Role of dietary fat type in the development of adiposity from dietary obesity-susceptible Sprague–Dawley rats

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The present study was designed to define how dietary fat type regulates body adiposity in dietary obesity-susceptible (DOS) Sprague–Dawley (SD) rats. Eighty-three SD rats received a purified diet containing 50 g maize oil (MO)/kg for 3 weeks and then thirty-nine of the rats, designated as the DOS rats, were allotted to diets containing 160 g MO (DOS-MO), beef tallow (DOS-BT) or fish oil (DOS-FO)/kg for 9 weeks. As a result of the experiment, the DOS-FO rats had significantly (P<0.05) reduced weight gain and abdominal and epididymal fat-pad mass than the DOS-MO and DOS-BT rats. Serum leptin level was also significantly (P<0.05) lower in the DOS-FO rats; however, hypothalamic leptin receptor (a and b) mRNA and neuropeptide Y expressions were not altered by dietary fat sources. A lower acetyl-CoA carboxylase mRNA expression in the liver was observed in the DOS-FO group, whereas hepatic peroxisome proliferator-activated receptor-γ mRNA and protein expressions were markedly elevated in the DOS-FO group compared with those in the other groups. We did not observe differences in acetyl-CoA carboxylase and peroxisome proliferator-activated receptor-γ expressions in epididymal fat of the DOS rats consuming MO, BT or FO. It is concluded from our present observations that dietary fat type, especially that rich in FO, plays a potential role in down-regulation of adiposity by altering hepatic lipogenic genes, rather than feeding behaviour, in the DOS-SD rats.

**Fat type:** Leptin: Hypothalamic leptin receptor: Neuropeptide Y: Acetyl carboxylase

**CoA:** Peroxisome proliferator-activated receptor-γ

There has been a considerable interest in the role of dietary fat type in the development of adiposity. However, the mechanisms by which dietary fat composition modulates body adiposity are still not conclusive, as several contradictory studies exist. It has been demonstrated that dietary fat type could have different regulatory effects on body-fat accumulation, adipogenesis and fat oxidation, although the development of adiposity depends in part on the strain, sex and age of animals (West & York, 1998; Jump & Clarke, 1999). However, the reported effect of dietary fat type, supplied by either polyunsaturated fatty acids (PUFA) or saturated fatty acids, on body weight, adipogenesis or lipolysis in rats varies (Awad et al. 1990; Hill et al. 1992; Cha & Jones, 1998). There is a lack of evidence on the responses of dietary n-3 and n-6 fatty acids to serum leptin level and leptin signalling pathway, although dietary PUFA can influence the composition of cellular membrane lipids and transmembrane receptor activities (Heshka & Jones, 2001). Moreover, dietary composition of PUFA varies significantly with respect to its action on adiposity, even though both n-3 and n-6 PUFA appear to regulate fat synthesis and subsequent fat accumulation in numerous studies (Sessler & Ntambi, 1998; Clarke, 2000). Thus, the goal of the present study was to investigate how dietary fatty acid composition modulates central and peripheral metabolic processes to regulate body adiposity.

**Abbreviations:** ACC, acetyl-CoA carboxylase; BT, beef tallow; DOS, dietary obesity-susceptible; FO, fish oil; MO, maize oil; NPY, neuropeptide Y; Ob-R, hypothalamic leptin receptor; PPARγ, peroxisome proliferator-activated receptor-γ; SD, Sprague–Dawley.

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Leptin, the circulating 16 kDa hormone secreted from adipocytes, is believed to regulate food intake and body weight via the hypothalamic leptin receptor (OB-R) and subsequently affects neuropeptide Y (NPY; Spiegelman & Flier, 1996). Obese animals had higher serum leptin levels compared with normal-weight animals, suggesting that leptin level could be an index of adiposity in animals (Caro et al. 1996). However, it has also been suggested that dietary fat type, independent of body-fat mass, plays a crucial role in the plasma leptin level in dietary obesity-induced rodents (Cha & Jones, 1998). As well as the central mechanism of leptin’s obesity control, peripheral tissue activated receptor-γ (PPARγ) are considered as the key adipogenic markers to influence body-fat mass (Clarke & Jump, 1994). It is reasonable to investigate the regulatory action of dietary fat type on the adipogenic genes, since ACC and PPARγ are directly modulated by dietary substrate in the liver and adipocytes (Jump & Clarke, 1999).

