Effects of Fatty Acid Quality and Quantity in the Japanese Diet on the Suppression of Lipid Accumulation

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Abstract: Japan has been known as a healthy country since its life expectancy became among the highest in the world in the 1980s. The influence of the Japanese diet is one of the factors explaining Japan's high life expectancy. Our recent study that fed representative freeze-dried and powdered Japanese diets from 1960, 1975, 1990, and 2005 based on National Health and Nutrition Research to mice showed the 1975 Japanese diet exhibited the strongest visceral fat accumulation suppression and overall health benefits. However, it is unclear why. We investigated the effects of the fatty acid composition in Japanese diets on visceral fat accumulation in mice. ICR mice were fed diets replicating the fatty acid composition and macronutrient ratios of Japanese diets from 1960, 1975, 1990, and 2005 for four weeks. The 1975 diet suppressed visceral fat accumulation and adipocyte hypertrophy. DNA microarray analysis showed the 1975 diet suppressed Acyl-CoA synthetase and prostaglandin D2 synthase mRNA expressions in white adipose tissue. As the effects of the 1975 diet are likely due to differences in fatty acid intake and/or composition, we investigated test diets that replicated only the fatty acid composition of Japanese diets. There were no significant differences in visceral fat mass. Therefore, both the quality and quantity of fatty acids are involved in the anti-obesity effects of the 1975 Japanese diet.

Key words: mice, fatty acid composition, Japanese diet, Japanese food, obesity, macronutrient ratio

1 INTRODUCTION

Japan has been known as a healthy country since its life expectancy became among the highest in the world in the 1980s¹⁾. The influence of the Japanese diet is one of the factors explaining Japan's high life expectancy. The Japanese diet is diverse and high in rice and traditional food such as fish, vegetables, and soy as well as meat, milk, oils, and fruits¹⁾. In addition, lipid and protein contents differ from those of Western diets, because of high consumption of fresh seafood. Thus, the characteristic components of the Japanese diet have been widely studied. For example, Jelinek *et al.* report that replacing a portion of the lipid content in high-fat diet to fish oil decreases cholesterol and neutral fat levels in the livers of mice²⁾. Moreover, Nakagawa *et al.* report that green tea catechins enhance antioxidant activity in plasma in healthy men³⁾. In meta-analysis of trials of blood lipids in humans, Anderson *et al.* found that replacing animal protein with soy protein decreases total cholesterol (TC), especially low-density lipoprotein

cholesterol, and neutral fat levels⁴⁾. However, the impact of the Japanese diet on human health has scarcely been examined. In our previous study, we cooked modern Japanese and American foods and replicated, freeze-dried, powdered, and fed them to rats for three weeks¹⁾; the results show lipid and peroxide lipid levels in the liver and plasma were lower in rats fed the Japanese diet than those fed the American diet. In particular, liver TC levels in rats fed the Japanese diet were approximately 70% of those in rats fed the American diet. Furthermore, DNA microarray analysis of gene expression levels in the liver showed the expressions of stress-response genes were lower while those of energy, glucose, and lipid metabolism-related genes were higher in rats fed the Japanese diet compared to those fed the American diet. This suggests the Japanese diet decreases stress, stimulates metabolism, and is less likely to cause obesity compared to the American diet. However, the contents of the Japanese diet have been greatly altered by Western influence in the last 50 years⁵⁾. In particular,

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rice consumption has halved, animal product consumption has quadrupled, and lipid consumption has tripled. Therefore, the Japanese diet may have been healthier in the past. However, it is unknown whether Japanese diets of any era are beneficial for maintaining health. A study that fed representative freeze-dried and powdered Japanese diets from 1960, 1975, 1990, and 2005 based on National Health and Nutrition Research to mice^{6,7)} showed the 1975 Japanese diet exhibited the strongest visceral fat accumulation suppression and overall health benefits. Furthermore, we investigated whether this effect of the 1975 Japanese diet depends on the macronutrient ratio, i.e., the ratio of proteins, lipids, and carbohydrates. To this end, purified diets that replicated the macronutrient ratio of the Japanese diet in each era were fed to ICR mice for four weeks. However, there were no differences in visceral fat quantity among groups; this suggests the effects of the 1975 Japanese diet do not solely depend on the differences in the macronutrient ratio. Therefore, in the present study, we focused on the quality of macronutrients, especially the fatty acid composition of lipids, in order to determine the influence of fatty acid composition in Japanese diets from various eras on visceral fat accumulation.

2 MATERIALS & METHODS

2.1 Test oil preparation

The fatty acid compositions of Japanese diets from various eras were measured to prepare corresponding test oils (mimic oils here after). The total lipids from powdered Japanese diet, which were prepared as described previously⁶⁾, were extracted according to the procedure of Bligh & Dyer⁸⁾. In brief, 400 μ L 0.9% NaCl solution (aq) containing 1 mM EDTA was added to 50–100 mg of each sample, followed by the addition of 1000 μ L MeOH and 500 μ L CHCl_3 containing butylated hydroxytoluene. The solution was then vortexed for 2 min. And, 400 μ L 0.9% NaCl solution (aq) and 500 μ L CHCl_3 were mixed, vortexed for 2 min, and centrifuged for 5 min at 3000 rpm at 4°C (Takara Tomy, Japan). The lower layer was transferred to another tube, and 1000 μ L CHCl_3 was added to the upper layer, vortexed for 2 min, and centrifuged for 5 min at 3000 rpm at 4°C; this step was repeated three times. The total lipids were dried under a stream of nitrogen gas. To prepare the fatty acid methyl esters, the total lipids and a known amount of heptadecanoic acid (17:0) (Sigma, St. Louis, MO, USA) as an internal standard were methylated by the addition of 1000 μ L 5% HCl/MeOH for 30 min at 60°C. Then, 3 mL hexane and 2 mL 2% KHCO_3 (aq) were added. The solution was vortexed for 2 min and centrifuged for 5 min at 3000 rpm at room temperature. The upper layer was transferred to another tube, 3 mL demineralized water was added, and the solution was vortexed for 2 min and centrifuged for 5

