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Shifts in Diet from High Fat to High Carbohydrate Improved Levels of Adipokines and Pro-inflammatory Cytokines in Mice Fed a High-fat Diet

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Abstract. High-fat diets induce an expansion of the adipose tissue (AT) that can be characterized by chronic low-grade inflammation. AT is an important source of adipokines and pro-inflammatory cytokines. The purpose of this study was to evaluate the effects of a shift from a high-fat diet to high-carbohydrate (CHO) diet on the blood levels of adipokines and pro-inflammation cytokines in mice fed a high-fat diet. Six-week-old male C57BL/6 mice were fed a high-fat diet (40% of the total calories) for 9 weeks to induce obesity, and then the diet was shifted to a high CHO diet (70% of the total calories) for 3 weeks. Body weight and organ weight as well as blood lipid levels were measured. The serum levels of adipokines and pro-inflammatory cytokines were analyzed. Shifting the diet from high fat to high CHO decreased significantly body weight, adipose tissues, and liver weight ($p < 0.05$). The lipid blood levels (TG, Total-chol, and LDL-chol) decreased. The leptin and resistin blood levels significantly decreased after the diet was shifted to a high-CHO diet ($p < 0.05$); however, the adiponectin concentrations did not change. The IL-6 levels were also significantly decreased by the high-CHO diet ($p < 0.05$). The IL-13 serum levels were significantly increased by the high-CHO diet ($p < 0.05$). Further, the serum levels of the TNF-α and supernatant IL-1β concentrations in mice fed a high-carbohydrate diet were significantly increased after the mice were shifted to a high-fat diet. On the other hand, the serum IL-4 and supernatant levels did not change. Conclusively, reduction of body weight and adipose tissues through shifts from a high-fat diet to a high-carbohydrate diet effectively improved low-grade inflammation states in mice fed a high-fat diet. Particularly, the reduction of body weight was associated with the levels of leptin, resistin, and IL-6.

Key words: High-fat diet, High-carbohydrate diet, Body fat, Adipokines, Pro-inflammatory cytokines

HIGH-FAT diets have contributed to the accumulation of adipose tissue, resulting in obesity, which is one of the most common metabolic disorders in developing and developed countries. Particularly, a shift from a diet low in fat to a diet high in fat in developing countries, including Korea, markedly raised the prevalence of obesity in the last two decades [1, 2]. It has been known that excessive intake of high-fat or low-CHO diets could induce insulin resistance. Ironically, over the past several years, low-fat and high-CHO diets have been used to treat obesity.

Adipose tissue is an important source of adipokines and pro-inflammatory cytokines [3, 4] and is not usually thought of as an endocrine or inflammatory organ. It has been known that long-term low-grade inflammation in obese individuals contributes to the development of metabolic diseases [5-8]. The discovery of the elevated secretion of these cytokines from the adipose tissue of obese individuals provided the first evidence of a direct connection between obesity and systemic inflammation [9, 10].

Leptin is an adipokine that protects T lymphocytes from apoptosis and modulates T-cell proliferation, increasing the proliferation of naive T cells while reducing the proliferation of memory T cells [11, 12]. Adiponectin, the most abundantly secreted adipokine

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from adipocytes, has potent vascular protective, angiogenic, anti-inflammatory, and anti-atherogenic properties. High concentrations of adiponectin are associated with a reduced risk of myocardial infarction in men, while low-serum adiponectin levels have been reported in obese individuals [13]. Another adipokine, resistin, confers resistance to insulin; thus, resistin has been implicated in the pathogenesis of diabetes mellitus [14, 15]. Altered levels of systemic and local adipokines have been reported in a variety of inflammatory states.

Pro-inflammatory cytokines are also secreted by adipose tissue and are associated with other adipokines such as adiponectin. TNF-α, which is increased in obese subjects, might downregulate adiponectin production [16, 17]. On the other hand, adiponectin reduces the production and activity of TNF-α [18]. A prototypical inflammatory cytokine, IL-1 is a critical early mediator of inflammation. The anti-inflammatory activities of adiponectin extend to inhibition of IL-6 production accompanied by induction of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist as well as IL-4 [19-21].

Many studies have highlighted the finding that plasma adipokines and pro-inflammatory cytokine concentrations increase in humans and animals with excess adiposity [3, 23]. Moreover, IL-6 and TNF-α levels in obese individuals also increase in the serum or adipose tissues [24, 25].

The increase in body fat mass puts individuals at risk for metabolic diseases for which a cluster of metabolic abnormalities is responsible [26]. These states also induce inflammatory conditions. Thus, body fat mass reduction via dietary intervention corrects metabolic parameters and increases life spans in humans and animals [27].

The purpose of this study was to investigate the effects of shifts of diet from high fat to high carbohydrate on the blood concentrations of lipids, adipokines, and pro-inflammatory cytokines in mice fed a high-fat diet.

Methods and Materials

Animals and Diet

The experimental protocol was approved by the Animal Care and Use Review Committee of Kyung Hee University. Five-week-old male C57BL/6 mice (n = 24) were purchased from Japan SLC, Inc. (Kyoto, Japan). Animals were housed (three per cage) in polycarbonate cages in temperature-controlled rooms (22 ± 2 °C) with a 12-h light/dark cycle, fed a pelleted chow diet, and given water *ad libitum* for an adaptation period of 1 week. All animals were weighed weekly, and food intake was measured two times per week. After a 1-week adaptation period, 12 mice were placed on a high-fat diet in which 45% of the calories were derived from lard and fed *ad libitum* for 9 weeks, whereas 12 mice were placed on a high-carbohydrate diet that was 70% carbohydrates (Table 1).