The discrepancy between dietary fat type and body-fat accumulation in many studies may be partly due to the genetic background of experimental animals. Therefore, care must be taken to consider the physiological relevance of the rodent models when we evaluate the effect of dietary fat type on the development adiposity. Among various animal models, Sprague–Dawley (SD) rats are able to reveal accurately the mechanisms that are applicable to polygenic animal obesity, because one-half of SD rats appear to develop obesity when fed diets moderately high in energy and fat (Lauterio et al. 1994). In an effort to minimize genetic variability in dietary obesity-susceptibility (DOS), therefore, the present study was undertaken with the selected DOS-SD rats as a model animal to investigate the effect of dietary fat type including maize oil (MO), beef tallow (BT) and fish oil (FO) as a sole fat source on serum leptin, hypothalamic OB-R and neuropeptide Y (NPY), and peripheral ACC and PPARγ expressions.

Methods

Experimental animals, diet and procedures

Male SD rats (Charles River, Atsugi, Japan) were maintained in polycarbonate cages under barrier-system-regulated temperature (23 ± 2°C), humidity (50 ± 10 %) and light–dark cycle conditions. Eighty-three rats, aged 6 weeks and weighing 160–200 g, received a purified diet containing 50 g MO/kg for 3 weeks; the SD rats were then divided into two categories (higher weight-gainer, lower weight-gainer) on the basis of body-weight gain. Then, the heaviest rats (n 39, aged 9 weeks) in the higher-weight-gain group were designated as DOS-SD rats. The remaining forty-four rats were not used for the present study. These DOS-SD rats were allotted to the DOS-MO (160 g MO/kg), DOS-BT (160 g BT/kg) and DOS-FO (160 g FO/kg) groups by body weight and were given the purified diet individually and continuously ad libitum for another 9 weeks. Weekly body weight and daily food intake were measured throughout all studies. All animal experiments were approved by our laboratory animal care and use committee (accredited by AAALAC, Rockville, MA, USA) and were performed in compliance with our committee guidelines.

The diets differed only in fat type, as shown in Table 1. The primary differences among the three dietary fat types were higher levels of linoleic acid (n-6) and oleic acid (n-9) in the MO diet, higher amounts of oleic acid and stearic acid in the BT diet and higher levels of eicosapentaenoic acid (n-3) and docosahexaenoic acid (n-3) in the FO diet. After the 9-week feeding trial, all rats were killed with anaesthetizing sodium pentobarbital (100 mg/kg), following 4–6 h food deprivation at a fixed time between 14.00–16.00 hours. Blood was drawn by heart puncture and serum was isolated. The liver, hypothalamus, abdominal fat and epididymal fat were collected, weighed and rapidly frozen in liquid N2. The tissues were stored at −70°C until further assay.

Semiquantification of hypothalamic leptin receptor, acetyl-CoA carboxylase and peroxisome proliferator-activated receptor-γ mRNA

Total RNA was isolated from the hypothalamus, liver and epididymal fat by the methods of RNAsol B (Tel-Test, Friendswood, TX, USA). Briefly, 100 mg tissue was removed from each organ and added to 1 ml RNAsol solution. The samples were cut with scissors and homogenized using a glass–glass homogenizer. The lysate was transferred to microcentrifuge tube and added to 0·1 vol. chloroform to remove protein extract. The aqueous phase was separated by

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DOS-MO</th>
<th>DOS-BT</th>
<th>DOS-FO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>MO oil*</td>
<td>16</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>BT†</td>
<td>−</td>
<td>16</td>
<td>−</td>
</tr>
<tr>
<td>FO‡</td>
<td>−</td>
<td>−</td>
<td>16</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Starch</td>
<td>49</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>α-Methionine</td>
<td>0·3</td>
<td>0·3</td>
<td>0·3</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0·2</td>
<td>0·2</td>
<td>0·2</td>
</tr>
<tr>
<td>AIN-76 mineral mix</td>
<td>3·5</td>
<td>3·5</td>
<td>3·5</td>
</tr>
<tr>
<td>AIN-76 vitamin mix</td>
<td>1·0</td>
<td>1·0</td>
<td>1·0</td>
</tr>
<tr>
<td>Energy (kJ/kg)</td>
<td>18,630</td>
<td>18,630</td>
<td>18,630</td>
</tr>
<tr>
<td>Fat (% energy)</td>
<td>32·34</td>
<td>32·34</td>
<td>32·34</td>
</tr>
</tbody>
</table>