min at 1000 rpm at room temperature. The upper layer was transferred to a centrifuge tube and dried by evaporator and a stream of nitrogen gas. The lipids were dissolved in 50 μ L hexane and subjected to gas chromatography (GC) (GC-2014, Shimadzu, Kyoto, Japan). Fatty acid composition was analyzed by GC using a flame ionization detector and a Supelco wax-10 fused silica capillary column (60 m $\times$ 0.32 mm, 0.25- μ m film thickness, Supelco, Bellefonte, PA, USA) as described previously^{9,10)}. On the basis of the results, the mimic oils were prepared by mixing soybean oil, lard, palm oil, fish oil, and high oleic safflower oil. Preparation focused on the ratio of saturated, monounsaturated, and polyunsaturated fatty acids (S:M:P ratio) as well as the ratio of ω 6 to ω 3 polyunsaturated fatty acids (ω 6: ω 3 ratio). The mimic oils were prepared to match the proportions of the various Japanese diets.

2.2 Test diet preparation

The test diets were prepared as purified diets based on AIN-93G. The mimic oil-balanced diets were test diets that replicated the macronutrient ratio and fatty acid composition of the Japanese diets from different eras. Over time, the proportions of lipids and carbohydrates in the Japanese diet have increased and decreased, respectively⁷⁾ (Table 1); the mimic oil-balanced diets were prepared on the basis of these values. Meanwhile, the macronutrient ratios were replicated by milk casein (protein); mimic oils (lipid); and cornstarch, α -cornstarch, and sucrose (carbohydrate) (Table 2). Moreover, the carbohydrate proportion was altered by changing only the amount of cornstarch.

On the other hand, the macronutrient ratios in the mimic oil diets were constant and based on the AIN-93G diet in all groups; the mimic oil diets only replicated fatty acid composition.

2.3 Animals

All animal procedures were performed in accordance with the Animal Experiment Guidelines of Tohoku University, and the animal protocol was approved by the Animal Use Committee at Tohoku University¹¹⁾. Male ICR mice (four weeks old) were obtained from Japan Clea (Tokyo, Japan). The mice had access to their respective diet and distilled water ad libitum in a temperature- and humidity-controlled room with a 12/12-h light/dark cycle. After accli-

Table 1 The macronutrient ratio of the Japanese food.

	1960	1975	1990	2005
	(%)			
Protein	12.2	15.1	16.9	16.1
Lipid	9.8	17.1	21.4	25.8
Carbohydrate	78.0	67.8	61.7	58.1

Table 2 The composition of the mimic oil-balanced diet.

	1960	1975	1990	2005	Normal
Cornstarch	50.98	44.19	39.76	37.76	39.75
Milk casein powder	11.61	15.01	17.30	16.95	20.00
α -cornstarch	13.20	13.20	13.20	13.20	13.20
Sucrose	10.00	10.00	10.00	10.00	10.00
Mimic oil	4.16	7.55	9.69	12.04	7.00
Cellulose powder (g/100 g)	5.00	5.00	5.00	5.00	5.00
Mineral mix (AIN-93G-MX)	3.50	3.50	3.50	3.50	3.50
Vitamin mix (AIN-93VX)	1.00	1.00	1.00	1.00	1.00
L-cystine	0.30	0.30	0.30	0.30	0.30
Choline bitartrate	0.25	0.25	0.25	0.25	0.25
Tertiary butylhydroquinone	0.0014	0.0014	0.0014	0.0014	0.0014
Energy (kcal/100 g)	381	398	408	420	395

matization to CE-2 powdered diet from Japan Clea for one week, 48 mice were randomly divided into four groups (two cages with six mice per cage) each fed the 1960, 1975, 1990, or 2005 mimic oil-balanced diet (mb60, mb75, mb90, and mb05 groups, respectively) for four weeks. In addition, a normal group was fed AIN-93G powdered diet as a reference. At nine weeks of age, the mice were weighed and blood samples were collected by decapitation. The brain, heart, lungs, liver, pancreas, spleen, kidneys, and white adipose tissues were removed and weighed. Sera and organs were stored at -80°C until use.

Moreover, the results of the test using the mimic oil diets were used to determine whether the effects of the diets were due to differences in the composition or amount of fatty acids. The 1960, 1975, 1990, or 2005 mimic oil diets or the reference diet were fed to the mice under the same conditions as mentioned above.

2.4 Histological analysis of white adipose tissue

Epididymal adipose tissue was fixed in 10% formalin and embedded in paraffin¹²⁾. Vertical sections (5 μm) were cut, mounted on glass slides, stained with hematoxylin & eosin, and observed under a microscope (BZ-9000, Keyence, Osaka, Japan). The mean area of adipocytes in each group was calculated and compared.

2.5 DNA microarray analysis

For DNA microarray analysis, total RNA was isolated from epididymal adipose tissue using the RNeasy Lipid Tissue Mini Kit (QIAGEN, Valencia, CA, USA)¹³⁾ after elution with 30 μL RNase-free water and stored at -80°C until use. DNA microarray analysis was performed using the SuperPrint G3 Mouse GE $8 \times 60\text{K}$ Microarray (Agilent Technology, Santa Clara, CA, USA) in the Takara Bio CDM Center (Mie, Japan).

