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Beneficial Effects of Dietary Fish-Oil-Derived Monounsaturated Fatty Acids on Metabolic Syndrome Risk Factors and Insulin Resistance in Mice

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ABSTRACT: The aim of this study was to elucidate the effect of fish-oil-derived monounsaturated fatty acids (MUFAs) containing large amounts of C20:1 and C22:1 isomers on metabolic disorders in mice. Male C57BL/6J mice were fed a 32% lard diet (control) or a 27% lard plus 5% saury-oil-derived MUFA diet for 6 weeks. Dietary MUFA improved insulin resistance and alleviated metabolic syndrome risk factors by reducing blood glucose and lipids. These favorable changes may be attributed to an improved adipocytokine profile. MUFA ingestion resulted in favorable changes in mRNA expression of genes involved in glucose/lipid metabolism (*SCD-1*, *CPT1a*, *UCPs*, and *CS*) as well as inflammation (*MAC1*, *MMP3*, and *SAA3*) and alterations in fatty acid composition. Our data suggest that marine MUFA improved glucose/lipid homeostasis and hindered the development of metabolic syndrome in obese mice.

KEYWORDS: Monounsaturated fatty acid, saury oil, insulin tolerance, glucose and lipid metabolism, adipocytokine, adipose inflammation, arachidonic acid

INTRODUCTION

Monounsaturated fatty acids (MUFAs), such as oleic acid (C18:1 *n*-9) and palmitoleic acid (C16:1 *n*-7), are abundant in naturally occurring food ingredients, such as olive oil and nuts. Studies have shown that diets rich in MUFA are effective in improving insulin sensitivity and the associated cardiometabolic risk and lead to improvements in lipid profile, whole body inflammation, and endothelial dysfunction.^{1,2} In addition to some marine mammals, such as whales and seals,^{3,4} consistently high levels of MUFA with longer aliphatic tails (>18 C atoms) are found in some pelagic surface fish species, such as saury,⁵ capelin,⁶ sprats,⁷ and herring.⁸ In comparison to the well-studied shorter chain MUFA, there is little documented information on the physiological effects of long-chain MUFA.

In healthy humans, it has been shown that seal/cod liver oil mixtures and whale oil increase high-density lipoprotein (HDL) cholesterol levels, and cod liver oil additionally reduces triglyceride levels.⁴ It has therefore been suggested that some of these findings may be attributable to long-chain MUFA that are present in large amounts in these oils. Our previous study demonstrated that blood glucose and lipid levels in mice were lowered by a saury-oil-rich diet, composed of the polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA; C20:5 *n*-3) and docosahexaenoic acid (DHA; C22:6 *n*-3), as well as high levels of the long-chain MUFA (C20:1 and C22:1).⁹ Dietary intervention studies have shown that *n*-3 PUFA supplementation reduces unfavorable risk parameters for type 2 diabetes mellitus and obesity.¹⁰ However, considering the relatively low content of *n*-3 PUFA in the saury oil-supplemented diet (1.8% EPA/DHA combined), we questioned whether *n*-3 PUFAs are the only active compounds responsible for the metabolic-syndrome-alleviating effect of saury oil. Mori et al.¹¹ showed that, in diet-induced obese C57BL/6J mice, the plasma levels of glucose, total

cholesterol (TC), and triglyceride did not change significantly in the fish oil diet group (with a relatively low *n*-3 PUFA content of 2% combined EPA/DHA) compared to the control diet group (a standard 30% triglyceride mixture of safflower, rapeseed, and perilla oils); whereas, our previous study showed that all of these values decreased significantly with a saury oil diet. In addition, Flachs et al.¹² showed that, in comparison to a control group of adult male C57BL/6J mice [fed a 35% (w/w) lipid diet with a very low *n*-3 PUFA content], plasma glucose levels were not significantly altered in the experimental group fed a high EPA/DHA diet (8.5% by weight), although their plasma triglyceride and insulin levels were lower after 5 weeks. In contrast, we observed significant decreases in all indicators, including plasma glucose, when diet-induced obese C57BL/6J mice were fed a saury oil diet, which had a lower EPA/DHA content.

To elucidate if marine animal MUFA have a beneficial impact on metabolic disorders, we concentrated MUFA from saury oil and investigated their effect on the metabolic and inflammatory parameters associated with metabolic syndrome in diet-induced obese C57BL/6J mice.

MATERIALS AND METHODS

Preparation of MUFA Concentrate Derived from Fish Oil.

