〈Article〉

A Japanese-style diet affects metabolic gene expression in young Japanese women

Rieko Mitamura

(Department of Food Science and Human Nutrition, Faculty of Human Life Sciences, Fuji Women's University)

Few studies have examined whether a Japanese-style diet provides health benefits to humans. In this study, we aimed to investigate the effects of a Japanese-style diet on the metabolic gene expression of young Japanese women. During an 8-week intervention period, 15 participants each consumed 40 (5 days x 8 weeks) Japanese-style diet meals as weekday lunches. We collected venous blood samples at baseline and after the intervention, assessed biochemical parameters, and performed DNA microarray analyses. The baseline lunch of the subjects consisted of 60% cooked rice, 20% noodles, and 20% bread as staple foods, and was generally accompanied by meat and eggs. In contrast, the Japanese-style diet lunch consisted of a rice and miso soup base, accompanied by plant-derived foods and fermented seasoning. Fish was also often included. After the intervention period, total serum cholesterol showed a decreasing tendency when compared with the baseline. The DNA microarray results showed that approximately 30% of the analyzed genes were differentially expressed in before and after intervention comparisons. Notably, genes associated with lipid metabolic parameters, including *ACADS*, *FASN* and *PPARD* were significantly down changed after the intervention period. The Japanese-style diet may affect metabolic gene expression due to its high contents of fish, plant-based foods, and fermented seasonings.

Keywords: Japanese-style diet; metabolic gene expression; young Japanese women

1. Introduction

The traditional Japanese-style diet consists of a soup and cooked rice as staple foods, and three side dishes, which typically contain fish, meat, beans, vegetables, mushrooms, and seaweeds. Compared with Western diets, the Japanese-style diet is rich in rice, fish, and vegetables and low in meat and eggs[1]. It also has a desirable food-intake profile that may provide protective effects against hypertension and obesity, and lead to a low prevalence of cardiovascular disease and metabolic syndrome[2–5]. The Japanese-style diet is nutritionally balanced and accords well with the nutritional recommendations, expressed in terms of dietary reference intakes (DRIs), in Japan[6–7].

Recently, however, there have been changes in the diet of the Japanese population. The intake of plant-based foods and fish has tended to decline and the Western-style diet, rich in lipids, has become increasing[8]. The fact that obesity and metabolic syndrome have become major health problems in Japan is undoubtedly related to this trend of diets.

Most previous studies that have examined the benefits of the Japanese-style diet have been observational, for example, cross-sectional studies in a Japanese population[2–8], or alternatively, have focused on individual ingredients and food components[9–11]. In contrast, there have been few interventional studies that have sought to determine whether the Japanese-style diet in itself confers health benefits to humans.

Recently, a randomized controlled trial study showed that the Japanese-style diet of 1975 improved

lipid metabolic parameters in young Japanese adults[12]. In this study, the subjects consumed the 1975 Japanese diet three times a day for 28 days. However, consuming a Japanese-style diet for every meal would be difficult considering current lifestyles. In our previous study, we cooked 40 Japanese-style diet meals and provided these as weekday lunches to young Japanese women during an 8-week intervention period to examine defecation conditions. Our previous study showed that the defecation quantity was significantly increased during weeks 5 to 8 of the intervention period[13]. In results of a food frequency questionnaire, the participants indicated that their intake of rice, vegetables, and seaweeds had significantly increased during the intervention compared with that of their normal daily diet, which could have led to the observed differences in defecation.

In this study, we compared the current daily diet to the Japanese-style diet in a before and after trial. As in our previous study, each of the subjects received a total of 40 Japanese-style diet meals as weekday lunches over the course of an 8-week intervention period. We analyzed the metabolic gene expression in the blood cells and blood lipid profiles before and after the intervention and considered the potential health benefits associated with the Japanese-style diet.

2. Materials and Methods

(1) Study design and subjects

This study was a before-after trial (Fig. 1) conducted in accordance with the Declaration of Helsinki and with the approval of the Fuji Women's University Ethics Committee (registration: April 25th, 2016). The participants were students of the Fuji Women's University, aged between 21 and 23 years. Exclusion criteria included the use of drug therapy for existing diseases, use of supplements, and severe food allergies. Fifteen student volunteers provided written informed consents for participation in the study.