After 9 weeks, the diet of the mice (n = 6) fed the high-fat diet shifted to a high-carbohydrate diet (the H-F/H-CHO group) and the diet of the mice (n = 6) fed the high-carbohydrate diet was shifted to a high-fat diet (the H-CHO/H-F group) until the 12th week. Twelve mice continued on the high-fat diet (the H-fat group) or the high-carbohydrate diet (the H-CHO group) until the 12th week. The experimental groups (H-CHO, H-Fat, H-CHO/H-F, and H-F/H-CHO) were killed at 12 weeks.

Body Weight and Food Consumption

Body weight and food consumption were measured weekly. The food efficiency ratio was calculated as the following formula: [weight gain (g)/day]/[amount of food consumed (g)/day].

Analysis of blood samples

Blood was collected at the 12th weeks of the experiment via retro-orbital sinus puncture of the anesthetized animals after 12 h of food deprivation. Serum TG, T-Chol, LDL-C, and glucose levels were determined using commercial kits (Asan Co Ltd, Asan, Korea).

Serum insulin, leptin, adiponectin, and resistin concentrations were determined using Millipore's MILLIPLEX mouse serum adipokine panels (Millipore, Billerica, MA). Because of the multiplex technology, the adipokines in the mouse panel were measured simultaneously in each sample. All assays were conducted according to the manufacturer's instructions. The plate was run on a Luminex 200 Instrument using Bio-Plex Manager 4.1 standard software (Bio-Rad Laboratories, Hercules, CA). Raw fluorescence data were analyzed by the software using a

Table 1. Composition of experiment diets

		High CHO diet		High fat diet	
Ingredients					
		gm%	kcal%	gm%	kcal%
Protein		19.2	20	24	20
Carbohydrate		67.3	70	41	35
Fat		4.3	10	24	45
	Total		**100**		**100**
	kcal/gm	**3.85**		**4.73**	
Ingredient					
		gm	**kcal**	**gm**	**kcal**
Casein, 80 Mesh		200	800	200	800
L-Cysteine		3	12	3	12
Corn Starch		315	1260	72.8	291
Maltodextrin 10		35	140	100	400
Sucrose		350	1,400	172.8	691
Cellulose, BW200		50	0	50	0
Soybean Oil		25	225	25	225
Lard		20	180	177.5	1,598
Mineral Mix S10026		10	0	10	0
DiCalcium Phosphate		13	0	13	0
Calcium Carbonate		5.5	0	5.5	0
Potassium Citrate, 1 H_2O		16.5	0	16.5	0
Vitamin Mix V10001[1)]		10	40	10	40
Choline Bitartrate		2	0	2	0
Total		**1,055**	**4,057**	**858.1**	**4,057**

Formulated by E.A. Ulman, Ph.D., Research Diets, Inc., 8/26/98 and 3/11/99.
*Typical analysis of cholesterol in lard = 0.95 mg/g.
RD - Cholesterol 18 mg/kg diet, HD - Cholesterol 196.5 mg/kg diet
[1)]Vitamin mix V10001(/kg diet): Vitamin A palmitate; 20,000 IU, vitamin D-3; 1,000 IU, vitamin E acetate; 50 IU, menadione sodium bisulfate; 0.5 mg, biotin; 0.3 mg, cyanocobalamin; 10 g, folic acid; 6 mg, nicotinic acid; 30 mg, calcium pantothenate; 30 mg, pyridoxine-HCl; 6 mg, riboflavin; 6 mg, thiamin-HCl; 6 mg, ascorbic acid; 500 mg.

5-parameter logistic method. The minimum detection concentration was 16 pg/mL for adipokines. The intra-assay and inter-assay precision of the mouse serum adipokine panel were <5% and <12%, respectively.

Pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and anti-inflammatory cytokines (IL-4 and IL-13) were measured in duplicate using Millipore's MILLIPLEX mouse cytokine panel (Millipore, Billerica, MA). Because of the multiplex technology, the cytokines in the mouse panel were measured simultaneously in each sample. All assays were conducted according to the manufacturer's instructions. The plate was run on a Luminex 200 Instrument using Bio-Plex Manager 4.1 standard software (Bio-Rad Laboratories, Hercules, CA). Raw fluorescence data were analyzed by the software using a 5-parameter logistic method. The minimum detection concentrations were 1.8, 1.0, 0.4, and 6.3 pg/mL for IL-6, TNF-α, IL-4, and IL-13, respectively. The intra-assay and inter-assay precision of the mouse cytokine panel were 2.16–9.12% and 3.11–5.86%, respectively.

Primary spleen cells culture

Spleens were isolated from the mice, and single-splenocyte suspensions were prepared and adjusted to 2×10^6 cells/mL in RPMI 1640 supplemented with 10% FBS, 1% 5×10^{-5} M 2-mercaptoethanol, and 10 mL/L antibiotic antimycotic solution (Sigma). Cell suspensions were transferred in a 1-mL aliquot to 24-well flat-bottom culture plates in the presence of optimal concentrations of LPS (5 μg/mL), and each culture was maintained for 24 h at 37 °C and 5% humidity [28].

