

Dietary Saury Oil Reduces Hyperglycemia and Hyperlipidemia in Diabetic KKAY Mice and in Diet-Induced Obese C57BL/6J Mice by Altering Gene Expression

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Abstract We investigated the effect of saury oil on the alleviation of metabolic syndrome in mice. Saury oil contains 18% (w/w) n-3 polyunsaturated fatty acids (n-3 PUFA) and 35% (w/w) monounsaturated fatty acids (MUFA). Diabetic KKAY mice were fed a 10% soybean oil diet (control) or a 10% saury oil diet for 4 weeks, and diet-induced obese C57BL/6J mice were fed a high-fat diet containing 32% lard (control) or 22% lard plus 10% saury oil for 6 weeks. After the intervention periods, the levels of glucose, insulin and lipids in plasma had decreased significantly for the saury oil diet group, and insulin sensitivity had improved. These favorable changes may be attributed to the increased adiponectin and decreased TNF α and resistin levels in plasma. The saury oil diet also resulted in downregulated expression of the lipogenic genes (*SREBP-1*, *SCD-1*, *FAS*, and *ACC*) as well as upregulation of the fatty acid oxidative gene, *CPT-1*, and the energy expenditure-related genes (*PGC1 α* and *PGC1 β*) in white adipose tissue for the diet-induced obese C57BL/6J mice. An increase in n-3 PUFA levels and the concomitant decrease in the n-6/n-3 PUFA level ratio in serum, white adipose tissue, and liver with a saury oil diet are likely to be involved in the beneficial changes to the metabolic indicators. MUFA may also play a positive role in remodeling lipid composition. Based on these mice models, our results suggest a potential use for saury oil for improving metabolic abnormalities.

Keywords Saury oil · n-3 PUFA · MUFA · Hypoglycemia · Hypolipidemia · Insulin resistance · Adipokine · Adipogenesis · Lipid composition

Abbreviations

ACC	Acetyl-CoA carboxylase
CPT-1	Carnitine palmitoyltransferase-1
FAS	Fatty acid synthase
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
MetS	Metabolic syndrome
MUFA	Monounsaturated fatty acids
NEFA	Nonesterified fatty acids
PGC-1 α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PGC-1 β	Peroxisome proliferator-activated receptor gamma coactivator 1-beta
PUFA	Polyunsaturated fatty acids
RT-PCR	Reverse transcription polymerase chain reaction
SAF	Saturated fatty acids
SCD-1	Stearoyl CoA desaturase-1
SREBP-1	Sterol regulatory element binding protein-1
TAG	Triacylglycerol
TC	Total cholesterol
WAT	White adipose tissue

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Introduction

Metabolic syndrome (MetS) is a major and growing public health problem and is characterized by a group of metabolic risk factors for cardiovascular disease and diabetes

that includes hypertension, glucose intolerance, dyslipidemia and abdominal obesity [1]. The causes of MetS are unknown, although they are considered to involve both genetic and environmental factors, including diet [2].

Alteration of the type of dietary lipids is an important way of preventing and treating obesity-associated MetS. Studies have demonstrated that the health benefits of fish oil, which is rich in n-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), include its ability to prevent heart disease, inflammation, dyslipidemia and diabetes via multiple mechanisms [3]. Studies in rats and mice fed a high-fat or lipogenic, sucrose-rich diet have shown that n-3 PUFA have beneficial effects on obesity and insulin resistance [3, 4]. Several studies have also demonstrated a decrease in adiposity in obese humans and improved glucose metabolism in human subjects after n-3 PUFA supplementation [5, 6].

Fish lipids generally contain varying amounts of different types of fatty acids in addition to n-3 PUFA [7]. Consistently high levels of monounsaturated fatty acids (MUFA) are found in the lipids of some pelagic surface fish species, such as saury [8], capelin [9], sprats [10], and herring [11], whose lipids originate from their food source, such as zooplankton [12, 13]. It has been established that ingestion of fish oil rich in MUFA increases peroxisomal beta-oxidation [14] and promotes the synthesis of long-chain essential fatty acids [15]. Additionally, Osterude et al. [16] demonstrated that a seal/cod liver oil mixture and whale oil both increased high-density lipoprotein cholesterol (HDL-C) levels, and furthermore, cod liver oil reduced triacylglycerol (TAG) levels in healthy humans. Because all these fish oils contain a considerable amount of MUFA in addition to n-3 PUFA, some of these effects may be attributable to MUFA. All of these studies suggested a possible beneficial effect of MUFA for the treatment of MetS.

Saury, a seawater fish from the family Scomberesocidae, is one of the most highly consumed fish in Japan. Saury oil contains n-3 PUFA, such as EPA and DHA, as well as considerable amounts of MUFA (C20:1 and C22:1). Given the positive effects on glucose and lipid homeostasis by n-3 PUFA, and the findings that MUFA may have an impact on the treatment of MetS, we evaluated the effect of saury oil on glucose and lipid metabolism using diabetic KKAY mice and diet-induced obese C57BL/6J mice for our animal model. Our research revealed that saury oil intake reduced insulin resistance and plasma levels of glucose, insulin, and lipids. This effect may be attributable to favorable changes in the plasma adipokine profile and lipid metabolism-related gene expression. Our research constitutes the first investigation of the effect of saury oil ingestion on glucose and lipid metabolism, and our findings suggest that saury oil may have a favorable impact on MetS.

