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Genetically Modified Rapeseed Oil Containing *cis*-9,*trans*-11,*cis*-13-Octadecatrienoic Acid Affects Body Fat Mass and Lipid Metabolism in Mice

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Punicic acid, one of the conjugated linolenic acid (CLN) isomers, exerts a body-fat reducing effect. Although punicic acid is found in pomegranate and *Tricosanthes kirilowii* seeds, the amount of this fatty acid is very low in nature. The goal of this study was to produce a transgenic oil containing punicic acid. A cDNA encoding conjugase that converts linoleic acid to punicic acid was isolated from *T. kirilowii*, and the plant expression vector, pKN-TkFac, was generated. The pKN-TkFac was introduced into *Brassica napus* by *Agrobacterium*-mediated transformation. As a result, a genetically modified rapeseed oil (GMRO) containing punicic acid was obtained, although its proportion to the total fatty acids was very low (approximately 2.5%). The effects of feeding GMRO in ICR CD-1 male mice were then examined. Wild-type rapeseed (*B. napus*) oil (RSO) containing no CLN was used as a control oil. For reference oils, RSO-based blended oils were prepared by mixing with different levels of pomegranate oil (PO), either 2.5% (RSO + PO) or 5.0% (RSO + 2PO) punicic acid. Mice were fed purified diets containing 10% of either RSO, RSO + PO, RSO + 2PO, or GMRO for 4 weeks, and dietary PO dose-dependently reduced perirenal adipose tissue weight with a significant difference between the RSO group and the RSO + 2PO group. GMRO, as compared to RSO, lowered the adipose tissue weight to the levels observed with RSO + 2PO. The liver triglyceride level of the RSO + 2PO and GMRO groups but not that of the RSO + PO group was lower than that of the RSO group. The RSO + 2PO and GMRO groups, but not the RSO + PO group, had increased carnitine-palmitoyltransferase activity in the liver and brown adipose tissue. These results showed that dietary GMRO, even at a dietary punicic acid level as low as 0.25 wt % of diet, reduced body fat mass and altered liver lipid metabolism in mice and was more effective than an equal amount of punicic acid from PO.

KEYWORDS: Conjugated linolenic acid; punicic acid; transgenic rapeseed; pomegranate seed; adipose tissue; mice

INTRODUCTION

Conjugated fatty acid is a collective term for those fatty acids that contain conjugated double bonds with multiple positional and geometric isomers. The most representative conjugated fatty acids are conjugated linoleic acid (CLA) and conjugated linolenic acid (CLN). CLA has been shown to exhibit various physiological functions (*1*, *2*). Among them, the body fat reducing effect attracts attention in terms of the evidence in humans, although results were not always unequivocal (*3*−*6*). We previously demonstrated that the body fat reducing effect of dietary CLN was at least comparable to or even stronger than that of CLA in rats (*7*). Because CLN used in the study was prepared by alkaline isomerization and consisted of a number of CLN isomers, it was difficult to draw any conclusion with respect to the active component(s). For CLA the physi-

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ological functions differ among the isomers, and 10t,12c-18:2 primarily exerts a reduction of body fat mass (*1*). In nature, there are several CLN isomers in specific plant seed oils as one of the major fatty acids. Bitter gourd (*Momordica charantia*) oil contains α-eleostearic acid (9c,11t,13t-18:3), pomegranate (*Punica granatum*) oil contains punicic acid (9c,11t,13c-18:3), catalpa (*Catalpa ovata*) oil contains catalpic acid (9t,11t,13c-18:3), and pot marigold (*Calendula officinalis*) oil contains calendic acid (8t,10t,12c-18:3) (*8*, *9*). Recently, we and others have reported that feeding of pomegranate seed oil containing punicic acid reduced the adipose tissue weight in rats (*10*, *11*), suggesting a possible role of punicic acid in the reduction of body fat mass. Although punicic acid is also known to be found abundantly [40% (w/w)] in *Tricosanthes kirilowii* seeds as well as pomegranate seeds (*12*), the availability of these oils is very limited.

With current gene recombination technology, it is possible to produce a genetically modified plant accumulating specific fatty acids. Recently, a gene encoding the conjugase that converts linoleic acid to punicic acid has been cloned from pomegranate and *T. kirilowii* (*12*, *13*), and a production of transgenic *Arabidopsis* accumulating punicic acid in the seed has been established (*12*). In the present study, we tried to introduce a conjugase gene isolated from *T. kirilowii* into *Brassica napus* by using an *Agrobacterium*-mediated transformation method and succeeded in producing a genetically modified rapeseed accumulating punicic acid, although its proportion to the total fatty acids was very low, approximately 2.5%. To evaluate the nutritional effects of the transgenic rapeseed oil, we compared in mice the physiological activities of this oil with that of untransformed rapeseed oil supplemented with pomegranate seed oil containing punicic acid.

MATERIALS AND METHODS

Production of Transgenic Rapeseed Accumulating Punicic Acid. We isolated a cDNA, TkFac, which encodes a conjugase associated with the formation of *trans*-Δ11,*cis*-Δ13 double bonds in the conjugated linolenic acid, punicic acid, from *T. kirilowii*. Detailed procedures for TkFac cDNA isolation and generation of the plant expression vector, pKN-TkFac, were described by Iwabuchi et al. (*12*). The TkFac gene was ligated downstream of a strong seed-specific promoter from the *B. napus* napin gene in the plant expression vector, pKN-TkFac.

We introduced pKN-TkFac into *B. napus* cv. Westar by *Agrobacterium*-mediated transformation, and the presence of the gene in regenerated plantlets was examined by PCR analysis. The detailed procedure for transformation of *B. napus* (rapeseed) was described previously (*14*). As a result, 11 PCR positive plants were obtained and grown in a growth chamber for harvest of the seeds.

Preparation of Transformed and Untransformed Rapeseed Oils and Their Fatty Acid Profiles. T_2 generations of the transformed rapeseed line accumulating punicic acid and untransformed rapeseed (*B. napus* cv. Westar) were grown in a growth chamber at 23/18 °C day/night and 18 h light/6 h dark cycle. The harvested seeds of both transformed and untransformed rapeseed were ground with a Waring blender, and the total lipid was extracted overnight with hexane. Hexane was removed completely from the total lipid extract by rotary evaporator. The residues originated from transformed and untransformed rapeseed, named as genetically modified rapeseed oil (GMRO) and wild-type rapeseed oil (RSO), respectively, were used for a feeding study.

For analysis of the fatty acid profile, the lipids were methylated with 0.5 M sodium methoxide in methanol (Sigma-Aldrich Inc.) at 50 °C for 1 h and were analyzed by gas chromatography (GC; Shimadzu GA18A) equipped with a TC-70 capillary column (60 m × 0.25 mm: 0.25 μm coating thickness; GL Science) (*12*).

