Effect of *Peucedanum japonicum* Thunb on the Expression of Obesity-Related Genes in Mice on a High-Fat Diet

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Abstract: The present study describes the effect of *Peucedanum japonicum* Thunb (PJT) intake on the expression of obesity-related genes in mice fed a high-fat diet. To explore the mechanism underlying the effect of PJT, This study focused on the expression of genes, especially those related to obesity and metabolism syndrome, in the liver and adipose tissues. In agreement with our previous observations, intake of 10 % PJT for 4 weeks significantly reduced serum triglyceride (TG), leptin, abdominal fat, and adipocyte size. PJT also significantly increased fecal excretion of TG, decreased that of bile acid, and tended to increase the fecal excretion of total cholesterol. Microarray analysis was used to monitor changes in 324 metabolic syndrome-related genes in the liver. Statistically significant upregulation of *PPP1R10*, *RORC*, and *PBEF1* genes and downregulation of *DUSP1*, *INSIG2*, and *SERPINA12* genes were noted and confirmed by real-time RT-PCR. These changes were indicative of increased fatty acid oxidation in the maintenance of lipid homeostasis and insulin sensitivity in the livers of PJT-fed mice. PJT increased the expression of *PPAR γ* , *FXR α* , *DGAT1*, and *ATGL* genes, suggesting an enhancement of adipocyte differentiation and normalization of functionality of adipose tissue.

Key words: metabolic syndrome, obesity, gene expression, *Peucedanum japonicum* Thunb

1 INTRODUCTION

In recent years, obesity has become one of the most prevalent health problem among developed countries, including Japan¹, and has been considered to be due to a high-fat diet and sedentary lifestyle². Obesity is not only an economic burden but also a risk factor for a variety of metabolic diseases, such as atherosclerosis, dyslipidemia, elevated blood pressure, and insulin resistance³⁻⁵. Therefore, it is very important to establish strategies for the prevention of obesity.

Peucedanum japonicum Thunb (PJT), a medicinal plant belonging to the family of Umbelliferae, grows on the cliffs of Okinawa Island, Japan. PJT leaves are traditionally consumed on Okinawa Island as a medicinal herb for the treatment of cough. The roots of this plant have been used

as a folk medicine for cold and neuralgic diseases in Taiwan⁶. There have been several reports on the biological activity of PJT⁷⁻¹⁰, which indicate the high potential of this plant in the development of natural agents for treatment of prevailing health problems. Our previous study reported for the first time that PJT inhibited high-fat-induced obesity in mice and resulted in a decrease in body weight of the mice¹¹. This anti-obesity activity of PJT appeared to be associated with decreased fat absorption, although the extent to which this mechanism is involved in the overall lipid-lowering activity of PJT is unknown. These findings also indicated an *in vivo* metabolic shift to inhibit obesity, and hence, more studies were required to explore the anti-obesity mechanism of PJT.

In this study, we investigated the effect of PJT on the

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expression of genes in liver and adipose tissues, which play a pivotal role in lipid metabolism. We focused on the expression of genes associated with the onset of obesity, and discussed the probable anti-obesity mechanism of PJT.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

PJT used in this study was harvested from Yonaguni island of Okinawa, Japan. PJT leaves and stems were freeze-dried and ground by Yonagunijima Yakusoen Co. (Okinawa, Japan). The dried powder of PJT was analyzed by the Japan food Research Laboratories (Tokyo, Japan), and its nutrient composition was found to be as follows: carbohydrate, 46.7%; fiber, 31.6%; mineral, 9.8%; protein, 7.6%; and fat, 4.4% (w/w). Casein, β -corn starch, cellulose, and mineral and vitamin mixes for AIN-76 rodent diets were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). DL-methionine and choline bitartrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2 Animals and diets