2.6 White adipose tissue, serum and liver biochemical analysis

Biochemical analyses of epididymal adipose tissue, serum and liver samples were performed as described previously^{1,14)}. The epididymal adipose tissue, serum and liver triacylglycerol (TG) were measured by using commercial enzyme kits (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instructions. The liver TC and Serum TC, phospholipid (PL) and glucose levels were measured by using commercial enzyme kits (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instructions. Serum insulin levels were determined by ELISA (Morinaga Institute of Biological Science, Kanagawa, Japan) according to the manufacturer's instructions. The liver PL levels were measured according to the procedure of Rouser *et al.*¹⁵⁾.

2.7 GC analysis of total lipid in white adipose tissue, serum and liver

The total lipids from epididymal adipose tissue, serum and liver were extracted according to the procedure of Bligh & Dyer⁸⁾. 50 mg of epididymal adipose tissue, 400 μL of serum or 150 mg of liver were used and performed in the same method as described above (2.1. *Test oil preparation*). The amount of serum samples were small, so they were pooled for each group. The methyl esterified lipids were dissolved in 1000 μL hexane and subjected to GC (GC-4000 Plus, GL Sciences, Tokyo, Japan). Fatty acid composition was analyzed by GC using a flame ionization detector and a Supelco wax-10 fused silica capillary column (60 m $\times$ 0.25 mm, 0.25- μm film thickness, Supelco, Bellefonte, PA, USA).

2.8 Statistical analysis

Data are shown mean $\pm$ SE. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison

test for comparisons among four groups. The level of significance was set at $p < 0.05$. The reference group was not included in the analysis. The stepwise multiple regression analysis was performed to explore determinants of total white adipose tissue weight. The fatty acids (14:0, 16:0, 16:1 n-7, 18:0, 18:1 n-9, 18:1 n-7, 18:2 n-6, 18:3 n-3, 20:1 n-9, 20:3 n-3, 20:4 n-6, 20:5 n-3 and 22:6 n-3) in epididymal adipose tissue were included as predictors in the original model. The level of significance was set at $p < 0.05$ for regression analysis.

3 RESULTS

3.1 Composition of the mimic oil-balanced diets

Preparation of the mimic oils focused on the fatty acid composition, especially the S:M:P and $\omega 6:\omega 3$ ratios. The lipids extracted from the Japanese diets were analyzed by GC, and respective mimic oils were prepared by using five kinds of oils (Table 3). GC analysis of the mimic oils demonstrated they replicated the fatty acid composition of the Japanese diets. The mimic oil-balanced diets, which replicated the macronutrient ratio and fatty acid composition of the Japanese diets by altering the amounts of purified diet and mimic oil, were subsequently prepared (Table 2). In addition, the fatty acid contents of test diets were shown in Supplemental table 1 and 2. As a result, the amount of increase in EPA and DHA (increased 0.09 g from 1975 year to 2005 year) were lower than that in saturated fatty acid (increased 1.05 g from 1975 year to 2005 year).

3.2 Effects of mimic oil-balanced diets on growth parameters

There were no significant differences in the final body

weight and body weight gain among the four groups (Table 4). Although food intake was significantly higher in the mb60 group than the others, there was no significant difference in energy intake among groups. The mesenteric and perirenal adipose tissue weights were significantly lower in the mb75 group than the mb90 and mb05 groups. The epididymal adipose tissue weight was significantly lower in the mb75 group than the mb90 group. In addition, the total adipose tissue weight was significantly lower in the mb75 group than the other groups. There were no significant differences in brain, heart, lung, liver, pancreas, spleen, or kidney weights among groups. Thus, the results suggest the 1975 mimic oil-balanced diet suppressed lipid accumulation in epididymal adipose tissue.

3.3 Histological analysis of white adipose tissue of mice fed mimic oil-balanced diets

The decreased adipose tissue weight suggests the 1975 mimic oil-balanced diet suppressed obesity. Thus, the epididymal adipose tissues were stained with hematoxylin & eosin and subjected to histological analysis. Adipocytes were smaller in mb75 group than the other groups (Fig. 1). In addition, the area of adipocytes in each group was calculated. With the normal group set as 1.00, the adipocyte areas in the mb60, mb75, mb90, and mb05 groups were 0.98 ± 0.06 , 0.72 ± 0.05 , 0.98 ± 0.08 , and 1.19 ± 0.13 , respectively; the adipocyte area was significantly smaller in the mb75 group than the mb05 group ($p = 0.009$). This result suggests adipocyte hypertrophy in white adipose tissue was suppressed by intake of the 1975 mimic oil-balanced diet.

Table 3 The fatty acid composition of mimic oil.

	1960	1975	1990	2005
Preparation of mimic oil	(%)			
Soybean oil	40.3	31.8	26.0	25.1
Lard	26.1	37.1	56.0	41.3
Palm oil	16.3	13.6	9.0	15.5
Fish oil	10.7	11.4	8.0	12.3
High oleic safflower oil	6.5	6.2	1.1	5.8
S:M:P ratio				
Japanese food	1.0:1.3:1.1	1.0:1.3:0.9	1.0:1.2:0.7	1.0:1.2:0.7
Mimic oil	1.0:1.3:1.1	1.0:1.3:0.9	1.0:1.2:0.7	1.0:1.3:0.8
$\omega 6:\omega 3$ ratio				
Japanese food	5.0	4.4	5.0	4.0
Mimic oil	5.0	4.5	5.0	4.0

S, saturated fatty acid; M, monounsaturated fatty acid; P, polyunsaturated fatty acid.