DOS, dietary obesity-susceptible rats; MO, maize oil; BT, beef tallow; FO, fish oil.
*MO contains (g/100 g total fat): linoleic acid (18:2 n-6) 57·4, palmitic acid 13·3, oleic acid (18:1 n-9) 26·0.
†BT contains (g/100 g total fat): oleic acid 41·0, palmitic acid 25·1, stearic acid 15·6.
‡FO (Pronova Biocare, CO., Aalesund, Norway) contains: eicosapentaenoic acid (20:5 n-3) 18·0, docosahexaenoic acid (22:6, n-3) 12·0.
centrifugation for 15 min at 15 000 rpm. Total RNA was pre-
cipitated with the same volume of isopropanol and cen-
trifuged for 15 min at 15 000 rpm. The precipitated total
RNA washed with ethyl alcohol (750 ml/l) was dried and
diluted with diethyl pyrocarboxylate-treated water. The con-
centration of isolated total mRNA was determined by spe-
trophotometer and confirmed on agarose (1-0 %) gel stained
with ethidium bromide. Semi-quantification of mRNA using
reverse transcription–polymerase chain reaction was per-
fomed to quantify mRNA of the leptin receptors, such as
extracellular region common to all isoforms (OB-Ra,
814 bp) and intracellular domain of the long form (OB-Rb,
511 bp), ACC (580 bp) and PPAR
814 bp) and intracellular domain of the long form (OB-Rb,
511 bp), ACC (580 bp) and PPAR

reverse transcription–polymerase chain reaction was per-
formed for thirty cycles of

denaturation at 94°C for 30 s, annealing at 62°C for 30 s
and extension at 72°C for 7 min with polymerase chain rea-
tion system in a reaction mixture containing 10 pmol primer,
0.5 µg cDNA, 2 mM-dNTP and 1 unit Taq polymerase.
The products were confirmed on agarose (1-0 %) gel stained with
ethidium bromide. The cDNA primers to amplify each gene
were as follows: 5'-TGGGTTGTCTATCTCTGAAAGTAAAG-
3’ and 5'-AATTGCTTCTTGGTTGGCTG-3’ for OB-Ra
(gene bank accession no U52966), 5’-TATGGAGGAGT-
TGAAAAACC-3’ and 5’-TAACCTGAGGGTTGACCTC-
TGAC-3’ for OB-Rb (gene bank accession no U52966), 5’-
GACAGGTTCAAGTGAAGTC-3’ for PPAR
5’-CTGTTCCAGAGCT-3’ and 5’-GTGGGGCGCCCCAGGCACCAGGGC-3’
for PPAR

\[\text{EDAS 120; Kodak, New Haven, CT, USA.}\]

Levels of all
mRNA were expressed as the value of signal intensity for
genes relative to that for β-actin.

Immunohistochemical assay of hypothalamic
neuropeptide Y

Immunohistochemical assays were performed as previously
described (Huh et al. 1997). In brief, male SD rats (n = 3)
were perfused with freshly prepared paraformaldehyde (40 ml/l
0-1 M-PBS, pH 7-4). The removed brains were post-fixed in
the same fixative overnight and continuously cryoprotected
with sucrose solution (200 g/l 50 mM-PBS, pH 7-4)
for 48 h. Frozen sections of 40 µm thickness in the coronal
plane were stained for immunohistochemical detection of
NPY using rabbit polyclonal antiserum (Incstar Corp.,
Stillwater, MN, USA). Free-floating sections were
incubated for 72 h in PBS (4°C) containing anti-NPY
antiserum (1:2000 dilution), Triton X-100 (3 ml/l), bovine
serum albumin (0.5 mg/ml) and normal goat serum
(15 ml/l). Sections were then incubated with goat anti-
rabbit secondary antibodies (1:100; Vector, Burlingame,
CA, USA) for 90 min and with avidin–biotin peroxidase
complex (1:100; Vector) for 1 h at room temperature.
Sections were reacted with 3,3′-diaminobenzidine tetry-
hydrochloride (0-2 g/l) and H2O2 (0-1 ml/l) for 3 min.