Saury oil was obtained from Nippon Suisan Kaisha, Ltd. (Tokyo, Japan). Ethyl esterification was carried out by refluxing the saury oil with sodium ethoxide/ethanol (2:8, w/w) at 80 °C for 110 min. The resulting product was dissolved in hexane, and a saturated aqueous saline solution

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Table 1. FA Composition of Lard (Control) and Fish-Oil-Derived MUFA Concentrate

	FA (g/100 g of FA)	
	lard ^a	MUFA ^a
C14:0	1.49	0.34
C14:1	0.05	0.03
C16:0	25.43	8.17
C16:1	2.35	0.33
C18:0	15.97	3.55
C18:1 <i>n</i> -9	40.64	1.59
C18:1 <i>n</i> -7	ND ^b	2.57
C18:2 <i>n</i> -6	10.81	0.17
C18:3 <i>n</i> -6	0.07	0.08
C18:3 <i>n</i> -3	1.00	0.03
C20:0	0.21	0.36
C20:1 <i>n</i> -9	0.80	22.78
C20:1 <i>n</i> -7	ND	5.44
C20:4 <i>n</i> -6	0.20	0.31
C20:4 <i>n</i> -3	ND	1.08
C20:5 <i>n</i> -3	0.02	0.12
C22:1 <i>n</i> -11	ND	38.98
C22:1 <i>n</i> -9	ND	2.47
C22:5 <i>n</i> -3	0.10	0.09
C22:6 <i>n</i> -3	0.03	1.22

^a Each value is the mean of three independently processed samples. ^b ND = not detected.

was added to give a hexane/saline solution ratio of 1:4. After a brief centrifugation, the hexane layer was evaporated and the resulting saury oil ethyl ester mixture was dehydrated by shaking with anhydrous magnesium sulfate and then clarified through a 1 μ m pore size filter. MUFA fractions were separated through an octadecylsilyl silica gel column with methanol as the eluent and reconstituted into triglycerides by adding glycerol (1:3 molar ratio of glycerol/ethyl ester products) and lipase (1:5 weight ratio of lipase/ethyl ester products). The resulting MUFA preparation (100% triglyceride) was used in subsequent experiments. Fatty acid (FA) composition (Table 1) of the MUFA concentrate, as well as that of lard, included in both diets, was determined after methylation of samples with 14% boron trifluoride/methanol solution (BF₃/methanol, Sigma) at 80 °C for 30 min. The fatty acid methyl esters were quantified by gas chromatography using an Agilent 6890N network gas chromatograph system (Agilent Technologies Japan, Ltd., Tokyo, Japan) equipped with a split injector, flame ionization detector (FID), and a fused silica capillary column (30 m \times 0.25 mm inner diameter \times 0.25 μ m film thickness, J&W Scientific, Agilent Technologies). Data were collected with GC Chemstation (Agilent Technologies). Identification of the methyl esters were made by a comparison of retention times of standard fatty acid methyl esters (Nu-Chek Prep, Inc., Elysian, MN). The oil was stored at -80 °C in air-free sealed tubes until analysis.

Animals and Diets. The 5-week-old male C57BL/6J mice (Charles River Laboratories Japan, Inc., Yokohama, Japan) were housed, one animal per cage, in a controlled environment at 23 ± 1 °C, with a 12 h light/12 h dark cycle with daylight at 06:00. Mice had free access to water and standard mouse chow CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan) for 1 week prior to the start of the experiment to stabilize their metabolism.

After the acclimatization period, mice were randomly assigned to one of two groups ($n = 10$) for a 6 week feeding period on one of two high-fat diets, both of which contained 60% of the calorific intake as fat. The

Table 2. Composition of the HF and HF-MUFA Diets

component (g/100 g of diet)	HF diet	HF-MUFA diet
casein	25.8	25.8
L-cysteine	0.4	0.4
maltodextrin 10	16.2	16.2
sucrose	8.9	8.9
cellulose	6.5	6.5
mineral mixture	1.3	1.3
vitamin mixture	1.3	1.3
choline bitartrate	0.3	0.3
soybean oil	3.2	3.2
lard	32	27
MUFA concentrate		5
energy (kcal/100 g)	597	597

standard high-fat (HF) diet contained 32% (w/w) lard (D12492 rodent diet with 60% kcal fat, Research Diets, Inc., New Brunswick, NJ), and the MUFA-supplemented high-fat (HF-MUFA) diet was the D12492 rodent diet supplemented with the MUFA concentrate, so that the total fat concentration was 27% (w/w) lard plus 5% (w/w) MUFA concentrate. The diet ingredients as well as composition are shown in Table 2. All feeds were stored at -20 °C and were provided fresh to the mice daily. Mice had free access to water and food during the experimental period. Body weight and food intake were monitored throughout the study every 3 or 4 days. All animal experiments were conducted in complete compliance with the National Institutes of Health: Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Nihon Bioresearch, Inc. (Gifu, Japan), where the animals were housed for the entire experimental period.

Measurement of FA Composition of Blood and Tissue Lipids. The FA composition of plasma, white adipose tissue (WAT), and liver in the C57BL/6J mice was determined as described previously.¹³ In brief, lipids were extracted in a 4:1 (v/v) methanol/hexane solution and then methylated with acetyl chloride at 80 °C for 1 h. FA methyl esters were separated using a capillary column, then quantified using gas chromatography, and compared to purified standards (Nu-Chek Prep).

Plasma Glucose Measurement. On days 1, 15, 29, and 42, blood samples were collected from the retro-orbital venous plexus of each mouse in a nonfasting state at 09:00. Each blood sample was centrifuged at 2000g for 15 min, and the glucose concentration of the plasma was measured using a glucose test kit (glucose CII test, Wako Pure Chemical Industries, Ltd., Japan).

