, hepatic lipid droplets, plasma glucose, total-cholesterol, nonHDL-cholesterol, total-cholesterol/HDL-cholesterol ratio, and hepatic lipid levels. Whereas it significantly increased the brown adipose tissue weight, plasma HDL-cholesterol, apolipoprotein A-I (apo A-I) concentrations, paraoxonase (PON) activity, and/or mRNA expression, compared to the HFHC control group. Plasma insulin and adiponectin levels were significantly lower and higher, respectively, in both P-I and P-II groups than the HFHC control group. Dietary pinitol significantly inhibited hepatic HMG-CoA reductase, acyl-CoA:cholesterol acyltransferase (ACAT), and cytochrome P4502E1 (CYP2E1) activities without altering their mRNA expressions compared to the control group. Pinitol significantly elevated the hepatic antioxidant enzyme activities, whereas it also significantly reduced the hepatic lipid peroxide and H_2O_2 production. Accordingly, these results indicate that both 0.05 and 0.1% pinitol supplementation may improve the lipid and antioxidant metabolism in HFHC diet-fed hamsters. In particular, pinitol supplementation was very effective on the elevation of antiatherogenic factors, including plasma HDL-cholesterol, apo A-I, adiponectin, and PON.

Keywords: Hamster / Hepatoprotection / High-fat and high-cholesterol diet / Lipid and antioxidant metabolism / Soy pinitol

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1 Introduction

It has been identified that inositol phosphoglycans (IPGs) are potentially important for secondary messengers of putative insulin [1]. IPGs are rapidly generated from lipid and/or protein precursors, glycosylphosphatidylinositols (GPIs), in cell membranes in response to insulin, and act as insulin-like factors *in vitro* and *in vivo* [2, 3]. D-*chiro*-inositol is one class of IPGs and is active *in vivo*; lowering elevated blood glucose when administered intravenously to diabetic rats [4]. D-pinitol (3-*O*-methyl-D-*chiro*-inositol) has been identified to one of separate classes of IPGs as a monomethylated form of D-*chiro*-inositol, which is the demethylated metabolite or chemical hydrolysis product of pinitol. It is a naturally occurring insulin-like compound, found in pine trees, legumes, and flowers and leaves of many plants, and is present about 1% of dry weight in soy [5]. Pinitol does not add any calories when consumed and,

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Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; **ANOVA,** analysis of variance; **BAT,** brown adipose tissue; **CHD,** coronary heart disease; **CYP2E1,** cytochrome P4502E1; **FFA,** free fatty acid; **GSH-Px,** glutathione peroxidase; **HFHC,** high fat and high cholesterol; **HMGR,** HMG-CoA reductase; **IR,** insulin resistance; **MDA,** malondialdehyde; **PON,** paraoxonase; **SOD,** superoxide dismutase; **TBARS,** thiobarbituric acid-reactive substance; **THR,** total-C/HDL-C ratio; **TNF-a,** tumor-necrosis factor-a; **WAT,** white adipose tissue

critically, and has been demonstrated to be extremely anabolic and has an antihyperglycemic effect as a powerful insulin mimicker [6]. Pinitol considerably increases the ability of muscle cells to absorb glucose, with consequent benefits for sports where there are requirements for strength and endurance, with the added benefit of shortened recovery times [7]. Moreover, many reports have shown that pinitol, as well as DCI and myo-INS, cause no adverse effects, even in severe doses [5, 8]. In our previous study [9, 10], pinitol did not exhibit the cytotoxicities despite treatment with severe overdose amounts in 3T3-L1 and THP-1 cell lines.

In general, chronic consumption of a high fat and high cholesterol diet (HFHC) may induce hyperlipidemia, hepatic lipid accumulation (nonalcoholic fatty liver disease), lipid peroxidation, and hepatotoxicity [11]. Hamsters are analogous to human in regards to lipid and/or bile acid metabolic patterns [12]. This study represents the first time hamsters as animal models in investigating the effects of pinitol on lipid and antioxidant metabolism. The purpose of this study was to investigate whether pinitol had lipid-lowering, antioxidant, and cytoprotective effects in dose-dependent manners in HFHC diet-induced hyperlipidemic hamsters.

2 Materials and methods

2.1 Animals and diets

All procedures involving animals were approved by the Kyungpook National University Guidelines for the Care and Use of Laboratory Animals.