Cytokine of splenocyte supernatant

Splenocyte supernatant concentrations of IL-1β,

Table 2. Body weights, calorie intake, and food efficiency ratio

	H-CHO[1)]	H-CHO/H-F[2)]	H-Fat[3)]	H-F/H-CHO[4)]
Initial body weight (g)	21.7 ± 1.0[5)]	21.1 ± 0.9	22.1 ± 1.0	22.1 ± 0.5
Weight at 9th week (g)	33.0 ± 2.1[b6)]	31.5 ± 1.0[b]	41.7 ± 1.9[a]	42.1 ± 2.0[a]
Final body weight (g)	33.0 ± 2.0[c]	36.4 ± 1.7[b]	41.8 ± 2.0[a]	37.5 ± 1.6[b]
Weight gain (g)				
9th week	11.0 ± 1.8[b]	9.7 ± 0.7[b]	19.5 ± 1.2[a]	20.4 ± 1.9[a]
12th week	2.0 ± 0.2[b]	5.7 ± 0.8[a]	1.9 ± 1.2[b]	-3.2 ± 0.5[c]
Calorie intake (kcal/day)				
9th week	10.2 ± 0.7[b]	9.7 ± 0.2[b]	11.8 ± 0.2[a]	12.1 ± 0.2[a]
12th week	10.9 ± 0.6[b]	12.4 ± 0.4[a]	10.8 ± 0.1[b]	9.1 ± 0.2[c]
FER[7)]				
9th week	0.017 ± 0.003[b]	0.016 ± 0.001[b]	0.027 ± 0.002[a]	0.027 ± 0.002[a]
12th week	0.009 ± 0.001[b]	0.022 ± 0.003[a]	0.008 ± 0.002[b]	- 0.017 ± 0.003[c]

[1)]H-CHO; fed high-carbohydrate diets (70% of calories derived from CHO) for 12 weeks.
[2)]H-CHO/H-F; fed high-CHO diets for 9 weeks followed by a high-fat diet (45% of calories derived from fat) for 3 weeks.
[3)]H-Fat; fed high-fat diets for 12 weeks.
[4)]H-F/H-CHO; fed high-fat diets for 9 weeks followed by a high-CHO diet for 3 weeks.
[5)]Values are expressed as mean ± SD (n = 6)
[6)]Different superscript letters within the same row are significantly different ($p < 0.05$) according to Duncan's multiple range test.
[7)]FER = Food efficiency ratio: [weight gain (g)/day]/[food consumed (kcal)/day]

IL-6, and TNF-α were measured in duplicate by using enzyme-linked immunoabsorbent assay (ELISA) kits (Endogen, US). Briefly, a plastic plate was coated overnight with a capture antibody for a specific cytokine, which was followed by washing and blocking of the plate. Diluted samples and standards were then added and incubated. An extensive wash was applied before the secondary antibody and enzyme conjugates were added. The plates then went through another round of incubation and extensive washing. Developing reagent was then added to the plate for 15 min. Color development was stopped during linear increases in substrate utilization by the addition of 0.5 M sulfuric acid to disrupt enzymatic activity. OD readings of samples were converted to concentrations based on the reference curve. The duplicate samples were analyzed for each cytokine ELISA. The minimum detection concentrations were 2.0, 0.4, and 6.3 pg/mL for IL-1β, IL-6, and TNF-α, respectively. The intra-assay and inter-assay precision of the high sensitivity cytokine panel was 2.16–9.12% and 3.11–5.86%, respectively.

Statistical analysis

All measurements were performed in triplicate, and statistical calculation was performed with Statistical Analysis System (SAS) version 8.0. Results are expressed as mean ± standard deviation. One-way ANOVA and Duncan's multiple range tests were used to examine the differences among the groups; statistical significance was considered at $p < 0.05$.

Results

Body weight, calorie intake, and food efficiency ratio

The effect of the shift in diet from high fat to high carbohydrate on weight gain, energy intake, and food efficiency ratio is shown in Table 2. The initial body weights were not significantly different among the experimental groups. Feeding of the high-fat diet for 9 weeks (H-Fat, H-F/H-CHO) resulted in a significantly higher body weight than in the mice fed the high-CHO diet (H-CHO, H-CHO/H-F). The H-CHO/H-F group showed a significant increase in final body weight compared to the H-CHO group (+11.0%, $p < 0.05$), whereas the H-F/H-CHO group showed a significant decrease in final body weight compared to that of the H-Fat group (–10.3%, $p < 0.05$).