Three-week-old male C57BL/6 mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed in a temperature-, humidity-, and light-controlled room (12-h light/dark cycle) and were allowed free access to water. The mice were maintained on commercial chow for 7 days to allow acclimatization, and were then fed 0% and 10% PJT diets (AIN76 diet containing 15% corn oil) for another 4 weeks. The composition of the experimental diets was as follows: carbohydrate, 53.0%; protein, 20.0%; fat, 15.0%; fiber, 7.0%; AIN-76 mineral mix, 3.5%; AIN-76 vitamin mix, 1.0%; DL-methionine, 0.3%; choline bitartrate, 0.2%; dried PJT powder, 0% or 10% (w/w); and energy, 427.0 kcal/100 g. In the dietary group with PJT powder, the additional protein, carbohydrate, fiber, and fat derived from PJT were compensated for by decreasing the corresponding nutrients: casein, sucrose, β -corn starch, cellulose, and corn oil. All animals were pair-fed on the diets and allowed free access to water. Mice were sacrificed after 6 h of fasting. The serum, liver tissue, and white adipose tissue (WAT) samples were collected, and stored at -80°C . Weights of liver tissue and WAT were measured immediately after collection.

2.3 Lipid concentrations and biochemical parameters in the serum and liver

Serum levels of total cholesterol (TC), triglyceride (TG), and glucose were determined using a commercial enzymatic kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum leptin, adiponectin, and insulin levels were measured using ELISA kits (leptin and insulin: Morinaga Insti-

tute of Biological Science, Inc., Yokohama, Japan; adiponectin: Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Total lipids were extracted from liver tissues by a previously described method¹²⁾. Liver levels of TC and TG were determined by the Shoenheimer-Sperry method¹³⁾ and Fletcher's method¹⁴⁾, respectively.

2.4 Lipid concentration in feces

During the last 3 days of the feeding period, fecal samples were collected and dried. The TC and TG in the feces were extracted by a previously described method¹²⁾, and their levels were determined using commercial enzymatic assay kits (Wako Pure Chemical, Osaka, Japan), as described previously¹⁵⁾. Total bile acids were extracted from the feces with 100% ethanol, and measured using the Total Bile Acid Test Wako kit (Wako Pure Chemical, Osaka, Japan).

2.5 Adipocyte size

The epididymal adipose tissue was fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin for microscopic measurement of cell sizes, as described elsewhere¹⁶⁾. The stained sections were viewed at $20\times$ magnification, and photographed with a digital camera (Olympus BX41, NY, USA); the images were converted into a binary format by using Adobe Photoshop 5.0.1 software (Adobe Systems, CA, USA), and incorporated into Scion Image for measurement of the cross-sectional area. The total cross-sectional areas of at least 200 adipocytes were manually measured for 4 microscopic fields, and used for calculation of the average cell surface area (μm^2).

2.6 Isolation of total RNA

Total RNA was extracted from 30 mg of liver tissue by using the Illustra RNA spin mini RNA isolation kit (GE Healthcare, NJ, USA), and also from 30 mg of epididymal adipose tissue by using the RNeasy lipid tissue mini kit (Qiagen, CA, USA). Purity of RNA was checked using agarose gel electrophoresis, and quantified by a UV spectrophotometer. Five micrograms of total RNA was reverse transcribed to produce cDNA by an oligo (dt) method using the PrimeScriptTM 1st strand cDNA synthesis kit (Takara BIO Inc., Shiga, Japan). cDNA samples were stored at -80°C in aliquots of 50 μL .

2.7 Microarray analysis

mRNA expression analyses of 333 genes (including 324 lifestyle disease-related genes as summarized in Table 1 and 9 housekeeping genes) were performed using a Gene SQUARE DNA array (Kurabo Industries Ltd., Osaka, Japan). Briefly, fluorescence-labeled cDNA synthesized from 10 μg of total RNA was mixed with standard saline citrate and sodium dodecyl sulfate in a volume of 20 μL .

Table 1 Microarray analysis of gene expression in the liver.