(2) Dietary assessment

Dietary habits during the preceding 2 weeks were self-reported in a food frequency questionnaire (FFQ) at baseline. Additionally, during a 5-day baseline period just before intervention, the subjects took photographs of their typical lunch. On the basis of these FFQ and photographs, registered dietitians estimated the food intake. At the end of intervention, dietary habits were checked again at FFQ, and these data were compared with those of the baseline.

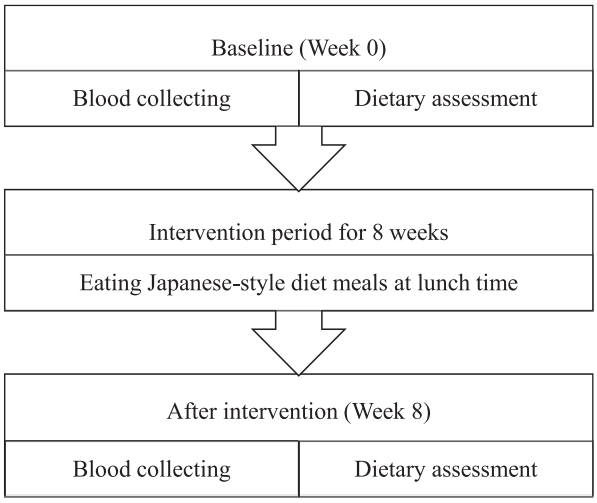

Figure 1. Flow chart of the study profile.

(3) Intervention

Over the course of the 8-week intervention period, each of the participants was given 40 Japanese-style diet meals as weekday lunches. The basic structure of the traditional Japanese-style diet lunch was a soup, cooked rice as a staple food, and three side dishes, which included fish, meat, beans, vegetables, mushrooms, seaweeds, and fruits. The meals were prepared by registered dietitians following the DRIs. The meals were prepared before 12 o'clock each weekday, and in compliance with the study requirements, the subjects ate these. The Japanese-style lunch frequently included soybeans, fish, and vegetables, as well as umami and fermented seasonings such as soy sauce, miso, sake, and vinegar. Apart from eating a Japanese-style lunch on weekdays, there were no restrictions on additional food consumed during the intervention period. During the weekends, the participants ate their customary lunches.

(4) Biochemical parameters in serum and DNA microarray analysis

We collected venous blood samples at baseline and at the end of the study and sent these to an external testing laboratory (SRL, Sapporo, Japan). Biochemical parameters including blood glucose, glycated hemoglobin (HbA1c), triglycerides (TG), total cholesterol

(TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), were analyzed using routine clinical chemistry methods. Total RNA was extracted from blood samples excluding red and white blood cells, and a DNA microarray analysis was performed using a fibrous DNA chip Genopal® (Mitsubishi Rayon, Yokohama, Japan). Biotinylated antisense RNA (aRNA) were synthesized and amplified from 1 μg of total RNA using the MessageAmpII biotin enhanced amplification kit (Applied Biosystems). After purification of the aRNA, 5 μg of the biotinylated aRNA were fragmented using 10X fragmentation regents by heating at 94℃ for 7.5 min. Hybridization, washing and fluorescent labelling were performed by Genopal instrument systems (UE-104, Mitsubishi Rayon) [14]. The fibrous DNA chip carries 194 genes related to metabolic processes such as lipid metabolism, glucose metabolism, energy production, signal transduction, cytokines, transcription factors, and redox reactions. The gene expression levels at baseline were set to 0 and compared with those recorded after the intervention. Comparisons were made by converting the expression levels of the corresponding individuals into a binary logarithm[14].

(5) Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 23 (Advanced Analytics, Inc, Tokyo, Japan). All reported P values are two-tailed, and a P value of <0.05 was considered statistically significant. The normality of each variable for continuous data was determined using the Kolmogorov-Smirnov test. The dietary intake information obtained from the photographs at baseline (Week 0) was compared with that of the Japanese-style diet lunch consumed during the intervention period (Week 8) using the Wilcoxon signed-rank test. The differences between the before and after biochemical parameters in serum were assessed using a paired t-test. Gene expression levels after intervention versus baseline were examined by Bonferroni correction.