Four-week-old male Golden-Syrian hamsters (*Mesocricetus auratus*) were purchased from Charles River Laboratories (Wilmington, MA) and fed a pelletized commercial chow diet for 1 wk after arrival. Then they were randomly divided into three groups ($n = 8$) and fed an HFHC diet (10% coconut oil plus 0.2% cholesterol w/w), either with pinitol (0.05%, P-I and 0.1%, P-II) or without pinitol. Pinitol was purchased from Amicogen (Jinju, Korea). The composition of the experimental diet was based on the AIN-76 semisynthetic diet. Diet compositions (%) were casein, 20; fat, 10 (10% coconut oil); corn starch, 15; sucrose, 44.8; cellulose, 5; mineral mixture (AIN-76-MX), 3.5; vitamin mixture (AIN76-MX), 1; D,L-methionine, 0.3; choline bitartrate, 0.2; cholesterol, 0.2. The animals were individually housed in stainless steel cages in an environmentally controlled room (20–22°C, 60% relative humidity, 12-h altering light/dark cycle), and had free access to food and water. Their body weight an food intake levels were monitored every 1 wk and every 2 days, respectively.

2.2 Sampling

The animals were fasted for 12 h before sacrifice. At the end of the experimental period, the hamsters were anesthetized with Ketamine-HCl. Blood was collected in heparinized tubes from inferior vena and centrifuged for 10 min at $2000 \times g$. The plasma samples were stored at −20°C to measure the plasma biomarkers. The liver, epididymal, and perirenal white adipose tissue (WAT) and brown adipose tissue (BAT) were weighed. The livers were then divided for the preparations of enzyme sources, for lipids measurements, and for RNA isolation and were stored at −70°C until analyzed.

2.3 Plasma analyses

Plasma lipids and glucose were measured by means of commercially available kits: total-cholesterol (total-C), HDL-cholesterol (HDL-C), triglyceride (TG) (Sigma, St. Louis, MO, USA), free fatty acids (FFA) (Wako Chemicals, Richmond, VA, USA), glucose (BioAssay Systems, USA), apolipoprotein A-I (apo A-I) (Nitto Boseki, Japan) and apo B (Nitto Boseki). The insulin and adiponectin levels were determined using an insulin RIA kit (Diagnostic Systems Laboratories, USA) and a sandwich ELISA kit (R&D Systems, MN, USA), respectively. Paraoxonase (PON) activity was assayed spectrophotometrically using the method described by Mackness *et al*. [13], which measured the increase in absorbance for 90 s at 405 nm and 25°C.

2.4 Liver analyses

The hepatic lipids were extracted using the procedure developed by Folch *et al*. [14]. The hepatic cholesterol and triglycerides were analyzed with the same enzymatic kit as used in the plasma analysis.

Hepatic cytosolic, mitochondrial, and microsomal preparations were performed according to Hulcher and Oleson's method [15] with a slightly modified procedure, and the protein concentration was determined using Bradford's method [16].

The HMG-CoA reductase (HMGR) activity was measured essentially as described by Shapiro *et al*. [17], which measured the formation of [^{14}C]mevalonic acid from [^{14}C]HMG-CoA (specific activity 2.142 GBq/mmol, NEN™ Life Science Product, Boston, MA, USA) using mevalonic acid as the internal standard. The acyl-CoA:cholesterol acyltransferase (ACAT) activity was determined according to the method of Gillies *et al*. [18], which assayed the conversion of [^{14}C]oleoyl-CoA (specific activity 2.0202 GBq/mmol, NEN Life Science Product) into [^{14}C]cholesteryl oleate. The activity of cytochrome P4502E1 (CYP2E1) in the liver microsome was estimated by colorimetrically measuring the formation of 4-nitrocatechol, a product from ρ-nitrophenol hydroxylation catalyzed by CYP2E1 [19]. The activities of the antioxidant enzymes were measured using the following methods. The superoxide dismutase (SOD) activity was estimated according to method of Marklund *et al*. [20] which utilizes the color

change due to the auto-oxidation of pyrogallol. Briefly, SOD was detected on the basis of its ability to inhibit superoxide-mediated reduction. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as unit *per* milligram protein. The catalase activity was measured using Aebi's method [21], wherein hydrogen peroxide decomposition to yield water and oxygen was measured. The absorbance of the samples was recorded for 5 min at a wavelength of 240 nm and the enzyme activities were expressed as the decrease in H_2O_2 nmol/min/mg protein. The glutathione peroxidase (GSH-Px) activity was assayed using Paglia and Valentine's method [22]. The conversion of NADPH to NADP, as a measure of the GSH-Px activity, was followed by recording the changes in absorbance at 340 nm for 5 min after the initiation of the enzyme reaction. The results are expressed as nmol NADPH/min/mg protein.