Gene group	Number of genes studied	Modulated gene name ($p < 0.05$) ^a
Adipogenesis	10	RORC ↑
Adipocyte related gene	18	
Regulators and adipocyte differentiation	44	SERPINA12 ↓
Lipid metabolism	47	CYP7A1 ↑
Lipid metabolism related gene	5	
Glucose and glycogen metabolism	19	
Insulin signal	54	PBEF1 ↑ PPP1R1 ↑ INSIG2 ↓
MAPK signal	20	
AMPK signal	4	
PPAR	18	
Wnt signal	15	
Beta cell related genes	5	
Diabetes	11	
Extra cellular matrix	4	
Exocytosis	10	
Immune response	11	
Transporters	25	
Others	4	DUSP1 ↓

a: Modulated genes are those showed statistically significant changes by the feeding of 10% *Peucedanum japonicum* Thunb (PJT) diet ($p < 0.05$ by Student's *t* test). Arrows denote upregulation or downregulation : ↑ upregulation; ↓ downregulation.

Eight microliters of this hybridization mix was applied to Gene SQUARE DNA array, and hybridized at 65°C for 15 h. The fluorescence profile was analyzed by a microarray scanner (GenePix 4000B with GenePix Pro 6.0.1.27; Filgen, CA, USA). The expression level of an individual gene was presented as an arbitrary unit, normalized to the house-keeping gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA.

2.8 Quantitative real-time RT-PCR

Quantitative real-time RT-PCR was performed on an Icyler (Bio-Red Laboratory, Tokyo, Japan) using SYBR Green I (Takara BIO Inc., Shiga, Japan) and Takara TaqTM Hot Start version (Takara BIO Inc., Shiga, Japan), according to the manufacturer's instructions. The primer sequences are listed in Table 2. Amplification of the target cDNA was checked by agarose gel electrophoresis and by identification of its DNA sequence, as described previously¹⁷⁾. A standard curve for each gene was constructed by performing PCR with serially diluted known concentrations of the template, and used for the estimation of target mRNA, as described elsewhere¹⁸⁾. Each sample was

assayed in duplicate and normalized to *GAPDH* expression in liver tissues and β -actin expression in adipose tissues.

2.9 Statistical analysis

Statistical significance of the mean values of the 2 groups was evaluated using Student's *t* test and the SAS statistical software program (SAS Institute, Tokyo, Japan).

3 RESULTS

3.1 Growth parameters and weights of liver and adipose tissues

Table 3 summarizes the growth parameters as well as the weights of the liver and adipose tissue samples. The mean final body weight of the 10% PJT diet group was significantly lower than that of the 0% PJT diet group. No significant differences were noted for food and energy intake values. The relative mean liver weight of the 10% PJT diet group was significantly higher than that of the 0% PJT diet group.

In the case of WAT, a significant decrease in the mean

Table 2 Primers sequences used for quantitative real-time RT-PCR.