Insulin Tolerance Test. An insulin tolerance test was performed for each mouse at the end of 5 weeks, after a 3 h period of fasting with free access to water. Each mouse received an intraperitoneal injection of 0.75 unit/kg of body weight of insulin (Humulin R U-100, Eli Lilly, Japan). Glucose concentrations in blood samples taken from the retro-orbital venous plexus were determined at 0, 20, 40, and 60 min after insulin administration.

Biochemical Analysis of Plasma. On the final day of the experiment, nonfasting mice were anesthetized with 4% sodium pentobarbital (Dainippon Sumitomo Pharma, Osaka, Japan). Blood was collected via the postcaval vein, and plasma was obtained by centrifugation at 2000g for 15 min. The plasma concentrations of TC, high-density lipoprotein (HDL) cholesterol, triglyceride, and free FA were measured using glucose CII test, cholesterol E test, HDL cholesterol E test, TG E test, and NEFA C test reagents, respectively (Wako), and low-density lipoprotein (LDL) cholesterol levels were calculated as TC $-$ HDL cholesterol $-$ 0.2 \times triglyceride. Plasma insulin levels were determined

Table 3. GenBank Accession Numbers and Primer Sequences used in Real-Time PCR Experiments^a

gene	primer sequences	accession number
SREBP-1	5'-GATGTGCGAACTGGACACAG-3' 5'-CATAGGGGGCGTCAACACAG-3'	NM_011480
SCD-1	5'-TTCTTGCATGACTCTGGTGC-3' 5'-CGGGATTGAATGTTCTTGTCTG-3'	NM_009127
FAS	5'-GGAGGTGGTGATAGCCGGTAT-3' 5'-TGGGTAATCCATAGAGCCAG-3'	NM_007988
Acaca	5'-ATGGGCGGAATGGTCTCTTTC-3' 5'-TGGGGACCTTGTCTTCATCAT-3'	NM_133360
CPT1a	5'-CTCCGCCTGAGCCATGAAG-3' 5'-CACCAGTGATGATGCCATTCT-3'	NM_013495
UCP-1	5'-AGGCTTCCAGTACCATTAGGT-3' 5'-CTGAGTGAGGCAAGCTGATTT-3'	NM_009463
UCP-2	5'-ATGGTTGGTTTCAAGGCCACA-3' 5'-CGGTATCCAGAGGGAAGTGAT-3'	NM_011671
UCP-3	5'-CTGCACCGCCAGATGAGTTT-3' 5'-ATCATGGCTTGAAATCGGACC-3'	NM_009464
CS	5'-TGCCACACAAGCCATTTG-3' 5'-CTGACACGTCTTTGCCAATT-3'	NM_026444
MAC1	5'-ATGGACGCTGATGGCAATACC-3' 5'-TCCCCATTACGCTCTCCA-3'	NM_008401
CD68	5'-GGACCCACAACGTCACTCAT-3' 5'-AAGCCCCACTTTAGCTTTACC-3'	NM_009853
MMP3	5'-ACATGGAGACTTTGTCCCTTTTG-3' 5'-TTGGCTGAGTGGTAGAGTCCC-3'	NM_010809
SAA3	5'-TGCCATCATTCTTGCATCTTGA-3' 5'-CCGTGAACCTTCTGAACAGCCT-3'	NM_011315

^a SREBP-1, sterol regulatory element binding protein 1; SCD-1, stearoyl CoA desaturase-1; FAS, fatty acid synthase; Acaca, acetyl-CoA carboxylase; CPT1a, carnitine palmitoyltransferase-1a; UCP, uncoupling protein; CS, citrate synthase; MAC1, macrophage-1 antigen; CD68, CD68 antigen; MMP3, matrix metalloproteinase 3; and SAA3, serum amyloid A 3.

using an insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Inc., Japan). Plasma concentrations of adiponectin and resistin were determined using a mouse/rat adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Japan) and a mouse resistin ELISA kit (Shibayagi Co. Ltd., Japan), respectively.

Analysis of mRNA Expression Using Quantitative Real-Time Polymerase Chain Reaction (PCR). After blood collection, mice were anesthetized with 4% sodium pentobarbital and killed by bleeding from the inferior vena cava. Portions of the mesenteric WAT, liver, and muscle were frozen in liquid nitrogen and maintained at -80°C until assayed. Total RNA was extracted from mesenteric WAT, liver, and muscle using TRIzol reagent (Qiagen, Tokyo, Japan) according to the protocol of the manufacturer, and RNA concentrations were determined by measuring absorbance at 260 nm. For cDNA synthesis, 1 μg of total template RNA and a PrimeScript first-strand cDNA synthesis kit (TaKaRa Bio, Inc., Otsu, Japan) were used. The resulting cDNA was used for real-time PCR with an ABI 7300 real-time PCR system (Applied Biosystems) and 18S rRNA as the internal control. The sequences of the PCR primers used are shown in Table 3.

Statistical Analysis. Each value is reported as the mean \pm standard error (SE). Statistical analyses were performed using the Student's *t* test. Data were considered statistically significant when $p < 0.05$.