At 9 and 12 weeks, the weight gain trends in the ex-

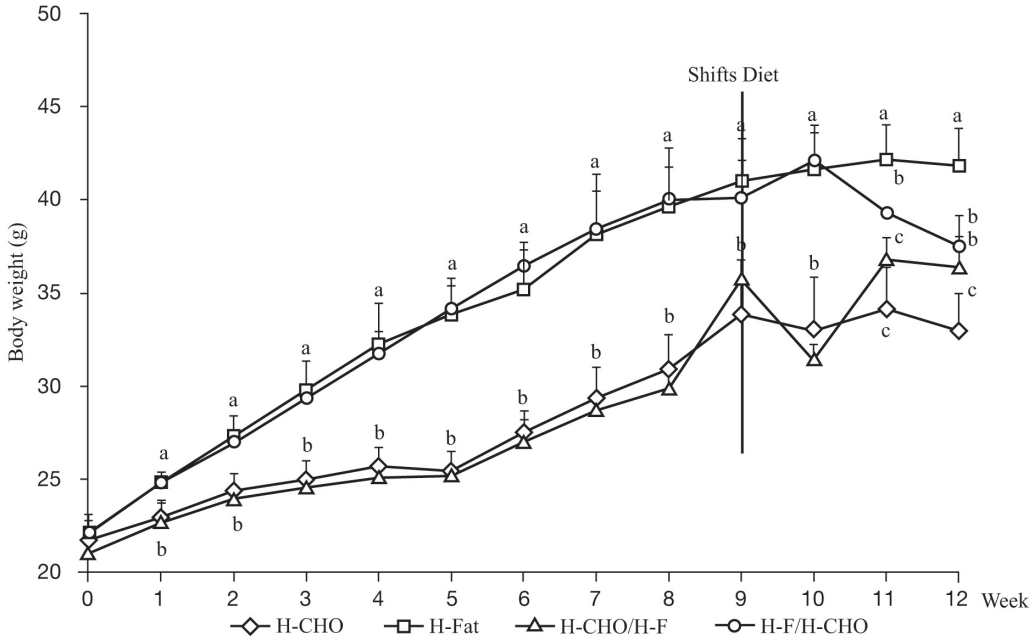

Fig. 1. Comparisons of body weight of the experimental groups during 12 weeks.
Each point represents the mean body weight of six animals.
Different superscript letters within the same row are significantly different ($p < 0.05$) according to Duncan's multiple range test.

perimental groups were similar for body weight. The caloric intake of the high-CHO diet (H-CHO, H-CHO/H-F) groups was significantly lower than that of the high-fat diet (H-Fat, H-F/H-CHO) groups at the ninth week. However, after 3 weeks, the caloric intake of the H-CHO/H-F groups was the highest and that of the H-F/H-CHO groups the lowest among the experimental groups. In addition, the food efficiency ratio trends in the experimental groups were similar to caloric intake at 9 and 12 weeks.

The comparisons of body weights among the groups during the 12 weeks of experiment are shown in Fig. 1.

Organ weights

The organ weights of the experimental groups are shown in Table 3. The average liver weight of the H-Fat group was significantly more than those of the H-CHO and H-CHO/H-F groups (1.59 ± 0.28 g *vs.* 1.29 ± 0.15 and 1.33 ± 0.11 g, $p < 0.05$). In contrast, the weights of the other organs (kidney, spleen, and testes) did not differ among the experimental groups. The weights of the epididymal and retroperitoneal adipose tissue of the H-CHO groups were the lowest ($p < 0.05$). Moreover, the weights of the epididymal and retroperitoneal adipose tissue of the H-Fat group were significantly higher than those of the H-F/H-CHO group ($p < 0.05$). The H-CHO/H-F group showed a significantly higher epididymal and retroperitoneal adipose tissue weight than the H-CHO group.

Serum and hepatic lipid levels

The serum and hepatic lipid levels are shown in Table 4. Levels of serum TG in the H-CHO groups were not altered after the shift to the high-fat diet (86.9 ± 10.7 mg/dL *vs.* 89.5 ± 6.3 mg/dL), whereas the levels in the H-Fat groups significantly decreased after the shift to the high-CHO diet (97.2 ± 9.6 mg/dL *vs.* 78.3 ± 5.1 mg/dL, $p < 0.05$). In contrast, the serum total cholesterol (TC) levels in the H-CHO group were significantly increased after the shift to the high-fat diet (96.6 ± 3.7 *vs.* 133.1 ± 19.7, $p < 0.05$), whereas the TC levels in the H-fat groups did not change after the shift to the high-CHO diet (128.6 ± 10.4 and 119.1 ± 15.0).

The serum LDL-C levels of the H-CHO group significantly increased after the shift to the high-fat diet (12.6 ± 7.2 mg/dL *vs.* 48.5 ± 19.0 mg/dL, $p < 0.05$), whereas those of the H-fat group diet were not altered by the high-CHO diet (33.9 ± 7.4 mg/dL *vs.* 27.5 ± 14.8 mg/dL). In addition, the serum LDL-C levels in

Table 3. Organ weights of experimental groups

	H-CHO[1)]	H-CHO/H-F[2)]	H-Fat[3)]	H-F/H-CHO[4)]
Liver (g)	1.29 ± 0.15[c,5)]	1.33 ± 0.11[bc]	1.59 ± 0.28[a]	1.55 ± 0.14[ab]
Kidney (g)	0.35 ± 0.24	0.36 ± 0.03	0.36 ± 0.03	0.35 ± 0.04
Spleen (g)	0.07 ± 0.02	0.07 ± 0.01	0.08 ± 0.02	0.07 ± 0.01
Testes (g)	0.25 ± 0.02	0.24 ± 0.02	0.23 ± 0.02	0.23 ± 0.02
Epididymal AT (g)	1.54 ± 0.20[c]	2.17 ± 0.22[b]	2.61 ± 0.19[a]	2.00 ± 0.15[b]
Retroperitoneal AT (g)	0.96 ± 0.12[c]	1.10 ± 0.16[bc]	1.54 ± 0.12[a]	1.21 ± 0.17[b]

[1)]H-CHO; fed a high-carbohydrate diet (70% of calories derived from CHO) for 12 weeks.
[2)]H-CHO/H-F; fed a high-CHO diet for 9 weeks followed by a high-fat diet (45% of calories derived from fat) for 3 weeks.
[3)]H-Fat; fed a high-fat diet for 12 weeks.
[4)]H-F/H-CHO; fed a high-fat diet fed for 9 weeks followed by a high-CHO diet for 3 weeks.
[5)]Values are expressed as mean ± SD (n = 6). Different superscript letters within the same row are significantly different ($p < 0.05$) according to Duncan's multiple range test.