Animal Feeding Study. *Animals and Diets.* The feeding study was conducted in accordance with the Guidelines for Animal Experiments approved by Siebold University of Nagasaki. GMRO was used as an experimental oil, and RSO was used as control oil. For reference, pomegranate seed oil (PO), containing approximately 80% of punicic acid, was added to RSO in order that the proportion of punicic acid in RSO became equal to that of punicic acid in GMRO (RSO + PO; RSO/PO = 32.5:1, by weight) or became double that of punicic acid in GMRO (RSO + 2PO; RSO/PO = 15.5:1, by weight). The fatty acid profiles of these oils are shown in **Table 1**.

Table 1. Fatty Acid Composition of Dietary Fats

	dietary fat[a] (% weight)			
fatty acid	RSO	RSO + PO	RSO + 2PO	GMRO
16:0	4.1	4.1	4.0	5.0
18:0	1.5	1.5	1.5	1.7
18:1n-9	62.5	60.8	59.0	68.4
18:2n-6	19.3	18.9	18.4	15.3
18:3n-3	9.8	9.5	9.2	3.8
9c,11t,13c-18:3	0.0	2.5	5.0	2.5
20:0	0.5	0.5	0.5	0.7
20:1	1.2	1.2	1.2	1.3

[a] RSO, untransformed rapeseed oil; PO, pomegranate seed oil; GMRO, genetically modified rapeseed oil.

Twenty-four male ICR CD-1 mice (6 weeks old) purchased from Clea Japan Inc. (Tokyo, Japan) were acclimatized for 5 days in a room maintained at 21−23 °C with a 12 h light/dark cycle. Animals were randomly assigned to four groups of six animals each according to the experimental oils: RSO, RSO + PO, RSO + 2PO, and GMRO. Diets were prepared according to the AIN-93G formula (*15*) and contained (by weight percent) casein, 20; experimental fat, 10; cornstarch, 36.7; dextrinized cornstarch, 13.2; sucrose, 10; cellulose, 5; mineral mixture (AIN-93G-MX), 3.5; vitamin mixture (AIN-93-VX), 1; L-cystine, 0.3; choline bitartrate, 0.25; and *tert*-butylhydroquinone, 0.0014. Animals were maintained on their respective diets ad libitum for 4 weeks. During the feeding period, body weight and food consumption were recorded every other day. After 4 weeks of feeding, mice in the fed state were anesthetized by intraperitoneal injection of pentobarbital sodium (0.05 mg/g of body weight) (Nembutal, Dainippon Sumitomo Pharma, Osaka, Japan), and blood was collected from the abdominal vena cava. Serum was separated by centrifugation. The liver and other tissues including perirenal and epididymal white adipose tissues and interscapular brown adipose tissue (BAT) were excised and weighed.

Serum Concentration of Lipids, Adipocytokine, Insulin, and Glucose. Serum total cholesterol, phospholipid, triglyceride, free fatty acid, and thiobarbituric acid reactive substances (TBARS) were measured using commercial assay kits (Cholesterol CII-, Phospholipid B-, Triglyceride G-, NEFA- and lipid peroxide-tests, Wako Pure Chemical Industries, Osaka, Japan).

The serum concentrations of leptin, adiponectin, and insulin were measured by enzyme-linked immunosorbent assay using a commercial kit [Mouse Leptin ELISA kit, Morinaga Institute of Biological Science, Yokohama, Japan, Mouse/Rat Adiponectin ELISA kit, Otsuka Pharmaceutical Co., Tokyo, Japan, and Mouse Insulin ELISA kit (TMB), Shibayagi Co. Ltd., Gunma, Japan]. The serum concentration of glucose was measured using a commercial kit (Glucose C-II test Wako, Wako Pure Chemical Industries).

Liver Lipid Analysis. Total liver lipids were extracted according to the method of Folch et al. (*16*). The concentrations of liver cholesterol, phospholipid, and triglyceride were measured according to the methods of Ide et al. (*17*), Rouser et al. (*18*), and Fletcher (*19*), respectively.

For the analysis of fatty acid profiles, total phospholipid and triglyceride fractions were separated from other lipid fractions by thin-layer chromatography using hexane/dimethyl ether/acetic acid (70:30:1, by volume) as the developing solvent. Each lipid fraction was then methylated with 0.5 M sodium methoxide in methanol, and the fatty acid profile was analyzed by GC (*12*).

Preparation of Hepatic Subcellular Fractions. A sample of freshly excised liver was homogenized in 7 volumes of 10 mM Tris-HCl buffer containing 0.25 M sucrose and 1 mM EDTA. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was

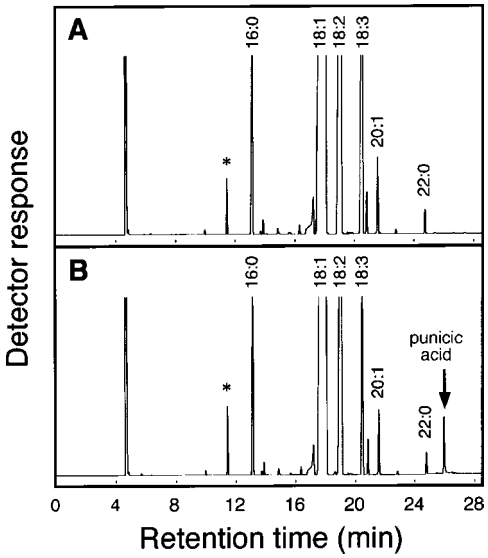

Figure 1. Gas chromatographic analysis of the fatty acid composition of total lipids from the mature T2 seeds: (**A**) untransformed rapeseed; (**B**) transformed rapeseed. *, unknown peak.

centrifuged at 16000*g* for 20 min to sediment mitochondria/peroxisomes. The pellet was suspended in the 10 mM Tris-HCl buffer. The resulting supernatant was ultracentrifuged at 125000*g* for 60 min to sediment microsomes, and the supernatant was used as cytosol fraction. The microsomal pellet was resuspended in the 10 mM Tris-HCl buffer and stored at −80 °C until used.

