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Site dependency of fatty acid composition in adipose triacylglycerol in rats and its absence as a result of high-fat feeding

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ABSTRACT

It is currently believed that metabolic syndrome, in general, and type 2 diabetes mellitus, in particular, depend more on visceral than on subcutaneous adipose tissue. However, the relationship between insulin resistance and fatty acid composition in visceral and subcutaneous adipose tissues remains to be clarified. In the present study, we extracted the triacylglycerol from visceral (epididymis and mesentery) and subcutaneous adipose tissues in normal and insulin-resistant, high-fat–fed (HFF) rats and determined the composition of each fatty acid. The concentrations of palmitoleic, docosapentaenoic, docosahexaenoic, dihomo-γ-linolenic, arachidonic, and docosatetraenoic acids were higher in epididymal adipose tissue than in mesenteric and subcutaneous adipose tissues; but no significant differences were detected between mesenteric and subcutaneous tissues in the normal group or among all the sites in the HFF rats. In the HFF group, stearic and oleic acid concentrations were higher, whereas n-3 and n-6 polyunsaturated ones were lower, than those in the normal group. Palmitoleic acid and some n-3 and n-6 polyunsaturated fatty acid compositions in adipose tissue triacylglycerol depend on anatomical location, which may affect the properties and/or function of adipose tissues. These results at least in part suggest that the properties of adipose tissue are difficult to distinguish based only on their "visceral" or "subcutaneous" sites. In addition, the absence of site dependence and/or difference in balance among saturated, monounsaturated, and polyunsaturated fatty acids may play an important role in the development of insulin resistance in the HFF rats.

All authors participated in the design and interpretation of the studies, analysis of the data, and review of the manuscript; DS, TN, GS, TK, TO, and ZF conducted the experiments; and DS, TN, KT, and MK wrote the manuscript.

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1. Introduction

In recent years, the increasing incidence of metabolic syndrome and type 2 diabetes mellitus has become a severe public health problem. A high-fat diet is considered to be one of the possible causes of the development of these diseases. Excess lipid and sugar intake results in an increase in cellular triacylglycerol (TAG) uptake and auxeses of adipocytes. Consequent lipid accumulation in visceral adipose tissue induces the development of insulin resistance in adipose tissue and skeletal muscle [1-3].

Triacylglycerol consists of saturated (SFA), monounsaturated (MUFA), and n-3 and n-6 polyunsaturated (PUFA) fatty acids. Serum SFA (as well as MUFA) concentration correlates with the homeostasis model assessment of insulin resistance index (HOMA-R) in type 2 diabetes mellitus patients [4]. Sucrose-fed obese rats having hyperglycemia, hyperinsulinemia, and hypertriglyceridemia had significantly higher proportions of palmitoleic (16:1n-7) and oleic (18:1n-9) acids in total lipid in the liver, skeletal muscle, and epididymal and subcutaneous adipose tissues [5]. These reports suggest that MUFA may be involved in the increase in insulin resistance. On the other hand, fish oil (n-3 PUFA rich) and arachidonic acid (n-6 PUFA, 20:4n-6) have been reported to prevent sucrose-fed [6] and high-fat–fed (HFF) rats [7], respectively, from high insulin resistance. In addition, high eicosapentaenoic acid (20:5n-3) has been shown to elevate adiponectin secretion in obese-model rats and obese humans [8]. These results could indicate that PUFA plays an important role in the prevention of high insulin resistance. Therefore, it is of particular concern to clarify the relationship between fatty acid composition in adipose tissues and insulin resistance induced by high-fat diet.

Several studies have been conducted on the function of the fatty acids that make up TAG [7-11], whereas the effects of fatty acid composition in TAG on the glucose and lipid metabolisms still remain to be clarified. Tran et al [12] reported that subcutaneous adipose tissue transplanted into the visceral cavity decreased body weight, total fat mass, plasma glucose and insulin levels, and insulin resistance during hyperinsulinemic-euglycemic clamps in mice, whereas visceral adipose tissue transplanted into the visceral cavity showed no effect. These results suggest the presence of intrinsic differences between visceral and subcutaneous adipose tissue and imply that subcutaneous adipose tissue plays a role in improving the glucose metabolism. If these differences are relevant to fatty acid composition in TAG, the composition of subcutaneous adipose tissue may differ from that in visceral one, enabling insulin sensitivity to be maintained.