Materials and Methods

Animals and Diets

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 at Nihon Bioresearch Inc. (Gifu, Japan), where the animals were housed for the entire experimental period. Five-week-old spontaneously diabetic male KKAY mice were obtained from CLEA Japan Inc. (Shizuoka, Japan), and 5-week-old male C57BL/6J mice were from Charles River Laboratories Japan Inc. (Yokohama, Japan). Mice were housed 1/cage at 23 ± 1 °C with a 12-h light/dark cycle, and provided with free access to water and standard mouse chow CRF-1 (Oriental Yeast Co. Ltd., Tokyo, Japan) for an acclimatization period of 1 week.

The fatty acid composition of the dietary oils is shown in Table 1. Saury oil (Nippon Suisan Kaisya, Ltd., Tokyo, Japan) contained 34.7% MUFA (20:1 and 22:1 isomers) and 18% of EPA and DHA combined. Following the acclimatization period, the KKAY mice were randomly assigned into two groups for a 4-week feeding experiment. The control group ($n = 10$) was fed on AIN-93G growth diet (Oriental Yeast Co., Tokyo, Japan) containing 10% soybean oil, and the saury oil group ($n = 10$) was fed the same basal diet supplemented with 10% saury oil. The two groups were pair-fed throughout the experiment. The

Table 1 Fatty acid composition of dietary oils

Fatty acids (%)	Soybean oil	Lard	Saury oil
C14:0	0.06	1.49	5.68
C16:0	9.49	25.43	9.23
C16:1	0.10	2.35	2.99
C18:0	3.86	5.97	1.66
C18:1	22.8	40.64	5.83
C18:2n-6	55.14	10.81	1.60
C18:3n-3	7.55	1.00	1.22
C20:0	0.33	0.21	0.19
C20:1n-9	0.20	0.80	12.13
C20:1n-7	ND	ND	3.11
C20:4n-6	ND	0.20	0.63
C20:5n-3	ND	0.02	6.11
C22:1n-11	ND	ND	18.49
C22:1n-9	0.05	ND	1.02
C22:5n-3	0.00	0.10	1.56
C22:6n-3	ND	0.03	11.82

Values correspond to the means of three separate samples processed independently

ND not detected

C57BL/6J mice were randomly divided into two groups for a 6-week pair-feeding period. The control group was fed on a high-fat diet (D12492 Rodent Diet with 60 kcal% Fat; Research Diets, Inc., New Brunswick, NJ, USA) containing 32% lard (corresponding to 60% energy from fat; $n = 10$), and the saury oil group was fed a saury oil-supplemented diet (22% lard plus 10% saury oil, corresponding to 60% energy from fat; $n = 10$). The compositions of the experimental diets are shown in Table 2. All diet feeds were stored at $-20\text{ }^{\circ}\text{C}$ and were provided fresh daily to the mice. Body weight and food intake were monitored throughout the study.

At the end of the intervention periods, the KKAY mice and C57BL/6J mice were anesthetized with 4% sodium pentobarbital (Dainippon Sumitomo Pharma, Osaka, Japan) in the early light phase of the light–dark cycle (fed condition), and blood was collected by abdominal vein puncture. Plasma was obtained by centrifugation at 3,000 rpm for 15 min and stored at $-80\text{ }^{\circ}\text{C}$ pending further analysis. Liver and mesenteric adipose tissue (WAT) were snap-frozen in liquid nitrogen after weighing for further analysis.

Lipid Extraction and Fatty Acid Analysis

The fatty acid composition of plasma, WAT, and liver in the C57BL/6J mice was determined as described [17]. Lipids were extracted by homogenizing the tissue samples in a 4:1 (v/v) methanol/hexane solution supplemented with 50 $\mu\text{g/ml}$ butylated hydroxytoluene (BHT) as an antioxidant. Fatty acids methyl esters were obtained by

transmethylation of the lipids (500 μl) with acetyl chloride (200 μl) and heating at $80\text{ }^{\circ}\text{C}$ for 1 h under a nitrogen atmosphere. Methyl docosatrienoate (22:3n-3) at a final concentration of 0.4 $\mu\text{g/mg}$ for WAT and liver, and methyl tricosanoate (23:0) at a final concentration of 0.2 $\mu\text{g}/\mu\text{l}$ for plasma were added to each sample as internal standards. Gas chromatographic analysis of fatty acid methyl esters was performed on an Agilent 6890N Network Gas Chromatograph System (Agilent Technologies Japan, Ltd., Japan) equipped with a split injector, FID detector and a fused silica capillary column (30 m \times 0.25 mm ID \times 0.25 μm film thickness, J & W Scientific, Agilent Technologies). Data were collected with GC Chemstation (Agilent Technologies). Fatty acid methyl esters were identified by co-chromatography with purified standard mixture (Nu-Chek Prep 462, Elysian, MN), and fatty acid data were expressed as the percentage peak area corresponding to the weight of individual fatty acids.