RESULTS

FA Composition of Plasma, WAT, and Liver. The compositions of total saturated FA, total MUFA, total *n*-6 PUFA, total *n*-3 PUFA, and *n*-6/*n*-3 PUFA ratio in plasma, WAT, and liver are shown in Figure 1A. The percentages of MUFA and PUFA were significantly different between the HF diet group (control) and the HF-MUFA diet group, although no noticeable differences were found in the levels of saturated FA between the two groups. Total MUFA levels increased significantly in WAT and liver in the HF-MUFA diet group by 6% ($p < 0.05$) and 18% ($p < 0.05$), respectively, compared to the HF diet group. MUFA ingestion decreased total *n*-6 PUFA levels significantly in plasma and liver by 11% ($p < 0.05$) and 9% ($p < 0.05$), respectively, and increased total *n*-3 PUFA levels in liver by 37% ($p < 0.05$). In liver, the decrease in *n*-6 PUFAs and concomitant increase in *n*-3 PUFAs with MUFA ingestion resulted in a significant decrease (34%, $p < 0.05$) in the *n*-6/*n*-3 PUFA ratio. With respect to long-chain MUFA composition (Figure 1B), the HF-MUFA diet resulted in significantly increased levels of C20:1 *n*-9 in plasma, WAT, and liver by 182% ($p < 0.05$), 679% ($p < 0.05$), and 63% ($p < 0.05$), respectively, and noticeably increased levels of C20:1 *n*-7 in plasma and WAT by 320% ($p < 0.05$) and 69% ($p < 0.05$), respectively. Ingestion of MUFA increased C22:1 *n*-11 levels significantly in plasma, WAT, and liver by 390% ($p < 0.05$), 620% ($p < 0.05$), and 290% ($p < 0.05$), respectively. In contrast to the significant increases in long-chain MUFA observed in the HF-MUFA diet group, there was no difference in the amounts of the shorter chain MUFA, such as oleic acid (C18:1 *n*-9) and palmitoleic acid (C16:1 *n*-7), between the HF and HF-MUFA diet groups (data not shown). Relative to *n*-6 PUFAs, the levels of the predominant *n*-6 PUFA, such as arachidonic acid (C20:4 *n*-6), decreased significantly in the plasma, WAT, and liver of the HF-MUFA diet group by 22% ($p < 0.05$), 41% ($p < 0.05$), and 24% ($p < 0.05$), respectively (Figure 2A). In contrast, the HF-MUFA diet resulted in significantly increased levels in the liver *n*-3 PUFA, such as EPA (C20:5 *n*-3) and DHA (C22:6 *n*-3), by 55% ($p < 0.05$) and 38% ($p < 0.05$), respectively (Figure 2B).

Effect of Dietary MUFA on Plasma Glucose Levels. For mice fed the HF-MUFA diet, plasma glucose concentrations were lower by 17% ($p < 0.05$), 8% ($p < 0.05$), and 10% ($p < 0.05$) on days 15, 29, and 42, respectively, compared to those fed the HF diet (Figure 3). According to the results of the insulin tolerance test, mice fed the HF-MUFA diet lowered blood glucose levels faster than the HF diet group (Figure 4); plasma glucose levels in the HF-MUFA diet group significantly decreased by 19% ($p < 0.05$) and 14% ($p < 0.05$) at 20 and 40 min, respectively, after injection of insulin.

Effect of Dietary MUFA on HF-Induced Metabolic Abnormalities. Body weight, mesenteric WAT mass, and plasma concentrations of insulin, lipids, and adipocytokines are shown in Table 4. There were no significant differences in food intake, body weight, and WAT weight between the HF and HF-MUFA diet groups. Ingestion of the MUFA-supplemented diet decreased the plasma concentrations of insulin by 46% ($p < 0.05$), TC by 38% ($p < 0.05$), LDL cholesterol by 67% ($p < 0.05$), and triglyceride by 42% ($p < 0.05$). Furthermore, in comparison to those of the HF diet group, plasma adiponectin levels increased by 26% ($p < 0.05$) and plasma resistin levels decreased by 14% ($p < 0.05$) in the HF-MUFA diet group.

Effect of Dietary MUFA on mRNA Expression in WAT, Liver, and Muscle. The HF-MUFA diet downregulated mRNA