3. Results

(1) Dietary assessment

Examples of the baseline lunches and the Japanese-style diet lunches consumed during the intervention period are shown in Fig. 2. The baseline lunches consisted of 60% cooked rice, 20% noodles, and 20% bread as staple foods. They also generally included meat and eggs. In contrast, the Japanese-style diet lunches contained staples of rice and miso soup, along with plant-based foods, umami, and fermented seasonings. Fish was also often included. A comparison between the food groups at baseline and in the Japanese-style diet is shown in Table 1. The data of food groups at baseline was estimated by 5-days photographs. Compared with the lunches consumed at baseline, the intake of plant-based foods, such as rice, vegetables, seaweeds, beans, and fruits, and fish was significantly increased during consumption of the Japanese-style diet (all $P<0.01$). The content of meat and eggs was lower in the Japanese-style diet than in the baseline meals ($P<0.01$).

Figure 2. Examples of baseline lunches (upper row) and the Japanese-style diet lunches (lower row).

Table 1. Comparison of food groups (g/lunch) at baseline and in the Japanese-style diet [Median (IQR)].

Food group	Baseline (Week 0)	Japanese-style diet	*P* value
Rice	45 (20, 50)	80 (80, 80)	0.001*
Potatoes	20 (0, 40)	30 (8, 46)	0.168
Green and yellow vegetables	10 (0, 20)	40 (20, 64)	0.001*
Light-colored vegetables	16 (8, 30)	85 (63, 110)	0.001*
Seaweeds	0 (0, 0)	0 (0, 1)	0.005*
Beans	0 (0, 10)	7 (7, 26)	0.001*
Fish	0 (0, 48)	10 (0, 60)	0.002*
Meat	48 (48, 64)	20 (0, 58)	0.005*
Eggs	25 (25, 50)	0 (0, 5)	0.002*
Fruit	0 (0, 10)	50 (0, 60)	＜0.001*

n=15. IQR, interquartile range. Comparisons were performed using the Wilcoxon signed-rank test.
* Significant differences between baseline and the Japanese-style diet.

Table 2. Serum biochemical parameters at baseline and the end of study (Mean values ± standard deviation).

Parameters	Baseline (Week 0)	After intervention (Week 8)	*P* value
Glucose (mg/dL)	82 ± 2.6	76 ± 2.8	0.105
HbA1c (%)	5.2 ± 0.1	5.3 ± 0.1	0.329
Triacylglycerol (mg/dL)	98 ± 14.0	99 ± 8.6	0.912
Total cholesterol (mg/dL)	191 ± 7.1	183 ± 5.7	0.082
Low-density lipoprotein cholesterol (mg/dL)	102 ± 6.4	97 ± 5.7	0.152
High-density lipoprotein cholesterol (mg/dL)	76 ± 3.8	72 ± 3.3	0.051

n=15. Comparisons were performed using a paired *t*-test.
Total and high-density lipoprotein cholesterol levels after intervention tended to be lower compared with those at baseline.

All subjects completed the study and consumed all the Japanese-style diet lunches provided. No serious or severe adverse events were observed. There were no appreciable differences in the body mass index (BMI) of the subjects before (20.3 ± 2.1 kg/m^2) and after (20.2 ± 2.1 kg/m^2) the intervention (P=0.637).

(2) Serum biochemical parameters

The serum biochemical parameters assessed in the present study are shown in Table 2. After the intervention period, the levels of TC and HDL-C were found to show a decreasing tendency (P=0.082 and P=0.051, respectively) when compared with those at baseline. For other parameters, there were no significant differences in the levels recorded before and after the intervention period.