Genes	Description	Primer sequence	Product	Accession no.
CYP7A1	cholesterol 7- α -hydroxylase	5'-AGC AAC TAA ACA ACC TGC CAG TAC TA-3' (forward primer, F) 5'-GTC CGG ATA TTC AAG GAT GCA-3' (reverse primer, R)	82	NM_007824
PBEF1 (visfatin)	pre-b-cell colony-enhancing factor 1	5'-GCT GAT CCC AAC AAA AGG TC-3' (forward primer, F) 5'-CTG CTC GAT GTT CAG CTG TG-3' (reverse primer, R)	204	NM_021524
PPP1R10	protein phosphatase 1, regulatory subunit 10	5'-TGA TGG AGG CCA CAG TCA TG-3' (forward primer, F) 5'-GGT AGA AGG CAC AGT TGT TC-3' (reverse primer, R)	104	NM_001163818
RORC	RAR-related orphan receptor γ	5'-CAA TTT GGA ACT GGC TTT CC-3' (forward primer, F) 5'- CTG AAG AGT TCC TTA TAG AG -3' (reverse primer, R)	189	NM_011281
DUSP1	dual specificity phosphatase 1	5'-TGA CAT GCG CGT ATG AGA G-3' (forward primer, F) 5'- CTG GCT TTG TCT GTC AGT GC-3' (reverse primer, R)	182	NM_013642
INSIG2	insulin induced gene 2	5'-CAG AGG TGG TTA TCA ACA TC-3' (forward primer, F) 5'-CTG TGA GCT TCA GTA GCC A -3' (reverse primer, R)	209	NM_133748
SERPINA12 (Vaspin)	serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antitrypsin, antitrypsin), member 12	5'-GCT CCG CAT CTC GTC TAC TT -3' (forward primer, F) 5'-CTT CCA TGC CTT TCT CAT CC -3' (reverse primer, R)	151	NM_026535
PPAR α	peroxisome proliferator activated receptor α	5'-CCT CAG GGT ACC ACT ACG GAG T-3' (forward primer, F) 5'-GCC GAA TAG TTC GCC GAA-3 (reverse primer, R)	69	NM_011144
PPAR γ	peroxisome proliferator activated receptor γ	5'-AGG CCG AGA AGG AGA AGC TGT TG-3' (forward primer, F) 5'-TGG CCA CCT CTT TGC TGT GCT C-3' (reverse primer, R)	276	NM_011146
FAS	fatty acid synthase	5'-TGC TCC CAG CTG CAG GC-3' (forward primer, F) 5'-GCC CGG TAG CTC TGG GTG TA-3' (reverse primer, R)	91	AF_127033
FXR α	farnesoid X receptor α	5'-TGG GCT CCG AAT CCT CTT AGA-3' (forward primer, F) 5'-TGG TCC TCA AAT AAG ATC CTT GG-3' (reverse primer, R)	72	NM_001163504
SREBP-1	sterol regulatory element-binding protein-1	5'-GCG CCA TGG ACG AGC TG-3' (forward primer, F) 5'-TTG GCA CCT GGG CTG CT-3' (reverse primer, R)	205	NM_011480
C/EBP α	CCAAT/enhancer binding protein α	5'-TGG ACA AGA ACA GCA ACG AGT AC-3' (forward primer, F) 5'-GCA GTT GCC CAT GGC CTT GAC-3' (reverse primer, R)	257	NM_007678
PEG1/MEST	paternally expressed gene 1(Peg1)/mesoderm specific transcript (Mest)	5'-GTT TTT CAC CTA CAA AGG CCT ACG-3' (forward primer, F) 5'-CAC ACC GAC AGA ATC TTG GTA GAA-3' (reverse primer, R)	52	NM_008590
DGAT1	diacylglycerol acyltransferase 1	5'-TCC GCC TCT GGG CAT TC-3' (forward primer, F) 5'-GAA TCG GCC CAC AAT CCA-3' (reverse primer, R)	65	NM_010046
ATGL	adipose triglyceride lipase	5'-AGG ACA GCT CCA CCA ACA TC-3' (forward primer, F) 5'-TGG TTC AGT AGG CCA TTC CT-3' (reverse primer, R)	165	AY894805
LPL	lipoprotein lipase	5'-AGG GCT CTG CCT GAG TTG TA-3' (forward primer, F) 5'-AGA AAT CTC GAA GGC CTG GT-3' (reverse primer, R)	199	NM_008509
UCP2	uncoupling protein-2	5'-CAG GTC ACT GTG CCC TTA CCA T-3' (forward primer, F) 5'-CAC TAC GTT CCA GGA TCC CAA G-3' (reverse primer, R)	101	NM_011671
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	5'-ACC CAG AAG ACT GTG GAT GG-3' (forward primer, F) 5'-ACA CAT TGG GGG TAG GAA CA-3' (reverse primer, R)	172	NM_008084
ACTB	actin, beta, cytoplasmic	5'-CAG AAG GAG ATT ACT GCT CTG GCT-3' (forward primer, F) 5'-GGA GCC ACC GAT CCA CAC A-3' (reverse primer, R)	93	NM_007393

weight of abdominal fat, including epididymal and perirenal fat, was noted for the 10% PJT diet group. The subcutaneous fat weight tended to decrease in the 10% PJT diet group. These results largely agree with our previous observations with PJT-fed mice¹¹.