Table 4. Serum and hepatic lipids

	H-CHO[1)]	H-CHO/H-F[2)]	H-Fat[3)]	H-F/H-CHO[4)]
Serum lipids				
Triglyceride (mg/dL)	86.9 ± 10.7[ab,5)]	89.5 ± 6.3[ab]	97.2 ± 9.6[a]	78.3 ± 5.1[b]
Total cholesterol (mg/dL)	96.6 ± 3.7[b]	133.1 ± 19.7[a]	128.6 ± 10.4[a]	119.1 ± 15.0[a]
LDL-cholesterol[6)](mg/dL)	12.6 ± 7.2[c]	48.5 ± 19.0[b]	33.9 ± 7.4[ab]	27.5 ± 14.8[bc]
HDL-cholesterol (mg/dL)	66.8 ± 6.3	73.4 ± 17.3	76.3 ± 12.0	75.9 ± 5.9
Hepatic lipids				
Triglyceride (mg/dL)	145.1 ± 15.2[a]	181.3 ± 21.3[ab]	207.4 ± 41.5[b]	181.3 ± 27.7[ab]
Total cholesterol (mg/dL)	60.9 ± 21.4[b]	52.2 ± 12.9[ab]	38.6 ± 5.3[a]	43.9 ± 0.7[ab]

[1)]H-CHO; fed a high-carbohydrate diet (70% of calories derived from CHO) for 12 weeks.
[2)]H-CHO/H-F; fed a high-CHO diet for 9 weeks followed by a high-fat diet (45% of calories derived from fat) for 3 weeks.
[3)]H-Fat; fed a high-fat diet for 12 weeks.
[4)]H-F/H-CHO; fed a high-fat diet for 9 weeks followed by a high-CHO diet for 3 weeks.
[5)]Values are expressed as mean ± SD (n = 6). Different superscript letters within the same row are significantly different ($p < 0.05$) according to Duncan's multiple range test.
[6)]LDL; low-density lipoprotein cholesterol was calculated by {Total Cholesterol – [HDL cholesterol + (TG/5)] }

the H-CHO group were significantly lower than those in the H-Fat group (12.6 ± 7.2 mg/dL *vs.* 33.9 ± 7.4 mg/dL $p < 0.05$), although the serum HDL-C levels were not significantly different among the experimental groups.

The hepatic TG and TC concentrations were not altered by the shift to a high-fat or high-CHO diet; however, the H-CHO and H-Fat groups had significantly different concentrations ($p < 0.05$). The TG concentration trends were the opposite of the TC concentration trends.

Blood glucose, insulin levels and HOMA-IR, and QUICKI

The blood glucose and insulin levels and HOMA-IR are shown in Table 5. The blood glucose levels in the H-CHO group were not altered after the shift to the high-fat diet (130.0 ± 19.1 mg/dL *vs.* 146.6 ± 14.0 mg/dL); moreover, the levels in the H-Fat group were not altered after the shift to the high-CHO diet (164.0 ± 24.3 mg/dL *vs.* 159.6 ± 20.5 mg/dL, $p < 0.05$). On the other hand, the blood glucose levels of the H-Fat group were significantly higher than those of the

Table 5. Blood glucose, insulin, and HOMA-IR

	H-CHO[1)]	H-CHO/H-F[2)]	H-Fat[3)]	H-F/H-CHO[4)]
Glucose (mg/dL)	130.0 ± 19.1[b,5)]	146.6 ± 14.0[ab]	164.0 ± 24.3[a]	159.6 ± 20.5[ab]
Insulin (mIU/mL)	14.3 ± 4.0[b]	19.2 ± 6.7[ab]	25.1 ± 7.9[a]	19.7 ± 6.7[ab]
HOMA-IR[6)]	4.1 ± 1.6[c]	6.9 ± 2.0[bc]	11.5 ± 3.2[a]	8.9 ± 1.9[ab]
QUICKI[7)]	0.31 ± 0.16[a]	0.29 ± 0.13[ab]	0.28 ± 0.13[b]	0.29 ± 0.12[ab]

[1)]H-CHO; fed high-carbohydrate diet (70% of calories derived from CHO) fed for 12 weeks.
[2)]H-CHO/H-F; fed high-CHO diet for 9 weeks followed by a high-fat diet (45% of calories derived from fat) for 3 weeks.
[3)]H-Fat; fed high-fat diet for 12 weeks.
[4)]H-F/H-CHO; fed high-fat diet for 9 weeks followed by a high-CHO diet for 3 weeks.
[5)]Values are expressed as mean ± SD (n=6). Different superscript letters within the same row are significantly different ($p < 0.05$) according to Duncan's multiple range test.
[6)]HOMA-IR = [Fasting insulin(uIU/mL) × Fasting glucose(mmol/L)]/22.5
[7)]QUICKI (Quantitative insulin sensitivity check index) = 1/[log fasting insulin (mg/dL) + log fasting glucose(mg/dL)]

H-CHO group (164.0 ± 24.3 mg/dL *vs.* 130.0 ± 19.1 mg/dL, $p < 0.05$).