Table 4 Body weight, food intake, energy intake and tissue weight in mice fed mimic oil-balanced diet.

	mb60	mb75	mb90	mb05	Normal
Final body weight (g)	40.9 ± 0.6	41.2 ± 0.8	42.3 ± 0.7	43.0 ± 1.6	40.5 ± 0.6
Body weight gain (g)	9.20 ± 0.27	9.41 ± 0.49	10.5 ± 0.4	10.9 ± 1.2	8.67 ± 0.20
Food intake (g/day)	4.91 ± 0.16 ^b	4.65 ± 0.13 ^a	4.51 ± 0.15 ^a	4.53 ± 0.18 ^a	4.76 ± 0.18
Energy intake (kcal/day)	18.7 ± 0.6	18.5 ± 0.5	18.4 ± 0.6	19.0 ± 0.8	18.8 ± 0.7
Tissue weight	(g/100 g B. W.)				
Brain	1.28 ± 0.02	1.24 ± 0.02	1.24 ± 0.02	1.20 ± 0.05	1.26 ± 0.02
Heart	0.47 ± 0.01	0.49 ± 0.02	0.46 ± 0.01	0.45 ± 0.01	0.45 ± 0.01
Lung	1.19 ± 0.13	1.08 ± 0.12	1.05 ± 0.10	0.89 ± 0.08	1.13 ± 0.15
Liver	3.91 ± 0.08	3.99 ± 0.05	3.85 ± 0.04	3.89 ± 0.08	4.01 ± 0.08
Pancreas	0.82 ± 0.03	0.84 ± 0.02	0.87 ± 0.02	0.80 ± 0.02	0.81 ± 0.02
Spleen	0.29 ± 0.01	0.32 ± 0.01	0.27 ± 0.01	0.29 ± 0.01	0.28 ± 0.01
Kidney	1.50 ± 0.03	1.59 ± 0.07	1.59 ± 0.06	1.69 ± 0.05	1.69 ± 0.04
White adipose tissue					
Mesenteric	0.88 ± 0.06 ^{ab}	0.64 ± 0.08 ^a	1.05 ± 0.10 ^b	1.04 ± 0.11 ^b	0.84 ± 0.08
Perinephric	1.15 ± 0.12 ^{ab}	0.68 ± 0.09 ^a	1.22 ± 0.13 ^b	1.43 ± 0.17 ^b	1.24 ± 0.14
Epididymal	2.25 ± 0.11 ^{ab}	1.59 ± 0.21 ^a	2.50 ± 0.23 ^b	2.33 ± 0.23 ^{ab}	2.17 ± 0.18
Total	4.39 ± 0.17 ^b	2.91 ± 0.33 ^a	4.76 ± 0.42 ^b	4.80 ± 0.45 ^b	4.26 ± 0.31

Data are shown as mean ± SE, n = 12. Different superscript letters indicate significant differences (^{a, b} $p < 0.05$). The mb60, mb75, mb90, and mb05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil-balanced diets, respectively, and the normal group was fed the AIN-93G powdered diet.

3.4 DNA microarray analysis of white adipose tissue of mice fed mimic oil-balanced diets

Because the lipid accumulation and hypertrophy of adipocytes in epididymal adipose tissue were suppressed by intake of the 1975 mimic oil-balanced diet, the differences in gene expression between the mb75 group and other three groups were evaluated by DNA microarray. A difference was considered to exist if the gene expression ratio of the mb75 group was more than 2 compared to that of the other three groups. All of the genes that expression ratio of the mb75 group was more than 2 compared to that of the other three groups were shown in **Supplemental table 3**. Genes whose expressions differed between groups were classified by function (**Table 5**); genes that did not have a metabolic-related function were classified as others. The expressions of 10 metabolism-related genes were significantly lower in the mb75 group than the other groups (**Table 6**). Carbohydrate metabolism-related genes included calmodulin-like protein 3 (*Calml3*), which is involved in glycogenesis suppression, solute carrier family 2 member 5 (*Slc2a5*), which is involved in fructose transport, NME/NM23 family member 5 (*Nme5*), which converts nucleotide diphosphate to the triphosphate form, and hepatic nuclear factor 4, alpha (*Hnf4a*), which promotes *G6Pase* transcription. Lipid metabolism-related genes included acyl-CoA

synthetase bubblegum family member 1 (*Acsbg1*), which synthesizes acyl-CoA, arylacetamide deacetylase (*Aadac*), which decomposes triacylglycerol, and prostaglandin D2 synthase (*Ptgds*), which synthesizes prostaglandin D2. Amino acid metabolism-related genes included d-amino acid oxidase (*Dao*), which converts d-amino acids to imino acids, pipecolic acid oxidase (*Pipox*), which synthesizes glycine from sarcosine, and butyrobetaine 2-oxoglutarate dioxygenase (*Bbox1*), which decomposes lysine. These results suggest the 1975 mimic oil-balanced diet suppressed obesity by decreasing the expression levels of the above mentioned metabolism-related genes.

3.5 Effects of mimic oil-balanced diets on white adipose tissue, serum and liver biochemical parameters

There were no significant differences in epididymal adipose tissue TG level, in serum TG, TC, PL glucose, or insulin levels and in liver TG, TC, PL levels among the four groups (**Table 7, 8, Supplemental table 4**).

3.6 Effects of mimic oil-balanced diets on the fatty acid composition in white adipose tissue, serum and liver

The fatty acid composition in epididymal adipose tissue, serum and liver were analyzed (**Table 9, Supplemental table 5, 6**). In the epididymal adipose tissue, 16:1 n-7 was

Fig. 1 Effects of the mimic oil-balanced diets on white adipose tissue in mice. Epididymal adipose tissue sections from representative mice of each group (hematoxylin & eosin, scale bar = 100 µm). The mb60, mb75, mb90, and mb05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil-balanced diets, respectively, and the normal group was fed the AIN-93G powdered diet.

Table 5 Functional classification of genes observed a difference in the expression ratio.