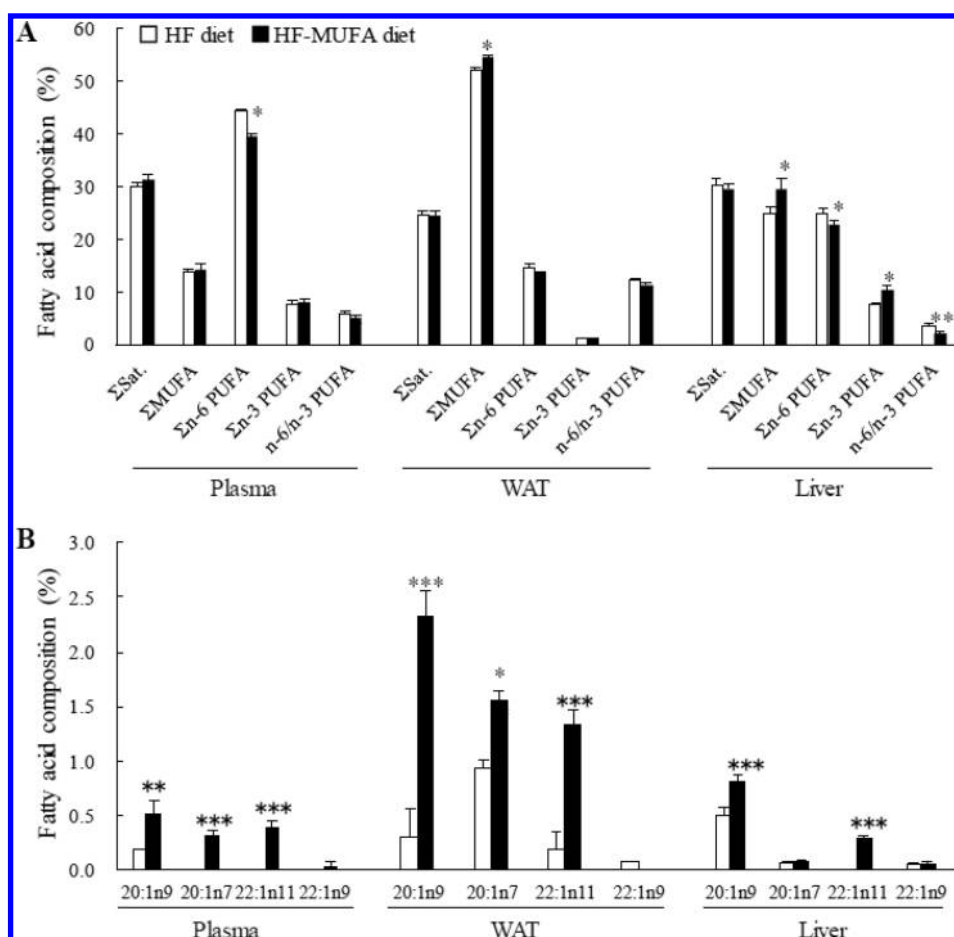


Figure 1. FA composition in plasma, WAT, and liver for C57BL/6J mice fed the HF diet or HF-MUFA diet for 6 weeks. (A) Total FA composition. (B) Long-chain MUFA composition. Each value is the mean \pm SE; $n = 10$. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ compared to the HF diet group. HF diet, high-fat diet; HF-MUFA diet, 5% MUFA-supplemented high-fat diet.

expression of the lipogenic gene *SCD-1* by 42% ($p < 0.05$) and 52% ($p < 0.05$) in mesenteric WAT and liver, respectively, compared to those of the HF diet group. In addition, *SREBP-1* mRNA in WAT and liver tended to decrease with the HF-MUFA diet ($p = 0.07$ and 0.1 , respectively) (Figure 5). Ingestion of MUFA upregulated the mRNA expression of the FA oxidation-related gene *CPT-1* by 65% ($p < 0.05$) and 84% ($p < 0.05$) in liver and muscle, respectively, and that of the UCP genes *UCP-1* by 421% ($p < 0.05$) in WAT, *UCP-2* by 63% ($p < 0.05$) in liver, and *UCP-3* by 55% ($p < 0.05$) in muscle. The mRNA levels of the citrate synthase (*CS*) gene were increased significantly by 117% ($p < 0.05$) and 97% ($p < 0.05$) in liver and muscle in the HF-MUFA diet group, respectively, compared to those of the HF diet group (Figure 6). Furthermore, MUFA ingestion suppressed mRNA expression of macrophage-marker *MAC-1* and inflammation-marker *MMP3* and *SAA3* in WAT by 39% ($p < 0.05$), 66% ($p < 0.05$), and 97% ($p < 0.05$), respectively (Figure 7).

DISCUSSION

This study demonstrates that fish-oil-derived MUFA ingestion reduces insulin resistance, as judged by the insulin tolerance test, and attenuates metabolic syndrome by improving hyperglycemia, hyperinsulinemia, and hyperlipidemia. The favorable change in adipocytokines possibly correlates with improved metabolic risk factors related to insulin resistance when MUFA was added

to the diet. Plasma adiponectin levels increased significantly for the HF-MUFA diet group compared to those with the HF diet group (Table 4). Adiponectin has been linked to insulin resistance in obesity and lipodystrophy.¹⁴ Blood levels of adiponectin were lower in obese subjects and those with type 2 diabetes mellitus compared to healthy controls,¹⁵ indicating a negative correlation between adiponectin and obesity as well as obesity-related disorders, e.g., atherosclerosis and diabetes. Therefore, the increased plasma adiponectin levels associated with the MUFA diet possibly contribute to the improved plasma metabolic profile. In contrast to the beneficial effect of adiponectin on metabolic risk factors, adipocytokine resistin has been variably associated with insulin resistance and inflammation as a proinflammatory cytokine.^{16,17} A noticeable decrease in plasma resistin (Table 4) may therefore possibly correlate with enhanced insulin sensitivity in the MUFA-supplemented diet group, compared to the HF diet group.