The serum insulin levels in the H-CHO group were not altered after the shift to the high-fat diet (14.3 ± 4.0 mIU/mL *vs.* 19.2 ± 6.7 mIU/mL), and the levels in the H-fat group were not altered after the shift to the high-CHO diet (25.1 ± 7.9 mIU/mL *vs.* 19.7 ± 6.7 mIU/mL). In contrast, the insulin levels of the H-Fat group were significant higher than those of the H-CHO group (25.1 ± 7.9 mIU/mL *vs.* 14.3 ± 4.0 mIU/mL, $p < 0.05$).

HOMA-IR, which is an index of insulin resistance, of the H-CHO group did not change after the shift to the high-fat diet (4.1 ± 1.6 *vs*. 6.9 ± 2.0) and that of the H-Fat group did not change after the shift to the high-CHO diet (11.5 ± 3.2 and 8.9 ± 1.9). Additionally, HOMA-IR of the H-Fat group was significantly higher than that of the H-CHO/H-F and H-CHO groups (11.5 ± 3.2, 4.1 ± 1.6 and 6.9 ± 2.0, $p < 0.05$).

Quantitative insulin sensitivity check index (QUICKI) were also calculated using the following formula : QUICKI = 1/[log fasting insulin (mg/dL) + log fasting glucose (mg/dL)]

Serum levels of adipokines and pro-inflammatory and anti-inflammatory cytokines.

The serum levels of selected adipokines and pro-inflammatory cytokines are shown in Table 6. The serum leptin levels of the H-fat groups were significantly higher than those of the H-CHO experimental groups (46.3 ± 15.4 *vs.* 15.9 ± 2.1, 23.4 ± 6.4 *vs.* 23.4 ± 2.6 ng/mL, $p < 0.05$); moreover, the serum leptin levels in the H-Fat group significantly decreased after the shift to the high-CHO diet (46.3 ± 15.4, *vs.* 23.4 ± 2.6 ng/mL, $p < 0.05$). However, the serum leptin levels in the H-CHO group did not increase after the shift to the high-fat diet.

The serum adiponectin levels of the experimental groups were not significantly different. The serum resistin levels of the H-CHO group significantly increased after the shift to the high-fat diet, whereas those in the H-Fat group decreased after the shift to the high-CHO diet. In addition, serum resistin levels were the highest in the H-Fat, H-F/H-CHO, and H-CHO groups in order (4.4 ± 1.6 ng/mL, 2.7 ± 0.6 ng/mL, 1.2 ± 0.11 ng/mL, $p < 0.05$).

The levels of serum IL-6, which is a pro-inflammatory cytokine, in the H-Fat group significantly decreased after the shift to the high-CHO diet (7.6 ± 3.5 *vs.* 2.1 ± 0.7, $p < 0.05$ pg/mL), whereas those in the H-CHO groups did not change after the shift to the high-fat diet. In addition, the levels of serum IL-6 in the H-Fat group were significantly higher than those in the H-CHO and H-CHO/H-F groups. The levels of TNF-α of the H-CHO group increased significantly after the shift to the high-fat diet (1.4 ± 0.2 pg/mL *vs.* 2.2 ± 0.5, $p < 0.05$ pg/mL), whereas those of the H-Fat group did not change after the shift to the high-CHO diet (2.5 ± 0.4 pg/mL *vs.* 2.1 ± 0.5 pg/mL).

The levels of serum IL-4, which is a representative anti-inflammatory cytokine, were not different among the experimental groups. Additionally, the levels of

Table 6. Serum levels of adipokines and pro- and anti-inflammatory cytokines

	H-CHO[1)]	H-CHO/H-F[2)]	H-Fat[3)]	H-F/H-CHO[4)]
Serum adipokines				
Leptin (ng/mL)	15.9 ± 2.1[b]	23.4 ± 6.4[b]	46.3 ± 15.4[a]	23.4 ± 2.6[b]
Adiponectin (ug/mL)	12.8 ± 2.7[5)]	11.3 ± 0.9	12.1 ± 0.4	11.8 ± 1.3
Resistin (ng/mL)	1.2 ± 0.1[c]	2.7 ± 0.6[b]	4.4 ± 1.6[a]	1.8 ± 0.3[bc]
Pro-inflammatory cytokines				
IL-6[6)](pg/mL)	2.3 ± 0.5[a]	2.0 ± 0.4[a]	7.6 ± 3.5[b]	2.1 ± 0.7[a]
TNF-α (pg/mL)	1.4 ± 0.2[b]	2.2 ± 0.5[a]	2.5 ± 0.4[a]	2.1 ± 0.5[ab]
Anti-inflammatory cytokines				
IL-4 (pg/mL)	2.3 ± 1.8	2.0 ± 1.5	2.5 ± 1.7	2.1 ± 1.6
IL-13 (pg/mL)	6.1 ± 4.5 [a]	10.6 ± 6.6 [ab]	10.0 ± 2.4[a]	17.2 ±3.1[b]