(3) DNA microarray analysis

The results of the DNA microarray analysis indicated that expression of approximately 30% of the 194 genes analyzed (60 genes in total) was significantly changed after the intervention when compared with the baseline values (Table 3). Notably, these changed genes are associated with lipid metabolic parameters [acyl-CoA dehydrogenase, C-2 to C-3 short chain (*ACADS*), fatty acid synthase (*FASN*), carnitine palmitoyltransferase 1A (liver) (*CPT1A*), carnitine palmitoyltransferase 2 (*CPT2*), peroxisome proliferator-activated receptor delta (*PPARD*), nuclear receptor subfamily 1 group H member 4 (*NR1H4*)]; energy production [e. g., NADH dehydrogenase (ubiquinone) 1 beta subcomplex 4 (*NDUFB4*), NADH dehydrogenase (ubiquinone) flavoprotein 2 (*NDUFV2*), SCO cytochrome oxidase deficient homolog 1 (*SCO1*)]; and redox reactions [e. g., NADPH oxidase 1 (*NOX1*)].

4. Discussion

In this study, we found that the expression of 60 metabolic genes was significantly changed after consumption of a Japanese-style diet lunch for 8 weeks (Table 3). We used blood sample for gene expression profiling because blood reaches every

Table 3. The 60 changed genes after intervention.

Gene Accession	Gene name	Relative expression values* vs.baseline	Function
NM_000017	*Homo sapiens* acyl-CoA dehydrogenase, C-2 to C-3 short chain (*ACADS*), transcript variant 1, mRNA	−2.61	Lipid metabolism
NM_000196	*Homo sapiens* hydroxysteroid (11-beta) dehydrogenase 2 (*HSD11B2*), mRNA	−1.63	
NM_004104	*Homo sapiens* fatty acid synthase (*FASN*), mRNA	−1.28	
NM_198834	*Homo sapiens* acetyl-CoA carboxylase alpha (*ACACA*), transcript variant 1, mRNA	−0.52	
NM_003501	*Homo sapiens* acyl-CoA oxidase 3, pristanoyl (*ACOX3*), transcript variant 1, mRNA	−0.43	
NM_000098	*Homo sapiens* carnitine palmitoyltransferase 2 (*CPT2*), nuclear gene encoding mitochondrial protein, mRNA	−0.43	
NM_001876	*Homo sapiens* carnitine palmitoyltransferase 1A (liver) (*CPT1A*), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA	−0.42	
NM_000151	*Homo sapiens* glucose-6-phosphatase, catalytic subunit (*G6PC*), mRNA	−1.05	Glucose metabolism
NM_002626	*Homo sapiens* phosphofructokinase, liver (*PFKL*), transcript variant 2, mRNA	−0.45	
NM_001168331	*Homo sapiens* NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa (*NDUFB4*), nuclear gene encoding mitochondrial protein, transcript variant 2, mRNA	−0.70	Energy production
NM_021074	*Homo sapiens* NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa (*NDUFV2*), nuclear gene encoding mitochondrial protein, mRNA	−0.66	
NM_004589	*Homo sapiens* SCO cytochrome oxidase deficient homolog 1 (yeast) (*SCO1*), nuclear gene encoding mitochondrial protein, mRNA	−0.66	
NM_003001	*Homo sapiens* succinate dehydrogenase complex, subunit C, integral membrane protein, 15kDa (*SDHC*), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA	−0.59	
NM_001696	*Homo sapiens* ATPase, H + transporting, lysosomal 31kDa, V1 subunit E1 (*ATP6V1E1*), transcript variant 1, mRNA	−0.50	
NM_001693	*Homo sapiens* ATPase, H + transporting, lysosomal 56/58kDa, V1 subunit B2 (*ATP6V1B2*), mRNA	−0.49	
NM_005006	*Homo sapiens* NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase) (*NDUFS1*), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA	−0.35	
NM_015941	*Homo sapiens* ATPase, H + transporting, lysosomal 50/57kDa, V1 subunit H (*ATP6V1H*), transcript variant 1, mRNA	−0.31	
NM_005205	*Homo sapiens* cytochrome c oxidase subunit VIa polypeptide 2 (*COX6A2*), nuclear gene encoding mitochondrial protein, mRNA	−0.28	
NM_004168	*Homo sapiens* succinate dehydrogenase complex, subunit A, flavoprotein (Fp) (*SDHA*), nuclear gene encoding mitochondrial protein, mRNA	−0.27	
NM_003366	*Homo sapiens* ubiquinol-cytochrome c reductase core protein II (*UQCRC2*), nuclear gene encoding mitochondrial protein, mRNA	−0.27	
NM_004542	*Homo sapiens* NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 3, 9kDa (*NDUFA3*), mRNA	−0.27	
NM_002490	*Homo sapiens* NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa (*NDUFA6*), nuclear gene encoding mitochondrial protein, mRNA	−0.27	
NM_001001973	*Homo sapiens* ATP synthase, H + transporting, mitochon-	−0.25	