To elucidate how MUFA ingestion improves metabolic syndrome risk factors, real-time PCR was performed to assess mRNA expression of genes related to glucose/lipid metabolism. WAT and hepatic mRNA expression of *SCD-1* was markedly suppressed by the MUFA diet (Figure 5). *SCD-1* acts as an enhancer of metabolic syndrome, and mice lacking *SCD-1* are largely protected against diet-induced and genetically induced obesity, hypertriglyceridemia, and insulin resistance.¹⁸ Furthermore,

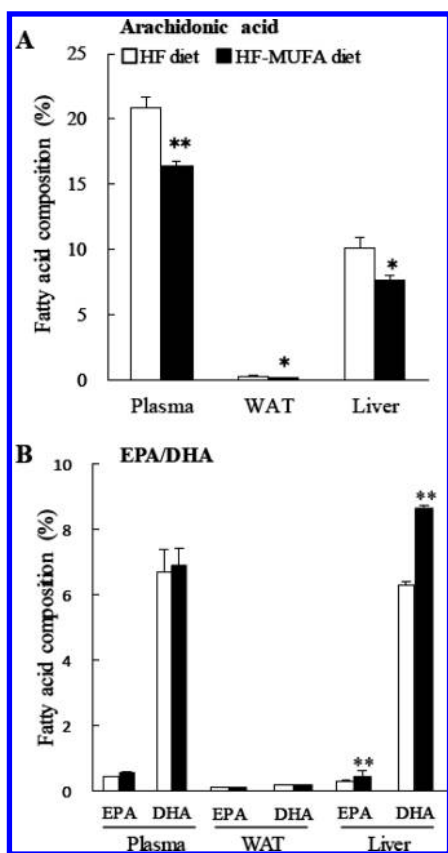


Figure 2. PUFA composition in plasma, WAT, and liver for C57BL/6J mice fed the HF diet or HF-MUFA diet for 6 weeks. (A) *n*-6 PUFA, such as arachidonic acid (C20:4 *n*-6). (B) *n*-3 PUFA, such as EPA (C20:5 *n*-3) and DHA (C22:6 *n*-3). Each value is the mean \pm SE; *n* = 10. (*) *p* < 0.05 and (**) *p* < 0.01 compared to the HF diet group. HF diet, high-fat diet; HF-MUFA diet, 5% MUFA-supplemented high-fat diet.

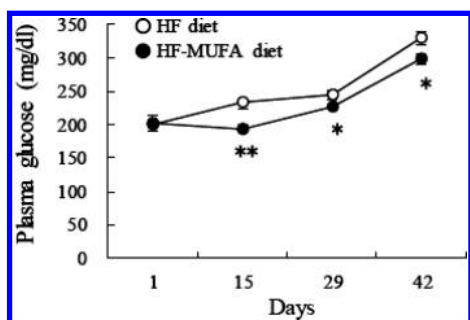


Figure 3. Effect of the MUFA on plasma glucose levels. Plasma from each animal was collected on days 1, 9, 15, and 42 of the experiment. Each value is the mean \pm SE; *n* = 10. (*) *p* < 0.05 and (**) *p* < 0.01 compared to the HF diet group. HF diet, high-fat diet; HF-MUFA diet, 5% MUFA-supplemented high-fat diet.

administration of an antisense oligonucleotide against *SCD-1* increased resistance to obesity by enhancing expression of β -oxidation enzymes and/or *UCPs*.¹⁹ Correspondently, we observed significant increases in mRNA expression of the gene encoding carnitine palmitoyltransferase-1a (CPT1a), a rate-limiting enzyme in the β -oxidation pathway,²⁰ and uncoupling proteins (UCPs) (Figure 6). UCPs influence the proton gradient across the inner mitochondrial membrane and, consequently, ATP

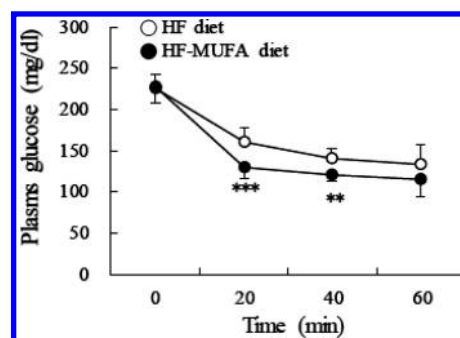


Figure 4. Effect of the MUFA on insulin sensitivity. An insulin tolerance test was performed at the end of the 5th week. Plasma from each animal was collected at 0, 20, 40, and 60 min. Each value is the mean \pm SE; *n* = 10. (**) *p* < 0.01 and (***) *p* < 0.001 compared to the HF diet group. HF diet, high-fat diet; HF-MUFA diet, 5% MUFA-supplemented high-fat diet.

Table 4. Effect of MUFA on Body Weight, WAT Mass, Plasma Markers of Glucose, Lipid Metabolism, and Adipocytokines^a

	HF diet	HF-MUFA diet
initial body weight (g)	24.1 \pm 0.17	24.1 \pm 0.14
final body weight (g)	30.9 \pm 0.29	30.8 \pm 0.54
food consumption (g/day)	2.6 \pm 0.041	2.6 \pm 0.038
mesenteric WAT mass (mg)	163 \pm 9.5	165 \pm 6.1
insulin (ng/mL)	4.6 \pm 0.8	2.5 \pm 0.4 ^b
TC (mg/dL)	176.4 \pm 23.6	110.2 \pm 7.9 ^c
HDL cholesterol (mg/dL)	59.2 \pm 7.6	66.5 \pm 6.4
LDL cholesterol (mg/dL)	94.7 \pm 13.1	30.6 \pm 14.4 ^d
triacylglycerol (mg/dL)	112.4 \pm 10.2	65.5 \pm 3.4 ^d
free fatty acid (mequiv/L)	0.68 \pm 0.14	0.53 \pm 0.09
adiponectin (μ g/mL)	14.14 \pm 0.47	17.86 \pm 0.94 ^b
resistin (ng/mL)	26.78 \pm 1.3	23.16 \pm 1.1 ^b