3.2 Adipocyte size

The mean cell size of the epididymal adipocytes for the

mice fed with 10% PJT diet was significantly smaller than that for the 0% PJT diet group, as exemplified in **Fig. 1-A**. The average adipocyte sizes were 4259 (227) μm^2 (mean (SD)) and 2694 (139) μm^2 for the 0% and 10% PJT diet group, respectively (**Fig. 1-B**).

3.3 Lipid metabolism-related parameters

Table 4 shows the serum levels of TC, TG, glucose,

Table 3 Effect of *Peucedanum japonicum* Thunb (PJT) on growth parameters, and weight of liver and adipose tissues in mice.

Parameters	Diet group ^a	
	0%	10%
Food intake (g/day)	2.9 ± 0.0	2.9 ± 0.0
Energy intake (kcal/day)	12.49 ± 0.06	12.44 ± 0.10
Initial body weight (g)	13.5 ± 0.4	13.5 ± 0.3
Final body weight (g)	22.4 ± 0.4	18.9 ± 0.4**
Liver weight (g/100g body weight)	4.2 ± 0.1	4.6 ± 0.1*
White adipose tissue (g/100 g body weight)	7.39 ± 0.68	5.78 ± 0.51
Abdominal fat	5.50 ± 0.24	3.87 ± 0.34**
Epididymal fat	2.76 ± 0.11	1.82 ± 0.13**
Perirenal fat	1.05 ± 0.07	0.55 ± 0.10**
Omental fat	1.70 ± 0.17	1.49 ± 0.13
Subcutaneous fat	2.35 ± 0.24	1.90 ± 0.17
Brown adipose tissue (g/100 g body weight)	0.67 ± 0.06	0.42 ± 0.04**

^a: C57BL/6 mice were fed modified-AIN76 diet containing 15 % corn oil with 0 % or 10 % PJT for 4 weeks. Values are mean ± SE of six mice. Asterisk shows significant difference from 0% PJT diet group by Student's t test (*: $p < 0.05$, **: $p < 0.01$).

insulin, adiponectin, and leptin; levels of TC and TG in the liver; and fecal excretion of TC, TG, and bile acid. Mice that were fed 10% PJT diet showed significantly lower serum TG and leptin levels than those fed 0% PJT diet. Moreover, the 10% PJT diet tended to increase the serum adiponectin level ($p = 0.155$). No significant differences were noted in the serum levels of TC, glucose, and insulin between the 2 dietary groups.

The liver TG level of the 10% PJT diet group was significantly lower than that of the 0% PJT diet group. As observed for the serum samples, PJT diet had no effect on the liver TC level.

PJT significantly increased the fecal excretion of TG, decreased that of bile acid, and tended to increase the fecal excretion of TC ($p = 0.08$).

3.4 Microarray analysis of gene expression in the liver

Table 1 summarizes the PJT diet-induced modulation of gene expression in the liver; only the significantly modulated genes ($p < 0.05$) are listed in this Table. Seven of the analyzed 324 genes showed significant changes in response to PJT administration. PJT diet upregulated the gene expression of *RORC*, *CYP7A1*, *PBEF1*, and *PPP1R10* by 1.5-2 fold, and downregulated *SERPINA12* (vaspin), *INSIG2*, and *DUSP1* by 0.2-0.3 fold. No significant changes were noted for other genes that are important in liver lipid metabolism, namely, *PPARα*, *FAS*, and *SREBP-1* genes.