Gene Accession	Gene name	Relative expression values* vs.baseline	Function
	drial F1 complex, gamma polypeptide 1 (*ATP5C1*), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA		
NM_004550	*Homo sapiens* NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase) (*NDUFS2*), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA	−0.22	
NM_006003	*Homo sapiens* ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1 (*UQCRFS1*), nuclear gene encoding mitochondrial protein, mRNA	−0.21	
NM_014247	*Homo sapiens* Rap guanine nucleotide exchange factor (GEF) 2 (*RAPGEF2*), mRNA	−1.12	Signal transduction
NM_006609	*Homo sapiens* mitogen-activated protein kinase kinase kinase 2 (*MAP3K2*), mRNA	−0.97	
NM_145160	*Homo sapiens* mitogen-activated protein kinase kinase 5 (*MAP2K5*), transcript variant 1, mRNA	−0.80	
NM_145185	*Homo sapiens* mitogen-activated protein kinase kinase 7 (*MAP2K7*), mRNA	−0.51	
NM_001654	*Homo sapiens* v-raf murine sarcoma 3611 viral oncogene homolog (*ARAF*), transcript variant 1, mRNA	−0.40	
NM_021158	*Homo sapiens* tribbles homolog 3 (Drosophila) (*TRIB3*), mRNA	−0.36	
NM_000572	*Homo sapiens* interleukin 10 (*IL10*), mRNA	−0.59	Cytokine
NM_001171623	*Homo sapiens* vascular endothelial growth factor A (*VEGFA*), transcript variant 1, mRNA	−0.58	
NM_001177800	*Homo sapiens* adiponectin, C1Q and collagen domain containing (*ADIPOQ*), transcript variant 1, mRNA	−0.36	
NM_021724	*Homo sapiens* nuclear receptor subfamily 1, group D, member 1 (*NR1D1*), mRNA	−0.83	Transcription
NM_006238	*Homo sapiens* peroxisome proliferator-activated receptor delta (*PPARD*), transcript variant 1, mRNA	−0.77	
NM_001206979	*Homo sapiens* nuclear receptor subfamily 1, group H, member 4 (*NR1H4*), transcript variant 1, mRNA	−0.67	
NM_001114123	*Homo sapiens* ELK1, member of ETS oncogene family (*ELK1*), transcript variant 1, mRNA	−0.65	
NM_003889	*Homo sapiens* nuclear receptor subfamily 1, group I, member 2 (*NR1I2*), transcript variant 1, mRNA	−0.30	
NM_007052	*Homo sapiens* NADPH oxidase 1 (*NOX1*), transcript variant NOH-1L, mRNA	−0.77	Redox related
NM_002084	*Homo sapiens* glutathione peroxidase 3 (plasma) (*GPX3*), mRNA	−0.59	
NM_001512	*Homo sapiens* glutathione S-transferase alpha 4 (*GSTA4*), mRNA	−0.23	
NM_002794	*Homo sapiens* proteasome (prosome, macropain) subunit, beta type, 2 (*PSMB2*), transcript variant 1, mRNA	−0.37	Proteasome
NM_002793	*Homo sapiens* proteasome (prosome, macropain) subunit, beta type, 1 (*PSMB1*), mRNA	−0.36	Proteasome
NM_148976	*Homo sapiens* proteasome (prosome, macropain) subunit, alpha type, 1 (*PSMA1*), transcript variant 1, mRNA	−0.30	
NM_002790	*Homo sapiens* proteasome (prosome, macropain) subunit, alpha type, 5 (*PSMA5*), transcript variant 1, mRNA	−0.28	
NM_003720	*Homo sapiens* proteasome (prosome, macropain) assembly chaperone 1 (*PSMG1*), transcript variant 1, mRNA	−0.27	
NM_002788	*Homo sapiens* proteasome (prosome, macropain) subunit, alpha type, 3 (*PSMA3*), transcript variant 1, mRNA	−0.26	
NM_001178	*Homo sapiens* aryl hydrocarbon receptor nuclear translocator-like (*ARNTL*), transcript variant 1, mRNA	−1.26	Circadian rhythm