Gene function	Expression ratio	
	Increase ↑	Decrease ↓
Carbohydrate metabolism	0	4
Lipid metabolism	0	3
Amino acid metabolism	0	3
The other	3	69
Total	3	79

Genes observed the differences in epididymal adipose tissue in the expression ratio were classified by function. Genes which did not have the metabolic-related function were classified as other.

significantly lower in the mb75 and mb05 groups than the mb60 group. 18:0 was significantly lower in the mb60 group than the mb90 and mb05 groups. 18:1 n-7 was significantly lower in the mb75, mb90 and mb05 groups than the mb60 group. 20:4 n-6 was significantly lower in the mb75 group than the mb60 group. In the liver, 16:1 n-7 was

significantly lower in the mb75 group than the mb60 group and higher than the mb05 group. 18:0 was significantly lower in the mb60 group than the mb05 group. 18:1 n-7 was significantly lower in the mb75, mb90 and mb05 groups than the mb60 group. 18:2 n-6 was significantly higher in the mb75 group than the mb60 group. 20:5 n-3 was significantly lower in the mb60, mb75 and mb90 groups than the mb05 group. 22:6 n-3 was significantly lower in the mb60 group than the mb75, mb90 and mb05 groups.

3.7 Multiple regression analysis

The best predictor of total white adipose tissue weight variation differing significantly, demonstrated by stepwise regression analysis of fatty acid composition, were shown in **Table 10**. 14:0 was an independent positive predictor of total white adipose tissue weights ($p < 0.01$). 18:0 was an independent positive predictor of total white adipose tissue weights ($p < 0.05$). 18:2 n-6 was an independent negative predictor of total white adipose tissue weights ($p < 0.01$).

Table 6 Carbohydrate metabolism, lipid metabolism and amino acid metabolism related gene.

Genebank ID	Gene Symbol	Expression ratio (vs mb60)			Pathway
		mb75	mb90	mb05	
NM_027416	<i>Calml3</i>	-7.72	-0.74	0.94	Carbohydrate metabolism
NM_008261	<i>Hnf4a</i>	-2.97	-0.86	0.79	
NM_080637	<i>Nme5</i>	-3.18	-0.51	0.85	
NM_019741	<i>Slc2a5</i>	-3.68	-0.92	1.66	
NM_023383	<i>Aadac</i>	-3.28	-0.46	1.10	Lipid metabolism
NM_053178	<i>Acsbg1</i>	-4.58	-1.16	0.75	
NM_008963	<i>Ptgds</i>	-3.18	-0.60	0.66	
NM_130452	<i>Bbox1</i>	-3.43	-0.54	0.80	Amino acid metabolism
NM_001286396	<i>Dao</i>	-7.58	-1.23	0.74	
NM_008952	<i>Pipox</i>	-3.47	-0.81	0.95	

The bottom was expressed as 2 in logarithm. It was determined that if gene expression ratio of mb75 group was more than 2 times compared to the other three groups in epididymal adipose tissue, the differences were observed. The mb60, mb75, mb90, and mb05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil-balanced diets, respectively. *Aadac*, arylacetamide deacetylase; *Acsbg1*, acyl-CoA synthetase bubblegum family member 1; *Bbox1*, butyrobetaine 2-oxoglutarate dioxygenase; *Calml3*, calmodulin-like protein 3; *Dao*, D-amino acid oxidase; *Hnf4a*, hepatic nuclear factor 4, alpha; *Nme5*, NME/NM23 family member 5; *Pipox*, pipecolic acid oxidase; *Ptgds*, prostaglandin D2 synthase; *Slc2a5*, solute carrier family 2 member 5.

Table 7 Biochemical parameters in serum in mice fed mimic oil-balanced diet.

		mb60	mb75	mb90	mb05	Normal
TG	(mmol/L)	1.09 ± 0.07	0.96 ± 0.07	0.93 ± 0.05	0.92 ± 0.08	1.58 ± 0.07
TC	(mmol/L)	2.44 ± 0.16	2.25 ± 0.14	2.28 ± 0.09	2.36 ± 0.13	2.68 ± 0.17
PL	(mmol/L)	2.09 ± 0.13	1.90 ± 0.10	1.97 ± 0.07	1.90 ± 0.06	2.29 ± 0.11
Glucose	(mmol/L)	4.64 ± 0.28	4.41 ± 0.30	4.32 ± 0.32	5.20 ± 0.30	4.51 ± 0.41
Insulin	(pmol/L)	34.5 ± 7.0	30.9 ± 5.7	37.9 ± 7.5	46.9 ± 7.7	35.3 ± 6.0

Data are shown as mean ± SE, n = 12. Different superscript letters indicate significant differences (^{a, b} $p < 0.05$). The mb60, mb75, mb90, and mb05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil-balanced diets, respectively, and the normal group was fed the AIN-93G powdered diet. TC, total cholesterol; TG, triacylglycerol; PL, phospholipid.

Table 8 Biochemical parameters in epididymal adipose tissue in mice fed mimic oil-balanced diet.

		mb60	mb75	mb90	mb05	Normal
TG	(mmol/g)	0.364 ± 0.017	0.365 ± 0.018	0.344 ± 0.021	0.391 ± 0.012	0.353 ± 0.018

Data are shown as mean ± SE, n = 8-12. Different superscript letters indicate significant differences ($p < 0.05$). The mb60, mb75, mb90, and mb05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil-balanced diets, respectively, and the normal group was fed the AIN-93G powdered diet. TG, triacylglycerol.