Insulin Tolerance Test

For the KKAY mice on the control diet and the saury oil diet, the insulin tolerance test was performed at the end of 3 weeks. Each mouse received an intraperitoneal injection of insulin (0.75 U/kg body weight, Humulin R U-100, Eli Lilly, Japan) after fasted for 6 h with free access to water. Blood samples were taken from the retro-orbital venous plexus prior to the insulin injection (0 min time point) and at indicated time points after the injection. The blood samples were centrifuged at 3,000 rpm for 15 min, and plasma glucose concentration was measured using a glucose test kit (Glucose CII-test, Wako Pure Chemicals Industries, Japan).

Biochemical Analysis of Plasma

The plasma concentrations of glucose, total cholesterol (TC), HDL-C, TAG, and nonesterified fatty acids (NEFA) were measured using a Glucose CII-Test, a Cholesterol E-Test, a HDL-Cholesterol E-Test, a TG E-Test and a NEFA C-Test, respectively (Wako), and low-density lipoprotein cholesterol (LDL-C) levels were calculated as $\text{TC} - \text{HDL-C} - \text{TGA} \times 0.2$. Plasma insulin levels were determined using an Insulin ELISA kit (Morinaga Institute of Biological Science, Inc., Japan). Plasma concentrations of adipokines, including adiponectin, resistin, tumor necrosis factor- α (TNF α), and leptin, were measured using the following respective enzyme immunoassay kits: mouse adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Japan), Mouse Resistin ELISA kit (Shibayagi Co. Ltd., Japan), Mouse TNF- α ELISA kit (Shibayagi) and Mouse Leptin ELISA kit (Morinaga).

Table 2 Composition of the diets for the KKAY and C57BL/6J mice

Component (%)	KKAY mice		C57BL/6J mice	
	Soybean oil diet	Saury oil diet	Lard diet	Saury oil diet
Casein	20	20	25.8	25.8
L-Cysteine	0.3	0.3	0.4	0.4
Corn starch	49.9	49.9	–	–
Maltodextrin 10	–	–	16.2	16.2
Sucrose	10	10	8.9	8.9
Cellulose	5	5	6.5	6.5
Mineral mixture	3.5	3.5	1.3	1.3
Vitamin mixture	1	1	1.3	1.3
Choline bitartrate	0.3	0.3	0.3	0.3
Soybean oil	10	–	3.2	3.2
Lard	–	–	32	22
Saury oil	–	10	–	10

Analysis of mRNA Expression Using Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)

For the C57BL/6J mice fed high-fat diets, total RNA was isolated from mesenteric WAT using TRIzol reagent (Qia-gen, Tokyo, Japan) according to the manufacturer's protocol. The first strand of cDNA was generated from total RNA using a PrimeScript II 1st strand cDNA Synthesis kit (TaKaRa Bio, Otsu, Japan) using oligo dT-adaptor primers, and 1–2 µg of total RNA as the template. The resulting cDNA pool was used for real-time RT-PCR amplification and specific sequence detection on an Applied Biosystems 7300 Real-Time PCR System (Life Technologies Co., Japan). The forward and reverse PCR primers shown in Table 3 were used at final concentrations of 10 µM. SYBR Premix Ex Taq (TaKaRa Bio) was also used. The PCR cycling parameters were: 30 s at 95 °C; followed by 40 cycles of 5 s, 95 °C, 34 s at 60 °C; and a final melting curve of 15 s at 95 °C, 1 min at 60 °C, 15 s at 95 °C. Gene expression was scaled to the expression of the housekeeping gene encoding 18S ribosomal RNA.

Statistical Analysis

All data are expressed as means ± SE. Statistical differences between two groups were determined using Student's *t* test. The value was considered to be significantly different for values of *P* < 0.05.

Results

Fatty Acid Composition of Plasma, WAT and Liver

Plasma, mesenteric WAT, and liver fatty acid compositions in C57BL/6J mice fed the control diet (lard) or

saury oil-supplemented diet are shown in Table 4. PUFA and MUFA percentages were significantly different in the saury oil group compared to the control group although there were no large differences in the levels of saturated fatty acids (SFA) between the two diet groups. The saury oil-supplemented diet resulted in reduced levels of arachidonic acid (C20:4 n-6) in plasma, WAT and liver by 33.8% (*P* < 0.05), 39.3% (*P* < 0.01), and 53.7% (*P* < 0.001) respectively, and reduced the total n-6 PUFA levels significantly in WAT and liver by 8% (*P* < 0.05) and 29.2% (*P* < 0.05), respectively. Ingestion of saury oil increased EPA levels significantly in both plasma and liver by 130% (*P* < 0.05) and 1118.5% (*P* < 0.001), respectively, and also increased DHA levels significantly in WAT and liver by 835.3% (*P* < 0.001) and 85% (*P* < 0.001), respectively. Compared to the control group, the total n-3 PUFA levels in the saury oil diet group increased significantly in plasma, WAT and liver by 35.9% (*P* < 0.05), 162.2% (*P* < 0.001), and 141.8% (*P* < 0.001), respectively. The decrease in n-6 PUFA and increase in n-3 PUFA with the saury oil diet resulted in significant decreases in n-6/n-3 PUFA ratios in plasma, WAT and liver by 39.7% (*P* < 0.05), 64.9% (*P* < 0.001), and 69% (*P* < 0.001), respectively. A different pattern of changes in lipid composition was observed for the MUFA levels with the saury oil diet. Compared to the control diet group, C22:1 levels in the saury oil group were significantly higher in plasma, WAT and liver by 110.3% (*P* < 0.05), 266.7% (*P* < 0.001), and 450% (*P* < 0.01), respectively. Also, C20:1 levels in WAT were significantly elevated (by 66.7%, *P* < 0.001) with the saury oil-supplemented diet. In contrast, saury oil intake decreased oleic acid (C18:1) levels markedly in WAT and liver, by 16.8% (*P* < 0.01) and 27.2% (*P* < 0.05), respectively.