Hepatic lipid peroxide contents were determined using the method of Ohkawa *et al*. [23] with a slight modification. A fresh malondialdehyde (MDA) solution by the hydrolysis of 1,1,3,3-tetramethoxypropane (TMP) was used as the standard. The results are expressed as the nmol MDA equivalent/mL plasma and nmol MDA/g liver. The cytosolic and mitochondrial hydrogen peroxide (H_2O_2) levels in the liver were measured by Wolff's method [24], and data were expressed as micromoles of H_2O_2 *per* milligram cytosolic or mitochondrial protein.

2.5 RNA isolation, cDNA synthesis, and quantitative real-time PCR analysis

Total RNA from liver tissue was isolated with TRIzol reagent (Invitrogen, Korea) according to the manufacturer's protocol. Five microgram of total RNA was used to produce cDNA, which was synthesized using the First Strand Synthesis System for the RT-PCR kit (Invitrogen). Gene-specific mouse primers were used for HMGR, 5′-CTCTGCTTGTGAGAAGGAAC-3′ (forward) and 5′-AGTCTCT GCTTCCACCACTA-3′ (reverse); ACAT, 5′-AGAAATCAAGCAAAGATCCA-3′ (forward), and 5′-AGGAGTCCTTGGGTAGTTGT-3′ (reverse); apo A-I, 5′-TGA ATCTCCTGGAAAACTG-3′ (forward) and 5-CCAATCTGTTTCTTTCTCCA-3′ (reverse); apolipoprotein B100 (apo B100), 5′-ACCTTCTCTAAGCAAAATGCAC-3′ (forward) and 5′-CTT GAGTTGAAAATGTTCTTGC-3′ (reverse); PON, 5′-GAACTTCCTAACTGTAATT TAG-3′ (forward) and 5′- CAAAATCAAATCCTTCTGCCAC-3′ (reverse); CYP-2E1, 5′-GTGGTTGGGAATATAGGACA-3′ (forward) and 5′-CCAATAGCTCAGGCTTC TTA-3′ (reverse). RNA expression was measured by real-time PCR. Taqman real-time RT-PCR reactions contained 10 ng of reverse-transcribed cDNA, 900 nM forward/reverse primer, and SYBR Green PCR Master Mix (Applied Biosystems, CA, USA) in a final volume of 10 μL. PCRs were carried out in 384-well plates by using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). All reactions were analyzed in triplicate. The amount of mRNA was calculated by the comparative cycle time method using the standard curve method. The mRNA levels of all genes were normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control.

2.6 Histopathological analysis

The liver and epididymal adipose tissue were removed and kept in a 10% formaldehyde solution for histopathological examination. The fixed tissues were stained with hematoxylin-eosin (H-E) and observed under a microscope at a magnification of 200.

2.7 Statistical analysis

All data were expressed as the mean ± SE and the statistical significance was analyzed with a one-way analysis of variance (ANOVA) using the SPSS package program. The results were only considered significant if the value of p was less than 0.05. Duncan's multiple-range test was performed if the differences between the groups were identified as $\alpha = 0.05$. Pearson's correlation coefficients were calculated to examine the association of plasma cholesterol with other parameters.

3 Results

3.1 Effects on body weight, food intake, and organ weights

Body weight gain, daily food intake, and liver weight were not influenced by either the HFHC diet and/or pinitol supplementation. Low and high dose pinitol (0.05 and 0.1%) supplementation, however, significantly lowered the weights of both epididymal and perirenal WAT when compared to the control group. And also, brown adipose tissue (BAT) weight was also significantly higher in both the P-I and P-II groups than in the control group, by 58.3 and 72.2%, respectively (Table 1). In these results, it seemed

Table 1. Effects of soy pinitol on body weight gain, food intake and organ weights in hamsters fed HFHC diet

