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ORIGINAL ARTICLE

High-Fat Diets Containing Different Amounts of n3 and n6 Polyunsaturated Fatty Acids Modulate Inflammatory Cytokine Production in Mice

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Abstract Dysregulation of adipokines is a hallmark of obesity. Polyunsaturated fatty acids in fish oil may exert anti-inflammatory effects on adipose tissue mitigating the dysregulation of adipokines thereby preventing obesity. This study investigated the effects of high-fat diets containing different amounts of n3 polyunsaturated fatty acids (PUFA) on adiposity and adipokine production in mice. Mice were fed a low-fat or a high-fat diet with 16 or 45 % of energy from corn oil (low n3 PUFA) in comparison with a high-fat diet containing soybean or high-oleic sunflower oil (adequate n3 PUFA) or flaxseed or fish oil (high n3 PUFA) for 11 weeks. High-fat diets, regardless of types of oils, significantly increased body fat mass and body weights compared to the low-fat diet. Adipose fatty acid composition and contents reflected dietary fatty acid profiles. The high-fat fish oil diet significantly increased adiponectin and reduced leptin concentrations in both plasma and adipose tissue; it did not elevate plasma insulin concentration compared to the high-fat corn oil diet. All high-fat diets elevated concentrations of plasminogen activator inhibitor-1 (PAI-1) and monocyte chemoattractant protein-1 (MCP-1) but lowered resistin concentrations in both plasma and adipose tissue. In conclusion, fish oil may be beneficial in improving insulin sensitivity by upregulation of adiponectin and downregulation of leptin production; n3 and n6 PUFA do not play a role at the dietary levels tested in reducing adiposity and production of pro-inflammatory cytokines (leptin, PAI-1, MCP-1 and resistin) and anti-inflammatory cytokine adiponectin.

Keywords Polyunsaturated fatty acids · Adiposity · Adipokines · Obesity · Mice

Abbreviations

ALA	α-Linolenic acid 18:3n3
DHA	Docosahexaenoic acid 22:6n3
DPA	Docosapentaenoic acid 22:5n3
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid 20:5n3
LNA	Linoleic acid 18:2n6
MCP-1	Monocyte chemoattractant protein-1
MUFA	Monounsaturated fatty acids
PAI-1	Plasminogen activator inhibitor-1
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids

Introduction

Obesity is a growing health concern worldwide; it leads to an increased risk of chronic morbidities including type-2 diabetes, cardiovascular diseases and certain types of cancer [1]. Adipose tissue is considered an endocrine organ that produces adipokines (inflammatory cytokines) that participate in the regulation of food intake, adipose storage, adipogenesis and energy homeostasis. For example, the pro-inflammatory cytokine leptin regulates body weight through its effects on food intake and energy expenditure [2], circulating leptin concentrations are proportional to visceral adiposity in the body [3]. Adiponectin, an anti-inflammatory cytokine, modulates glucose regulation and fatty acid catabolism [4], plasma levels of adiponectin are

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reduced in obesity and elevated in those with normal body weights [5]. Furthermore, adipose tissue produced pro-inflammatory cytokines contribute to low-grade systemic inflammation and play important roles in the pathogenesis of obesity and obesity-related diseases. Overexpression of pro-inflammatory adipokines such as leptin [3], plasminogen activator inhibitor-1 (PAI-1) [6], monocyte chemoattractant protein-1 (MCP-1) [7], and resistin [8] are reported in obesity. Blood levels of these adipokines are elevated in obese humans [3, 6–8] and laboratory rodents fed a high-fat diet [9, 10]; weight loss reduces blood concentrations of these adipokines in humans [7, 11–13] and animals [14–16].

A growing body of evidence suggests that different types of fats in a diet may have differences in contributing to the development of obesity and regulating adipokine production. Diets high in saturated fat promote obesity [17, 18]. Diets high in α-linolenic acid (ALA; 18:3n3) or eicosapentaenoic acid (EPA; 20:5n3)/docosahexaenoic acid (DHA; 22:6n3) upregulate production of adiponectin [19–22] and downregulate production of pro-inflammatory cytokines such as PAI-1 and MCP-1 [23, 24]. *In vitro* studies showed that palmitic acid downregulates adiponectin production [25, 26] and that EPA increases adiponectin secretion in 3T3-L1 adipocytes [25]. The n3:n6 ratio is suggested to be important in disease development, for example, cardiovascular disease [27] and cancer [28, 29] in rodent models fed a high-fat diet.

Corn oil is a source of dietary fat commonly used in nutrition research, particularly in studies where a low quantity of n3 polyunsaturated fatty acids (PUFA) in a diet is preferred [30–32]. We reported that significant increases in plasma concentrations of pro-inflammatory adipokines (e.g., leptin, PAI-1, and MCP-1) and decreases in anti-inflammatory adipokines (adiponectin) are accompanied with metabolic disturbance in mice fed a corn oil based high-fat diet [9]. However, it remains to be determined whether a lack of ALA in corn oil contributes to the elevation in pro-inflammatory cytokine production in mice fed a high-fat corn oil diet. We hypothesized that differences in n3 PUFA in diet affect inflammatory cytokine production. To test this hypothesis, we compared high-fat diets containing different amounts of n3 PUFA on adiposity and adipokine production in mice.

Materials and Methods

Reagents and Assay Kits

Five different oils were used in the high fat diets. Corn oil was obtained from ACH Food Companies (Memphis, TN), fish oil was from MP Biomedicals, LLC (Solon, OH), flaxseed oil was from Dyets, Inc. (Bethlehem, PA), soybean oil was from ConAgra Foods, Inc. (Omaha, NE) and sunflower oil was from Natural Oils International, Inc. (Simi Valley, CA). All reagents for gas chromatography were obtained from Sigma Aldrich (St. Louis, MO) unless otherwise detailed. Ketamine and xylazine were obtained from Midwest Veterinary Supply (Lakeville, MN). Enzyme-linked immunosorbent assay (ELISA) kits for leptin, resistin, and MCP-1 were obtained from R&D Systems (Minneapolis, MN), that for PAI-1 was from Molecular Innovations, Inc. (Novi, MI) and those for adiponectin and insulin were from EMD Millipore Corp. (Billerica, MA).