Measurement of Enzyme Activities. Hepatic fatty acid β-oxidation activity was measured in both mitochondria and peroxisomes. As an index of mitochondrial β-oxidation, the activity of carnitine-palmitoyltransferase (CPT) was measured spectrophotometrically according to the method of Lieber et al. (*20*). Peroxisomal β-oxidation was also measured spectrophotometrically according to the method of Lazarow (*21*). The lipogenic enzyme activities were measured in liver cytosol and microsomes. The cytosolic activities of malic enzyme, glucose-6-phosphate dehydrogenase (G6PDH), and fatty acid synthase (FAS) were measured spectrophotometrically according to the methods of Ochoa (*22*), Kelley and Kletzien (*23*), and Kelley et al. (*24*), respectively. The microsomal activity of phosphatidic acid phosphohydrolase (PAP) was measured according to the method of Surette et al. (*25*). The CPT activity was also measured in BAT. The protein concentrations were measured according to the method of Lowry et al. (*26*).

Statistical Analysis. Results were expressed as mean ± SEM. All data were analyzed by the one-way ANOVA followed by the Tukey−Kramer test to determine the dietary fat dependent difference. All analyses were performed by SuperANOVA software (Abacus Concepts, Berkeley, CA).

RESULTS

Fatty Acid Profiles of Transformed and Untransformed Rapeseed Oil. We measured the fatty acid composition of the mature seeds of the transformed and untransformed rapeseeds. Although there were no other differences in the fatty acid composition of the seeds between untransformed and transformed plants, a peak of fatty acid methyl esters, which was not present in seeds from untransformed plants, was found in seeds from transformed plants (**Figure 1**). The peak displayed a GC retention time identical to that of the methyl ester of *cis*-9,*trans*-11,*cis*-13-18:3, punicic acid, in *Punica granatum* seed (*12*). Then, we multiplied the transgenic and untransformed seeds and extracted total lipids for feeding experiment from T3 seeds (set seeds in T2 plants). Fatty acid profiles of the oils are shown in **Table 1**. The proportion of punicic acid in the transgenic seeds (GMRO) was 2.5% (by weight), whereas it was undetectable in the untransformed rapeseed (RSO).

Body Weight Gain, Food Intake, and Tissue Weights. Mice were fed the purified diets containing 10% dietary fat, either RSO, RSO + PO, RSO + 2PO, or GMRO for 4 weeks. During the feeding period, food intake and final body weight were not affected by the difference of dietary fat (**Table 2**).

Supplementation of PO to the RSO diet reduced dose-dependently the relative weight of perirenal adipose tissue. The same trend was observed in the epididymal adipose tissue weight. As compared with the RSO diet, the GMRO diet also significantly reduced the perirenal and epididymal adipose tissue weights to an extent that was comparable with or even lighter than that of the RSO + 2PO group (**Table 2**). On the other hand, dietary fat did not affect the relative weight of BAT. No effects were observed in relative weights of other tissues, including liver (**Table 2**).

Serum and Liver Lipid Concentrations. The concentrations of serum cholesterol, phospholipid, triglyceride, and free fatty acid were comparable among the groups (**Table 3**). Concentrations of TBARS in mice fed the RSO + 2PO diet were significantly higher than in those fed the other diets.

Supplementation of PO to the RSO diet to the 0.5% level of punicic acid (the RSO + 2PO diet) but not to the 0.25% level of punicic acid (the RSO + PO diet) significantly decreased the liver triglyceride concentration (**Table 3**). The GMRO diet, containing 0.25% punicic acid, also significantly decreased the concentration in comparison with the RSO diet. The concentrations of liver cholesterol and phospholipid were not affected by the type of dietary fats.

Fatty Acid Profiles of Liver Lipids. The fatty acid profile of liver phospholipids is shown in **Table 4**. Supplementation of PO containing punicic acid to the RSO diet dose-dependently increased not only the proportion of 9c,11t,13c-CLN but also that of 9c,11t-CLA. However, dietary PO did not affect the proportion of nonconjugated fatty acids. As compared with the RSO diet, feeding of the GMRO diet significantly increased the proportions of 9c,11t,13c-CLN and 9c,11t-CLA. The magnitude of the increase was comparable to that observed with the RSO + 2PO diet. Also, the GMRO diet significantly increased the proportions of 18:1 and 20:4n-6 at the expense of other major fatty acids. The increase in 20:4n-6 was more marked with the GMRO group than with two PO-added groups. As a result, the ratio of 18:1/18:0, a Δ9 desaturation index, was significantly higher in mice fed the GMRO diet than in those fed the other three diets. The ratio of (20:3n-6 + 20:4n-6)/18:2n-6, a Δ6 desaturation index, also was significantly higher in mice fed the GMRO diet than in those fed the RSO and RSO + 2PO diets.

The fatty acid profile of liver triglyceride is shown in **Table 5**. Supplementation of PO to the RSO diet again dose-dependently increased the proportions of 9c,11t,13c-CLN and 9c,11t-CLA. The GMRO diet also increased the proportion of these conjugated fatty acids, and the magnitude of the increase was intermediate to those of the two PO-added groups. Adding PO to the RSO diet increased the proportion of n-3 polyunsaturated fatty acids, whereas the GMRO diet decreased the proportions of 18:2n-6 and 18:3n-3. The proportion of 18:1 tended to decrease with dietary PO, although this trend was not statistically significant, but it was not affected by dietary GMRO. Because the proportion of 18:0 was comparable among the groups, the Δ9 desaturation index of RSO + PO and RSO + 2PO groups, but not the GMRO group, tended to be lower than that of RSO.

Lipogenic Enzyme and β-Oxidation Activities. As shown in **Table 6**, neither dietary supplementation of PO to the RSO

Table 2. Effect of Genetically Modified Rapeseed Oil Containing Punicic Acid on Body Weight, Food Intake, and Tissue Weights in Mice

	experimental group[a]			
growth parameter and tissue weight	RSO	RSO + PO	RSO + 2PO	GMRO
body weight (g)				
initial	30.1 ± 0.5	30.1 ± 0.5	30.0 ± 0.6	30.0 ± 0.5
final	38.3 ± 1.2	38.4 ± 1.6	38.5 ± 1.6	37.8 ± 1.5
food intake (g/day)	5.39 ± 0.25	5.42 ± 0.22	5.07 ± 0.11	5.36 ± 0.07
tissue weights (g/100 g of body weight)				
white adipose tissue				
perirenal	0.94 ± 0.09 a	0.83 ± 0.09 ab	0.66 ± 0.06 b	0.61 ± 0.07 b
epididymal	2.79 ± 0.34 a	2.63 ± 0.28 a	2.27 ± 0.22 ab	1.89 ± 0.17 b
brown adipose tissue	0.65 ± 0.12	0.60 ± 0.09	0.72 ± 0.10	0.69 ± 0.06
liver	5.33 ± 0.17	5.16 ± 0.11	5.12 ± 0.14	5.34 ± 0.22
kidney	1.79 ± 0.12	1.64 ± 0.08	1.69 ± 0.06	1.69 ± 0.05
spleen	0.34 ± 0.02	0.33 ± 0.03	0.32 ± 0.02	0.34 ± 0.02
heart	0.49 ± 0.03	0.48 ± 0.02	0.46 ± 0.01	0.46 ± 0.01
lung	0.44 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	0.47 ± 0.02

[a] RSO, rapeseed oil; PO, pomegranate seed oil; GMRO, genetically modified rapeseed oil. All data are expressed as means ± SEM of five or six mice. Values not sharing a common letter are significantly different at $p < 0.05$.