Gene Accession	Gene name	Relative expression values* vs.baseline	Function
NM_002616	*Homo sapiens* period homolog 1 (Drosophila) (*PER1*), mRNA	−1.10	
NM_002518	*Homo sapiens* neuronal PAS domain protein 2 (*NPAS2*), mRNA	−0.64	
NM_004898	*Homo sapiens* clock homolog (mouse) (*CLOCK*), mRNA	−0.54	
NM_001352	*Homo sapiens* D site of albumin promoter (albumin D-box) binding protein (*DBP*), mRNA	−0.36	
NM_000789	*Homo sapiens* angiotensin I converting enzyme (peptidyl-dipeptidase A) 1 (*ACE*), transcript variant 1, mRNA	−1.31	Angiogenic/ inflammatory marker
NM_139314	*Homo sapiens* angiopoietin-like 4 (*ANGPTL4*), transcript variant 1, mRNA	−0.42	
NM_021135	*Homo sapiens* ribosomal protein S6 kinase, 90kDa, polypeptide 2 (*RPS6KA2*), transcript variant 1, mRNA	−0.31	
NM_014874	*Homo sapiens* mitofusin 2 (*MFN2*), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA	−0.42	Others
NM_000331	*Homo sapiens* serum amyloid A1 (*SAA1*), transcript variant 1, mRNA	−0.36	
NM_001845	*Homo sapiens* collagen, type IV, alpha 1 (*COL4A1*), mRNA	−0.30	
NM_000089	*Homo sapiens* collagen, type I, alpha 2 (*COL1A2*), mRNA	−0.21	

n=15.

* Relative expression values after intervention compared with the gene expression levels at baseline (log2-transformed values).

Bonferroni correction was used.

The gene expression ratio indicates a significant decrease in expression after intervention compared with that at baseline ($P<0.05$).

living cell in the body, and approximately 85% of the genes expressed in various tissues are expressed in blood[15]. It is worth noting that many of these changed genes are associated with lipid metabolism, and the observed reduction in their expression might be attributable to the fact that the Japanese-style diet has low-fat and high-carbohydrate contents compared with the baseline diet. In this regard, it has been demonstrated that a diet rich in vegetables, fruits, and fish is associated with a reduced prevalence of metabolic syndrome in the Japanese population[2–5].

The Japanese-style diet might suppress fatty acid synthesis by reducing the expression of *ACADS*, *FASN*, *PPARD*, and *NR1H4*. Although the differences were not significant, the expression of *SREBP1c* was reduced in 12 subjects (data not shown, average expression rate −0.88). Furthermore, serum TC and HDL-C levels tended to be lower during the intervention than at baseline, although TG did not differ significantly (Table 2). Other hand, fatty acid decomposition also suppressed by the Japanese-style diet (Table 3). It is considered that Japanese-style diet had good energy balance for this subjects, and both synthesis and decomposition of fatty acids were suppressed. Indeed there were no appreciable differences in the body mass index (BMI) of the subjects before (20.3 ± 2.1 kg/m^2) and after (20.2 ± 2.1 kg/m^2) the intervention (P=0.637). Since the degradation of fatty acids was suppressed, it is considered that no effect was observed on serum TG levels. Another fact that we did not observe a significant effect on serum biochemical parameters may be because the intervention period was too short. In this study, the intervention period lasted for 8 weeks and included only weekday lunches. In the study conducted by Sugawara *et al.*[12], young adult volunteers were supplied with three Japanese-style diet meals every day for 4 weeks, and the authors observed a significant decrease in the body weight, BMI, and serum TG and LDL-C levels of these young adults. However, the contribution of the Japanese-style diet to the total daily intake of volunteers in our study was considerably lower than that examined in previous studies. Therefore, future trials with longer intervention periods should be considered.