	Control	P-I	P-II
Body weight gain (g/wk)	3.66 ± 0.30	3.74 ± 0.30	3.49 ± 0.26
Food intake (g/day)	8.71 ± 0.66	8.97 ± 0.32	9.17 ± 0.34
Liver (g)	6.55 ± 0.19	6.43 ± 0.20	6.91 ± 0.36
Epididymal WAT (g)	2.50 ± 0.10^a	1.99 ± 0.12^b	1.86 ± 0.10^b
Perirenal WAT (g)	1.93 ± 0.05^a	1.63 ± 0.13^b	1.38 ± 0.09^b
BAT (g)	0.36 ± 0.03^a	0.57 ± 0.05^b	0.62 ± 0.03^b

Mean ± SE.
Means not sharing a common letter are significantly different among groups at $p < 0.05$ as determined by a one-way ANOVA test.

Table 2. Effects of soy pinitol on plasma and hepatic lipid and lipoprotein profiles in hamsters fed HFHC diet

	Control	P-I	P-II
Plasma			
Triglyceride (mmol/L)	2.28 ± 0.34	1.79 ± 0.27	1.67 ± 0.29
FFA (mmol/L)	1.30 ± 0.14	1.22 ± 0.08	1.07 ± 0.10
Total-cholesterol (mmol/L)	9.08 ± 0.41[a]	7.64 ± 0.30[b]	7.14 ± 0.47[b]
HDL-cholesterol (mmol/L)	1.53 ± 0.09[a]	1.89 ± 0.09[b]	2.07 ± 0.15[b]
nonHDL-cholesterol (mmol/L)	7.55 ± 0.31[a]	5.75 ± 0.33[b]	5.07 ± 0.31[b]
THR	5.93 ± 0.61[a]	4.04 ± 0.42[b]	3.45 ± 0.53[b]
Glucose (mg/dL)	335.7 ± 13.4[a]	293.5 ± 15.1[b]	231.2 ± 8.82[c]
Apo A-I (g/L)	1.16 ± 0.03[a]	1.44 ± 0.01[b]	1.40 ± 0.02[b]
Apo B (g/L)	0.26 ± 0.01	0.29 ± 0.01	0.26 ± 0.01
Adiponectin (pg/mL)	120.61 ± 8.85[a]	169.11 ± 13.9[b]	157.43 ± 9.71[b]
Insulin (ng/mL)	7.31 ± 0.82[a]	5.26 ± 0.39[b]	5.71 ± 0.18[b]
Liver			
Triglyceride (mg/g liver)	98.31 ± 11.41[a]	75.4 ± 3.92[b]	75.7 ± 2.13[b]
Cholesterol (mg/g liver)	78.04 ± 2.21[a]	68.6 ± 1.84[b]	59.94 ± 2.34[b]

Mean ± SE.
Means not sharing a common letter are significantly different among groups at $p < 0.05$ as determined by a one-way ANOVA test.

Table 3. Effects of soy pinitol on plasma and hepatic cholesterol regulating and antioxidant enzyme activities and on hepatic TBARS and H_2O_2 contents in hamsters fed HFHC diet

	Control	P-I	P-II
HMGR (pmol/mg protein/min)	115.8 ± 12.1[a]	69.2 ± 10.9[b]	74.0 ± 4.9[b]
ACAT (pmol/mg protein/min)	381.5 ± 29.2[a]	229.8 ± 7.3[b]	229.9 ± 9.1[b]
PON (pmol/mL plasma)	1.26 ± 0.12[a]	2.58 ± 0.24[b]	2.41 ± 0.19[b]
SOD (Unit/mg protein)	0.82 ± 0.08[a]	0.88 ± 0.06[ab]	1.07 ± 0.06[b]
Catalase (nmol/min/mg protein)	8.93 ± 0.77[a]	13.81 ± 1.23[b]	12.8 ± 1.10[b]
GSH-Px (nmol/min/mg protein)	16.92 ± 0.50[a]	18.01 ± 1.31[ab]	21.23 ± 1.35[b]
CYP2E1 (nmol/min/mg protein)	2.01 ± 0.02[a]	1.27 ± 0.01[b]	1.25 ± 0.02[b]
TBARS (nmol/g liver)	13.63 ± 1.17[a]	9.81 ± 1.54[b]	9.31 ± 0.99[b]
Mitochondrial H_2O_2 (nmol/mg protein)	10.87 ± 1.42[a]	8.12 ± 0.84[b]	7.22 ± 0.91[b]
Cytosolic H_2O_2 (nmol/mg protein)	3.14 ± 0.37[a]	2.44 ± 0.13[b]	2.47 ± 0.11[b]

Mean ± SE.
Means not sharing a common letter are significantly different among groups at $p < 0.05$ as determined by a one-way ANOVA test.

that high-dose (0.1%) supplementation was more effective than low-dose supplementation on increasing BAT weight for heat production.