Western immunoblot assay of peroxisome
proliferator-activated receptor-γ

Briefly, protein extracts were prepared from the liver and
epididymal fat by polytron homogenization in an extraction
buffer containing 40 g SDS/l and 10000 g centrifugation at
4°C for 30 min. Protein concentration was assessed by
bicinchoninic acid kit (Pierce, Rockford, IL, USA). Protein
(50 µg) was applied to SDS (100 g/l)–PAGE gel and the
fractionated proteins were transferred to Hynond-P nitrocel-
 lulose membranes (Amersham, Piscataway, NJ, USA).
Membranes were first incubated with antibody against poly-
clonal PPARγ, which recognized only N-terminal portion of
PPARγ (1-400; Santa Cruz Biotech, Santa Cruz, CA, USA),
for 1 h at 25°C, washed immediately three times and then
incubated with a secondary goat anti-mouse immunoglobulin
G horseradish (Armoracia rusticana) peroxidase (1:1000; Amersham). The protein band was visualized by
ECL Western blotting system (Amersham).

Statistical analysis

One-way ANOVA, followed by Tukey’s multiple range
test, was applied to analyse the results obtained from the
three groups of the DOS rats fed MO, BT and FO. The
level of probability for statistical difference was estab-
lished at P<0.05.

Results

Weight gain, feed intake and body-fat mass

Body-weight gain and food intake in response to dietary fat
type were monitored in all groups of the DOS-SD rats
throughout the experimental period (Table 2, Fig. 1). When
the effect of dietary fat type on weight gain after 9
weeks of feeding was examined, the DOS-FO rats (266.5
(SE 7.24) g) showed 12.3 and 11.3 % lower (P<0.05)
weight gain compared with the DOS-MO (299.3 ( SE
0.53) g) and DOS-BT (296.7 ( SE 7.23) g) groups fed MO
and BT respectively (Table 2). However, body weight and
mean daily food intake on a cumulative and weekly basis
were not statistically different among the three dietary
groups (Table 2, Fig. 1). Fig. 2 shows changes in abdominal
and epididymal fat-mass obtained from the DOS-SD rats fed
MO, BT and FO. Total abdominal fat-pad weights were
31.5 and 25.1 % lower (P<0.05) in the DOS-FO rats (68.6
(SE 0.27) g) compared with the DOS-MO (90.2 ( SE 0.53) g)
and the DOS-BT (85.8 ( SE 0.34) g) rats respectively. Total
epididymal fat-pad weights were also 61.2 and 44.5 %
decreased in the DOS-FO group (7.38 ( SE 0.43) g) compared
with the DOS-MO (11.90 ( SE 0.37) g) and DOS-BT
(12.4 (SE 1.01) g) rats respectively. When body-fat mass was expressed per 100 g body weight, the statistically significant differences remained ($P, 0.05$). The decreased abdominal and epididymal fat deposits in the DOS-FO rats partly reflected a lower weight gain.

**Serum biochemical analyses**

The DOS-FO group showed a 29.6% lower ($P<0.05$) circulating leptin level (10.56 (SE 0.91) ng/ml) in the absence of considerable change in insulin concentration compared with the DOS-MO group (18.14 (SE 1.51) ng/ml; Fig. 3). Moreover, the DOS-FO rats had a 45.7% decrease ($P<0.05$) in serum leptin level with a significantly lower serum insulin ($238$ %) in comparison with the DOS-BT rats (17.03 (SE 1.58) ng/ml).