Table 9 The fatty acid composition in epididymal adipose tissue in mice fed mimic oil-balanced diet.

	mb60	mb75	mb90	mb05	Normal
	(%)				
14:0	1.3 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	0.9 ± 0.0
16:0	24.8 ± 0.4	24.7 ± 0.4	25.1 ± 0.5	25.3 ± 0.5	19.2 ± 0.4
16:1 n-7	6.4 ± 0.4 ^b	4.7 ± 0.3 ^a	5.0 ± 0.5 ^{ab}	4.1 ± 0.2 ^a	4.3 ± 0.3
18:0	2.4 ± 0.1 ^a	3.1 ± 0.1 ^{ab}	3.4 ± 0.1 ^b	3.5 ± 0.1 ^b	1.7 ± 0.2
18:1 n-9	38.9 ± 0.8	39.0 ± 0.7	39.8 ± 0.4	40.1 ± 0.4	25.6 ± 3.2
18:1 n-7	3.0 ± 0.2 ^b	2.6 ± 0.1 ^a	2.4 ± 0.1 ^a	2.2 ± 0.0 ^a	6.7 ± 2.9
18:2 n-6	20.3 ± 0.7	21.9 ± 0.6	20.1 ± 0.4	20.7 ± 0.6	37.7 ± 0.8
18:3 n-6	n.d.	n.d.	n.d.	n.d.	n.d.
18:3 n-3	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.0	1.3 ± 0.0	2.9 ± 0.2
20:1 n-9	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
20:3 n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
20:4 n-6	0.4 ± 0.1 ^b	0.2 ± 0.0 ^a	0.3 ± 0.0 ^{ab}	0.2 ± 0.0 ^{ab}	0.4 ± 0.0
20:5 n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	n.d.
22:6 n-3	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.1 ± 0.0

Data are shown as mean ± SE, n = 11-12. Different superscript letters indicate significant differences (^{a, b} $p < 0.05$). The mb60, mb75, mb90, and mb05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil-balanced diets, respectively, and the normal group was fed the AIN-93G powdered diet. n.d., not detected.

Table 10 Best predictors of variation in total white adipose tissue weight in a stepwise regression analysis.

	Predictor	beta coefficient	P	Adjusted R ²
Total white adipose tissue weight	14:0	0.4048	0.0009	
	18:0	0.2990	0.0175	0.4370
	18:2 n-6	-0.5068	0.0001	

n = 11-12.

3.8 Effects of mimic oil diets on growth parameters

In order to elucidate the effects of the 1975 mimic oil-balanced diet with respect to differences in the amounts of fatty acids or their composition, the experiment was performed by using the mimic oil diet that replicated only fatty acid composition. There were no significant differences in final body weight and body weight gain among the four groups (Table 11). Food intake was significantly lower in the m90 group than the m60 and m05 groups. There were no significant differences in the mesenteric, perirenal, and epididymal adipose tissue weights among groups. Moreover, there were no significant differences in the brain, heart, lung, liver, pancreas, spleen, or kidney weights. These results suggest the suppression of lipid accumulation by intake of the 1975 mimic oil-balanced diet does not depend solely on its fatty acid composition.

3.9 Histological analysis of white adipose tissue in mice fed mimic oil diets

Epididymal adipose tissue sections were stained with hematoxylin & eosin and subjected to histological analysis. There was no significant difference among the four groups with respect to adipocyte size (Fig. 2): when the adipocyte size of the normal group was set at 1.00, the adipocyte sizes of the m60, m75, m90, and m05 groups were 1.12 ± 0.05 , 1.11 ± 0.04 , 1.15 ± 0.07 , and 1.08 ± 0.03 , respectively. Furthermore, there was no significant difference among the groups with respect to adipocyte area. These results further suggest the suppression of adipocyte hypertrophy by intake of the 1975 mimic oil-balanced diet does not depend solely on its fatty acid composition.

Table 11 Body weight, food intake and tissue weight in mice fed mimic oil diet.

	m60	m75	m90	m05	Normal
Final body weight (g)	42.7 ± 0.8	42.8 ± 1.2	41.0 ± 0.7	42.4 ± 0.9	42.9 ± 1.1
Body weight gain (g)	10.7 ± 0.7	10.8 ± 0.8	8.98 ± 0.52	10.3 ± 0.6	10.9 ± 0.8
Food intake (g/day)	4.61 ± 0.36 ^b	4.58 ± 0.23 ^{ab}	4.39 ± 0.40 ^a	4.75 ± 0.29 ^b	4.87 ± 0.28
Tissue weight	(g/100 g B. W.)				
Brain	1.22 ± 0.03	1.16 ± 0.02	1.21 ± 0.03	1.19 ± 0.02	1.20 ± 0.03
Heart	0.47 ± 0.02	0.48 ± 0.01	0.48 ± 0.02	0.48 ± 0.02	0.50 ± 0.02
Lung	1.02 ± 0.09	0.96 ± 0.06	0.98 ± 0.09	1.10 ± 0.08	1.13 ± 0.09
Liver	3.97 ± 0.07	3.88 ± 0.05	4.00 ± 0.09	3.92 ± 0.05	4.02 ± 0.08
Pancreas	0.92 ± 0.03	0.84 ± 0.03	0.83 ± 0.03	0.88 ± 0.04	0.85 ± 0.03
Spleen	0.27 ± 0.01	0.33 ± 0.01	0.37 ± 0.06	0.32 ± 0.02	0.36 ± 0.03
Kidney	1.59 ± 0.05	1.53 ± 0.04	1.62 ± 0.07	1.66 ± 0.05	1.73 ± 0.04
White adipose tissue					
Mesenteric	0.96 ± 0.07	0.81 ± 0.09	0.85 ± 0.08	0.87 ± 0.06	0.88 ± 0.12
Perinephric	1.28 ± 0.11	1.33 ± 0.18	1.18 ± 0.12	1.23 ± 0.10	1.23 ± 0.18
Epididymal	2.27 ± 0.14	2.22 ± 0.24	1.88 ± 0.22	2.22 ± 0.20	1.98 ± 0.25
Total	4.59 ± 0.29	4.46 ± 0.49	3.91 ± 0.38	4.32 ± 0.31	4.09 ± 0.47

Data are shown as mean ± SE, n = 12. Different superscript letters indicate significant differences (^{a, b} $p < 0.05$). The m60, m75, m90, and m05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil diets, respectively, and the normal group was fed the AIN-93G powdered diet.