The aims of the present study were to compare the fatty acid composition of TAG in epididymal, mesenteric, and subcutaneous adipose tissues in normal and HFF rats and to clarify the site dependency of fatty acid composition and the relationship between this possible site dependency and insulin resistance.

2. Materials and methods

2.1. Experimental animals

Male Wistar rats, obtained from an in-house breeding colony, were randomized at 6 weeks of age into normal and HFF groups for collection of adipose tissue (normal, n = 5; HFF, n = 5) and evaluation of glucose-insulin index (normal, n = 7; HFF, n = 8). The normal group received standard laboratory chow (CE-2; CLEA, Tokyo, Japan) for 5 weeks, whereas an additional beef tallow was provided to the HFF group. All the animals were allowed free access to food and tap water and were housed in a room illuminated daily from 8:00 AM to 8:00 PM (a 12:12-hour light/dark cycle) and maintained at 21°C ± 1°C. All of the surgical and experimental procedures described below have been approved by the Yamagata University Animal Research Committee.

2.2. Surgical procedures

At the end of the 5-week feeding period, the rats were anesthetized with intraperitoneal administration of sodium pentobarbital (50 mg/kg, Tokyo Chemical Industry, Tokyo, Japan) after overnight fast and placed in a supine position. The right jugular vein and the left common carotid artery were cannulated, and the catheters were filled with heparinized saline. Additional doses of sodium pentobarbital were intermittently administered via the venous line to keep the animals in a stable condition. Blood sampling was performed via the arterial line to determine blood glucose (BG) and immunoreactive serum insulin (IRI), TAG, and nonesterified fatty acid (NEFA) levels. The blood samples were centrifuged (approximately 8000*g*), and the serum fraction was stored at −20°C in a freezer until analysis. After blood sampling, the rats were killed by intravenous overdose administration of sodium pentobarbital; and visceral (epididymal [EPI] and mesenteric [MES]) and subcutaneous (SUB) adipose tissues were collected immediately. All the tissues were weighed, freeze-clamped in liquid nitrogen, and then stored at −80°C until subsequent analyses.

2.3. Blood analysis

Blood glucose and IRI were determined using standard enzymatic and enzyme-linked immunosorbent assay methods (Glutest Ace; Sanwa Kagaku Kenkyusho, Aichi, Japan, and Rat Insulin ELISA Kit, AKRIN-010; Shibayagi, Gunma, Japan, respectively). Serum lipids (TAG and NEFA) were assayed using standard enzymatic kits (Triglyceride E and NEFA C, respectively; Wako Pure Chemical Industries, Osaka, Japan).

2.4. Fatty acid analysis

The total lipids in each tissue were extracted by modifying the method proposed by Folch et al [13]. Approximately 40 to 50 mg of tissues was homogenized (T18 basic ULTRA-TURRAX; IKA Japan KK, Nara, Japan) in a mixture of 2 mL of chloroform and methanol (2:1, vol/vol). After centrifugation at 1500*g* for 10 minutes, the lower chloroform layer was collected. Triacylglycerol was extracted by modifying the method of Hamilton and Comai [14] using prepacked silica columns (Sep-Pak Vac 1 mL/100 mg; Waters, Milford, MA). The fatty acid components in the TAG fraction were determined via gas chromatography at SRL, Tokyo, Japan. Total lipid was also extracted from the diet given to each group, and fatty acid component were measured.

2.5. Glucose-insulin index

An intravenous glucose tolerance test (IVGTT) was performed in both groups to obtain a glucose-insulin index as an insulin resistance parameter at the end of the 5-week feeding period. Under the fast and stable anesthesia condition described above and immediately after the measurement of basal BG and IRI, a bolus of 50% glucose solution (0.25 mL) was injected intravenously; and BG and IRI were measured several times thereafter over a period of 120 minutes. The glucose-insulin index was calculated as the product of areas under the curves (AUCs) of BG and IRI from 0 to 120 minutes [15,16].