3.2 Effects on plasma and hepatic lipids, plasma glucose, apolipoproteins, adipocytokines, and hormone levels

Table 2 shows the lipid-lowering effects of pinitol. P-I and P-II groups were significantly lower in the plasma total-C, non-HDL-C, glucose concentrations, total-C/HDL-C ratio (THR) as well as hepatic TG and cholesterol contents compared to the control group ($p < 0.05$). Conversely, the concentrations of plasma HDL-C and apo A-I were significantly higher in the two pinitol groups than in the control group. Pinitol supplementation, however, did not significantly affect plasma TG, FFA and apo B concentrations, although dietary pinitol tended to lower the levels of plasma TG and FFA.

The plasma adiponectin concentration (Table 2) from adipocytes was significantly increased in both the P-I and P-II groups compared to the control group, whereas pinitol supplementation significantly decreased the plasma insulin level compared to the control diet (Table 2).

Altogether, hypoglycemic effect was significantly higher in high-dose pinitol group than in low-dose group, and P-II group showed somewhat similar and/or better results than P-I group in plasma and hepatic lipid levels although no significant differences.

3.3 Effects on hepatic and plasma cholesterol-regulating, antioxidant, and cytotoxic enzyme activities and hepatic lipid peroxide and H_2O_2 production

Of the cholesterol-regulating enzymes, both HMGR and ACAT activities were significantly lowered by both 0.05

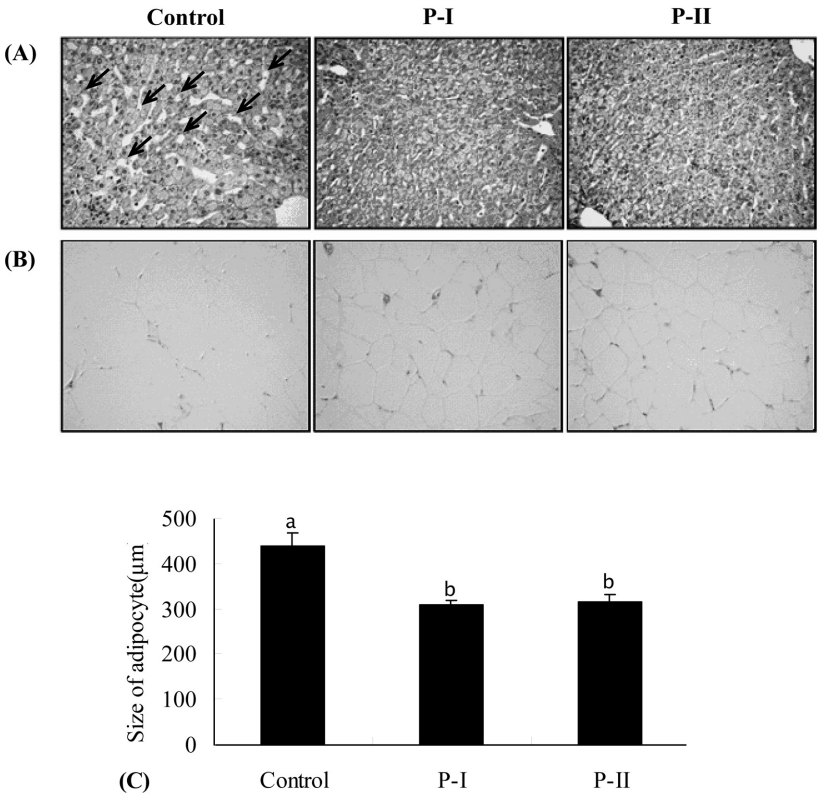

Figure 1. Effects of soy pinitol on (A) liver and (B) epididymal adipose tissue morphologies and size of (C) epididymal adipocyte in hamsters fed HFHC diet. Arrows indicate hepatic lipid droplets. H-E staining, 200×. The results were means ± SE ($n = 5$). [ab]Means not sharing a common letter are significantly different among groups at $p < 0.05$ as determined by a one-way ANOVA test.

and 0.1% pinitol supplement compared to the control group (Table 3).