3.10 Effects of mimic oil diets on serum biochemical parameters

There were no significant differences in serum TG levels among groups. Serum TC level was significantly lower in the mb05 group than the mb75 group. There were no significant differences among groups with respect to serum PL, glucose, or insulin levels (Table 12).

4 DISCUSSION

The present study investigated the effects of diets replicating the fatty acid composition and macronutrient ratio of Japanese diets at different times on visceral fat accumulation in growing mice. The results show the 1975 mimic oil-balanced diet suppressed visceral fat accumulation and adipocyte hypertrophy compared to the 1960, 1990, and 2005 mimic oil-balanced diets. To investigate the underlying mechanism, gene expressions in epididymal adipose tissue were analyzed by DNA microarray. The expression levels of 10 metabolism-related genes were significantly lower in the mice fed the 1975 mimic oil-balanced diet than those fed the other mimic oil-balanced diets; therefore, these genes were examined in detail. Genes involved in the inhibition of adipocyte hypertrophy included *Acsbg1* and *Ptgds*. *Acsbg1* synthesizes acyl-CoA from fatty acids, promotes TG synthesis, and induces adipocyte hypertrophy¹⁶.

On the other hand, *Ptgds* synthesizes prostaglandin D2 from prostaglandin H2, which is a metabolite of arachidonic acid, and acts in the pathway to synthesize 15-deoxy prostaglandin J2 which is endogenous ligands on PPAR γ ^{17, 18}. The activation of PPAR γ by its endogenous ligand promotes adipocyte hypertrophy¹⁹. In addition, although the gene expression ratio of the mb75 group was less than 2 compared to that of the other three groups, the major lipid metabolism-related genes were shown in Table 13. As a result, the modest difference was found in Fatty acid synthase (*Fasn*) and Uncoupling protein 1 (*Ucp1*). The expression level of *Fasn* who promotes the lipid accumulation²⁰ was lowest in mb75 group. The expression level of *Ucp1* who promotes the energy consumption²¹ was highest in mb75 group. In the present study, the 1975 mimic oil-balanced diet strongly inhibited the functions of these molecules, thus inhibiting adipocyte hypertrophy and visceral fat accumulation.

The observed anti-obesity effect of the 1975 mimic oil-balanced diet was considered to be due to differences in the intake levels of fatty acids or differences in fatty acid composition. Therefore, we investigated a mimic oil diet that replicated only the fatty acid composition of Japanese food. There were no significant differences in visceral fat mass or adipocyte size in mice fed the mimic oil diet. These results indicate the suppression of fat accumulation in white adipose tissue caused by the 1975 mimic oil-balanced

Fig. 2 Effects of the mimic oil diets on white adipose tissue in mice. Epididymal adipose tissue sections from representative mice of each group (hematoxylin & eosin, scale bar = 100 μ m). The m60, m75, m90, and m05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil diets, respectively, and the normal group was fed the AIN-93G powdered diet.

Table 12 Biochemical parameters in serum in mice fed mimic oil diet.

		m60	m75	m90	m05	Normal
TG	(mmol/L)	1.40 $\pm$ 0.12	1.45 $\pm$ 0.13	1.19 $\pm$ 0.10	1.16 $\pm$ 0.09	1.41 $\pm$ 0.10
TC	(mmol/L)	2.70 $\pm$ 0.19 ^{ab}	3.13 $\pm$ 0.24 ^b	2.94 $\pm$ 0.18 ^{ab}	2.37 $\pm$ 0.09 ^a	2.64 $\pm$ 0.19
PL	(mmol/L)	2.30 $\pm$ 0.14	2.63 $\pm$ 0.16	2.47 $\pm$ 0.13	2.27 $\pm$ 0.13	2.40 $\pm$ 0.12
Glucose	(mmol/L)	4.15 $\pm$ 0.16	4.50 $\pm$ 0.25	4.03 $\pm$ 0.21	4.41 $\pm$ 0.36	4.63 $\pm$ 0.31
Insulin	(pmol/L)	46.5 $\pm$ 9.4	43.1 $\pm$ 8.2	27.3 $\pm$ 4.3	28.2 $\pm$ 1.9	46.1 $\pm$ 7.8

Data are shown as mean $\pm$ SE, n = 12. Different superscript letters indicate significant differences (^{a, b} $p < 0.05$). The m60, m75, m90, and m05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil diets, respectively, and the normal group was fed the AIN-93G powdered diet. TC, total cholesterol; TG, triacylglycerol; PL, phospholipid.

diet does not solely depend on its fatty acid composition. In addition, the amounts of lipids are reported to not significantly affect visceral fat⁷⁾. Thus, the results indicate the effect of 1975 mimic oil-balanced diet depends on both the composition and amounts of fatty acids.

What of the 1975 mimic oil-balanced diet affected the suppression of fat accumulation was discussed. The tran-

scription of *Ptgds*, whose expression was decreased in the mice fed the 1975 mimic oil-balanced diet, is controlled by Srebp-1c²³⁾. Srebp-1c is activated by saturated fatty acids and inhibited by polyunsaturated fatty acids, particularly EPA and DHA²³⁾. Therefore, fatty acid intake levels in the two experiments of mimic oil-balanced diet and mimic oil diet were compared. The intake levels of saturated fatty

Table 13 Lipid metabolism-related gene.