Animals and Diets

Three-week-old male C57BL/6 mice were purchased from Harlan Laboratories (Madison, WI). Mice were housed, three per cage, in a pathogen-free room under a 12:12 h light–dark cycle and maintained at 22 ± 1 °C. Six diets were compared and gross energy of each diet (Table 1) was quantified using bomb calorimetry (Model 6200, oxygen bomb calorimeter, Parr Instrument, Moline, IL). The AIN93G diet [33] with 16 % of energy from corn oil was used as the low-fat control diet (0.001 mol ALA/kg, 0.13 mol LNA/kg). The high-fat diets, modified from the AIN93G diet, contained 45 % of energy from various oils to obtain different contents of ALA (18:3n3) or linoleic acid (LNA; 18:2n6). These diets were (1) corn oil diet: 45 % of energy from corn oil (0.005 mol ALA/kg, 0.5 mol LNA/kg); (2) fish oil diet: 35 % of energy from fish oil and 10 % of energy from corn oil (0.007 mol ALA/kg, 0.17 mol long-chain n3 fatty acids/kg, 0.11 mol LNA/kg); (3) flaxseed oil diet: 18 % of energy from flaxseed oil and 27 % of energy from corn oil (0.17 mol ALA/kg, 0.33 mol LNA/kg); (4) soybean oil diet: 45 % of energy from soybean oil (0.05 mol ALA/kg, 0.4 mol LNA/kg); (5) sunflower oil diet: 39 % of energy from high-oleic sunflower oil and 6 % of energy from flaxseed oil (0.057 mol ALA/kg, 0.103 mol LNA/kg). All diets were powder diets; stored at −20 °C before being provided to the mice. The amounts and composition of fatty acids of dietary oils and energy provided by fatty acids from each oil are presented in Tables 2 and 3, respectively.

Experimental Design

This study was approved by the Animal Care and Use Committee of the U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center. The procedures followed the National Institutes of Health guidelines for the care and use of laboratory animals [34]. Following a one-week acclimation with the low-fat diet, mice were randomized into six groups of 12 each (3 per cage). Mice had free access to diets and

Table 1 Composition of experimental diets

	Low-fat control	High-fat diets				
	Corn oil	Corn oil	Fish oil	Flaxseed oil	Soybean oil	Sunflower oil
Ingredient (g)						
Corn starch	397.5	40.1	40.1	40.1	40.1	40.1
Casein	200	239.4	239.4	239.4	239.4	239.4
Sucrose	100	119.7	119.7	119.7	119.7	119.7
Dyetrose	132	239.4	239.4	239.4	239.4	239.4
Corn oil	70	241.2	54.2	144.7		
Soybean oil					241.2	
Sunflower oil						207.4
Flaxseed oil				96.5		33.8
Menhaden oil			187.0			
Cellulose	50	59.8	59.8	59.8	59.8	59.8
AIN93G mineral mix	35	41.9	41.9	41.9	41.9	41.9
AIN93G vitamin mix	10	12	12	12	12	12
L-Cysteine	3	3.6	3.6	3.6	3.6	3.6
Choline bitartrate	2.5	3	3	3	3	3
tert-butylhydroquinone	0.014	0.02	0.02	0.02	0.02	0.02
Total	1000	1000	1000	1000	1000	1000
Energy (%)						
Carbohydrates	64.2	34.8	34.8	34.8	34.8	34.8
Fat	15.6	45.0	45.0	45.0	45.0	45.0
Protein	20.2	20.2	20.2	20.2	20.2	20.2
Gross energy (kcal/g)[a]	4.4 ± 0.3	5.4 ± 0.2	5.4 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.4 ± 0.2

[a] Values are means ± SEM, $n = 3$ per diet

Table 2 Composition of fatty acids of dietary oils

Fatty acids	Corn oil		Fish oil		Flaxseed oil		Soybean oil		Sunflower oil	
	mol/kg	mol%	mol/kg	mol%	mol/kg	mol%	mol/kg	mol%	mol/kg	mol%
18:1n9	1.0	28.6	0.4	10.2	0.6	17.0	0.8	23.6	2.4	68.4
16:1n7	0	0	0.5	15.9	0	0	0	0	0.02	0.6
14:1n5	0	0	0.01	0.2	0	0	0	0	0	0
Monounsaturated	1.0	28.6	0.9	27.2	0.6	17.1	0.9	25.7	2.4	69.5
16:0	0.4	12.8	0.8	24.2	0.3	10.0	0.4	11.6	0.3	8.6
18:0	0.05	1.5	0.1	3.5	0.1	3.5	0.1	4.2	0.1	2.4
Saturated	0.5	14.3	1.4	38.6	0.5	15.6	0.6	16.0	0.6	17.8
18:2n6	1.9	56.6	0.05	1.3	0.6	16.8	1.8	53.2	0.4	12.7
18:3n6	0	0	0	0	0.004	0.1	0.02	0.5	0	0
n6 polyunsaturated	1.9	56.6	0.2	5.2	0.6	16. 9	1.8	53.7	0.4	12.7
18:3n3	0.02	0.5	0.03	1.0	1.7	50.4	0.2	4.6	0.002	0.04
20:5n3	0	0	0.5	15.6	0	0	0	0	0	0
22:5n3	0	0	0.1	2.6	0	0	0	0	0	0
22:6n3	0	0	0.3	9.4	0	0	0	0	0	0
n3 polyunsaturated	0.02	0.5	0.98	28.5	1.73	50.4	0.16	4.6	0.002	0.04

Sources of oils: corn oil (ACH Food Companies; Memphis, TN), fish oil (MP Biomedicals, LLC.; Solon, OH), flaxseed oil (Dyets, Inc.; Bethlehem, PA), soybean oil (ConAgra Foods, Inc.; Omaha, NE), high oleic sunflower oil (Natural Oils International, Inc.; Simi Valley, CA)