In the antioxidant enzyme analyses, hepatic catalase and plasma PON activities were significantly higher in the two pinitol groups than in the control group. On the other hand, SOD and GSH-Px activities were the highest in the P-II group compared to the control group (Table 3). The hepatic CYP-2E1 activity was determined to identify whether the pinitol supplement was effective to provide hepatocyte protection from HFHC diet. Supplementation of 0.05 and 0.1% pinitol significantly lowered the hepatic CYP2E1 activity by 36.8 and 37.8%, respectively, compared to the HFHC control diet (Table 3).

The hepatic thiobarbituric acid-reactive substance (TBARS) content was significantly lower in the two pinitol groups than the control group. Both hepatic mitochondrial or cytosolic H_2O_2 contents were also significantly lowered in the two pinitol groups compared to the control group (Table 3). In general, there were no differences between P-I and P-II groups in the above results.

3.4 Histopathologies of hepatocytes and epididymal adipocytes

Hepatic morphology exhibited that pinitol supplement suppressed formation of lipid droplets, whereas the HFHC-control group showed evidence of noticeable fatty liver (Fig. 1A). Adipocyte sizes of the two pinitol groups were significantly smaller than the HFHC-control group (Figs. 1B and C), and these were similar between P-I and P-II groups.

3.5 Effects on hepatic gene expressions of variable enzymes and apolipoproteins

The gene expressions of hepatic HMGR, ACAT (Fig. 2A), apo A-I, apo B (Fig. 2B), and CYP2E1 (Fig. 2C) were not affected by either pinitol supplementation and/or the HFHC diet. The PON mRNA expressions, however, were significantly upregulated in the two pinitol groups compared to the control group (Fig. 2C, $p < 0.05$), and there was no significant difference between P-I and P-II groups in the PON mRNA expression.

4 Discussion

Our previous *in vitro* study showed the inhibitory effects of soy pinitol on cholesterol-laden foam cell formation in THP-1 macrophages [9]. In another *in vitro* study [10], it was reported that pinitol played a role as an insulin sensitizer in insulin-mediating adipocyte differentiation in 3T3-L1 preadipocytes. The present study suggests that pinitol supplementation with HFHC diet for 10 wks played important roles on improving both lipid and antioxidant metabolism in hamsters. Supplementation of both 0.05 and 0.1%