3.5 Analysis of gene expression in liver and adipose tissues by real-time RT-PCR

Analysis by real-time RT-PCR confirmed the increase in expression of *RORC*, *PPP1R10*, and *PBEF1* in the liver due to the PJT diet, as shown in Fig. 2-A. Decreases in the expression of *DUSP1*, *INSIG2*, and *SERPINA12* to variable extents were also noted (Fig. 2-B). There were increasing tendencies of *FXRα* and *SREBP-1* expression in the livers of PJT-fed mice, and no difference was found in *PPARα* and *FAS* expression (Fig. 2-C).

Figure 3 shows the gene expression in adipose tissue. Genes that are important in lipid metabolism in lipid metabolism were selected for analyses, based on a review of literature. Compared to the 0% PJT diet, the 10% PJT diet significantly upregulated the gene expression of *PPARγ*, *FXRα*, *DGAT1*, and *ATGL*, and downregulated the gene expression of *PEG1/MEST*. No significant differences were noted for the expression levels of *PBEF1*, *RORC*, *C/EBPα*, *SREBP-1*, *LPL*, and *UCP2* in adipose tissue.

4 DISCUSSION

The present study confirmed the anti-obesity activity of PJT in mice. The observed influences of PJT on lipid parameters and body weight gain in the present study were in agreement with the observations in our previous study; PJT reduced serum TG, leptin, abdominal fat, and adipocyte size, with a concomitant decrease in body weight (Tables 3 and 4, Fig. 1). Furthermore, increased fecal TG

Fig. 1 Effect of *Peucedanum japonicum* Thunb (PJT) on adipocyte size.

Microscopic observation (A) and cell size of epididymal adipocytes (B) in mice fed with (10%) or without (0%) PJT. Values in B were the mean $\pm$ SE of 6 mice. Asterisks denote a statistically significant difference from the 0% PJT diet group (Student's *t* test; *: $p < 0.05$, **: $p < 0.01$).

and TC excretion, and decreased bile acid excretion were noted in the PJT-fed mice.

It has been reported that supplementation with persimmon leaf powder decreased TC and TG levels in the plasma and liver, and increased daily fecal weight and fecal excretion of TC and TG in rats that were fed a high-fat diet¹⁹. This study suggested that fiber might be responsible for an improvement of fat metabolism. However, the total fiber level of all experimental diets in our study was adjusted to the same level by using cellulose. It is, therefore, unlikely that the increased fecal lipid excretion in the PJT diet group is due to a difference in the fiber content of the diet. Instead, a dietary component, other than fiber, may be responsible for the decreased lipid absorption. Our preliminary results showed that PJT was rich in polyphenol, which inhibits lipase to reduce lipid absorption, as suggested for tea catechins²⁰. Furthermore, several lines of *in vitro* studies have demonstrated the occurrence of hypolipidemic compounds in PJT leaves^{9, 10}. It is, therefore, likely that these active components may influence the lipid dynamics, in concert, to prevent the mice from developing obesity.

In order to gain insight into the mechanism of action of PJT, its effect on gene expression in liver and adipose tissues were studied. Of the upregulated genes in the liver, increases in *RORC* and *PBEF1* levels were prominent. The protein encoded by the *RORC* gene is a DNA-binding transcription factor and is a member of the NR1 subfamily of the nuclear hormone receptors. The specific functions of this protein are not fully understood. However, a study in mouse skeletal muscle cells suggested that *RORC* increased the expression of the retinoid-related orphan receptor alpha (*RORα*) gene, and thereby modulated the expression of genes involved in the control of lipid homeostasis, such as fatty acid binding protein, fatty acid translocase, lipoprotein lipase, and uncoupling protein 3²¹. Furthermore, *RORα* directly regulates the transcription of carnitine palmitoyltransferase 1²². Therefore, it is likely that PJT increased the β -oxidation of fatty acids via the elevation of the *RORC* level and subsequent activation of *RORα*. This explanation is in line with our previous results that demonstrated increased carnitine palmitoyltransferase activity in the livers of PJT-fed mice¹¹.