Table 3. Effect of Genetically Modified Rapeseed Oil Containing Punicic Acid on Serum and Liver Lipid Concentrations

	experimental group[a]			
serum and liver lipid	RSO	RSO + PO	RSO + 2PO	GMRO
serum lipids (mmol/L)				
cholesterol	3.07 ± 0.17	2.99 ± 0.15	3.34 ± 0.34	3.50 ± 0.28
phospholipid	3.13 ± 0.13	3.14 ± 0.14	3.23 ± 0.26	3.59 ± 0.24
triglyceride	1.12 ± 0.10	1.19 ± 0.20	1.43 ± 0.22	1.25 ± 0.15
free fatty acid	1.03 ± 0.07	1.03 ± 0.10	1.22 ± 0.11	1.11 ± 0.09
TBARS[b] ($\times 10^{-3}$)	7.84 ± 1.25 a	8.95 ± 0.63 a	14.9 ± 3.1 b	8.49 ± 0.81 a
liver lipids (μmol/g)				
cholesterol	6.07 ± 0.28	5.76 ± 0.26	5.68 ± 0.39	6.07 ± 0.41
phospholipid	30.7 ± 1.3	32.4 ± 0.7	32.6 ± 1.4	31.7 ± 1.2
triglyceride	25.3 ± 2.8 a	25.2 ± 1.2 ab	17.1 ± 1.7 c	19.2 ± 1.8 bc

[a] RSO, rapeseed oil; PO, pomegranate seed oil; GMRO, genetically modified rapeseed oil. All data are expressed as means ± SEM of five or six mice. Values not sharing a common letter are significantly different at $p < 0.05$. [b] TBARS, thiobarbituric acid reactive substances.

Table 4. Effect of Genetically Modified Rapeseed Oil Containing Punicic Acid on Fatty Acid Profile in Liver Phospholipid

	experimental group[a] (weight %)			
fatty acid	RSO	RSO + PO	RSO + 2PO	GMRO
16:0	34.1 ± 0.8	30.8 ± 1.7	31.1 ± 1.3	31.1 ± 0.6
18:0	17.6 ± 0.9	17.7 ± 2.1	15.5 ± 1.4	15.4 ± 0.4
18:1	9.4 ± 0.4 a	9.5 ± 0.9 a	9.3 ± 0.5 a	12.1 ± 0.4 b
18:2n-6	12.9 ± 0.5	13.3 ± 1.1	13.2 ± 0.7	11.3 ± 0.4
20:3n-6	1.8 ± 0.1	2.0 ± 0.2	1.8 ± 0.2	2.1 ± 0.1
20:4n-6	8.9 ± 0.3 a	11.5 ± 0.8 c	10.0 ± 0.6 ac	13.2 ± 0.5 b
20:5n-3	1.6 ± 0.1 a	1.8 ± 0.1 a	1.5 ± 0.2 a	0.6 ± 0.1 b
22:6n-3	10.0 ± 0.4	10.7 ± 0.8	10.3 ± 0.7	10.4 ± 0.4
9c,11t-18:2	0.00 ± 0.00 a	0.12 ± 0.03 b	0.34 ± 0.08 c	0.30 ± 0.03 c
9c,11t,13c-18:3	0.00 ± 0.00 a	0.05 ± 0.02 b	0.12 ± 0.01 c	0.09 ± 0.01 d
Δ9 DI[b]	0.55 ± 0.12 a	0.55 ± 0.20 a	0.58 ± 0.14 a	0.78 ± 0.10 b
Δ6 DI	0.83 ± 0.06 a	1.05 ± 0.25 ab	0.76 ± 0.35 a	1.36 ± 0.22 b

[a] RSO, rapeseed oil; PO, pomegranate seed oil; GMRO, genetically modified rapeseed oil. All data are expressed as means ± SEM of five or six mice. Values not sharing a common letter are significantly different at $p < 0.05$. [b] DI, desaturation index; Δ9 DI, 18:1/18:0; Δ6 DI, (20:3n-6 + 20:4n-6)/18:2n-6.

diets nor dietary GMRO affected the activity of hepatic lipogenic enzymes, cytosolic malic enzyme, G6PDH, FAS, and microsomal PAP.

The β-oxidation activity was examined in liver mitochondria and peroxisomes. Supplementation of PO to the RSO diet dose-dependently increased the CPT activity, a key enzyme in the fatty acid β-oxidation pathway in mitochondria, and it was significantly higher in mice fed the RSO + 2PO diet than in those fed the RSO diet. CPT activity of the GMRO group was also significantly higher than that of the RSO group and was comparable to that of the RSO + 2PO group. On the other hand, no significant differences were observed in hepatic peroxisomal β-oxidation among the groups.

CPT activity was also measured using the BAT homogenate. As shown in **Table 6**, supplementation of PO to the RSO diet dose-dependently increased the CPT activity in BAT, and the difference of the activity was significant between the RSO + 2PO group and the RSO group. The GMRO diet as compared to the RSO diet significantly increased the CPT activity to the level observed with the RSO + 2PO diet.

Serum Concentration of Adipocytokines, Insulin, and Glucose. As shown in **Table 7**, serum concentrations of leptin were not affected by adding PO to the RSO diet. Although serum leptin concentrations tended to be lower in mice fed the GMRO diet than in those fed the RSO diet, the difference was not