Table 3 GenBank accession numbers and primer sequences used in real-time RT-PCR experiments

Gene	Primer sequences	Accession number
SREBP-1c	5'-GATGTGCGAACTGGACACAG-3' 5'-CATAGGGGGCGTCAAACAG-3'	NM_011480
SCD-1	5'-TTCTTGCGATACACTCTGGTGC-3' 5'-CGGGATTGAATGTCTTGTTCGT-3'	NM_009127
FAS	5'-GGAGGTGGTGATAGCCGGTAT-3' 5'-TGGGTAATCCATAGAGCCCAG-3'	NM_007988
ACC	5'-ATGGGCGGAATGGTCTCTTTC-3' 5'-TGGGGACCTTGTCTTCATCAT-3'	NM_133360
CPT-1	5'-CTCCGCCTGAGCCATGAAG-3' 5'-CACCAGTGATGATGCCATTCT-3'	NM_013495
PGC-1α	5'-GAAGTGGTGTAGCGACCAATC-3' 5'-AATGAGGGCAATCCGTCTTCA-3'	NM_008904
PGC-1β	5'-TCCTGTAAAAGCCCGGAGTAT-3' 5'-GCTCTGGTAGGGCAGTGA-3'	NM_133249

SREBP-1 sterol regulatory element binding protein 1, *SCD-1* stearoyl CoA desaturase-1, *FAS* fatty acid synthase, *ACC* acetyl-CoA carboxylase, *CPT-1* carnitine palmitoyltransferase-1, *PGC-1α* peroxisome proliferator-activated receptor gamma coactivator 1-alpha, *PGC-1β* peroxisome proliferator-activated receptor gamma coactivator 1-beta

Table 4 Serum, mesenteric WAT and liver fatty acid composition (%) for diet-induced obese C57BL/6J mice

Fatty acid	Serum		Mesenteric WAT		Liver	
	Control	Saury oil	Control	Saury oil	Control	Saury oil
14:0	1.18 ± 0.2	2.2 ± 0.24	1.17 ± 0.02	2 ± 0.03*	0.72 ± 0.01	0.71 ± 0.01
16:0	15.81 ± 0.21	15.32 ± 0.26	22.47 ± 0.19	22.73 ± 0.23	18.96 ± 0.52	19.56 ± 0.24
18:0	29.34 ± 0.6	28.54 ± 0.48	4.29 ± 0.11	5.26 ± 0.17*	16.9 ± 0.52	16.86 ± 0.32
SAF	48.58 ± 1.42	47.7 ± 1.35	27.94 ± 1.42	30 ± 1.35	38.26 ± 1.17	38.39 ± 0.77
12:1	11.89 ± 1.5	10.27 ± 1.42	0.07 ± 0	0.07 ± 0	11.69 ± 0.6	13.41 ± 0.73
16:1	0.88 ± 0.05	0.85 ± 0.04	4.92 ± 0.16	3.85 ± 0.2*	1.34 ± 0.1	0.93 ± 0.05
18:1	23.31 ± 0.08	23.56 ± 0.19	47.84 ± 0.38	39.78 ± 0.58**	17.86 ± 1.24	13 ± 0.55*
20:1	0.21 ± 0.02	0.19 ± 0.01	0.69 ± 0.01	1.15 ± 0.03***	0.36 ± 0.03	0.35 ± 0.02
22:1	0.29 ± 0.03	0.61 ± 0.05*	0.03 ± 0	0.11 ± 0.01***	0.02 ± 0	0.11 ± 0.01**
MUFA	36.58 ± 1.4	35.48 ± 1.35	53.55 ± 0.36	44.96 ± 0.54**	31.26 ± 2.09	27.81 ± 1.42
18:2n-6	3.99 ± 0.27	3.75 ± 0.16	13.61 ± 0.18	12.72 ± 0.25*	10.05 ± 0.24	9.64 ± 0.21
18:3n-6	0.26 ± 0.07	0.21 ± 0.02	0.07 ± 0	0.03 ± 0	0.3 ± 0.01	0.18 ± 0*
20:2n-6	0.15 ± 0.01	0.16 ± 0.01	0.25 ± 0.01	0.23 ± 0	0.19 ± 0.01	0.14 ± 0
20:3n-6	0.29 ± 0.02	0.25 ± 0.02	0.13 ± 0.02	0.08 ± 0	0.81 ± 0.07	0.63 ± 0.04
20:4n-6	3.22 ± 0.5	2.13 ± 0.35*	0.28 ± 0.01	0.17 ± 0.01**	9.86 ± 0.24	4.57 ± 0.11***
22:4n-6	0.2 ± 0.03	0.16 ± 0.01	0.08 ± 0	0.04 ± 0	0.34 ± 0.01	0.09 ± 0.01**
n-6 PUFA	8.11 ± 0.86	6.66 ± 0.65	14.42 ± 0.19	13.27 ± 0.26*	21.55 ± 0.57	15.25 ± 0.4*
18:3n-3	0.16 ± 0.01	0.18 ± 0.01	0.77 ± 0.02	1.01 ± 0.02*	0.32 ± 0.02	0.43 ± 0.03*
20:5n-3	0.63 ± 0.22	1.45 ± 0.28*	0.16 ± 0.01	0.09 ± 0.01	0.27 ± 0.01	3.29 ± 0.1***
22:5n-3	2.25 ± 0.02	0.26 ± 0.01	0.09 ± 0	0.43 ± 0.01**	0.45 ± 0.02	1.36 ± 0.06***
22:6n-3	1.94 ± 0.19	2.16 ± 0.13	0.17 ± 0.01	1.59 ± 0.05***	6.47 ± 0.17	11.97 ± 1***
n-3 PUFA	2.98 ± 0.46	4.05 ± 0.54*	1.19 ± 0.03	3.12 ± 0.07***	7.51 ± 0.22	17.05 ± 1.2***
n-6/n-3	2.72 ± 0.42	1.64 ± 0.43*	12.11 ± 0.15	4.25 ± 0.08***	2.87 ± 0.04	0.89 ± 0.09***