[1)]H-CHO; fed high-carbohydrate diet (70% of calories derived from CHO) for 12 weeks.
[2)]H-CHO/H-F; fed high-CHO diet for 9 weeks followed by a high-fat diet (45% of calories derived from fat) for 3 weeks.
[3)]H-Fat; fed high-fat diet for 12 weeks.
[4)]H-F/H-CHO; fed high-fat diet for 9 weeks followed by a high-CHO diet for 3 weeks.
[5)]Values are expressed as mean ± SD (n = 6), and different superscript letters within a row are significantly different ($p < 0.05$) according to Duncan's multiple range test.
[6)]IL-6; interleukin-6, TNF-α; tumor necrosis factor-alpha, IL-4; interleukin-4, IL-13; interleukin-13

Table 7. Pro-inflammatory cytokines in the supernatant of isolated spleen lymphocytes

	H-CHO[1)]	H-CHO/H-F[2)]	H-Fat[3)]	H-F/H-CHO[4)]
IL-1β[2)] (pg/mL)	3.9 ± 2.7[b,5)]	6.8 ± 1.0[a]	1.5 ± 1.0[c]	1.2 ± 1.2[c]
IL-6 (pg/mL)	12.8 ± 2.7	11.3 ± 0.9	12.1 ± 0.4	11.8 ± 1.3
TNF-α (pg/mL)	1.2 ± 0.1[c]	2.7 ± 0.6[b]	4.4 ± 1.6[a]	1.8 ± 0.3[bc]

[1)]H-CHO; fed high-carbohydrate diet (70% of calories derived from CHO) for 12 weeks.
[2)]H-CHO/H-F; fed high-CHO diet for 9 weeks followed by a high-fat diet (45% of calories derived from fat) for 3 weeks.
[3)]H-Fat; fed high-fat diet for 12 weeks.
[4)]H-F/H-CHO; fed high-fat diet for 9 weeks followed by a high-CHO diet for 3 weeks.
[5)]Values are expressed as mean ± SD (n = 6), and different superscript letters within a row are significantly different ($p < 0.05$) according to Duncan's multiple range test.
[6)]IL-1β; interleukin-1beta, IL-6; interleukin-6, TNF-α; tumor necrosis factor-alpha

IL-13 in the H-CHO groups did not change by the high-fat diet (6.1 ± 4.5 pg/mL *vs.* 10.6 ± 6.6 pg/mL); however, those in the H-Fat group were elevated after the shift to the high-CHO diet (10.0 ± 2.4 pg/mL *vs.* 17.2 ± 3.1 pg/mL, $p < 0.05$).

Pro-inflammatory cytokines in the supernatant of isolated spleen lymphocytes

Pro-inflammatory cytokines in the supernatant of isolated spleen lymphocytes are shown in Table 7. IL-1β concentrations in the H-Fat group significantly increased after the shift to the high-CHO diet (3.9 ± 2.7 pg/mL *vs.* 6.8 ± 1.0 pg/mL, $p < 0.05$), whereas those in the H-CHO groups were not changed by the high-fat diet (1.5 ± 1.0 pg/mL *vs.* 1.2 ± 1.2 pg/mL). In addition, the IL-1β concentrations of the H-CHO and H-CHO/H-F groups were significantly higher than those of the HF and HF/C groups. In contrast, the IL-6 concentrations were not changed by the experimental diets. TNF-α concentrations in the H-CHO groups significantly increased after the shift to the

high-fat diet (1.2 ± 0.1 pg/mL *vs.* 2.7 ± 0.6 pg/mL, $p < 0.05$); moreover, those in the H-CHO group decreased after the shift to the high-CHO diet (4.4 ± 1.6 pg/mL *vs.* 1.8 ± 0.3 pg/mL, $p < 0.05$).

Discussion

The present study showed the effects of the shift from a high-fat diet to a high-carbohydrate diet on body weight and adipose tissues (epididymal and retroperitoneal) in mice fed a high-fat diet. The mice fed the high-fat diet had significantly increased body weight, liver weight, and epididymal and retroperitoneal fats.

A previous study investigated the effects of a low-fat diet on body weight and fat mass [29]. According to that study, weight loss induced by a low-fat diet concomitantly improved multiple metabolic risks associated with obesity. In contrast, a high-fat diet caused the expansion of adiposity in animals and humans resulting in increased metabolic risks [30, 31].

Many studies have investigated the effects of diet on blood and liver lipid levels in animals and humans [31-33]. In accordance with the previous study, the current study found that blood glucose and serum insulin levels increased by a high-fat diet for 12 weeks. HOMA-IR was also affected by the contents of fat in the experimental diets. The dietary shifts did not affect the glucose and insulin levels as well as insulin sensitivity. The 3-week duration after the shift in the diets might not be enough to improve the blood glucose and insulin levels even though body weight and body fats decreased significantly during this period of time.

The levels of leptin secreted by the adipocytes are positively correlated with the amount of adipose tissue [34]. The primary role of leptin is not only control of appetite but also regulation of immunity [35]. In this study, the levels of leptin in the obese mice fed the high-fat diet were significantly higher than in the mice fed the high-CHO diet. The switch from the high-fat diet to the high-CHO diet reduced body fat as well as the levels of leptin while switching from the high-CHO diet to the high-fat diet did not influence the serum leptin levels even though body fat had been significantly increased.