Table 3 Energy provided by fatty acids in experimental diets

	Low-fat control	High-fat diets				
	Corn oil	Corn oil	Fish oil	Flaxseed oil	Soybean oil	Sunflower oil
Total oils (g)	70	201.5	201.5	201.5	201.5	201.5
Total energy, fat (%)	15.6	45.0	45.0	45.0	45.0	45.0
Energy from oils (%)	15.6	45.0	45.0	45.0	45.0	45.0
Energy (%)						
18:1n9	4.5	12.9	6.5	10.8	10.6	27.6
16:1n7	0	0	5.6	0	0	0.3
14:1n5	0	0	0.1	0	0	0
Monounsaturated	4.5	12.9	12.4	10.8	11.6	28.0
16:0	2.0	5.7	9.7	5.2	5.2	4.0
18:0	0.2	0.7	1.4	1.0	1.9	1.1
Saturated	2.2	6.4	14.9	6.7	7.2	7.9
18:2n6	8.9	25.5	6.2	18.3	23.9	6.0
18:3n6	0	0	0	0	0.2	0.0
n6 polyunsaturated	8.9	25.5	7.6	18.3	24.2	6.0
18:3n3	0.1	0.2	0.4	9.2	2.1	3.2
20:5n3	0	0	5.4	0	0	0
22:5n3	0	0	0.9	0	0	0
22:6n3	0	0	3.3	0	0	0
n3 polyunsaturated	0.1	0.2	10.0	9.2	2.1	3.2
n3:n6 ratio	0.01	0.01	1.33	0.50	0.09	0.53

deionized water throughout the experiment; they were weighed weekly. Four weeks after the initiation of experimental feeding, six mice from each group were individually housed and their food intake was recorded daily (5 days per week) for 4 consecutive weeks. Caloric intake was calculated on the basis of average daily food intake and diet caloric content (Table 1). The duration of the experiment was 11 weeks. At week 10, body composition analysis of fat and lean mass of conscious, immobilized mice was performed by using quantitative magnetic resonance imaging (Echo whole-body composition analyzer, Model 100, Echo Medical System, Houston, TX). At termination, mice were fasted for 8 h and euthanized with an intraperitoneal injection of a mixture of ketamine and xylazine. Blood was collected via cardiac puncture into a tube containing 0.05 mM EDTA; plasma was obtained by centrifuging the blood at 10,000*g* for 10 min. Plasma and epididymal adipose tissue collected were stored at −80 °C for quantification of adipokines and insulin.

Quantification of Fatty Acids in Dietary Oils and Adipose Tissue

Epididymal adipose tissue (50 mg) was homogenized with 1 mL of ice-cold 3:2 hexane:isopropanol solution containing 1 mM butylated hydroxytoluene (Sigma Aldrich, St. Louis, MO) and centrifuged to remove insoluble materials. A 200 µL portion of the supernatant was mixed with 100 µL of internal standard solution [nonadecanoic acid (2.94 mg/mL) and heneicosanoic acid (0.21 mg/mL) (Nu-Chek Prep Inc., Elysian, MN) in methanol], and the solvent was evaporated under a stream of nitrogen at 35 °C. Dried samples were refluxed at 80 °C for 2 h in 2 mL of anhydrous methanol containing 5 % acetyl chloride (v/v) [35]. After heating, samples were returned to room temperature, neutralized with saturated sodium bicarbonate solution (0.5 mL) and methyl esters were extracted in hexane (1 mL) for analysis. Dietary oils (Table 2) and adipose tissue (Table 5) were quantified for fatty acids by gas chromatography on a Thermo Trace-1310 equipped with a TriPlus RSH Autosampler (Thermo Fisher Scientific, Waltham, MA) and a Supelco SP-2560 capillary column (Supelco, Belfonte, PA). Instrument parameters were adapted from Masood *et al.* [36]. Samples were injected twice, first with a 100:1 split ratio to allow for accurate quantitation of the major fatty acid species with respect to the nonadecanoic acid internal standard, and a second time with a 20:1 split ratio to permit quantitation of the minority species with respect to the heneicosanoic acid internal standards.