Each value represents the mean ± SE ($n = 10$). C57BL/6J mice were fed a 32% lard diet (control) or a 32% lard plus 10% saury oil diet (saury oil group) for 6 weeks

SAF saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to controls

Effect of Saury Oil on Plasma Glucose Levels in an Insulin Tolerance Test

The plasma glucose concentrations in an insulin tolerance test are shown in Fig. 1 for KKAY mice fed a soybean oil (control) or saury oil diet. Plasma glucose levels in the saury oil group declined ($P = 0.14$) at 60 min, and then significantly decreased by 25.3% ($P < 0.05$) at 80 min after insulin injection, compared to the control group.

Effect of Saury Oil on Metabolic Variables in Plasma

The body weight, mesenteric WAT mass, and plasma concentrations of glucose, insulin, and lipids in KKAY mice and diet-induced obese C57BL/6J mice are shown in Table 5. After 4 weeks of feeding KKAY mice the saury oil diet, the mesenteric WAT mass in the saury oil group was 10.4% ($P < 0.05$), plasma concentrations of glucose were 13.7% ($P < 0.05$), insulin were 45.4% ($P < 0.01$), TC were 39.4% ($P < 0.001$), LDL-C were 76.4% ($P < 0.001$),

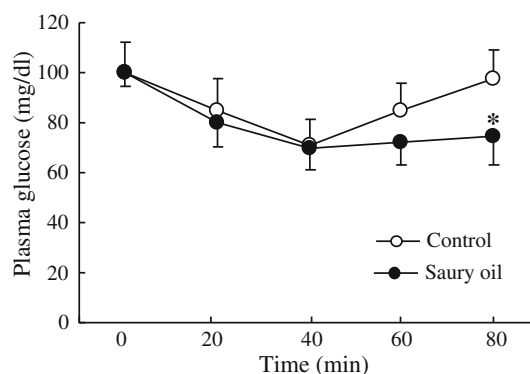


Fig. 1 The effect of saury oil on insulin sensitivity. An insulin tolerance test was performed at the end of 3 weeks to KKAY mice fed a 10% soybean oil diet (control) or 10% saury oil diet. Values are mean ± SE ($n = 10$) and are normalized relative to the control group. * $P < 0.05$ compared to controls

and NEFA were 23.1% ($P < 0.01$) lower than in the control soybean oil group. Plasma TAG concentrations also tended to be lowered by the saury oil diet ($P = 0.14$).

Table 5 The effect of saury oil on body weight, mesenteric WAT mass, and plasma markers of glucose and lipid metabolism in KKAY and diet-induced obese C57BL/6J mice

	KKAY mice		C57BL/6J mice	
	Soybean oil diet (Control)	Saury oil diet	Lard diet (Control)	Saury oil diet
Body weight (g)	37.9 ± 0.6	36.9 ± 0.4	31.6 ± 1.3	32.1 ± 2.1
Body weight gain (g)	7.7 ± 0.4	6.9 ± 0.3	10.3 ± 0.9	9.8 ± 1.8
WAT mass (g)	2.4 ± 0.1	2.17 ± 0.1*	1.7 ± 0.09	1.7 ± 0.13
Glucose (mg/dl)	541.5 ± 20.1	467.3 ± 8.2*	301.4 ± 11.2	258.9 ± 19.8**
Insulin (ng/ml)	40.9 ± 4.4	22.3 ± 2.3**	4.7 ± 0.6	3.1 ± 0.4*
TC (mg/dl)	109.3 ± 3.7	65.9 ± 2.6***	134.7 ± 4.2	107.2 ± 5.1**
LDL-C (mg/dl)	45.9 ± 2.4	18.5 ± 1.2***	28.2 ± 6.4	20.5 ± 3.4*
TAG (mg/dl)	67.6 ± 9.1	54.1 ± 4.6	76.6 ± 5	60.5 ± 4.9***
NEFA (mequiv/l)	1.1 ± 0.07	0.8 ± 0.04**	0.5 ± 0.03	0.48 ± 0.06