PBEF1 (visfatin) is an adipokine that is involved in glucose homeostasis through its glucose-lowering effect²³. It was initially thought that visfatin had insulin-mimicking functions, stimulating glucose utilization in the peripheral tissues²³. However, visfatin has now been identified as the extracellular form of the enzyme nicotinamide phosphoribosyltransferase (NAMPT), which is essential in the NAD biosynthesis pathway and critical for glucose-stimulated insulin secretion in β -cells. Furthermore, a recent study demonstrated that overexpression of visfatin altered whole body insulin sensitivity and lipid profile in rats²⁴. Thus, the increased expression of visfatin in PJT-fed mice may represent their improved insulin sensitivity. The precise mechanism underlying the aforementioned beneficial activity of visfatin is not fully understood. More studies are needed to explore the physiological significance of increased visfatin gene expression in PJT-fed mice.

The PJT diet significantly lowered the expression of *INSIG2* and *DUSP1* genes in the liver. The decreased expression of *INSIG2* gene may represent sensitized insulin signaling in PJT-fed mice, which could occur because the diet ensures the endoplasmic supply of *SREBP-1* to the Golgi. In this sense, it is also notable that the reduced gene expression of *DUSP1*, which inactivates MAP kinase in insulin-mediated signaling, may contribute to the anti-obesity activity of PJT. Consistent with this scenario, downregulation of MAP kinase phosphatase-1 enhanced resistance to diet-induced obesity in mice²⁵.

PJT upregulated the expression of *PPARγ*, *FXRα*, *DGAT1*, and *ATGL* genes in adipose tissue (Fig. 3). Of these genes, the nuclear hormone receptors (*PPARγ* and *FXRα*) are important transcription factors involved in the regulation of essential metabolic functions, including

Table 4 Effect of *Peucedanum japonicum* Thunb (PJT) on lipid metabolism-related parameters in mice.

Parameters	Diet group ^a	
	0%	10%
Serum		
Total cholesterol (mg/dL)	137.3 ± 3.2	165.4 ± 12.6
Triglyceride (mg/dL)	55.7 ± 5.3	35.0 ± 5.8*
Adiponectin (ng/mL)	2.37 ± 0.08	2.53 ± 0.07
Leptin (ng/mL)	7.52 ± 0.53	3.55 ± 0.92*
Glucose (mg/dL)	68.4 ± 16.3	61.3 ± 19.6
Insulin (pg/mL)	197.0 ± 35.0	152.0 ± 48.0
Liver tissue		
Total cholesterol (mg/g liver)	3.83 ± 0.20	3.88 ± 0.21
Triglyceride (mg/g liver)	65.8 ± 5.4	47.4 ± 3.0**
Fecal excretion		
Total cholesterol (mg/day)	3.33 ± 0.20	4.24 ± 0.21
Triglyceride (mg/day)	0.32 ± 0.08	0.51 ± 0.04*
Bile acid (mg/day)	1.28 ± 0.09	0.77 ± 0.08**

^a: C57BL/6 mice were fed modified-AIN76 diet containing 15 % corn oil with 0 % or 10 % PJT for 4 weeks. Values are mean ± SE of six mice. Asterisk shows significant difference from 0% PJT diet group by Student's t test (*: $p < 0.05$, **: $p < 0.01$).

glucose and lipid metabolism²⁶⁾.

PPAR γ gene is a master regulator of adipogenesis in adipose tissue. The physiological significance of the current findings is rather ambiguous and somewhat controversial. *PPAR γ* belongs to the nuclear receptor superfamily of ligand-activated transcription factors. The earliest events occurring in adipogenesis are a brief burst of mesenchymal precursor cells, followed by cell-cycle arrest and terminal differentiation²⁷⁾. *PPAR γ* expression is induced at the stage of terminal differentiation in adipogenesis, and assists in the completion of the process involved in the production of mature adipocytes²⁷⁾. Thus, the lipid-lowering activities of several natural compounds have been explained, in part, by the downregulation of the *PPAR γ* gene²⁸⁾. In contrast to these observations, in this study, PJT increased the expression of the *PPAR γ* gene without causing any detrimental obesity. Obesity is essentially due to over-nutrition or positive energy balance. The functionality of adipose tissue is associated with its ability to buffer the accumulation of the excess energy consumed in the diet, and to provide energy as free fatty acids when the energy expenditure exceeds intake. Therefore, the decreased adipose tissue weight in the PJT diet may simply be a consequence of reduced lipid levels available for adipogenesis.