2.6. Statistical analyses

Data were expressed as mean ± SE. The statistical significance of fatty acid concentration data obtained from different anatomical locations in the same dietary group was tested by 1-way analysis of variance (ANOVA) followed by the Tukey post hoc test for multiple comparisons. Comparisons between normal and HFF data were conducted with the 2-sided, unpaired Student *t* test. The significance level was set at $P < .05$.

3. Results

3.1. Body composition and glucose- and lipid-metabolism parameters

Averaged daily fatty acid intake, calculated from the food intake and fatty acid composition of the diet (Table 1), is shown in Table 2. The normal group received approximately 58% and 13% of their calories as carbohydrate and fat, respectively, whereas the HFF group received approximately 21% and 69%, respectively. The body weight was not significantly different between the 2 groups, whereas adipose tissue weights in EPI, MES, and SUB in the HFF group were 1.8-, 1.8-, and 1.6-fold, respectively, of those in the normal rats ($P < .01$, Table 3).

The BG in the HFF group was slightly higher than that in the normal group, although the *P* value was just at the significance level ($P = .05$). The IRI in the HFF group was significantly higher than that in the normal group ($P < .05$, Table 3). The serum TAG in the HFF group was somewhat lower (approximately 67%) than that in the normal group (not significant). No clear difference was detected in NEFA between the groups.

During IVGTT, temporal BG and IRI seemed higher in the HFF group than in the normal group (Fig. 1). Consequently, the glucose-insulin index computed from blood glucose AUC (BG-AUC) and serum insulin AUC (IRI-AUC) was significantly higher in the HFF group than in the normal group ($P < .01$, Table 3).

Table 1 – The fatty acid composition in standard chow and beef tallow

Fatty acid	Standard chow	Beef tallow
12:0	0.6	2.4
14:0	1.5	82.5
16:0	23.8	522.3
16:1n-7	1.6	61.2
18:0	4.6	250.4
18:1n-9	26.1	658.4
18:2n-6	51.1	35.7
18:3n-6	ND	1.2
18:3n-3	4.0	1.8
20:0	0.3	1.6
20:1n-9	0.8	3.5
20:2n-6	ND	0.3
20:3n-6	ND	0.6
20:4n-6	0.2	0.3
20:5n-3	1.9	0.9
22:0	ND	0.3
22:1n-9	ND	0.4
22:6n-3	1.7	ND

Data are expressed in micromoles per gram. ND indicates not detected.

Table 2 – Averaged fatty acid intake from diet per day

Fatty acid	Normal (n = 5)	HFF (n = 5)
12:0	13.0 ± 0.3	20.9 ± 0.4
14:0	33.4 ± 0.7	532.5 ± 8.8
16:0	533.2 ± 11.1	3505.5 ± 56.3
16:1n-7	35.9 ± 0.8	399.5 ± 6.5
18:0	102.0 ± 2.1	1615.7 ± 26.6
18:1n-9	584.2 ± 12.2	4381.3 ± 70.6
18:2n-6	1145.0 ± 23.9	707.9 ± 31.2
18:3n-6	NA	7.5 ± 0.1
18:3n-3	89.3 ± 1.9	49.0 ± 2.4
20:0	6.3 ± 0.1	12.5 ± 0.2
20:1n-9	17.6 ± 0.4	29.3 ± 0.6
20:2n-6	NA	1.6 ± 0.0
20:3n-6	NA	3.5 ± 0.1
20:4n-6	4.3 ± 0.1	3.9 ± 0.1
20:5n-3	41.8 ± 0.9	23.0 ± 1.1
22:0	NA	2.0 ± 0.0
22:1n-9	NA	2.4 ± 0.0
22:6n-3	39.1 ± 0.8	16.5 ± 1.1

Data are expressed as mean ± SE in micromoles per day. NA indicates not applicable.