Each value represents the mean ± SE ($n = 10$). KKAY mice were fed a 10% soybean oil diet (control) or a 10% saury oil diet for 4 weeks. C57BL/6J mice were fed a 32% lard diet (control) or a 22% lard plus 10% saury oil diet (saury oil diet) for 6 weeks

WAT white adipose tissue, TC total cholesterol, LDL-C low-density lipoprotein cholesterol, TAG triacylglycerol, NEFA nonesterified fatty acid
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to controls

There was no difference in body weight between the saury oil group and the control group. Similarly, ingestion of saury oil also decreased the plasma concentrations of glucose in C57BL/6J mice by 13.6% ($P < 0.01$), of insulin by 35.2% ($P < 0.05$), of TC by 18.6% ($P < 0.01$), of LDL-C by 27.3% ($P < 0.05$), and of TAG by 19.4% ($P < 0.001$), although the mesenteric WAT mass did not differ between the saury oil group and the control group.

Effect of Saury Oil on Adipokine Levels in Plasma

Intake of saury oil increased the concentration of adiponectin in plasma by 28.7% ($P < 0.01$) in KKAY mice and by 23.6% ($P < 0.01$) in C57BL/6J mice (Fig. 2a). Plasma resistin concentrations were decreased by 28.5% ($P < 0.05$) and 9.5% ($P < 0.05$) with a saury oil diet in KKAY mice and C57BL/6J mice, respectively (Fig. 2b). Ingestion of saury oil also decreased plasma TNF α concentrations by 59.5% ($P < 0.01$) in KKAY mice (Fig. 2c) and plasma leptin concentrations by 66.9% ($P < 0.01$) in C57BL/6J mice (Fig. 2d).

Effect of Saury Oil on the Expression of mRNAs Related to Lipid Metabolism in WAT

The saury oil-supplemented diet downregulated the mRNA expression of lipogenic genes *SREBP-1* (sterol regulatory element binding protein 1), *SCD-1* (stearoyl-coenzyme A desaturase 1), *FAS* (Fatty acid synthase), and *ACC* (Acetyl-CoA carboxylase) by 65% ($P < 0.05$), 72% ($P < 0.01$), 70% ($P < 0.05$), and 74% ($P < 0.05$), respectively, compared to the control lard diet group (Fig. 3a), and upregulated expression of the oxidation-related gene *CPT-1*

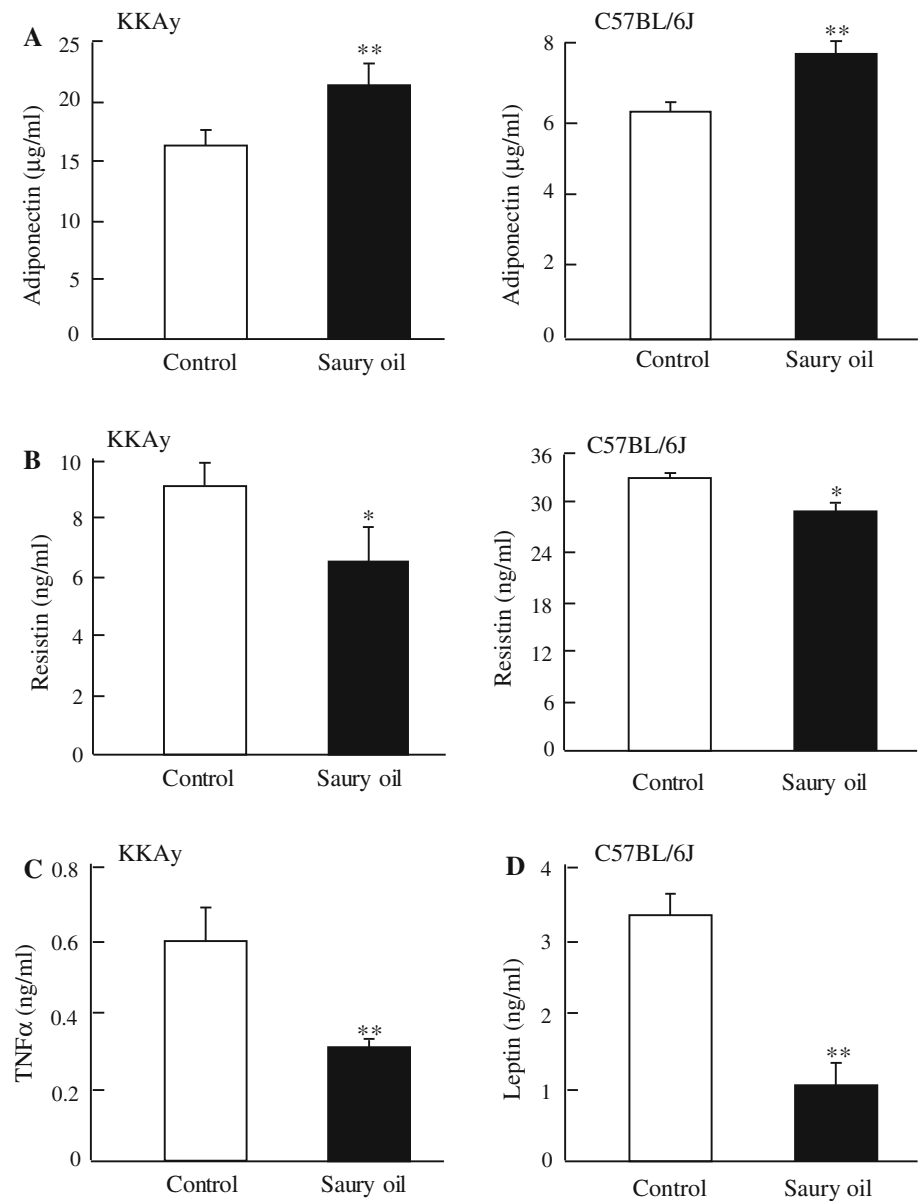
(Acetyl-CoA carboxylase Carnitine palmitoyltransferase-1), the energy consumption-related genes *PGC1 α* (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha) and *PGC1 β* (Peroxisome proliferator-activated receptor gamma coactivator 1-beta) by 123% ($P < 0.05$), 171% ($P < 0.05$), and 176% ($P < 0.05$), respectively (Fig. 3b), in diet-induced obese C57BL/6J mice.