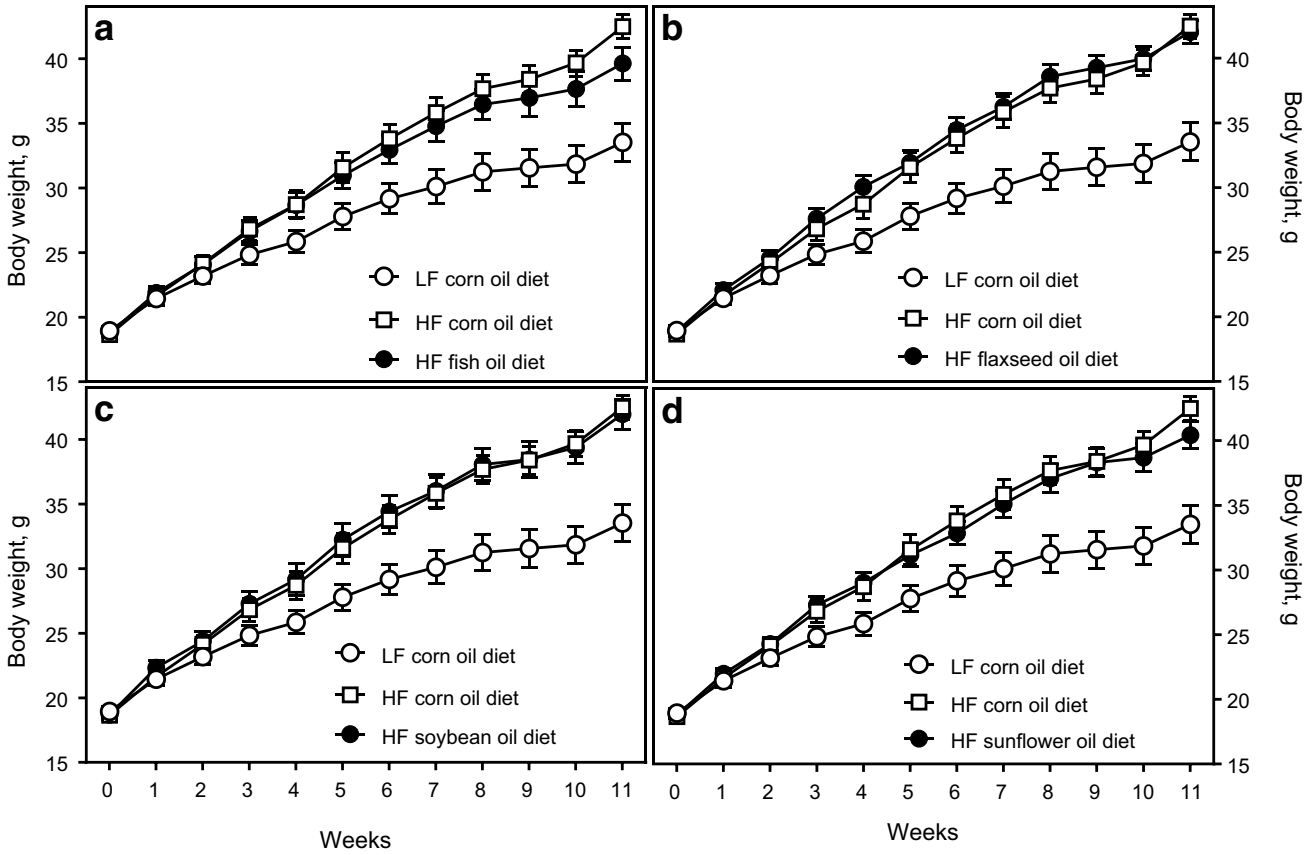

Fig. 1 Body weight changes in mice fed different high-fat diets. One-way ANOVA and Tukey's contrasts were performed to test for differences among the groups. Values are means ± SEM ($n = 12$ per group). Mice fed the high-fat diets were heavier than those fed the low-fat control diet, and the difference was significant starting 5 weeks after the initiation of the experimental feeding ($p \leq 0.05$). *LF* low-fat diet, *HF* high-fat diet

Quantification of Adipokines and Insulin

Enzyme-linked immunosorbent assays were used to quantify insulin in plasma and adipokines, leptin, adiponectin, PAI-1, MCP-1, and resistin, in both plasma and adipose tissue following manufacturers' protocols. Ten samples were randomly chosen from each group for analysis, and samples were read within the linear range of the assay, and the accuracy of the analysis was confirmed by the controls provided in each kit.

Statistical Analyses

One-way analysis of variance (ANOVA) and Tukey's contrasts were used to compare differences among the groups. All data are presented as means ± standard errors of the mean (SEM). Differences with a p value of 0.05 or less are considered significant. All statistical analyses were performed using SAS software (version 9.3, SAS Institute, Cary, NC).

Results

Body Weight, Body Composition and Caloric Intake

Feeding mice a high-fat diet, regardless of amounts of n3 PUFA, significantly increased body weight compared to mice fed the low-fat control diet ($p < 0.05$); the magnitude of increase was similar among all groups fed the high-fat diets (Fig. 1a–d).

All high-fat diets significantly increased the percentage body fat mass ($p < 0.05$) and correspondingly reduced the percentage body lean mass ($p < 0.05$) compared to mice fed the low-fat diet; there were no significant differences in body fat mass among the high-fat diet-fed groups (Table 4). There were no significant differences in absolute lean mass weight among all dietary groups, except mice fed high-fat soybean oil diet which exhibited a small but significant increase compared to mice fed the low-fat diet ($p < 0.05$, Table 4). There were no significant differences in caloric intake among all groups regardless of diets

Table 4 Body composition, and caloric and food intake of mice fed different high-fat diets

	Low-fat control	High-fat diets				
	Corn oil	Corn oil	Fish oil	Flaxseed oil	Soybean oil	Sunflower oil
Fat mass ratio (%)	26.3 ± 2.3^{b}	37.6 ± 0.9^{a}	32.7 ± 1.5^{a}	36.7 ± 0.7^{a}	33.6 ± 1.2^{a}	37.2 ± 1.0^{a}
Lean mass ratio (%)	65.2 ± 2.0^{a}	57.0 ± 0.9^{b}	59.8 ± 1.3^{b}	56.5 ± 0.6^{b}	59.6 ± 0.8^{b}	57.2 ± 0.8^{b}
Lean mass weight (g)	20.6 ± 0.5^{b}	22.0 ± 0.7^{ab}	22.1 ± 0.5^{ab}	22.4 ± 0.6^{ab}	23.0 ± 0.7^{a}	21.8 ± 0.5^{ab}
Caloric intake (Kcal/day)	15.6 ± 0.6	16.1 ± 0.3	14.5 ± 0.5	16.0 ± 0.8	15.9 ± 0.3	15.6 ± 0.4
Food intake (g/day)	3.6 ± 0.1^{a}	3.0 ± 0.1^{b}	2.7 ± 0.1^{b}	3.0 ± 0.1^{b}	3.0 ± 0.1^{b}	2.9 ± 0.1^{b}

One-way ANOVA and Tukey's contrasts were performed to test for differences among the groups

Values (mean ± SEM) with different letters are significantly different at $p \leq 0.05$ ($n = 12$ per group; $n = 6$ per group for caloric intake)