Table 3 – Body composition and glucose- and lipid-metabolic parameters

	Normal		HFF	
	n		n	
Body weight (g)	5	328 ± 13	5	334 ± 10
Adipose tissue weight (g/100 g body weight)				
EPI	5	1.21 ± 0.09	5	2.17 ± 0.18†
MES	5	1.00 ± 0.06	5	1.81 ± 0.14†
SUB	5	1.62 ± 0.07	5	2.56 ± 0.10†
BG (mg/dL)	5	60 ± 2	5	68 ± 3
Serum				
IRI (ng/mL)	5	0.9 ± 0.1	5	1.5 ± 0.2*
TAG (mg/dL)	5	92 ± 20	5	59 ± 8
NEFA (μEq/L)	5	890 ± 146	5	751 ± 57
BG-AUC (mg/dL·min)	7	9168 ± 161	8	12298 ± 1417
IRI-AUC (ng/mL·min)	7	229 ± 31	8	321 ± 26*
Glucose-insulin index ($U \cdot 10^4$)	7	210 ± 28	8	393 ± 51†

Data are expressed as mean ± SE.
* $P < .05$ vs corresponding data in normal group.
† $P < .01$ vs corresponding data in normal group.

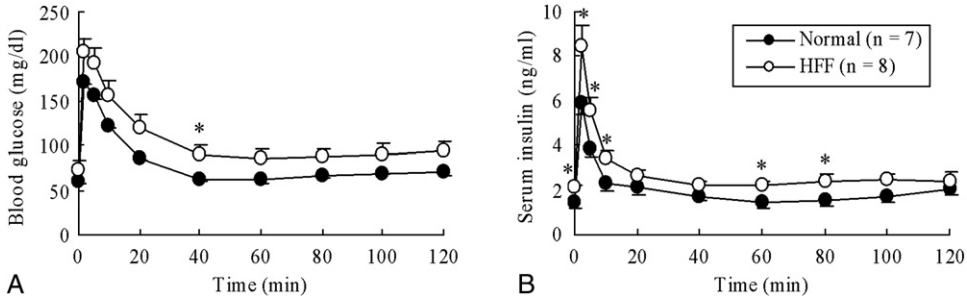

Fig. 1 – Temporal measurements of blood glucose and serum insulin levels. Blood glucose (A) and serum insulin (B) response during 120-minute IVGTT. A bolus of 50% glucose solution (0.25 mL) was injected intravenously at time 0. The data at time 0 were measured immediately before glucose administration. Data are expressed as mean ± SE. Significant differences were detected by *t* test. *P < .05 vs data in the normal group at the same time.

3.2. *Major SFA and MUFA contents in adipose tissues*

In the normal group, 16:1n-7 content in EPI was higher than that in SUB ($P < .05$) (Fig. 2). The other SFA and MUFA did not show any significant differences among the sites. In the HFF group, no differences were observed in any SFA or MUFA among the sites.

In general, stearic acid (18:0) and 18:1n-9 contents in all the sites in the HFF group were higher than those in the normal group ($P < .01$ or $P < .05$, except for 18:0 SUB), whereas palmitic acid (16:0) and 16:1n-7 contents were not significantly different between the 2 groups.

3.3. *n-3 PUFA contents in adipose tissues*

Docosapentaenoic (22:5n-3) and docosahexaenoic acid (22:6n-3) contents in EPI were higher in comparison with those in SUB and MES ($P < .01$) in the normal group (Fig. 3). Similar differences were also seen in linolenic (18:3n-3) and eicosapentaenoic (20:5n-3) acids, although no statistic significance was observed (18:3n-3: EPI, 44.1 ± 1.1; MES, 33.6 ± 7.7; SUB, 28.9 ± 5.9 μmol/g tissue and 20:5n-3: EPI, 14.8 ± 3.9; MES, 4.8 ± 1.9; SUB, 5.4 ± 2.1 μmol/g tissue). Meanwhile, no differences in any of the fatty acids were observed among the sites in the HFF group.

As a whole, 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3 contents in the HFF group were lower than those in the normal group ($P < .01$ or $P < .05$, except for 20:5n-3 contents in MES and SUB).