Discussion

The present study has demonstrated that ingestion of saury oil alleviates MetS in type II diabetic KKAY mice and diet-induced obese C57BL/6J mice by improving glycemic and lipid control. To understand the possible mechanisms of the hypoglycemic and hypolipidemic effects of saury oil, we investigated adipokine levels. As a key adipokine, adiponectin has received considerable attention due to its anti-inflammatory, antiatherogenic and antidiabetic properties [18, 19]. There is increasing evidence supporting the positive association of higher adipocyte-derived adiponectin with glycemic control, lipid profile, and inflammation [20]. By contrast, elevated levels of circulating NEFA and other adipokines such as TNF α , resistin, and leptin are associated with insulin resistance [21–26]. Therefore, the increase in plasma levels of adiponectin, as well as the decreases in plasma levels of NEFA, TNF α , resistin, and leptin with a saury oil diet are possibly associated with the improvement of hyperglycemia, hyperinsulinemia, and hyperlipidemia relative to insulin resistance.

Saury oil intake decreased mesenteric WAT mass significantly in KKAY mice, and several lines of evidence indicate that a lower content of WAT is associated with a

Fig. 2 The effect of saury oil on plasma adipokines. Plasma adipokine concentrations in KKAY mice (*left*) and in diet-induced obese C57BL/6 J mice (*right*) for: **a** adiponectin, **b** resistin, **c** TNF α , and **d** leptin. KKAY mice were fed a 10% soybean oil diet (control) or a 10% saury oil diet for 4 weeks. C57BL/6J mice were fed a 32% lard diet (control) or 22% lard plus 10% saury oil diet (saury oil group) for 6 weeks. After the feeding period, plasma adipokine levels were measured in duplicate by ELISA. Values are mean \pm SE ($n = 10$). * $P < 0.05$, ** $P < 0.01$ compared to controls



favorable profile of adipokines as well as a lower MetS risk [27]. Saury oil intake did not change WAT mass in diet-induced obese C57BL/6J mice, however, although there were favorable changes in plasma adipokines associated with a favorable metabolic profile. It is therefore suggested that adipose tissue mass loss is not the only essential factor for the improvement of metabolic disarrangement in diet-induced obese C57BL/6J mice fed a saury oil diet. Notably, Saraswathi et al. [28] demonstrated that by feeding fish oil to LDL receptor-deficient mice, WAT-specific inflammation and insulin sensitivity were improved and macrophage infiltration was reduced despite an increase in adipose tissue mass.

The n-3 PUFA levels in plasma, WAT and liver increased significantly in the saury oil group compared to

the control group for C57BL/6J mice. It has been demonstrated that EPA increases adiponectin secretion in rodent models of obesity and in human obese subjects, in part through TNF α downregulation in macrophages [29, 30]. n-3 fatty acids also decrease TNF α , resistin and leptin levels, all of which are implicated in insulin insensitivity [31–33]. The beneficial effect of saury oil in producing a more favorable adipokine profile and easing the onset of MetS may be partly attributed to the increase in n-3 PUFA.

SREBP-1 and its target genes *SCD-1*, *FAS* and *ACC* are involved in adipogenesis, and *SREBP-1* has a key role in regulating fatty acid synthesis [34]. Insulin-induced expression of *SREBP-1* mRNA can be prevented by the n-3 PUFA [35]. n-3 PUFA also prevented insulin induction of the downstream lipogenic enzyme targets *FAS* and *ACC*,