Table 5. Effect of Genetically Modified Rapeseed Oil Containing Punicic Acid on Fatty Acid Profile in Liver Triglyceride

	experimental group[a] (weight %)			
fatty acid	RSO	RSO+PO	RSO+2PO	GMRO
16:0	25.0 ± 1.9	26.3 ± 1.3	24.8 ± 1.3	28.3 ± 1.4
16:1	1.9 ± 0.3	1.7 ± 0.1	1.9 ± 0.1	1.9 ± 0.2
18:0	1.7 ± 0.2	2.0 ± 0.1	1.5 ± 0.1	1.7 ± 0.2
18:1	47.4 ± 2.0	44.1 ± 0.8	42.3 ± 2.2	48.0 ± 3.1
18:2n-6	14.2 ± 1.2 a	12.4 ± 1.3 ab	14.0 ± 1.5 a	9.9 ± 1.2 b
18:3n-3	2.7 ± 0.4 a	2.2 ± 0.3 a	2.5 ± 0.3 a	0.7 ± 0.1 b
20:4n-6	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	0.6 ± 0.1
20:5n-3	0.4 ± 0.0 a	0.5 ± 0.1 a	0.7 ± 0.0 b	0.3 ± 0.1 a
22:5n-6	0.8 ± 0.2 a	0.5 ± 0.1 ab	0.8 ± 0.1 a	0.3 ± 0.1 b
22:6n-3	1.2 ± 0.1 ab	1.7 ± 0.3 bc	2.0 ± 0.2 c	0.8 ± 0.2 a
9c,11t-18:2	0.02 ± 0.01 a	0.58 ± 0.04 b	1.73 ± 0.11 c	0.95 ± 0.08 d
9c,11t,13c-18:3	0.02 ± 0.01 a	0.43 ± 0.02 b	0.89 ± 0.07 c	0.47 ± 0.06 b
Δ9 DI[b]	31.9 ± 4.9	22.7 ± 0.9	26.1 ± 1.6	30.9 ± 3.6
Δ6 DI ($\times 10^{-1}$)	0.58 ± 0.05 a	0.63 ± 0.06 a	0.67 ± 0.41 a	0.94 ± 0.09 b

[a] RSO, rapeseed oil; PO, pomegranate seed oil; GMRO, genetically modified rapeseed oil. All data are expressed as means ± SEM of five or six mice. Values not sharing a common letter are significantly different at $p < 0.05$. [b] DI, desaturation index; Δ9 DI, 18:1/18:0; Δ6 DI, (20:3n-6 + 20:4n-6)/18:2n-6.

Table 6. Effect of Genetically Modified Rapeseed Oil Containing Punicic Acid on Hepatic Lipogenic Enzyme and β-Oxidation Activities and Brown Adipose Tissue β-Oxidation Activity

enzyme activity	experimental group[a]			
	RSO	RSO + PO	RSO + 2PO	GMRO
liver lipogenic enzyme[b] (nmol/min/mg of protein)				
ME	60.8 ± 6.5	79.8 ± 10.0	59.7 ± 5.2	67.5 ± 5.1
G6PDH	14.5 ± 1.7	13.4 ± 0.5	12.2 ± 0.9	12.6 ± 0.6
FAS	15.7 ± 0.9	16.5 ± 2.6	13.9 ± 2.1	17.6 ± 1.4
PAP	18.2 ± 1.6	20.3 ± 2.0	16.4 ± 0.7	18.6 ± 1.2
liver β-oxidation activity (nmol/min/mg of protein)				
mitochondrial (CPT[c])	13.3 ± 0.9 a	15.5 ± 1.3 ab	18.5 ± 1.5 b	17.4 ± 0.5b
peroxisomal	57.6 ± 4.7	59.9 ± 11.7	65.2 ± 5.4	61.9 ± 7.6
brown adipose enzyme activity (nmol/min/mg of protein)				
CPT	0.69 ± 0.04 a	0.79 ± 0.12 ab	0.85 ± 0.07 b	0.98 ± 0.15 b

[a] RSO, rapeseed oil; PO, pomegranate seed oil; GMRO, genetically modified rapeseed oil. All data are expressed as means ± SEM of five or six mice. Values not sharing a common letter are significantly different at $p < 0.05$. [b] ME, cytosolic malic enzyme; FAS; cytosolic fatty acid synthase; G6PDH, cytosolic glucose-6-phosphate dehydrogenase; PAP, microsomal phosphatidic acid phosphohydrolase. [c] CPT, carnitine-palmitoyltransferase.

Table 7. Effect of Genetically Modified Rapeseed Oil Containing Punicic Acid on Serum Adipocytokine, Insulin, and Glucose Concentrations

	experimental group[a]			
	RSO	RSO + PO	RSO + 2PO	GMRO
leptin (ng/mL)	7.00 ± 2.31	6.88 ± 2.12	7.03 ± 1.57	4.79 ± 0.54
adiponectin (μg/mL)	11.6 ± 2.0	9.84 ± 0.98	11.5 ± 1.2	9.32 ± 0.88
insulin (ng/mL)	1.15 ± 0.30	1.48 ± 0.24	1.38 ± 0.26	1.01 ± 0.14
glucose (mg/mL)	2.45 ± 0.18	3.07 ± 0.27	2.85 ± 0.24	2.79 ± 0.12

[a] RSO, rapeseed oil; PO, pomegranate seed oil; GMRO, genetically modified rapeseed oil. All data are expressed as means ± SEM of four to six rats.

statistically significant. Serum adiponectin concentrations were not affected by the difference of dietary fat. Both serum insulin and glucose concentrations were comparable among the experimental groups.

DISCUSSION

In the present study, we produced transgenic rapeseed containing punicic acid and evaluated the nutritional effect of the transgenic rapeseed oil through a feeding study in mice. Results in **Figure 1** indicate that transformed rapeseed, but not a wild-type plant, can synthesize one particular fatty acid, which exhibits a GC retention time identical to that of *cis*-9,*trans*-11,*cis*-13-CLN, punicic acid. In our previous observations with transgenic *Arabidopsis* carrying pKN-TkFac, accumulation of punicic acid in the seeds was confirmed by GC-MS analysis (*12*). Then, we concluded that punicic acid was produced and accumulated in the seed of *B. napus*. Although the proportion of the punicic acid was very low at approximately 2.5%, we examined whether dietary GMRO affects body fat mass and lipid metabolism in mice. For this purpose, the effect of GMRO was compared with that of RSO supplemented with PO containing punicic acid in the present study.