Adiponectin is secreted by adipocytes and circulates at the highest levels (in the microgram per milliliter range) [36]. In humans, adiponectin concentrations are reduced as adiposity increases [37, 38]. However, various studies have reported increased adiponectin concentrations in mice and rats fed a high-fat diet, and it has been proposed that this increase may represent an initial response to counteract diet-induced obesity and insulin resistance [39-41]. In this study, the adiponectin concentrations did not change in accordance with weight reduction; therefore, it can be speculated that adiponectin might not be a contributing factor for a reduction in body fat induced by dietary modification.

Many studies have reported that levels of resistin convey resistance to insulin and have been implicated in the pathogenesis of diabetes [14, 15]. The trends of the blood glucose and insulin levels as well as HOMA-IR were similar to the resistin response. Although the study showed that the blood glucose and insulin concentrations were not improved by a high-carbohydrate diet, the levels of serum resistin in the mice fed the high-fat diet decreased after the switch to the high-CHO diet. Therefore, levels of serum resistin in mice were more sensitively responsive to dietary modification than the blood glucose and insulin levels.

Generally, obesity is associated with low-grade inflammation disease such as type 2 diabetes mellitus and dyslipidemia. Low-grade inflammation is controlled by a balance between pro-inflammatory cytokines and anti-inflammatory cytokines. A few studies reported that IL-6 and IL-8 levels increased in obese individuals and individuals with type 2 diabetes mellitus [42, 43]. In accordance with previous studies, this study also found that the levels of IL-6 in the obese mice fed the high-fat diet were significantly higher than those in the mice fed the high-CHO diet. Moreover, the levels of IL-6 in the mice fed the high-fat diet decreased after the shift to the high-CHO diet concomitantly reduced body weight.

Additionally, another study reported that IL-6 has anti-inflammatory effects as well as pro-inflammatory effects [44]. Particularly, IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-α. TNF-α levels increased in diet-induced obese mice compared to normal-weight mice [45]. The serum levels of TNF-α of the obese mice fed high-fat diet were the highest among the groups. Increased concentration of TNF-α in that group was not lowered by the modification of fat contents in the diet even though we could observe the reduction of body fat by low fat diet. On the other hand, the shift

from high-CHO to high-fat diet significantly increased TNF-α concentration and body fat. The present study showed that diet-induced weight gain significantly raised the levels of TNF-α however, weight reduction through the shift from the high fat to the high-CHO diet did not affect the levels of TNF-α.

The levels of IL-6 in the supernatant of isolated spleen lymphocytes did not change, while TNF-α concentration in supernatant decreased by the shift to the high-CHO diet. In contrast with previous studies [46], this study showed that IL-1β concentrations in supernatant in the mice fed the high-fat diet were significantly higher than those in the mice fed the high-CHO diet. However, the high-fat diet increased IL-1β concentration in the supernatant of isolated spleen lymphocytes. The reasons for this discrepancy are unclear. Thus, a high-CHO diet may improve low-grade inflammation by reducing body fat.

IL-4 and IL-13 are well-known anti-inflammatory cytokines [19-21], whose levels in overweight and obese subjects decreased compared to the levels in the normal-weight group. In the case of IL-13, the current study showed that a high-CHO diet increased serum IL-13 concentrations in the mice fed the high-fat diet for a short period of time. The increase in the IL-13 concentrations is affected by the increase in IL-6. A normal state of inflammation is maintained by a balance between pro-inflammatory and anti-inflammatory cytokines in the acute phase. The present study showed that weight reduction through a shift to a high-CHO diet significantly increased IL-13 concentrations. However, the serum IL-4 concentrations did not change. These results suggest that weight reduction due to diet affected the concentration of anti-inflammatory cytokines. Particularly, the state of inflammation in the mice fed the high-fat diet was affected by the shift to the high-CHO diet.

TNF-α, IL-1β, and IL-6 contribute to inflammatory diseases such as diabetes mellitus [47]. Adipokines, including leptin, adiponectin, and resistin, also affect the inflammatory condition [3, 4]. For instance, leptin and resistin have pro-inflammatory effects, whereas adiponectin has an anti-inflammatory effect [13]. In our study, reduction in body weight and adipose tissues improved the inflammatory state through a decrease of pro-inflammatory factor (leptin, resistin, serum IL-6, and TNF-α in supernatant of isolated spleen lymphocytes) and an increase in anti-inflammatory factor (IL-13).

There are some limitations in this study. The duration after the shift in diet in the present study was somewhat short although body weight and adipose tissues were significantly decreased by the shift to the high-CHO diet. Additionally, even if the weight reduction was induced by the high-CHO diet, the reasons for the weight reduction are unclear because the experimental animals were fed a high-CHO diet and a low-calorie diet compared with a high-fat diet at the same time. Dietary consumption was not controlled. If dietary consumption had been controlled, the mice would have been affected by diet as well as other effects such as stress and starvation. Another limitation of this study was that it could not be confirmed whether serum cytokines were secreted from adipose tissue or other organs and whether the amount of adipose tissue caused inflammation or weight reduction through the high-CHO diet caused inflammation.

In conclusion, dietary modification influenced the energy intake, body weight and adipose tissues, as well as the levels of adipokines and inflammatory cytokines. Particularly, the reduction of body weight and adipose tissues is associated with a change in pro-inflammatory factors as leptin, resistin, IL-6, and TNF-α. Future studies are necessary to investigate the signals or mechanisms that affect the regulation of secretion of adipokines or cytokines from adipose tissues.

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