Table 5 Fatty acid composition of epididymal adipose tissue

Fatty acids	Low-fat control	High-fat diets				
	Corn oil (mol%)	Corn oil (mol%)	Fish oil (mol%)	Flaxseed oil (mol%)	Soybean oil (mol%)	Sunflower oil (mol%)
18:1n9	23.4 ± 4.5^{b}	21.1 ± 3.6^{b}	20.0 ± 1.9^{b}	16.4 ± 4.8^{b}	23.6 ± 3.2^{b}	56.9 ± 7.3^{a}
16:1n7	7.5 ± 0.4^{b}	4.6 ± 1.1^{c}	10.2 ± 0.5^{a}	1.9 ± 0.2^{d}	2.1 ± 0.1^{d}	2.6 ± 0.1^{cd}
14:1n5	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.5 ± 0.1^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.1^{b}
Total monounsaturated	33.7 ± 4.2^{b}	35.8 ± 0.7^{b}	35.5 ± 1.5^{b}	34.7 ± 0.8^{b}	31.8 ± 0.6^{b}	69.0 ± 0.5^{a}
16:0	24.9 ± 1.5^{b}	19.5 ± 2.3^{bc}	30.8 ± 1.0^{a}	13.3 ± 1.4^{d}	15.7 ± 0.2^{cd}	13.3 ± 0.4^{d}
18:0	1.5 ± 0.1^{b}	2.0 ± 0.3^{b}	2.9 ± 0.2^{a}	1.4 ± 0.2^{b}	2.0 ± 0.0^{b}	1.3 ± 0.0^{b}
12:0	0.1 ± 0.0^{b}	0.1 ± 0.0^{bc}	0.2 ± 0.0^{a}	0.04 ± 0.01^{c}	0.02 ± 0.01^{c}	0.01 ± 0.01^{c}
Total saturated	26.6 ± 1.7^{b}	21.7 ± 2.6^{bc}	34.0 ± 0.9^{a}	14.8 ± 1.5^{d}	17.8 ± 0.2^{cd}	14.7 ± 0.4^{d}
18:2n6	38.0 ± 2.7^{b}	40.6 ± 3.5^{ab}	21.0 ± 0.6^{c}	39.1 ± 0.9^{ab}	46.5 ± 0.4^{a}	12.7 ± 0.1^{d}
18:3n6	0.1 ± 0.0^{c}	0.2 ± 0.0^{b}	0.3 ± 0.0^{a}	0.2 ± 0.0^{b}	0.4 ± 0.04^{a}	0.02 ± 0.01^{c}
20:4n6	0.3 ± 0.1^{c}	0.4 ± 0.1^{b}	0.6 ± 0.1^{a}	0.1 ± 0.1^{e}	0.2 ± 0.1^{d}	0.1 ± 0.1^{e}
Total n6 polyunsaturated	39.2 ± 2.6^{b}	41.7 ± 3.4^{ab}	22.3 ± 0.6^{c}	39.7 ± 0.9^{b}	47.3 ± 0.4^{a}	13.0 ± 0.1^{d}
18:3n3	0.45 ± 0.03^{c}	0.69 ± 0.10^{c}	1.19 ± 0.04^{c}	10.45 ± 0.63^{a}	2.60 ± 0.19^{b}	3.05 ± 0.18^{b}
20:5n3	0.01 ± 0.01^{b}	0.01 ± 0.01^{b}	2.62 ± 0.22^{a}	0.06 ± 0.01^{b}	0.18 ± 0.16^{b}	0.03 ± 0.01^{b}
22:5n3	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.99 ± 0.05^{a}	0.13 ± 0.02^{b}	0.09 ± 0.04^{b}	0.06 ± 0.18^{b}
22:6n3	0.04 ± 0.02^{b}	0.05 ± 0.01^{b}	3.01 ± 0.14^{a}	0.13 ± 0.02^{b}	0.22 ± 0.14^{b}	0.12 ± 0.01^{b}
Total n3 polyunsaturated	0.53 ± 0.04^{d}	0.77 ± 0.10^{d}	7.90 ± 0.39^{b}	10.81 ± 0.64^{a}	3.10 ± 0.44^{c}	3.28 ± 0.20^{c}

Values (mean ± SEM) with different letters are significantly different at $p \leq 0.05$ ($n = 12$ per group)

(Table 4). Food intake of high-fat diet-fed groups were approximately 17–25 % lower than that of the low-fat control group ($p < 0.05$), there were no significant differences among groups fed the high-fat diets (Table 4).

Fatty Acid Concentrations of Epididymal Adipose Tissue

Fatty acid concentrations of epididymal adipose tissue (Table 5) reflected the fatty acid composition of the diets (Tables 2 and 3). Oleic acid contributed the highest concentrations of monounsaturated fatty acids (MUFA) to the adipose tissue. The highest concentrations of oleic acid were detected in mice fed the high-fat sunflower oil diet compared to all other groups ($p < 0.05$, Table 5). There were no differences in adipose oleic acid concentrations in any of the other dietary groups compared.

There were no significant differences in concentrations of saturated fatty acids (SFA) in adipose tissue between groups fed the low-fat and high-fat corn oil diets (Table 5). Palmitic acid contributed the highest concentration of SFA to adipose tissue, and the highest concentration of palmitic acid was detected in the high-fat fish oil group (Table 5). Compared to the high-fat fish oil diet, concentrations of

palmitate were significantly lower in mice fed the corn oil and soybean oil diets by 37 and 49 % and flaxseed oil and sunflower oil diets each by 57 % ($p < 0.05$, Table 5). Similarly, stearic acid and lauric acid concentrations were significantly higher in mice fed the high-fat fish oil diet compared to all the other groups (Table 5).

Concentrations of n6 PUFA in adipose tissue were contributed largely by the high percentage of LNA in diets. Concentrations of LNA were significantly higher in mice fed the high-fat soybean oil diet compared to all other groups except the high-fat corn oil and flaxseed oil groups, and the lowest concentrations were observed in the sunflower oil group ($p < 0.05$, Table 5). Compared to the high-fat corn oil and soybean oil diets, LNA concentrations were 48 and 55 % lower respectively, in the fish oil group ($p < 0.05$) and 69 and 73 % lower respectively, in the sunflower oil group ($p < 0.05$, Table 5). Contributions of arachidonic acid and γ-linolenic acid to total n6 PUFA concentrations were small; however, mice fed the fish oil diet had the highest amounts of both compared to all other groups ($p < 0.05$, Table 5). Addition of corn oil to the fish oil and flaxseed oil diets contributed to concentrations of LNA observed in adipose tissue of mice fed those diets.

Concentrations of n3 PUFA in adipose tissue were largely contributed by ALA in all diets except the fish oil diet in which contributing fatty acids were EPA, docosapentaenoic acids (DPA; 22:5n3) and DHA (Table 5). There were no significant differences in adipose ALA concentrations among groups fed the low-fat corn oil, high-fat corn oil and high-fat fish oil diets (Table 5). Concentrations of ALA in adipose tissue were highest in mice fed the high-fat flaxseed oil diet, followed by mice fed the high-fat soybean oil or high-fat sunflower oil diet (Table 5). The significant elevation of ALA in mice fed the sunflower oil diet was due to the addition of flaxseed oil to the diet. Mice fed the fish oil diet had the highest concentrations of DHA, DPA and EPA in adipose tissue compared to all other groups ($p < 0.05$, Table 5). There were no significant differences in concentrations of DHA, DPA and EPA among all other dietary groups (Table 5).

Insulin and Adipokine Concentrations in Plasma and Adipose Tissue

Adipose-produced inflammatory cytokines participate in energy metabolism and contribute to obesity [3, 5] and obesity-related diseases [9, 30, 31]. To understand roles of high-fat diets containing different amounts of n3 PUFA in adipokine production, we quantified leptin, adiponectin, PAI-1, MCP-1 and resistin in plasma and adipose tissue in mice fed different diets.