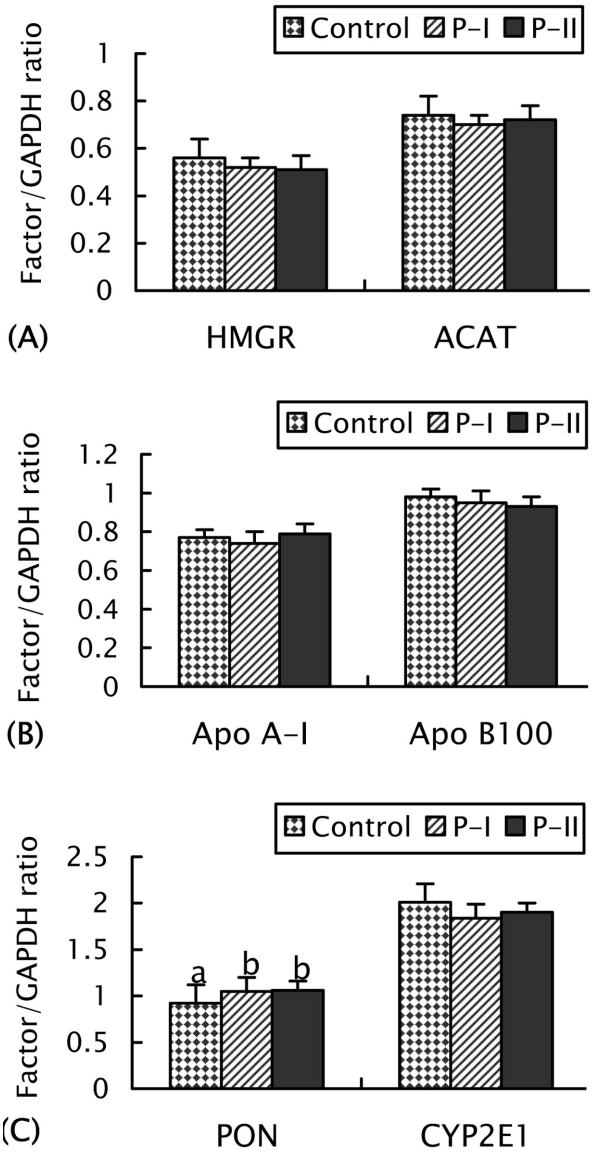

Figure 2. Real time RT coupled PCR (RT-PCR) analysis for several enzymes and apolipoproteins. The mRNA for these factors was quantified using GAPDH as the internal standard. The results were means ± SE ($n = 5$). [ab]Means not sharing a common letter are significantly different among groups at $p < 0.05$ as determined by a one-way ANOVA test.

pinitol significantly lowered the hepatic cholesterol and TG levels as well as plasma total-C concentration, THR level. Furthermore, antiatherogenic factors including the plasma HDL-C and apo A-I concentrations and PON activity/gene expression were simultaneously elevated and hepatic gene expression of PON by pinitol supplementation compared to the HFHC group. These results are in agreement with Kim *et al*.'s report [25] in which pinitol supplementation significantly elevated the plasma HDL-C concentration, whereas decreased plasma total-C and THR without altering plasma TG concentration in type II diabetic patients. In human, a THR of less than 3.5 indicates a state of low coronary heart disease (CHD) risk, whereas a ratio of 5 places a patient at average risk [26]. An increase in the plasma apo A-I concentration was accompanied with an HDL-C-raising effect by pinitol supplement. Apo A-I is required for the formation of HDL, and is highly correlated with plasma HDL-C concentrations and inversely correlated with CHD risk. Prospective epidemiological study has consistently indicated that high levels of plasma HDL-C protect against the development and progression of atherosclerosis [27]. HDL may also modify atherogenesis by carrying an antioxidant enzyme, PON, which probably contributes to its antiatherogenic properties by degrading the oxidized LDL [28]. Mackness *et al*. [29] suggested that PON plays a pivotal role in the antioxidative/anti-inflammatory/antiatherosclerotic properties of HDL. Even a small increase in HDL concentration greatly influences atherogenecity, and this effect could partially be related to increased PON activity. Our results strongly support others' finding [30] regarding strong positive correlations between the PON activity and the concentrations of HDL-C and apo A-I. Accordingly, the current study on soy pinitol supplementation in HFHC-fed hamsters seems to provide an evidence of the beneficial effect of pinitol for CHD risk reduction, as suggested by changes in various biomarkers related to lipid parameters.

Feeding a high cholesterol diet or inducing hypercholesterolemia has been generally shown to increase the plasma and hepatic levels of lipid peroxides, which have been shown to inhibit PON activity [31]. It is entirely feasible that a high cholesterol diet caused an increase in lipid peroxide level, resulting in the inhibition of PON activity and mRNA expression as shown in this investigation and our previous study [32]. In general, naturally occurring plant substances decrease cardiovascular disease (CVD) risk and cellular damage by lowering oxidative stress as antioxidants. Soy pinitol (D-methyl-*chiro*-inositol) supplement, in present study, increased hepatic SOD, catalase, GSH-Px, and plasma PON activities along with corresponding decreases in hepatic CYP2E1 activity and hydrogen peroxide levels. Nobre *et al*. [33] showed a similar finding that quebrachitol (2-*O*-methyl-L-inositol) has both antioxidant and cytotoxicity-preventive effects. CYP2E1 is a form of P-450 under the CYP2E gene superfamily which is present in rodents. It is induced by several xenobiotics (ethanol, acetone, isoniazid, and pyrazole), a high-fat diet [9] and physiological conditions such as fasting [34] and diabetes [35]. CYP2E1 induction has been also recognized as a major pathogenic feature for the liver disease observed in both alcoholic and nonalcoholics. In 1998, however, the FDA had authorized the use of pinitol as a very safe component (Docket No. 95S-0316). There are currently no known examples of any adverse side effects with the use of pinitol at recommended dosage levels (10 mg/kg body weight/day), and even severe overdose treatment [9, 10, 36].

Accordingly, our data suggest that soy pinitol is safe as a functional food and an effective material in reducing a hepatic cytotoxicity, which can be inducible by HFHC feeding.