After 4 weeks of feeding, supplementation of PO into the RSO diet dose-dependently decreased the perirenal adipose tissue weight, and the tissue weight difference between the RSO group and the RSO + 2PO group was statistically significant ($p < 0.05$). This trend also was observed in the epididymal adipose tissue weight. The results were consistent with our earlier findings in rats (*11*). Also, Arao et al. conducted a 2 week feeding study in the Otsuka−Long-Evans Tokushima Fatty (OLETF) rats and reported that 5% supplementation of pomegranate seed oil, but not 2% supplementation, to the diet significantly reduced omental adipose tissue weight (by 27%) (*10*). On the other hand, Yamasaki et al. recently showed that feeding diets supplemented with pomegranate seed oil (0.1 and 1.0% as punicic acid) did not exhibit significant effects on the perirenal and epididymal adipose tissue weight in male C57BL/6N mice (*27*). The discrepancy between the findings reported by Yamasaki et al. and ours could be due to the difference in the diet composition. In the present study, we fed mice using rapeseed oil-based diets supplemented with PO for 4 weeks, whereas Yamasaki et al. gave soybean oil-based diets supplemented with pomegranate seed oil for 3 weeks. Taking these observations together, the more saturated rapeseed oil-based diet and 4 weeks of feeding period might induce significant effects of dietary punicic acid on adipose tissue weights, although the dietary level of punicic acid was low in our study. Also, the discrepancy could be due to the difference in the animal strain; ICR-CD 1 was used in this study, whereas C57BL/6N was used in their study. Because the C57BL/6J strain was suggested to develop obesity, hyperinsulinemia, and hyperglycemia when the animals were allowed free access to a high-fat diet (*28*), suppression of accumulating body fat mass by feeding of PO might be less effective in C57BL/6N. In the present study, the GMRO diet also exhibited a significant reduction of the perirenal and epididymal adipose tissue weights, and the magnitude of the weight reduction was comparable with or even more than that observed with the RSO + 2PO diet, which contained twice as much punicic acid as the GMRO diet (**Table 2**). Therefore, the finding suggests that the body fat reducing effect of GMRO is more effective than that of punicic acid in PO. It seems plausible that the difference in the fatty acid composition of the dietary fats may modify the body fat lowering effect of punicic acid.

Dietary punicic acid was less effective on the serum lipid concentrations, except TBARS level in the present study. The serum TBARS level exhibited a dose-dependent increase, and the difference between the RSO group and the RSO + 2PO group was statistically significant ($p < 0.05$). This could be due to a lower oxidation stability of conjugated fatty acids (*29*). However, as far as the dietary level of punicic acid was 0.25% (RSO + PO and GMRO), an increase of TBARS level was not statistically significant, irrespective of the source of punicic acid, PO, or GMRO. On the other hand, serum triglyceride concentrations slightly increased with PO supplementation, although the effects were not significant enough. Dietary GMRO did not affect serum triglyceride concentrations either. The study of Yamasaki et al. cited above showed that dietary pomegranate seed oil (0.1 and 1% as punicic acid) significantly increased

serum triglyceride concentration in C57BL/6N mice (*27*). In rats, on the other hand, the serum triglyceride concentrations were not statistically affected by feeding of pomegranate seed oil (*10*, *11*), whereas they increased when the rats were fed with CLN prepared by alkaline isomerization containing various isomers of conjugated dienes and trienes (*7*). Therefore, the effect of dietary punicic acid on serum triglyceride concentration seems unequivocal, possibly depending on experimental protocols such as diet composition, feeding period, and animal species and strains.

Rather, dietary punicic acid affected liver triglyceride concentrations in the present study. The concentration was significantly decreased by supplementation of PO (0.5% as punicic acid) to the RSO diet ($p < 0.05$). Because dietary supplementation of PO to the RSO diet dose-dependently increased the activity of CPT in hepatic mitochondria and BAT homogenates, the increase of fatty acid β-oxidation could be at least a possible factor of the reduction of liver triglyceride concentration. An increase in the hepatic β-oxidation activity by dietary pomegranate seed oil or synthetic CLN also was observed in our previous study, although the response to liver triglyceride concentration was not necessarily the same (*7*, *11*). Although dietary PO did not affect the activity of liver microsomal PAP in the present study, Arao et al. showed in in vitro study that incubation of human hepatoma HepG2 cells with punicic acid significantly reduced apoB 100 secretion and triglyceride synthesis from [1-^{14}C]oleate (*30*). All of these findings support the decrease in the concentration of liver triglyceride. In our study, dietary GMRO also affected liver triglyceride concentration and hepatic enzyme activities in the same manner as dietary PO. Also, the liver triglyceride concentration and hepatic CPT activity in the GMRO group were comparable to those of the RSO + 2PO group. Besides, the activity of CPT in brown adipose tissue tended to be higher in the GMRO group than in the RSO + 2PO group. Therefore, it was suggested that the magnitude of the effects of punicic acid as GMRO were greater than those of the same amount of punicic acid as PO. It is not clear why punicic acid as GMRO exhibited more potent effects on body fat mass and liver lipid metabolism. However, in our preliminary analysis, punicic acid was not detected at all in the free fatty acid fraction after hydrolysis of GMRO with *sn*-1 or -3 specific lipase (data not shown). This strongly suggested that the punicic acid in GMRO was distributed exclusively in the *sn*-2 position. On the other hand, because PO contained over 80% of punicic acid, the punicic acid in PO is plausibly located in all positions of the triglyceride molecule. Therefore, the positional difference of punicic acid in the triglyceride molecule could be one of the factors to cause the difference between the GMRO and PO. However, Tsuzuki et al. recently showed that lymphatic recovery of punicic acid was roughly quantitative (approximately 90%) after 24 h in rats administered pomegranate seed oil, even though punicic acid is located randomly in the triglyceride molecule (*31*). Thus, the advantage of the specific distribution of punicic acid in GMRO remains unclear.

As shown in **Tables 4** and **5**, dietary PO dose-dependently increased the proportion of punicic acid and another conjugated fatty acid, 9c,11t-CLA, in liver phospholipid and more evidently in triglyceride. In all cases, the proportion of 9c,11t-CLA was more than that of punicic acid, suggesting that punicic acid was effectively Δ13-saturated to 9c,11t-CLA (*8*, *31*, *32*). Dietary GMRO also increased the proportion of these conjugated fatty acids to the level between the RSO + PO and PSO + 2PO groups. These results at least indicated that dietary punicic acid in PO and GMRO was absorbed, incorporated, and metabolized in mice.

It has been recognized that monounsaturated fatty acid, such as 18:1n-9, is a major fatty acid form in adipose tissues (*27*, *33*). Conversion of 18:0 to 18:1n-9, Δ9 desaturation, is therefore considered to be implicated in various diseases including obesity (*34*, *35*). In the present study, the ratio of 18:1n-9/18:0, Δ9 desaturation index of liver triglyceride, tended to be decreased by dietary PO. The result was not inconsistent with a previous paper, which showed that dietary punicic acid significantly decreased the Δ9 desaturation index in serum triglyceride (*10*). The decrease of Δ9-desaturase activity, stearoyl-CoA desaturase, was also reported in mouse liver microsomes incubated with 10t,12c-CLA, which is known as an active CLA isomer of antiobese effect (*36*). In the present study, dietary GMRO did not affect the Δ9 desaturation index in liver triglyceride. The proportions of several polyunsaturated fatty acids, 18:2n-6, 18:3n-3, and 22:5n-6, were significantly decreased by feeding of the GMRO diet ($p < 0.05$). Because the concentration of liver triglyceride was significantly decreased by dietary GMRO ($p < 0.05$), it seems likely that the amounts of these fatty acids were low in mice fed GMRO. The results suggested that the effect of punicic acid on fatty acid composition observed with GMRO was at least not necessarily the same as that with punicic acid in PO.