Compared to the low-fat corn oil diet, the high-fat corn oil diet increased concentrations of leptin in plasma by 2.1-fold ($p < 0.05$, Fig. 2a) and in adipose tissue by 2.5-fold ($p < 0.05$, Fig. 2b). The high-fat fish oil diet, in comparison to the high-fat corn oil diet, significantly reduced concentrations of leptin by 27 % in plasma ($p < 0.05$, Fig. 2a) but not in adipose tissue (Fig. 2b). The high-fat soybean oil diet reduced leptin in plasma by 23 % ($p < 0.05$, Fig. 3a) but not in adipose tissue (Fig. 2b).

The high-fat corn oil diet, in comparison to the low-fat corn oil diet, significantly reduced concentrations of adiponectin in plasma by 17 % ($p < 0.05$, Fig. 2c) but not in adipose tissue (Fig. 2d). Compared to the high-fat corn oil diet, the high-fat fish oil diet significantly increased concentrations of adiponectin in plasma by 43 % ($p < 0.05$, Fig. 2c) and in adipose tissue by two-fold ($p < 0.05$, Fig. 2d); the high-fat flaxseed oil diet significantly increased adiponectin concentrations in adipose tissue by 63 % ($p < 0.05$, Fig. 2d) but not in plasma (Fig. 2c).

All high-fat diets significantly increased concentrations of PAI-1 in plasma compared to the low-fat diet (Fig. 3a). In adipose tissue, there was no significant difference in PAI-1 between groups fed the low-fat and high-fat corn oil diets (Fig. 3b); the high-fat flaxseed oil diet increased PAI-1 concentration by 2.3-folds compared to the high-fat corn oil diet ($p < 0.05$, Fig. 3b).

Both the high-fat corn oil and fish oil diets elevated plasma MCP-1 concentrations by twofold compared to the low-fat corn oil diet ($p < 0.05$, Fig. 3c). There was no significant difference in adipose MCP-1 concentration between groups fed the low-fat and high-fat corn oil diets; the high-fat fish oil diet significantly elevated MCP-1 concentration by 85 % compared to the high-fat corn oil diet ($p < 0.05$, Fig. 3d).

The high-fat diets, regardless of types of oils, lowered plasma concentrations of resistin compared to the low-fat diet; the reduction by the high-fat corn oil diet (28 %) and flaxseed oil diet (34 %) were statistically significant ($p < 0.05$, Fig. 3e). In adipose tissue, all high-fat diets significantly reduced resistin concentrations compared to the low-fat diet (Fig. 3f).

Feeding mice a high-fat diet significantly increased plasma concentrations of insulin compared to the low-fat diet, except the high-fat fish oil diet maintained concentrations of insulin unchanged compared to the low-fat diet (Fig. 4).

Discussion

The present study investigated the effects of high-fat diets containing different amounts of n3 PUFA on adiposity and production of inflammatory cytokines in mice. Consumption of a high-fat diet, regardless of the type of oil, increased body fat mass, which was responsible for

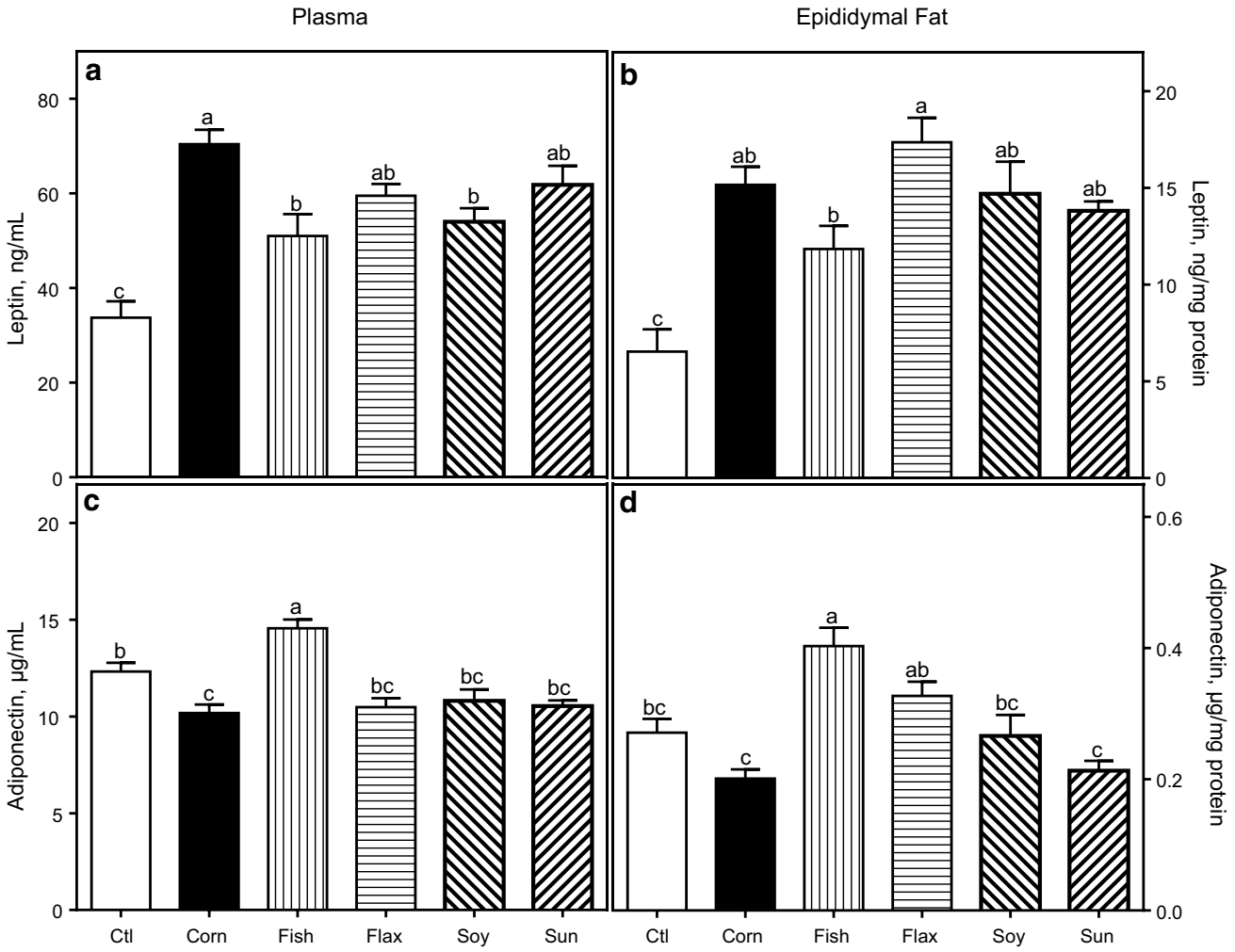

Fig. 2 Plasma and adipose concentrations of leptin (**a**, **b**) and adiponectin (**c**, **d**) of mice fed different high-fat diets. One-way ANOVA and Tukey's contrasts were performed to test for differences among the groups. Values (mean ± SEM) with different letters are significantly different at $p \leq 0.05$ ($n = 10$ per group). ctl: low-fat corn oil control, corn: corn oil, fish: fish oil, flax: flaxseed oil, soy: soybean oil, sun: sunflower oil