We also noticed that the expression of redox-related genes, such as NADPH oxidase 1 (*NOX1*), was changed after the intervention period. NADPH

oxidase enzymes are major producers of reactive oxygen species (ROS), which are eliminated by antioxidant enzymes. Angiotensin II, vascular endothelial growth factor (*VEGF*), and tumor necrosis factor alpha stimulate signaling pathways that lead to the upregulation of NADPH oxidase enzymes. However, polyphenols, which are abundant in fruits and vegetables, regulate a number of major inflammatory and ROS-dependent signaling pathways, suggesting that these foods could play a protective role against cardiovascular diseases by reducing LDL-C, TC, TG and inflammatory molecules[16]. The Japanese-style diet assessed in the present in study served to increase the daily intake of vegetables and fruits among the volunteers (Table 1), which is considered to be beneficial in terms of a tendency to lower serum TC levels (Table 2) and reduce the expression of inflammatory molecules (Table 3). The Japanese-style diet contains a large proportion of plant-based foods that are rich in polyphenols, vitamins, and fiber that can potentially provide protective effects against hypertension and obesity, and lead to a low prevalence of cardiovascular disease and metabolic syndrome.

Angiotensin I converting enzyme (*ACE*) catalyzes the conversion of angiotensin I to angiotensin II in the circulatory system, leading to an increase in oxidative stress and an elevation in blood pressure. In the present study, we observed that not only *NOX1* but also *VEGF* and *ACE* were significantly changed after consuming the Japanese-style diet when compared with pre-intervention levels. A daily intake of polyphenols such as quercetin, which is mainly provided by onions, green peppers, and tomatoes[17], in the Japanese-style diet might have led to a reduction in *NOX1*, *VEGF*, and *ACE* gene expression, as observed in the present study.

A previous study has shown that the traditional Japanese diet, which is associated with a high intake of miso, soy sauce, vegetables, beans, potatoes, and mushrooms, has a beneficial impact on blood pressure and LDL-C and HDL-C levels in Japanese individuals[18]. Soybean-based foods in the form of fermented miso, soy sauce, and tofu, which are common items in traditional Japanese diets, are known to reduce blood pressure by inhibiting *ACE* activity[19–21]. In Japan, meals that include soy-sauce and miso also typically contain vegetables and fruits that contain high amounts of potassium, which in known to reduce blood pressure[22–23]. In the present study, the Japanese-style diet lunch invariably contained rice and miso soup as well as plant-based foods and fermented seasonings such as miso and soy sauce. This combination of food items could have contributed to a reduction in the expression of *ACE* and genes associated with lipid metabolism.

The present study has some limitations. Firstly, it was a before-after trial, not a randomized controlled trial. Secondly, the intervention period was relatively short (8 weeks), which might be too short to have a detectable effect on biochemical parameters, and in total, volunteers consumed only 40 Japanese-style diet lunches, Therefore, trials with longer intervention periods should be considered in the future. Thirdly, we did not investigate blood pressure and serum antioxidant capacity. Finally, the present study had a small sample size.

However, our study does provide evidence that the traditional Japanese diet, which is characterized by a high consumption of fish and soybean products and a low intake of animal fat and meat, may be correlated with a reduction in the expression of cellular factors that are associated with disorders such as cardiovascular disease and metabolic syndrome. Further studies are required to obtain more conclusive evidence on the effects of the Japanese-style diet.

5. Conclusions

In this study, we found that consumption of a Japanese-style diet lunch reduced the expression of genes involved with lipid metabolism, such as *ACADS*, *FASN*, and *PPARD*, and also that of redox-related genes such as *NOX1*, thereby indicating that a high intake of fish and plant-based food and the use of fermented seasonings, which are characteristic of the Japanese-style diet, may have beneficial effects through modifying the expression of specific metabolic genes.

Acknowledgments: We would like to thank Kikkoman Corporation, for advice regarding investigational methodology; and Editage (www. editage. jp) for English language editing.

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