It has been established that adipocytokines, such as leptin and adiponectin, secreted from adipocytes can modulate the amount of adipose tissue (*37*, *38*). Therefore, serum concentrations of these adipocytokines were measured. Although supplementation of PO to the RSO diet did not affect the serum leptin concentration, the GMRO diet tended to decrease it (by 32%). The decreasing trend of serum leptin concentration in the GMRO group could be the result of reduced white adipose tissue weights in the present study (*7*).

It was demonstrated that dietary CLA is far more effective in reducing body fat mass in mice as compared to rats (*39*). This effect accompanied a drastic reduction of both the serum concentration of leptin and adiponectin in mice (*40*, *41*) and induced lipodystrophy characterized by insulin resistance (*42*, *43*). In the present study, however, serum leptin and adiponectin concentrations did not significantly change, and serum insulin and glucose concentrations were not affected by feeding of punicic acid. It is, therefore, plausible that the dietary punicic acid not only in PO, but also in GMRO, did not cause unfavorable results in mice under the experimental conditions in the present study.

In conclusion, dietary GMRO as well as PO containing punicic acid decreased adipose tissue weights and hepatic triglyceride concentration in mice, which could in part be due to an increase of hepatic fatty acid β-oxidation activity. The effect of GMRO was estimated to be more potent than that of PO. To our knowledge, this is the first report to show the dietary effect of gene-modified plant oil containing punicic acid. Further studies are required to elucidate the mechanism for the effects. Also, efforts to produce a transformed plant accumulating higher levels of punicic acid are expected.

ABBREVIATIONS USED

BAT, brown adipose tissue; CLA, conjugated linoleic acid; CLN, conjugated linolenic acid; CPT, carnitine-palmitoyltransferase; DI, desaturation index; FAS, fatty acid synthase; GC, gas chromatography; GMRO, genetically modified rapeseed oil; G6PDH, glucose-6-phosphate dehydrogenase; ME, malic en-

zyme; OLETF, Otsuka−Long-Evans Tokushima Fatty; PAP, phosphatidic acid phosphohydrolase; PO, pomegranate seed oil; RSO, wild-type rapeseed oil; TBARS, thiobarbituric acid reactive substances.

LITERATURE CITED

(1) Pariza, M. W.; Park, Y.; Cook, M. E. The biologically-active isomers of conjugated linoleic acid. *Prog. Lipid Res.* **2001**, *40*, 283−298.

(2) Belury, M. A. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. *Annu. Rev. Nutr.* **2002**, *22*, 505−531.

(3) Zambell, K. L.; Keim, N. L.; Van Loan, M. D.; Gale, B.; Benito, P.; Kelley, D. S.; Nelson, G. J. Conjugated linoleic acid supplementation in humans: effects on body composition and energy expenditure. *Lipids* **2000**, *35*, 777−782.

(4) Smedman, A.; Vessby, B. Conjugated linoleic acid supplementation in humans−metabolic effects. *Lipids* **2001**, *36*, 773−781.

(5) Petridou, A.; Mougios, V.; Sagredos, A. Supplementation with CLA: isomer incorporation into serum lipids and effect on body fat of women. *Lipids* **2003**, *38*, 805−811.

(6) Gaulliet, J.-M.; Halse, J.; Hoye, K.; Kristiansen, K.; Fagertun, H.; Vik, H.; Gudmundsen, O. Conjugated linoleic acid supplementation for 1y reduces body fat mass in healthy overweight humans. *Am. J. Clin. Nutr.* **2004**, *79*, 1118−1125.

(7) Koba, K.; Akahoshi, A.; Yamasaki, M.; Tanaka, K.; Yamada, K.; Iwata, T.; Kamegai, T.; Tsutsumi, K.; Sugano, M. Dietary conjugated linolenic acid in relation to CLA differently modifies body fat mass and serum and liver lipid levels in rats. *Lipids* **2002**, *37*, 343−350.

(8) Noguchi, R.; Yasui, Y.; Suzuki, R.; Hosokawa, M.; Fukunaga, K.; Miyashita, K. Dietary effects of bitter gourd oil on blood and liver lipids of rats. *Arch. Biochem. Biophys.* **2001**, *396*, 207−212.

(9) Suzuki, R.; Noguchi, R.; Ota, T.; Abe, M.; Miyashita, K.; Kawada, T. Cytotoxic effect of conjugated trienoic fatty acids on mouse tumor and human monocytic leukemia cells *Lipids* **2001**, *36*, 477−482.

(10) Arao, K.; Wang, Y.-M.; Inoue, N.; Hirata, J.; Cha, J.-Y.; Nagao, K.; Yanagita, T. Dietary effect of pomegranate seed oil rich in 9cis, 11trans, 13cis conjugated linolenic acid on lipid metabolism in obese hyperlipidemic OLETF rats. *Lipids Health Dis.* **2004**, *3*, 24.

(11) Koba, K.; Akahoshi, A.; Tanaka, K.; Miyashita, K.; Iwata T.; Kamegai, T.; Yamamoto, T.; Sugano, M. Dietary conjugated linolenic acid modifies body fat mass, and serum and liver lipid levels in rats. In *Dietary Fats and Risk of Chronic Disease*; Huang, Y.-S., Yanagita, T., Knapp, H. R., Eds.; AOCS Press: Champaign, IL, 2006; pp 92−105.

(12) Iwabuchi, M.; Kohno-Murase, J.; Imamura, J. Δ12-Oleate desaturase-related enzymes associated with formation of conjugated trans-Δ11, cis-Δ13 double bonds. *J. Biol. Chem.* **2003**, *278*, 4603−4610.

(13) Hornung, E.; Pernstich, C.; Feussner, I. Formation of conjugated Δ11Δ13-double bonds by Δ12-linoleic acid (1,4)-acyl-lipid-desaturase in pomegranate seeds. *Eur. J. Biochem*. **2002**, *269*, 4852−4859.

(14) Kohno-Murase, J.; Murase, M.; Ichikawa, H.; Imamura, J. Effects of an antisense napin gene on seed storage compounds in transgenic *Brassica napus* seeds. *Plant Mol. Biol.* **1994**, *26*, 1115−1124.

(15) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939−1951.

(16) Folch, J.; Lees, M.; Slone-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−506.

(17) Ide, T.; Oku, H.; Sugano, M. Reciprocal responses to clofibrate in ketogenesis and triglyceride and cholesterol secretion in isolated rat liver. *Metabolism* **1982**, *31*, 1065−1072.