Gene Accession	Gene Symbol	Expression ratio (vs mb60)			Pathway
		mb75	mb90	mb05	
NM_007643	<i>Cd36</i>	-0.05	0.02	-0.08	transport
NM_008509	<i>Lpl</i>	0.03	0.12	-0.05	
NM_007988	<i>Fasn</i>	-0.52	-0.25	-0.23	lipogenesis
NM_008062	<i>G6pdh</i>	0.06	0.14	0.04	
NM_001198933	<i>Me1</i>	0.01	0.13	0.17	
NM_145494	<i>Me2</i>	-0.10	-0.05	-0.07	
NM_181407	<i>Me3</i>	0.08	0.30	0.31	
NM_011146	<i>Pparg</i>	0.08	-0.13	-0.33	
NM_011480	<i>Srebp-1c</i>	0.10	0.08	0.33	lipolysis
NM_010719	<i>Hsl</i>	0.12	0.06	-0.03	
NM_011145	<i>Ppard</i>	0.01	0.05	-0.13	
NM_009463	<i>Ucp1</i>	0.55	-0.62	-1.13	energy expenditure

The bottom was expressed as 2 in logarithm. The lipid metabolism-related gene expression ratio of mb75 group was less than 2 times compared to the other three groups in epididymal adipose tissue. The mb60, mb75, mb90, and mb05 groups were fed the 1960, 1975, 1990, and 2005 mimic oil-balanced diets, respectively. *Cd36*, Cluster of differentiation 36; *Fasn*, Fatty acid synthase; *G6pdh*, Glucose-6-phosphate dehydrogenase; *Hsl*, hormone-sensitive lipase; *Lpl*, lipoprotein lipase; *Me1*, Malic enzyme 1; *Me2*, Malic enzyme 2; *Me3*, Malic enzyme 3; *Ppard*, Peroxisome proliferator-activated receptor δ ; *Pparg*, Peroxisome proliferator-activated receptor γ ; *Srebp-1c*, Sterol regulatory element-binding protein-1c; *Ucp1*, Uncoupling protein 1.

acid in the mice fed the 1960, 1975, 1990, and 2005 mimic oil-balanced diets were 49.1 ± 2.4 , 79.0 ± 3.4 , 120 ± 6.0 , and 125 ± 7.0 mg/day, respectively; the intake levels of EPA + DHA were 4.21 ± 0.21 , 6.80 ± 0.29 , 6.53 ± 0.32 , and 10.9 ± 0.6 mg/day, respectively. Whereas the intake levels of EPA and DHA did not change substantially, those of saturated fatty acids increased significantly over time. Thus, the greater visceral fat accumulation in the 1990 and 2005 mimic oil-balanced diet groups is likely due to higher saturated fatty acid intake than that in the 1975 mimic oil-balanced diet group. Furthermore, visceral fat accumulation in the 1960 mimic oil-balanced diet group is considered to be due to high carbohydrate intake. Low-fat/high-carbohydrate diets are reported to enhance lipid synthesis²⁴⁾. Therefore, a similar phenomenon likely occurred in the rats fed the 1960 mimic oil-balanced diet. In addition, the stepwise multiple regression analysis was performed by using the total white adipose tissue weights as the objective variable and the fatty acid composition as the explanatory variable. The positive correlation was observed between the saturated fatty acids, especially 14:0 and 18:0, and total white adipose tissue weights. In addition, the negative correlation was observed between linoleic acid (18:2 n-6) and total white adipose tissue weights. It was suggested that white adipose tissue weights are affected of the saturated fatty acid. As shown **Table 9**, the rate of linoleic acid was large and the rate of arachidonic acid (20:4

n-6) was lowest in mb75 group, so it was considered that the $\Delta 6$ -desaturase activity in mb75 group is low¹⁴⁾. Hence, it was suggested that the generation of endogenous ligands on PPAR γ is less^{19, 20)}. Moreover, these changes in fatty acid composition was also observed in serum and liver.

On the other hand, in the mimic oil diet experiment, mean saturated fatty acid intake was 77.0 ± 3.3 , 71.8 ± 1.7 , 83.6 ± 4.4 , and 75.6 ± 2.4 mg/day in the mice fed the 1960, 1975, 1990, and 2005 mimic oil diets, respectively; EPA + DHA intake was 6.60 ± 0.28 , 6.18 ± 0.14 , 4.56 ± 0.24 , and 6.62 ± 0.21 mg/day, respectively. Because there were no significant differences in intake levels of saturated fatty acids and EPA + DHA, the diets were considered to have no effect on visceral fat mass.

The S:M:P ratios of the Japanese diets were similar. Also, the $\omega 6:\omega 3$ ratio was not lowest in the 1975 diet; nevertheless, intake of the 1975 mimic oil-balanced diet suppressed fat accumulation. This suggests there are better indices of assessing the effect of lipid composition on health maintenance than the S:M:P and $\omega 6:\omega 3$ ratios that are currently published. In the future, we will analyze the composition and quantities of fatty acids in the 1975 diet in greater detail in order to clarify way to take fat effectively to the health maintenance.

5 CONCLUSION

The present study shows that both the quality and quantity of fatty acids are important for the previously reported anti-obesity effects of the 1975 Japanese diet. However, this diet does not improve sugar metabolism or the inhibitory effect of liver lipid accumulation. Thus, the results suggest this diet affects components other than fatty acids, including unsaponifiables, proteins, carbohydrates, minerals, vitamins, etc. Accordingly, we are planning to investigate components of this diet other than fatty acids in detail.

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SUPPORTING INFORMATION

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