^a C57BL/6J mice were fed the HF diet or HF-MUFA diet for 6 weeks. Each value is the mean \pm SE; *n* = 10. ^b *p* < 0.05 compared to the HF diet group. ^c *p* < 0.01 compared to the HF diet group. ^d *p* < 0.001 compared to the HF diet group. HF diet, high-fat diet; HF-MUFA diet, 5% MUFA-supplemented high-fat diet.

production *in vitro*, and they may influence energy expenditure by diverting energy from fat synthesis to heat production.^{21,22} MUFA ingestion also upregulated citrate synthase (CS) mRNA in liver and muscle (Figure 6). CS has been extensively used as a metabolic marker when assessing oxidative and respiratory capacity because it is the first, key enzyme of the tricarboxylic acid cycle.²³ Mitochondrial dysfunction is associated with insulin resistance, and mitochondrial respiration is reduced in type 2 diabetes mellitus subjects.²⁴ Enhancement of this key mitochondrial enzyme activity via mRNA expression may therefore correlate to the increased insulin sensitivity. As observed by changes in mRNA levels, improved glucose and lipid metabolism associated with the HF-MUFA diet may be caused, in part, by suppression of adipogenesis, promotion of lipid/glucose oxidative capabilities, and energy expenditure.

Chronic inflammation of hypertrophied adipocytes within adipose tissue induced by obesity is known to interfere with insulin signaling and induces insulin resistance.²⁵ Conversely, an increase in macrophage infiltration has been associated with adipose

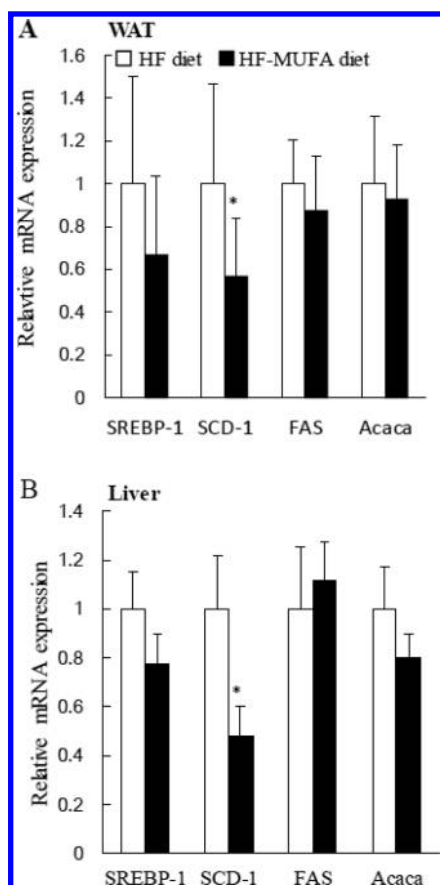


Figure 5. Effect of the MUFA on lipogenic gene expressions in WAT and liver. mRNA levels of sterol regulatory element binding protein 1 (*SREBP-1*), stearoyl CoA desaturase-1 (*SCD-1*), fatty acid synthase (*FAS*), and acetyl-CoA carboxylase (*Acaca*) in mesenteric WAT and liver were measured by real-time PCR at the end of the 6 week intervention period. (A) mRNA expressions in mesenteric WAT. (B) mRNA expressions in liver. Each value is the mean \pm SE; $n = 10$. (*) $p < 0.05$ compared to the HF diet group. HF diet, high-fat diet; HF-MUFA diet, 5% MUFA-supplemented high-fat diet.

tissue inflammation.²⁶ In addition, macrophages are probably the major source of resistin.²⁷ In the present study, MUFA ingestion significantly suppressed mRNA expression of genes encoding markers for macrophages and WAT inflammation (Figure 7), which possibly then decreased secretion of proinflammatory adipocytokines and improved insulin sensitivity. Moreover, *n*-6 PUFA arachidonic acid (C20:4 *n*-6), through its myriad metabolites, is involved in inflammation,²⁸ and any increase in inflammatory tendencies can exacerbate the effects of insulin insensitivity. FA composition analysis revealed that plasma and organ *n*-6 PUFA, in particular, arachidonic acid, decreased remarkably when mice were fed the MUFA diet (Figure 2A), which may partly contribute to the improvement in systemic inflammation and the further alleviation of metabolic syndrome.