(18) Rouser, G.; Siakotos, A. N.; Fleischer, S. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. *Lipids* **1966**, *1*, 85−86.

(19) Fletcher, M. J. A calorimetric method for estimating serum triglycerides. *Clin. Chim. Acta* **1968**, *22*, 393−397.

(20) Lieber, L. L.; Abraham, T.; Helmrath, T. A rapid spectrophotometric assay for carnitine palmitoyltransferase. *Anal. Biochem.* **1972**, *50*, 509−518.

(21) Lazarow, P. B. Assay of peroxisomal β-oxidation of fatty acids. *Methods Enzymol.* **1981**, *72*, 315−319.

(22) Ochoa, S. "Malic" enzyme. *Methods Enzymol.* **1955**, *1*, 739−753.

(23) Kelley, D. S.; Kletzien, R. F. Ethanol modulation of the hormonal and nutritional regulation of glucose 6-phosphate dehydrogenase activity in primary cultures of rat hepatocytes. *Biochem. J.* **1983**, *217*, 543−549.

(24) Kelley, D. S.; Nelson, G. J.; Hunt, J. E. Effect of prior nutritional status on the activity of lipogenic enzymes in primary monolayer cultures of rat hepatocytes. *Biochem. J.* **1986**, *235*, 87−90.

(25) Surette, M. E.; Whelan, J.; Broughton, K. S.; Kinsella, J. E. Evidence for mechanisms of the hypotriglyceridemic effect of n-3 polyunsaturated fatty acids. *Biochim. Biophys. Acta* **1992**, *1126*, 199−205.

(26) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

(27) Yamasaki, M.; Kitagawa, T.; Koyanagi, N.; Chujo, H.; Maeda, H.; Kohno-Murase, J.; Imamura, J.; Tachibana, H.; Yamada, K. Dietary effect of pomegranate seed oil on immune function and lipid metabolism in mice. *Nutrition* **2006**, *22*, 54−59.

(28) Collins, S.; Martin, T. L.; Surwit, R. S.; Robidoux, J. Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. *Physiol. Behav.* **2004**, *81*, 243−248.

(29) Miyazawa, T.; Tsuzuki, T.; Nakagawa, K.; Igarashi, M. Fatty acids with conjugated unsaturation: relationship between oxidative stability and physiological activities. *Lipid Technol.* **2005**, *17*, 221−225.

(30) Arao, K.; Yotsumoto, H.; Han, S.-Y.; Nagao, K.; Yanagita, T. The 9cis, 11trans, 13cis isomer of conjugated linolenic acid reduces apolipoprotein B100 secretion and triacylglycerol synthesis in HepG2 cells. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 2643−2645.

(31) Tsuzuki, T.; Kawakami, Y.; Abe, R.; Nakagawa, K.; Koba, K.; Imamura, J.; Iwata, T.; Ikeda, I.; Miyazawa, T. Conjugated linolenic acid is slowly absorbed in rat intestine, but quickly converted to conjugated linoleic acid. *J. Nutr.* **2006**, *136*, 2153−2159.

(32) Tsuzuki, T.; Igarashi, M.; Komai, M.; Miyazawa, T. Significant occurrence of 9,11-conjugated linoleic acid (18:2, n-7) in the liver and plasma lipids of rats fed 9,11,13-eleostearic acid (18:3, n-5)-supplemented diet. *J. Nutr. Sci. Vitaminol.* **2003**, *49*, 195−200.

(33) Insull, W.; Bartsch, G. E. Fatty acid composition of human adipose tissue related to age, sex and race. *Am. J. Clin. Nutr.* **1967**, *20*, 13−23.

(34) Jones, B. H.; Maher, M. A.; Banz, W. J.; Zemel, M. B.; Whelan, J.; Smith, P. J.; Moustaid, N. Adipose tissue stearoyl-CoA desaturase mRNA is increased by obesity and decreased by polyunsaturated fatty acids. *Am. J. Physiol.* **1996**, *271*, E44−E49.

(35) Pan, D. A.; Hulbert, A. J.; Storlien, L. H. Dietary fats, membrane phospholipids and obesity. *J. Nutr.* **1994**, *124*, 1555−1565.

(36) Park, Y.; Storkson, J. M.; Ntambi, J. M.; Cook, M. E.; Sih, C. J.; Pariza, M. W. Inhibition of hepatic stearoyl-CoA desaturase activity by trans10, cis-12 conjugated linoleic acid and its derivatives. *Biochim. Biophys. Acta* **2000**, *1486*, 285−292.

(37) Halle, M.; Berg, A.; Northoff, H.; Keul, J. Importance of TNF-α and leptin in obesity and insulin resistance: a hypothesis on the impact of physical exercise. *Exerc. Immunol. Rev.* **1998**, *7*, 77−94.

(38) Nagasawa, A.; Fukui, K.; Funahashi, T.; Maeda, N.; Shimomura, I.; Kihara, S.; Waki, M.; Takamatsu, K.; Matsuzawa, Y. Effects of soy protein diet on the expression of adipose genes and plasma adiponectin. *Horm. Metab. Res.* **2002**, *34*, 635−639.

(39) Pariza, M. W. Perspective on the safety and effectiveness of conjugated linoleic acid. *Am. J. Clin. Nutr.* **2004**, *79* (Suppl.), 1132S−1136S.

(40) Tsuboyama-Kasaoka, N.; Takahashi, M.; Tanamura, K.; Kim, H.-J.; Tange, T.; Okuyama, H.; Kasai, M.; Ikemoto, S.; Ezaki, O. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes* **2000**, *49*, 1534−1542.

(41) Tsuboyama-Kasaoka, N.; Miyazaki, H.; Kasaoka, S.; Ezaki, O. Increasing the amount of fat in a conjugated linoleic acid-supplemented diet reduces lipodystrophy in mice. *J. Nutr.* **2003**, *133*, 1793−1799.

(42) Ohashi, A.; Matsushita, Y.; Shibata, H.; Kimura, K.; Miyashita, K.; Saito, M. Conjugated linoleic acid deteriorates insulin resistance in obese/diabetic mice in association with decreased production of adiponectin and leptin. *J. Nutr. Sci. Vitaminol.* **2004**, *50*, 416−421.

(43) Poirier, H.; Rouault, C.; Clement, L.; Niot, I.; Monnot, M. C.; Guerre-Millo, M.; Besnard, P. Hyperinsulinemia triggered by dietary conjugated linoleic acid is associated with a decrease in leptin and adiponectin plasma levels and a pancreatic beta cells hyperplasia in the mouse. *Diabetologia* **2005**, *48*, 1059−1065.

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