3.4. *n-6 PUFA contents in adipose tissues*

In the normal group, dihomo-γ-linolenic acid (20:3n-6), 20:4n-6, and docosatetraenoic acid (22:4n-6) contents in EPI were higher than those in SUB and MES ($P < .01$ or $P < .05$, except for 20:3n-6 and 22:4n-6 MES) (Fig. 4) as seen in n-3 PUFAs. No significant differences were observed in the other n-6 PUFAs (ie, linoleic acid [18:2n-6], γ-linolenic acid [18:3n-6], and eicosadienoic acid [20:2n-6]).

In the HFF group, n-6 PUFA contents in general were lower than those in the normal group ($P < .01$ or $P < .05$), as observed for the n-3 PUFA contents, with the exception of 18:3n-6 content in MES and SUB.

4. Discussion

It has been suggested so far that fat accumulation influenced the development of insulin resistance [1,17,18]. In the present study, although no significant difference was detected in body

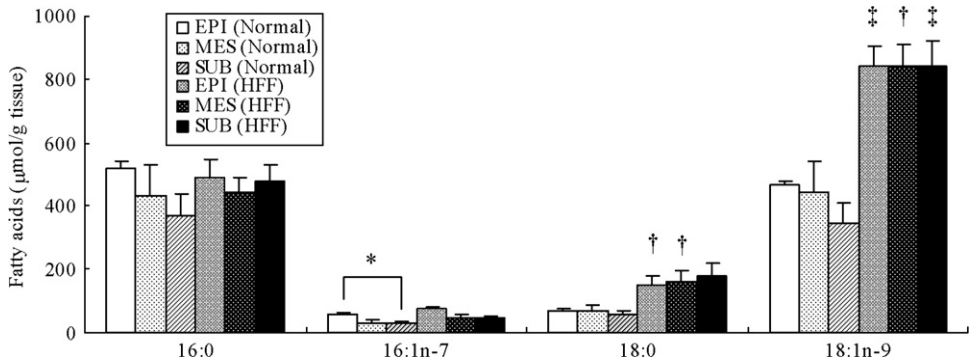

Fig. 2 – Major saturated and monounsaturated fatty acid contents in TAG in adipose tissues. Triacylglycerol fraction was obtained using solid-phase extraction. Fatty acid concentrations were determined with gas chromatography. Data are expressed as mean ± SE (n = 5). Significant differences among anatomical sites in the same group were detected by ANOVA. Comparisons of data between the normal and HFF groups were conducted with *t* test. ‡P < .01; †P < .05 vs corresponding parameter in the normal group. *P < .05.

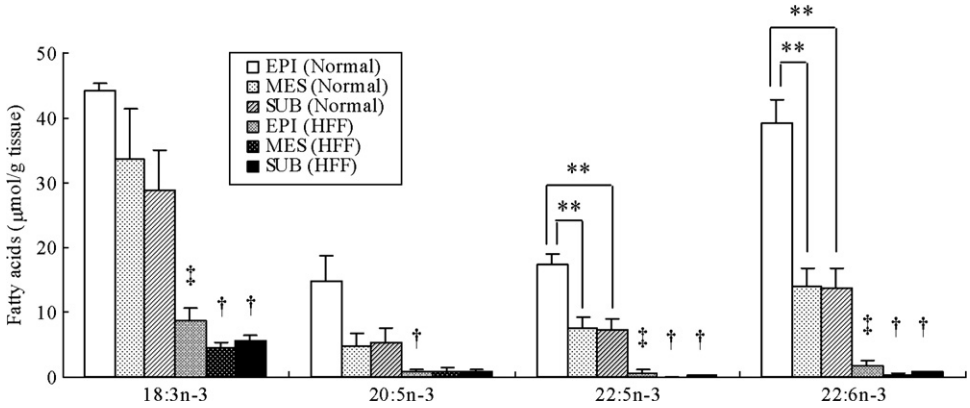

Fig. 3 – The n-3 PUFA contents in adipose tissues. Triacylglycerol fraction was obtained using solid-phase extraction. Fatty acid concentrations were determined with gas chromatography. Data are expressed as mean ± SE (n = 5). Significant differences among anatomical sites in the same group were detected by ANOVA. Comparisons of data between the normal and HFF groups were conducted with *t* test. ‡P < .01; †P < .05 vs corresponding parameter in the normal group. **P < .01.

weight, the adipose tissue weights in the HFF group were significantly higher than those in the normal group (Table 3). These results are consistent with the findings reported by Peyron-Caso et al [6] and Wu et al [7]. The glucose-insulin index (an insulin resistance parameter) was also significantly higher in the HFF group than in the normal one (Table 3). These results indicate that the fat accumulation in adipose tissues as a result of high-fat feeding increased the insulin resistance, which is consistent with the reports by Chalkley et al [19] and Kraegen et al [20]. In addition to the gross amount of adipose tissue, effects of the composition of fatty acid—not only in serum but also in adipose tissue—on the regulation of insulin function were evaluated in the present study.