**Expressions of hypothalamic leptin receptor, neuropeptide Y, acetyl-CoA carboxylase and peroxisome proliferator-activated receptor-γ**

To examine the possibility of altered regulation of leptin’s signalling pathway in the hypothalamus in response to dietary fat type (a lower circulating leptin level by FO), we investigated the expressions of hypothalamic OB-R mRNA and NPY by reverse transcription–polymerase chain reaction and immunohistochemical assay respectively (Figs 4 and 5). Dietary fat type did not noticeably affect hypothalamic OB-Ra and -Rb mRNA expressions with the DOS-MO and DOS-BT groups. When the response of NPY to dietary fat type in the arcuate and paraventricular nuclei of the hypothalamus was examined by immunohistochemical staining, the distributions of NPY expression in both nuclei were similar among treatments (Fig. 5). Therefore, dietary fatty acid composition (or lower serum leptin) did not significantly modulate hypothalamic OB-R and neuropeptide Y in the DOS-rats.

In order to examine the effect of fat type on peripheral adipogenesis in our DOS-SD model, ACC and PPARγ expressions in the liver and epididymal adipose tissue are compared in Figs 6 and 7. ACC is one of important key enzymes for synthesis of fatty acids in the liver and adipose tissue, and PPARγ appears to play a primary role in adipocyte differentiation and the storage of lipids. As a result, hepatic ACC mRNA expression was markedly ($P<0.05$)

**Table 2.** Effect of dietary fat type on body weight, weight gain and food intake over entire period from the dietary–obesity susceptible (DOS)-Sprague–Dawley rats fed the diets containing 160 g maize oil (DOS-MO), beef tallow (DOS-BT) or fish oil (DOS-FO)* (Mean values with their standard errors for thirteen rats per group)

<table>
<thead>
<tr>
<th></th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Weight gain over entire period (g)</th>
<th>Daily food intake over entire period (g/d)</th>
<th>Cumulative food intake over entire period (g/9 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>DOS-MO</td>
<td>314.03</td>
<td>5.48</td>
<td>613.33</td>
<td>15.51</td>
<td>299.30</td>
</tr>
<tr>
<td>DOS-BT</td>
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<td>5.29</td>
<td>610.50</td>
<td>10.83</td>
<td>296.70</td>
</tr>
<tr>
<td>DOS-FO</td>
<td>311.19</td>
<td>5.37</td>
<td>577.69</td>
<td>7.74</td>
<td>266.50</td>
</tr>
</tbody>
</table>

*a,bMean values in the same column with unlike superscript letters were significantly different ($P<0.05$).

* For details of diets and procedures, see p. 430.
lower in the DOS-FO as compared with that in the DOS-MO and DOS-BT rats. In contrast, hepatic PPARγ mRNA expression was significantly (P < 0.05) higher in FO-fed DOS rats than in the other fat-fed DOS rats (Fig. 6). Western immunoblot assay of PPARγ also showed that hepatic PPARγ was noticeably greater in the DOS-FO rats (Fig. 7). However, we could not observe marked alterations in ACC and PPARγ mRNA expressions in the epididymal fat of the DOS-SD rats fed various fat sources including MO, BT and FO (Fig. 6). In addition, Western immunoblot of epididymal fat PPARγ protein was not markedly changed by dietary fat sources (Fig. 7).

**Discussion**

The responses of dietary fat type to the regulation of body adiposity are not consistent in the literature in which opposite results have sometimes been reported (Cha & Jones, 1998; Loh et al., 1998; Reseland et al., 2001). The reason for this discrepancy may be due to dietary factors and to the genetic backgrounds of the animals. To overcome these limitations, we used the DOS-SD rat to investigate the role of dietary fat type in the development of adiposity.

Dietary fat type, as well as amount of fat, are also thought to be important factors in determining body adiposity and adipogenesis (Takahashi & Ide, 2000). However, several studies have reported that dietary fat supplied by either PUFA or saturated fatty acids did not markedly affect body weight, feed efficiency and lipogenesis in rats (Awad et al., 1990; Hill et al., 1992). SD rats fed FO and safflower oil had a markedly increased plasma leptin level compared with rats fed BT, despite smaller body-fat mass in the rats fed FO (Cha & Jones, 1998). In our present study, in contrast to the studies mentioned earlier, the DOS-FO rats had a marked decrease in weight gain, fat-pad mass and serum leptin level compared with the DOS-MO and DOS-BT groups. It has been reported that a diet enriched with n-3 fatty acids decreased plasma leptin level and leptin mRNA in rat epididymal adipose tissue (Reseland et al., 2001). Several studies have indicated that a high-fat diet increases body fat, with a substantial increase in serum leptin level (Ahren et al., 1997; Bahceci et al., 1999). Circulating leptin level was generally positively correlated with adiposity in human subjects and rodents (Frederich et al., 1995; Caro et al., 1996), although energy balance other than body-fat mass is likely to influence leptin production and secretion (Havel, 1998). It is thus reasonable to conclude that decreased body-fat deposition by feeding FO rich in n-3 fatty acids...
reduces synthesis and secretion of leptin under normal physiological status in the DOS rats.