increases in body weights in mice. These results indicate that different types and amounts of n3 or n6 PUFA at dietary levels tested do not affect adipogenesis and that high-fat diet-induced adiposity is independent from dietary n3 and n6 PUFA.

It is well documented that leptin regulates satiety and energy intake [2] and that concentrations of leptin in blood increase in obesity and decline in weight loss [2]. On the other hand, adiponectin regulates lipid and glucose metabolism, increases insulin sensitivity and protects against chronic inflammation [37, 38]. We found that the high-fat fish oil diet significantly reduced leptin and increased adiponectin concentrations in both plasma and adipose tissue; furthermore it did not elevate insulin concentration in plasma. These results indicate that fish oil or fish oil components attenuate high-fat diet-induced metabolic disturbance and improve insulin sensitivity. Previous studies showed that replacing corn oil with fish oil elevated plasma adiponectin concentrations in sucrose-fed, insulin resistant rats [39]. Consumption of a high-fat diet enriched with EPA/DHA increased plasma adiponectin concentrations in C57BL/6 mice [20] and increased adiponectin mRNA expression and improved insulin tolerance in ob/ob mice [21]. Dietary supplementation with EPA/DHA negatively correlated to plasma leptin concentrations in men, and intake of a diet containing long-chain n3 PUFA reduced leptin concentrations in plasma and leptin mRNA expression in adipose tissue in rats [17]. *In vitro* studies showed that EPA and DHA inhibited insulin–stimulated leptin secretion in isolated white adipocytes [40] and leptin mRNA expression in trophoblast cells [17]. Our results agree with these reports that fish oil improves insulin

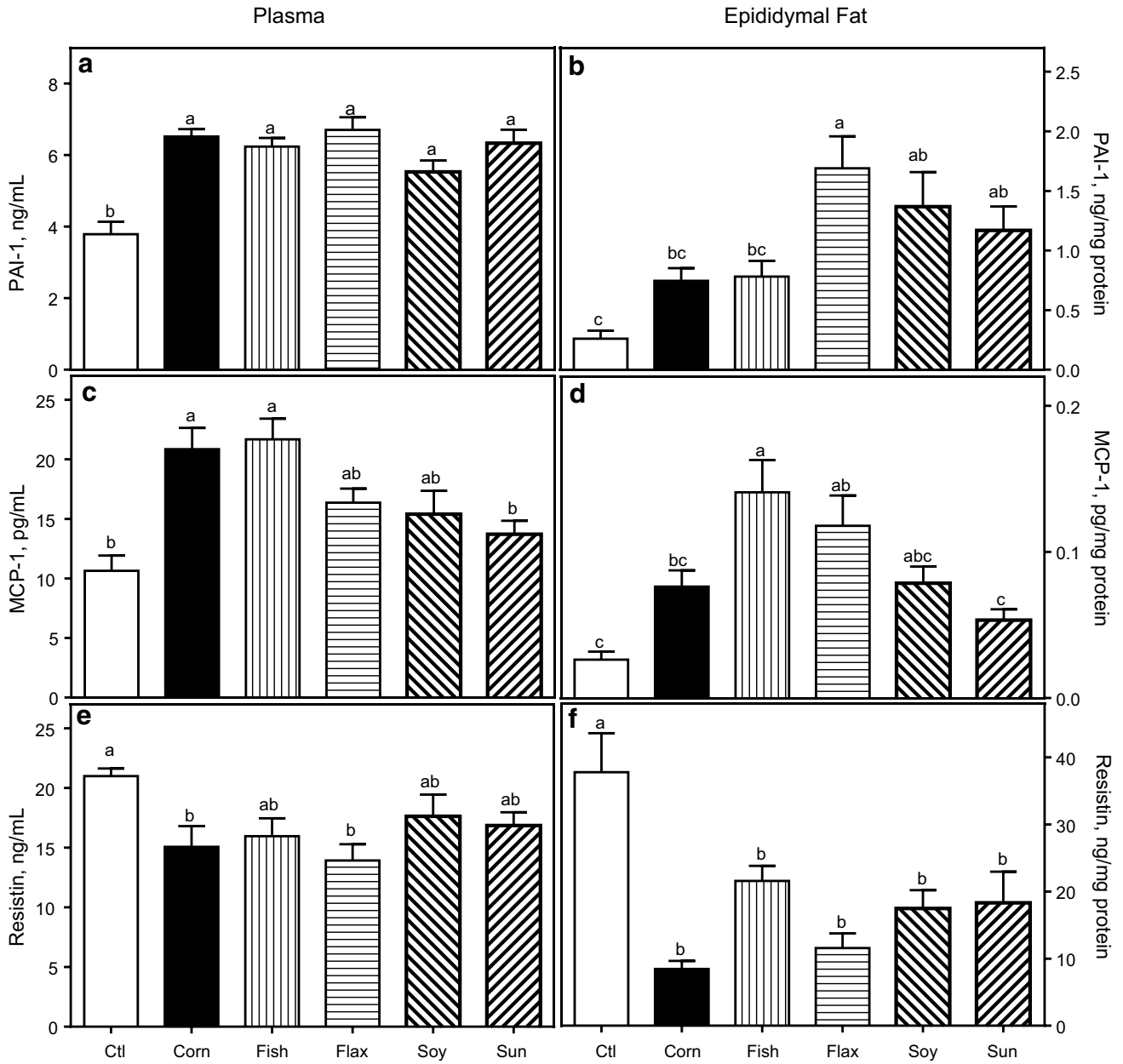

Fig. 3 Plasma and adipose concentrations of PAI-1 (**a**, **b**), MCP-1 (**c**, **d**) and resistin (**e**, **f**) of mice fed different high-fats diets. One-way ANOVA and Tukey's contrasts were performed to test for differences among the groups. Values (mean ± SEM) with different letters are significantly different at $p \leq 0.05$ ($n = 10$ per group). ctl: low-fat corn oil control, corn: corn oil, fish: fish oil, flax: flaxseed oil, soy: soybean oil, sun: sunflower oil

sensitivity by positively regulating adipokine profiles and support that n3 PUFA may be responsible for such beneficial effects. Our results of significant increases in adipose concentrations of adiponectin in the flaxseed oil diet-fed mice further support this concept. However, we do not agree with the reports suggesting that such improvements by fish oil were associated with its reduction in adipose mass [41–43], since we did not observe reductions in body fat mass in the fish oil diet-fed mice in the present study.