A chronic HFHC diet can induce insulin resistance (IR)/hyperinsulinemia [37], which is strongly correlated with either visceral fat accumulation [38] or high plasma FFA concentration [39], and also may be an independent cardiovascular risk factor [40]. An association between IR and hepatic lipid accumulation (nonalcoholic fatty liver disease) has been found [41]. Insulin promotes both glucose and FFAs transport into peripheral cells, by accelerating the removal of glucose and FFA from blood, and maintains energy homeostasis by coordinating the storage of fat in liver, muscle, and adipose tissues. Pinitol, a methylated derivative of D-*chiro*-inositol, was hypothesized to mediate the insulin signaling pathways that stimulate glucose transport *via* insulin-like action or postreceptor pathways of insulin action [6]. Pinitol supplements altered some metabolic features in HFHC-fed hamsters as indicated by decreased glucose and insulin concentrations (Fig. 1A) as well as reduced visceral fat weight (epididymal and perirenal WAT) and hepatic lipid contents as well as lipid droplets (Fig. 2A) compared to the HFHC control group. In our previous *in vitro* study using the 3T3-L1 cell line [10], pinitol treatment stimulated the adipogenesis by acting as a PPARγ agonist, which acts as an insulin sensitizer. Current *in vivo* study, however, exhibited different results that pinitol supplementation with HFHC diet significantly decreased the weight of abdominal fat (epididymal and perirenal WAT) without altering body weight. These results seemingly correspond with others' finding [42] that the PPARγ agonist increases overall adiposity by favoring lipid deposition in subcutaneous fat while reducing or maintaining visceral fat mass, and this plays a role in insulin-sensitizing activity. In addition, it was observed that the PPARγ agonist recruited BAT *in vivo* [43], and pinitol supplementation dramatically increased the BAT weight in current study. It is plausible that stimulation of glucose and/or FFA transport by insulin-like pinitol supplementation resulted in both the decrease of liver and visceral fat storage and the increase in BAT weight for the efficient heat production in HFHC diet-fed hamsters.

Adipocytokines or adipokines secreted from adipocytes can affect the function of other organs, including the vascular walls throughout the whole body. Of the adipokines, tumor-necrosis factor-α (TNF-α) can induce IR [44]. On the other hand, adiponectin can improve IR and metabolic syndrome by its antiatherogenic, antidiabetic and/or anti-inflammatory function [45]. The plasma adiponectin level is negatively correlated with fasting insulin concentration and insulin sensitivity, respectively. A negative correlation between adiponectin level and visceral adiposity has been reported to be stronger than between adiponectin level and subcutaneous adiposity [46]. In our study, pinitol supplement contributed to the elevation of plasma adiponectin level and the reduction of plasma insulin concentration and visceral fat weight. Accordingly, negative correlations were apparently observed between adiponectin and insulin, and between adiponectin and visceral fat weight.

Cholesterol homeostasis must be constantly maintained by cholesterol absorption, excretion, and endogenous biosynthesis. The liver plays an essential role in keeping this cholesterol balance. For example, a high cholesterol intake elevates blood cholesterol concentration, which induces the downregulation of HMGR, a rate-limiting enzyme of the cholesterol biosynthetic pathway, and increase hepatic ACAT activity. In this study, pinitol with HFHC diet resulted in the significant reduction of these two enzyme activities without altering the mRNA expressions compared with the HFHC group. These changes in cholesterol regulating enzyme activities seemingly contribute to decrease the plasma and hepatic cholesterol concentration. Our results also showed a general tendency of disagreement between enzyme activity and its gene expression.

In conclusion, pinitol improves the lipid and antioxidant metabolism by lowering visceral fat weight, hepatic lipid accumulation and insulin secetion, and by increasing the BAT weight and adiponectin levels. Pinitol also improves HDL metabolism as well as exhibits antioxidant and cytoprotective effects in HFHC diet-fed hamsters. These results support the fact that pinitol has a potential capacity for prevention and/or improvement of CVD, and suggest that soy pinitol is safe as a functional food since it reduced a hepatotoxicity which can be induced by HFHC feeding. Also, there were no significant differences between 0.05 and 0.1% pinitol supplementation in all factors except plasma glucose, and it is considered that recommended dosage of pinitol is allowed to upgrade because two dosages were five- and ten-fold of human recommended dosage. Nonetheless, it remains to be elucidated whether pinitol can regulate nonshivering thermogenesis in diet induced obese animal model.

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