Leptin regulates food intake and body weight via hypothalamic OB-R (Flier & Maratos-Flier, 1998) and consequently modulates NPY in the hypothalamus. Whether dietary fatty acid profiles would affect leptin’s signalling pathway has rarely been investigated, although dietary fatty acid composition is known to alter membrane fluidity in various tissues and to alter serum leptin levels, which could lead to changes in body-fat accumulation. We thus tried to determine whether dietary fat type and a lower circulating leptin level would affect hypothalamic OB-R and NPY in the DOS rats. In the present study, dietary n-3 and n-6 fatty acids did not affect the expression of

![Image](https://www.cambridge.org/core/journals/Kumar/434.png)

**Fig. 5.** Neuropeptide Y immunohistochemical assay in the hypothalamus (A, arcuate nucleus; B, paraventricular nucleus) of the dietary obesity-susceptible (DOS) Sprague–Dawley rats fed diets containing 160 g maize oil (DOS-MO), beef tallow (DOS-BT) or fish oil (DOS-FO)/kg as a major fat source. For details of diets and procedures, see Table 1 and p. 430.

![Image](https://www.cambridge.org/core/journals/Kumar/434.png)

**Fig. 6.** Semi-quantification of mRNA expressions of acetyl-CoA carboxylase (ACC) and peroxisome proliferator-activated receptor-γ (PPARγ) in the liver (A) and epididymal fat (B) of dietary obesity-susceptible (DOS) Sprague–Dawley rats fed diets containing 160 g maize oil (DOS-MO, ■), beef tallow (DOS-BT, □) and fish oil (DOS-FO, ▼)/kg. For details of diets and procedures, see Table 1 and p. 430. Levels of all mRNA were expressed as the value of signal intensity for genes relative to that for β-actin. Values are means for five rats per group with standard errors shown by vertical bars. a,bMean values with unlike superscript letters were significantly different among dietary groups (P<0.05).
hypothalamic OB-R and NPY, although the role of fat type in OB-R functions, such as leptin binding and transport in the hypothalamus, remains to be elucidated. In addition, a lower circulating leptin level in the DOS rats fed FO did not induce leptin action on hypothalamic OB-R mRNA; this is in agreement with a study suggesting that increased serum leptin was not enough to provoke up-regulation of OB-R expression (Lin et al. 2000). It is likely that circulating serum leptin in the DOS rats is not involved in the regulation of hypothalamic OB-R expression. It is possible that the OB-R binding capability or signalling pathway of leptin in the hypothalamus of the DOS rats is impaired. Caro et al. (1996) reported that the high concentration of serum leptin in obesity was not reflected by a proportional increase in cerebrospinal fluid leptin in numerous animals. Burguer et al. (2000) also reported that leptin levels in cerebrospinal fluid were similar among lean Zucker, dietary-induced obese and genetically obese Zucker rats, although circulating plasma leptin levels were much higher in both obese rat groups. With DOS Osborne–Mendel rats, mRNA levels of OB-Ra and -Rb expression were not altered by a high-fat diet, but post-transcriptional expression of OB-R was markedly decreased by a high-fat diet (Madiehe et al. 2000). However, Wistar rats fed a high-fat diet (340 g/kg) for 14 weeks showed up-regulation of OB-Ra mRNA in the blood–brain barrier, although plasma leptin level was not changed by a high-fat diet (Boado et al. 1998). The NPY regulation by dietary fatty acid composition has not been studied in detail so far, although NPY expression is known to be highly regulated by dietary fat amount and energy restriction. Several studies have demonstrated that dietary fat amount did not modulate compensatory NPY expression in DOS rats and mice compared with counterpart animals (Bergen et al. 1999; Lauterio et al. 1999). Thus, it seems that DOS rats have NPY already expressed at a high level and this could lead to dysregulation of NPY expression in response to dietary fat (Levin & Dunn-Meynell, 1997). Overall, dietary fatty acid composition did not promptly affect hypothalamic OB-R and NPY expression in the DOS animals after prolonged feeding periods.