Griffin et al [21] reported an abnormal insulin signaling cascade in skeletal muscle in high-NEFA rats. Kabayama et al [22] reported that adipocytes play a role in increasing insulin resistance via the inhibition of insulin signaling. Therefore, fat accumulation may result in high insulin resistance via impairment of the insulin signaling pathway.

Tremblay et al [23] reported that fatty acid content in human plasma TAG was involved in high insulin resistance—independent of body fat mass and visceral fat accumulation. In our study, serum TAG in the HFF group tended to be lower than that in the normal group (Table 3). In several studies conducted thus far, plasma TAG tended to decrease after short-term (<3 weeks) [20] or long-term (10 months) [19] exposure to high-fat feeding in rats, whereas a high-carbohydrate diet caused an elevation in serum TAG [24]. The results of the present study are consistent with these previous reports and not with the results of Tremblay et al. This discrepancy may suggest a very important topic in diet therapy for diabetic patients although the effects of high-fat intake on serum TAG in rats may differ from those in humans. Similarly, no elevation was found in serum NEFA.

Regarding the fatty acid composition within the adipose tissues, when the HFF rats had their intake of 18:0, 18:1n-9, 16:0, and 16:1n-7 augmented by 17-, 8-, 7-, and 12-fold, respectively (Table 2), the contents of 18:0 and 18:1n-9 in the HFF group were generally higher than those in the normal group, whereas those of 16:0 and 16:1n-7 were not (Fig. 2). Recent studies have demonstrated that C12-16 fatty acids are elongated to C18 SFA and MUFA by Elovl6 in mice [25,26]. Therefore, Elovl6 might have converted 16:0 and 16:1n-7 into longer-chain fatty acids, ameliorating any difference in 16:0 or 16:1n-7 contents between the normal and HFF groups (Fig. 2). Consequently, an excess of 18:0 and

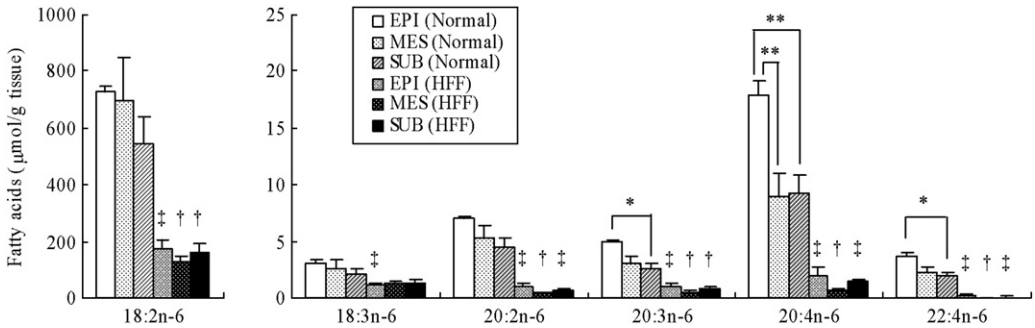

Fig. 4 – The n-6 PUFA contents in adipose tissues. Triacylglycerol fraction was obtained using solid-phase extraction. Fatty acid concentrations were determined with gas chromatography. Data are expressed as mean ± SE (n = 5). Significant differences among anatomical sites in the same group were detected by ANOVA. Comparisons of data between the normal and HFF groups were conducted with *t* test. ‡P < .01; †P < .05 vs corresponding parameter in the normal group. **P < .01; *P < .05.

18:1n-9 might have accumulated in the adipose tissue, especially in the HFF group.