Elevation of PAI-1 and MCP-1 are observed in obese humans [7, 44] and laboratory rodents fed a high-fat diet [9, 45]. In the present study, feeding mice high-fat diets containing different amounts of n3 PUFA did not result in reduction in either PAI-1 or MCP-1. Changes in fatty acid composition and contents in adipose tissue corresponded to dietary fatty acid profiles. These changes indicate that n3 PUFA at levels tested in the present study do not down-regulate pro-inflammatory cytokines PAI-1 and MCP-1 or

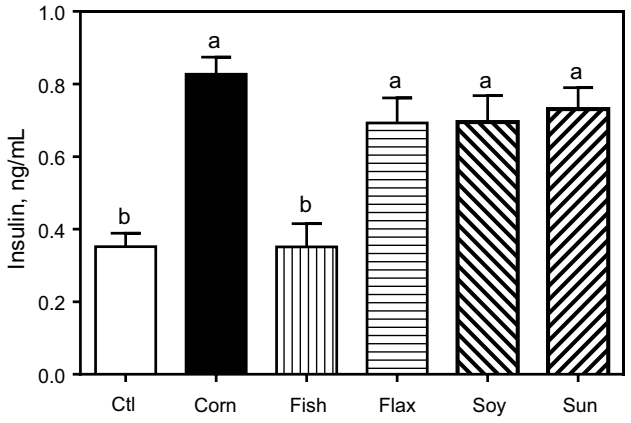

Fig. 4 Plasma concentrations of insulin from mice fed different high-fat diets. One-way ANOVA and Tukey's contrasts were performed to test for differences among the groups. Values (mean ± SEM) with different letters are significantly different at $p \leq 0.05$ ($n = 10$ per group). ctl: low-fat corn oil control, corn: corn oil, fish: fish oil, flax: flaxseed oil, soy: soybean oil, sun: sunflower oil

they are insufficient at the levels tested for such a downregulation. Furthermore, the lack of reductions in plasma and adipose PAI-1 and MCP-1 concentrations in mice fed the fish oil diet indicates that mechanisms regulating PAI-1 and MCP-1 may be different from those regulating adiponectin and leptin in these mice. It is important to note that the production of inflammatory cytokines, e.g. PAI-1 and MCP-1, can be up-regulated by inflammation. For example, concentrations of PAI-1 in mouse liver are relatively low [46]; however, its gene expression in liver is up-regulated by inflammatory mediators, e.g. endotoxin [46, 47]. Adipose produced PAI-1 and MCP-1 contributed to their concentrations in plasma. However, systemic inflammation related to increases in body fat mass in high-fat diet-fed groups may up-regulate production of PAI-1 and MCP-1 in other organs, e.g. liver, which may further increase plasma concentrations of these inflammatory cytokines.

Resistin, a pro-inflammatory adipokine, is associated with insulin resistance and inflammation in obesity [48–50]. Serum concentrations of resistin are elevated in human obese subjects [8] and in laboratory rodents of diet-induced and genetic forms of obesity [10]. The findings that concentrations of resistin were lower in both plasma and adipose tissue of high-fat diet-fed groups than in low-fat diet-fed controls do not support those observations. However, our results are in agreement with the reports that obesity reduces resistin levels. Feeding mice a high-fat fish oil diet reduced serum concentrations of resistin and leptin and improved the obesity phenotype [51]. Resistin expression in adipose tissue is severely depressed in genetic obese models including ob/ob, db/db and tub/tub mice [50]. While reasons for the different results from available studies on obesity and resistin remain to be elucidated, our results indicate that the mode of action for reductions in resistin across all groups fed high-fat diets may be independent from n3 and n6 PUFA at the dietary levels tested.

Results of adipose fatty acid analyses are consistent with the existing knowledge that fatty acids in adipose tissue correlate with types of fatty acids consumed from a diet [52, 53]. Adipose MUFA, SFA and n6 PUFA were contributed mainly by dietary oleate, palmitate, and linoleate, respectively. Similarly, adipose n3 PUFA were contributed mainly by dietary ALA and by EPA, DHA and DPA in the fish oil diet-fed mice. The amounts of LNA in adipose tissue of mice fed the flaxseed oil and fish oil diets were largely due to the addition of corn oil to the diets to adjust the amounts of n6 PUFA. Similarly, concentrations of ALA in adipose tissue of mice fed the sunflower oil diet were due to the addition of flaxseed oil to the diet to adjust the amounts of n3 PUFA.

In summary, consumption of high-fat diets significantly increased body fat mass and body weights. Adipose fatty acid composition and contents reflected dietary fatty acid profiles. Fish oil had favorable effects on downregulation of leptin and upregulation of adiponectin, which may be responsible for its improvement in insulin sensitivity compared to other types of oils. The lack of reductions in pro-inflammatory cytokines, PAI-1 and MCP-1, in groups fed the high-fat diets containing different amounts of n3 and n6 PUFA, in comparison to mice fed the high-fat corn oil diet containing lower amounts of n3 PUFA, suggests that n3 or n6 PUFA do not play a role in downregulation of PAI-1 and MCP-1 or they are insufficient at the dietary levels tested for such a downregulation. Together with the finding of lower concentrations of resistin in all groups fed the high-fat diets, we conclude that the actions of the high-fat diets on adiposity and regulation of inflammatory cytokine production are independent from dietary contents of n3 PUFA and n6 PUFA.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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