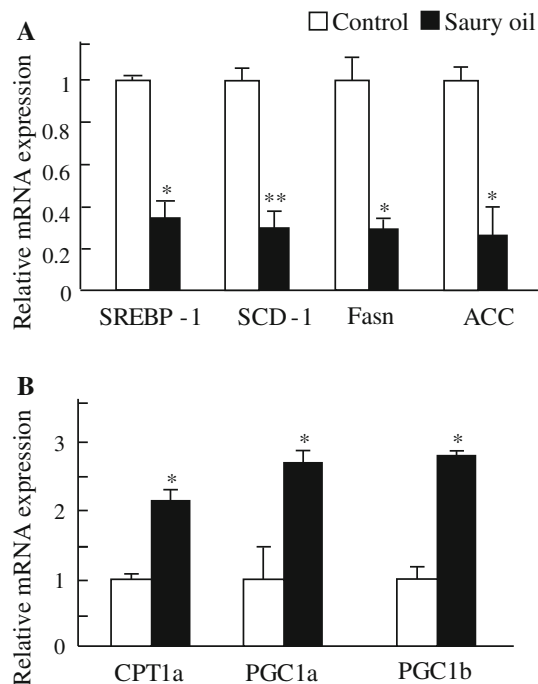


Fig. 3 The effect of saury oil on the mRNA expression levels of genes involved in lipid metabolism in mesenteric WAT. **a** mRNA expression of markers of lipogenesis (*SREBP1*, *SCD-1*, *FAS* and *ACC*). **b** mRNA expression of markers of lipid oxidation (*CPT1a*), and energy expenditure (*PGC1 α* and *PGC1 β*). C57BL/6J mice were fed a 32% lard diet (control) or a 22% lard plus 10% saury oil diet for 6 weeks. After the intervention period, mRNA levels in mesenteric WAT were measured by real-time RT-PCR. Values for expression levels were normalized to that of 18S ribosomal RNA and expressed relative to the control group. Values are means \pm SE ($n = 10$). * $P < 0.05$, ** $P < 0.01$ compared to controls

and reduced de novo lipogenesis [36]. Enzyme CPT-1 is rate limiting for fatty acid β -oxidation in mitochondria [37], and PGC1 α is a molecular marker of energy expenditure via regulatory function for mitochondrial biogenesis and oxidative metabolism [38–40]. Flachs et al. [41] demonstrated that the antiadipogenic effect of an EPA/DHA concentrate (6% EPA and 51% DHA) may involve a metabolic switch in adipocytes that includes enhancement of β -oxidation with increase in mRNA expression of *CPT-1* and upregulation of mitochondrial biogenesis with an increase in the expression of genes encoding PGC1 α and nuclear respiratory factor-1 (Nrf-1). Suppression of adipogenesis and promotion of fatty acid oxidation, as well as energy expenditure in mRNA levels with a saury oil diet are associated with improvement of glucose and lipid metabolism, and this is potentially derived from a significant increase in n-3 PUFA with an intake of saury oil.

In contrast to the increase in n-3 PUFA, there were marked reductions in n-6 PUFA including arachidonic acid, along with a corresponding decrease in the n-6/n-3 PUFA ratio, with the saury oil diet. n-6 PUFA, which compete with n-3 PUFA for several physiological

processes, can increase inflammatory signals and have been associated with metabolic/cardiovascular disorders and cancer [42–44]. A decrease in the n-6/n-3 PUFA ratio can enhance glucose tolerance in healthy animals [45], and prolong survival of type I diabetes model non-obese diabetic mice by retaining the beta cell mass [46]. Thus, a decrease in n-6 PUFA, as well as in the n-6/n-3 PUFA ratio, contributes to the beneficial effects of saury oil on glucose and lipid metabolism in a saury oil diet.

In addition to n-3 PUFA, diets rich in MUFA are recommended for individuals with type 2 diabetes mellitus, and studies suggest the beneficial effect of dietary oleic acid on insulin sensitivity [47]. Levels of 18:1 in WAT and liver decreased significantly with a saury oil diet, however, suggesting a minor role for 18:1 in the improvement of MetS with saury oil treatment. Because a considerable fraction of the fatty acids in saury oil is comprised of MUFA (35% of C20:1 and C22:1), some of the beneficial effects of saury oil on metabolic control of hyperglycemia and hyperlipidemia observed in the present study may be associated with MUFA.

Whale oil is rich in MUFA—28% of it is comprised of C20:1 and C22:1 aliphatics—but contains only 54% of combined EPA/DHA found in cod liver oil and 68% of that found in seal oil. Despite this difference, Osterud et al. [16] demonstrated that the consumption of these three oils by healthy subjects increased the content of n-3 fatty acids in serum lipids significantly to a comparable degree and also reduced the arachidonic acid levels similarly for all three oils. Similarly, in another study by Halvorsen et al. [15] feeding rats fish oil fractions rich in MUFA (80% of C20:1 and C22:1), the level of EPA in the plasma lipids was increased while the content of arachidonic acid was reduced compared with that of rats fed lard. The increase in n-3 PUFA and the improved ratio of n-6 PUFA to n-3 PUFA, which contributes to the improvement of glycemic and lipid metabolism in diet-induced obese C57BL/6J mice fed a saury oil-supplemented diet, may therefore be partly attributable to MUFA. In addition, it has been demonstrated that MUFA-enriched whale oil supplementation reduces TNF α generation in lipopolysaccharide-stimulated blood [16]; this supports our finding that reduced blood TNF α levels correlated with improved insulin resistance in KKAY mice fed a saury oil diet. Taken together, these results are interesting and suggest a possible synergistic effect between n-3 PUFA and MUFA in the improvement of MetS.

Very limited data exist defining the health beneficial actions of saury oil, and the current study showed the beneficial effects of saury oil in reducing risk factors for metabolic syndrome in spontaneously diabetic mice and diet-induced obese mice. On the other hand, the mechanism by which MUFA boosts the effect of n-3 PUFA and whether

MUFA has a specific effect on metabolic disorders themselves still needs to be elucidated. This is of importance if the development of functional lipids from fish and MetS treatment with a diet rich in saury oil are to be pursued.

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