On the other hand, 18:2n-6 content in adipose tissues in the HFF group was significantly lower than that in the normal group (Fig. 4). The intake of 18:2n-6 in the HFF group was two thirds of that in the normal group, whereas the content was approximately one third. 18:2n-6 is known to be the primary material for synthesizing 20:4n-6 [27]. 20:4n-6 is one of the sources of eicosanoids that plays an important role in improving insulin sensitivity. Therefore, the maintenance of insulin sensitivity at the normal level requires 20:4n-6, at least [7]. The results obtained in the present study may indicate that low intake of 18:2n-6 is related to a deficiency in 20:4n-6 and that diet composition may not directly correspond to the fatty acid composition of adipose tissues.

In addition to n-6 PUFA, n-3 PUFA contents in the HFF group were generally lower than those in the normal group (Fig. 3). However, the mechanism responsible for the relationship between low n-3 PUFA and insulin resistance has not been documented in sufficient detail thus far.

It has been reported that n-3 PUFA regulates PPARs and SREBP1c activities [9,28,29] and that 20:5n-3 and 22:6n-3, in particular, suppress the production of tumor necrosis factor–α, interleukin-1, and interleukin-6, which were involved in increasing the insulin resistance in an in vitro study and in humans [10,11]. Therefore, the insulin resistance may be attributed to the lack of not only n-6 but also n-3 PUFA.

Surprisingly, significant differences in the contents of 16:1n-7 (Fig. 2), 22:5n-3, 22:6n-3 (Fig. 3), 20:4n-6, and 22:4n-6 (Fig. 4) were observed between EPI and the other adipose tissues (MES and SUB) in the normal group. Thus, the composition of at least these fatty acids appears to depend on anatomical location, which may affect the function and/or properties of adipose tissue—probably including insulin sensitivity. Unlike our original expectation, however, the fatty acid composition in SUB was similar to that in MES. Although the detailed mechanisms of site dependency were not determined, the results at least suggest that it is difficult to distinguish the properties of adipose tissue based only on their "visceral" or "subcutaneous" sites.

Interestingly, the clear site difference observed in the normal group was not observed in the HFF group. As the 5 fatty acids discussed above are known to play an important role in regulating insulin sensitivity [27,30], the absence of difference may be one of the causes of lower insulin sensitivity. Despite the shortage of polyunsaturated fatty acid intake, more PUFA might have been consumed to maintain the insulin sensitivity at its normal level in the HFF condition. As a result, the normal balance of fatty acid distribution might have been disrupted. The findings reported by Tran et al [12] mentioned in the "Introduction" (ie, the functional difference between subcutaneous and visceral adipose tissues) may support our results. To the best of our knowledge, this is the first report of the site dependency of fatty acid composition in TAG composing adipose tissue and the disappearance of this site dependency in HFF rats.

In conclusion, high-fat feeding in rats caused increases in BG, IRI, and glucose-insulin index, along with elevation of 18:0 and 18:1n-9 contents in adipose tissue, and decreased the contents of n-3 and n-6 PUFA. Significant differences in some PUFA contents were observed among the anatomical locations in the normal group but not in the HFF group, which suggests that the fatty acid distribution is site dependent and that this difference may be important for maintaining the insulin sensitivity. The findings obtained in the present study are summarized in Table 4.

Table 4 – Summary of the present study

	Normal rats	HFF rats
SFA and MUFA		
Contents	–	High
Site dependency	EPI ≈ MES ≈ SUB	EPI ≈ MES ≈ SUB
PUFA		
Contents	–	**Very low**
Site dependency	**EPI > MES ≈ SUB**	**EPI ≈ MES ≈ SUB**
BG, IRI, and glucose-insulin index	–	High

In the present study, we focused on the properties and composition of fatty acids in TAG in adipose tissue and the relationship with insulin function because 90% to 99% of adipose tissue lipid is TAG [31]. In addition to the TAG, the fatty acid composition in phospholipid could be associated with insulin sensitivity in humans [32,33]. It may, therefore, be of interest also to study the phospholipid and cholesterol, although their fractions were considerably smaller than that of TAG.

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Conflicts of Interest

The authors report no conflicts of interest.

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