Intriguingly, the percentages of liver EPA and DHA were significantly increased in the mice fed the MUFA diet (Figure 2B), although the amounts of these *n*-3 PUFA were very low in the MUFA concentrate (Table 1). Given the beneficial effect of *n*-3 PUFA on the improvement of lipidemic metabolism in obesity, increased *n*-3 PUFA levels, induced by MUFA ingestion, may therefore at least partially contribute to the beneficial changes in

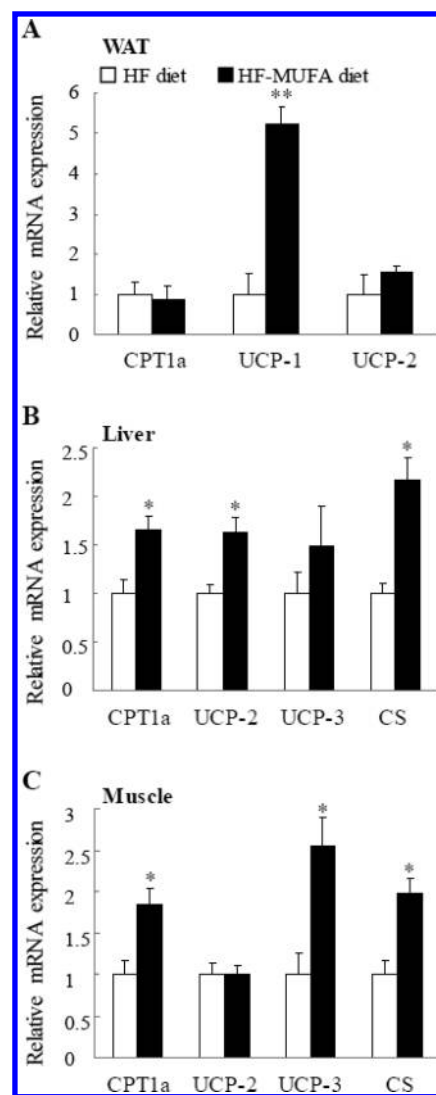


Figure 6. Effect of the MUFA on energy metabolism-related gene expressions in WAT, liver, and muscle. The mRNA levels of carnitine palmitoyltransferase-1a (*CPT1a*), uncoupling proteins (*UCPs*), and citrate synthase (*CS*) in mesenteric WAT, liver, and muscle were measured by real-time PCR at the end of the 6 week intervention period. (A) mRNA expressions in mesenteric WAT. (B) mRNA expressions in liver. (C) mRNA expressions in skeletal muscle. Each value is the mean \pm SE; $n = 10$. (*) $p < 0.05$ and (**) $p < 0.01$ compared to the HF diet group. HF diet, high-fat diet; HF-MUFA diet, 5% MUFA-supplemented high-fat diet.

the metabolic parameters found in the present study. Conversely, MUFA intake dramatically increased levels of long-chain MUFA C20:1 isomers and C22:1 *n*-11 in plasma, WAT, and liver (Figure 1B), although the percentages of shorter chain MUFA, e.g., palmitoleic acid and oleic acid, did not differ between the HF and HF-MUFA diet groups. Notably, the percentages of long-chain MUFA, as well as total MUFA, found in WAT were predominant over those found in plasma and liver (Figure 1) when mice were fed the MUFA diet, suggesting that MUFA may positively correlate with improved WAT lipid metabolism and decreased WAT inflammation, thereby attenuating metabolic syndrome risk factors.

In conclusion, the present study showed that plasma glucose, insulin, and lipid concentrations were reduced and insulin

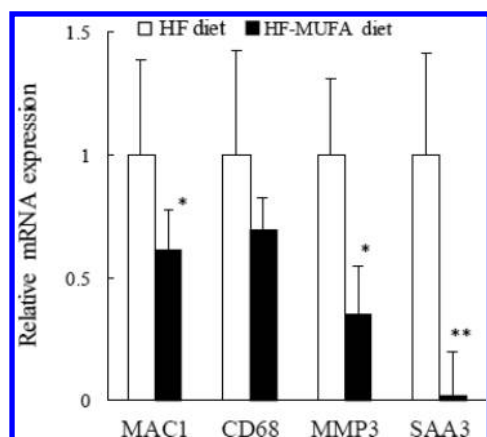


Figure 7. Effect of the MUFA on inflammation-related gene expressions in WAT. The mRNA levels of macrophage-1 antigen (MAC1), CD68 antigen (CD68), matrix metalloproteinase 3 (MMP3), and serum amyloid A 3 (SAA3) were measured by real-time PCR at the end of the 6 week intervention period. Each value is the mean \pm SE; $n = 10$. (*) $p < 0.05$ and (**) $p < 0.01$ compared to the HF diet group. HF diet, high-fat diet; HF-MUFA diet, 5% MUFA-supplemented high-fat diet.

sensitivity was improved in mice fed a fish-oil-derived MUFA diet. The favorable changes in plasma adipocytokines and lipid/glucose metabolism-related mRNA levels might be attributed to improved lipid and glucose metabolism. Furthermore, intake of MUFA decreased obesity-induced inflammation by suppressing WAT inflammatory marker genes and arachidonic acid levels, thereby possibly, in turn, reducing insulin resistance in diet-induced obese mice.

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ABBREVIATIONS USED

Acaca, acetyl-coenzyme A carboxylase α ; CPT1a, carnitine palmitoyltransferase-1a; CD68, CD68 antigen; CS, citrate synthase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FAS, fatty acid synthase; MAC1, macrophage-1 antigen; MMP3, matrix metalloproteinase 3; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SAA3, serum amyloid A 3; SCD-1, stearoyl-coenzyme A desaturase 1; SREBP-1, sterol regulatory element binding protein 1; UCP, uncoupling protein

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