As dietary fat type and a lower leptin level did not alter in hypothalamic OB-R and NPY expressions, we investigated changes in lipogenic gene expression in the liver and epididymal fat. Dietary fat type may regulate expression of key lipogenic enzymes such as ACC (Kim et al. 1999). PPARγ, especially PPARγ2, has been shown to be induced by high dietary fat and PUFA, leading to changes in lipogenic gene expression and fatty acid metabolism in the liver and adipose tissue (Tugwood et al. 1996; Sessler & Nambi, 1998). In the DOS-FO rats fed 160 g FO/kg there was a decrease in ACC mRNA expression in the liver, but not in the adipocyte. Such a reduced ACC mRNA expression could result in down-regulation of de novo lipogenesis in the liver and consequently reduced serum triacylglycerol (Clarke & Jump, 1994; Harris et al. 1997). The mechanism by which n-3 fatty acids present in FO regulate lipogenic enzymes has been extensively studied. Several studies have indicated that n-3 fatty acids modulated lipogenic enzymes through both PPARγ-dependent and -independent mechanisms of controlling adipogenesis (Clarke & Jump 1994; Kliwer et al. 1997). Physiological concentrations of n-3 fatty acids have been associated directly with enhanced PPARγ to activate adipocyte differentiation, and increased PPARα to induce peroxisomal β-oxidation (Reddy & Mannaehts, 1994). Even if enhanced adipogenesis is commonly associated with obesity, increased adipogenesis by a greater PPARγ response to dietary FO is strongly linked with a greater increase in insulin sensitivity and insulin receptors in peripheral tissues (Spiegelman & Flier, 1996). In particular, induction of the phosphorylated form of PPARγ has been considered as an anti-adipogenic factor acting by down-regulating transcriptional activity (Hu et al. 1996; Qian et al. 1998). However, feeding dietary MO to the DOS-SD rats did not induce changes in body-fat mass, leptin and hepatic lipogenic genes compared with feeding BT, although dietary fat enriched in n-6 fatty acids has been reported as a potent inhibitor of hepatic lipogenesis (Jump & Clarke, 1999). In particular, induction of the phosphorylated form of PPARγ has been considered as an anti-adipogenic factor acting by down-regulating transcriptional activity (Hu et al. 1996; Qian et al. 1998). However, feeding dietary MO to the DOS-SD rats did not induce changes in body-fat mass, leptin and hepatic lipogenic genes compared with feeding BT, although dietary fat enriched in n-6 fatty acids has been reported as a potent inhibitor of hepatic lipogenesis (Jump & Clarke, 1999). In particular, induction of the phosphorylated form of PPARγ has been considered as an anti-adipogenic factor acting by down-regulating transcriptional activity (Hu et al. 1996; Qian et al. 1998). However, feeding dietary MO to the DOS-SD rats did not induce changes in body-fat mass, leptin and hepatic lipogenic genes compared with feeding BT, although dietary fat enriched in n-6 fatty acids has been reported as a potent inhibitor of hepatic lipogenesis (Jump & Clarke, 1999). In particular, induction of the phosphorylated form of PPARγ has been considered as an anti-adipogenic factor acting by down-regulating transcriptional activity (Hu et al. 1996; Qian et al. 1998). However, feeding dietary MO to the DOS-SD rats did not induce changes in body-fat mass, leptin and hepatic lipogenic genes compared with feeding BT, although dietary fat enriched in n-6 fatty acids has been reported as a potent inhibitor of hepatic lipogenesis (Jump & Clarke, 1999).
rather than leptin’s central signalling pathway. Our present observations also suggest that dietary fat type is an important and feasible means to regulate body-fat stores in DOS animals. Much intensive work is still needed to define the role of dietary fatty acid profiles involved in leptin’s biological actions on appetite, energy expenditure and nutrient absorption in obesity.

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