It is noteworthy that the activation of the *PPAR γ* gene in adipose tissue prevents adipocyte hypertrophy and metabolic abnormalities during obesity²⁹⁾. Adipose tissue regulates the metabolism of other fat cells or cells located in

the brain, liver, muscle, or pancreas via the secretion of adipocytokines. Adipocyte enlargement is associated with disruption in the adipokine profile and dysfunction of fat cells³⁰⁾. In this regard, increased differentiation by *PPAR γ* may result in the production of small adipocytes with normal functionality. Hypoxia in adipose tissue induced by adipocyte enlargement is an emerging physiological event that may initiate the inflammatory response of adipose tissue³¹⁾. PJT may accelerate adipocyte differentiation to yield small adipocytes, which may offset the adverse effects of fat accumulation in adipose tissue. This explanation may be supported by the observation that the PJT diet decreased the adipocyte size (Fig. 1) and the expression of the *PEG1/MEST* gene (Fig. 3), an adipocyte size marker³²⁾.

FXR is a nuclear receptor and bile acid sensor expressed in the liver, intestine, adrenal gland, and adipose tissue³³⁾. It has been shown that activation of *FXR* in adipose tissue increased insulin sensitivity and led to phosphorylation of the insulin receptor substrate-1 (IRS-1) and Akt, resulting in increased expression of the glucose transporter type-4 (Glut-4), adiponectin, and fatty acid binding-protein³⁴⁾. Therefore, as observed in the liver, PJT may enhance the insulin response of adipose tissue via *FXR* activation.

In conclusion, a lipid-lowering mechanism for PJT is proposed as follows: PJT inhibits the absorption of TG and TC, which may be the first and most critical step of the lipid-lowering activity of this medicinal food. The inhibition of lipid absorption may subsequently limit the excess energy

Fig. 2 Effect of *Peucedanum japonicum* Thunb (PJT) on gene expression in liver tissue determined by quantitative real-time RT-PCR.

A, genes upregulated in the foregoing microarray analysis; B, genes downregulated in the microarray analysis; C, other well-documented and important genes in lipid metabolism. For descriptions of genes, see Table 2. Closed and open columns, respectively, show values for 0% and 10% PJT diet groups. Values are in arbitrary units, expressed as the mean $\pm$ SE of 6 mice. Asterisk denotes a statistically significant difference from 0% PJT diet group (Student's *t* test; $p < 0.05$).

required for fat storage in adipose tissue. Decreased bile acid excretion, caused by PJT, may increase the return flow of bile acid from the small intestine to the liver and affect lipid metabolism in the whole body, including liver and adipose tissues. In addition, PJT may enhance insulin sensitivity of both liver and adipose tissues via the normalization of the functionality of adipose tissue. Although no changes in serum glucose and insulin levels were noted in this study, the possibility of increased insulin sensitivity associated with PJT should be evaluated by glucose tolerance tests in future studies. In this context, future studies also should focus on the effects of PJT on the muscle tissue, which is important for the energy metabolism of the whole

Fig. 3 Effect of *Peucedanum japonicum* Thunb (PJT) on gene expression in adipose tissues determined by quantitative real-time RT-PCR.

For descriptions of genes, see Table 2. Closed and open columns, respectively, show the value for 0% and 10% PJT diet groups. Values are arbitrary units, expressed as the mean $\pm$ SE of 6 mice. Asterisk denotes a statistically significant difference from 0% PJT diet group (Student's *t* test; $p < 0.05$).

body.

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