EXPERIMENTAL STUDY

Differential effects of dietary saturated and trans-fatty acids on expression of genes associated with insulin sensitivity in rat adipose tissue

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Abstract

Objective: Trans-fatty acids (TFAs) are formed during partial hydrogenation of vegetable oils and are shown to be more atherogenic than saturated fatty acids (SFAs). Our previous study showed that dietary TFAs decrease adipose tissue insulin sensitivity to a greater extent than SFAs in rats. We hypothesized that the effects of these fatty acids on insulin sensitivity could be mediated through an alteration in gene expression. In the current study we have investigated the effects of dietary TFAs or SFAs on expression of genes associated with insulin sensitivity in rat adipose tissue.

Design and methods: Male weanling Wistar/NIN rats were divided into four groups and fed one of the following diets containing 10% fat (g/100 g diet) differing only in the fatty acid composition for 3 months: control diet (3.7% linoleic acid (LA)), SFA diet (5% SFA), TFA diet 1 (1.5% TFA + 1% LA) and TFA diet 2 (1.5% TFA + 2% LA). The mRNA expression of peroxisome proliferator-activated receptor γ (PPARγ), lipoprotein lipase (LPL), glucose transporter-4 (GLUT4), resistin and adiponectin was analyzed in epididymal fat using RT-PCR. The effects of TFA were studied at two levels of LA to understand the beneficial effects of LA over the effects of TFA.

Results: Both dietary SFA and TFA upregulated the mRNA levels of resistin. Dietary SFA downregulated adiponectin and GLUT4 and upregulated LPL, while TFA downregulated PPARγ and LPL. The effects of dietary TFA on PPARγ and resistin were not counteracted by increased LA (TFA diet 2).

Conclusion: The effects of SFAs on the aforementioned genes except PPARγ could be extrapolated towards decreased insulin sensitivity, while only the alteration in the mRNA levels of PPARγ and resistin could be associated with insulin resistance in TFA-fed rats. These findings suggest that dietary SFAs and TFAs alter the expression of different genes associated with insulin sensitivity in adipose tissue.

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Introduction

Dietary fatty acids influence both stored and structural lipids, which in turn alter several physiological functions such as the physical state of the membrane (fluidity), hormone binding, signal transduction (1) and eicosanoid production (2). Recent reports show that long-chain derivatives of dietary n-6 and n-3 polyunsaturated fatty acids (PUFAs) regulate the proteins involved in lipid and carbohydrate metabolism at the level of gene expression (3–7).

The oxidative products of n-6 PUFAs are effective ligands for the activation of peroxisome proliferator-activated receptor γ (PPARγ) (8). PPARγ has been shown to exert profound effects on insulin sensitivity (9, 10). PPARγ regulates the expression of adipocyte-derived circulating hormones (adipocytokines) such as adiponectin (11) and resistin (12, 13) through the PPAR response element. Serum adiponectin levels are decreased in type 2 diabetes and obesity (14–19). However, the levels of adiponectin are increased in insulin-resistant subjects treated with PPARγ-dependent insulin sensitizers (20, 21). Resistin is a recently identified protein which is associated with insulin resistance in murine models (22). However, human studies have failed to correlate the levels of resistin with the development of insulin resistance or obesity (18, 19, 23–25). Reports showed that ligand-activated PPARγ and dietary n-3 PUFAs upregulate the expression of glucose transporter-4 (GLUT4), an insulin-responsive membrane protein which is involved in facilitative glucose transport (26, 27). Lipoprotein lipase (LPL) is the rate-limiting enzyme in lipoprotein metabolism, which acts on lipoproteins and releases diacylglycerol and free fatty acids.
(FFAs), which are known to decrease insulin sensitivity (28). In a recent study, overexpression of the LPL gene has been implicated in insulin resistance in rat skeletal muscle and liver (29).

Our previous observations showed that feeding both saturated fatty acids (SFAs) and trans-fatty acids (TFAs) decreased insulin sensitivity in rat adipocytes, as evidenced by increased plasma levels of insulin, triglycerides and the homeostatic model of assessment of insulin resistance, although food intake and body weight gain were similar among different experimental groups. Both dietary SFAs and TFAs decreased the levels of total n – 6 PUFAs (linoleic acid (LA) + arachidonic acid) in adipocyte plasma membrane phospholipids; however, when compared with the control or SFA group, dietary TFAs decreased adipocyte plasma membrane fluidity in rats. The changes observed in the adipocyte plasma membrane phospholipid composition in both TFA- and SFA-fed groups were accompanied by decreased adipose tissue insulin sensitivity as evidenced by increased lipolysis, decreased antilipolysis and decreased glucose uptake in rat adipocytes. However, the effects of dietary TFAs on adipocyte insulin resistance were greater than the effects of dietary SFAs, which could be attributed to the decreased adipocyte plasma membrane fluidity in TFA-fed rats (30).

The present work is the follow-up study of the previous work and therefore the aim was to understand the association between the effects of these dietary fatty acids on insulin resistance and the mRNA expression of PPARγ, LPL, GLUT4, adiponectin and resistin, which are involved in adipose tissue insulin sensitivity in rats. Since both dietary SFAs and TFAs induced insulin resistance in rat adipose tissue the mRNA levels of PPARγ was analyzed. It has been shown that the expression of resistin and adiponectin is influenced by PPARγ. In the previous study both the dietary fatty acids decreased the levels of arachidonic acid in adipocyte plasma membrane phospholipids, which is the precursor for the PPARγ ligands. Therefore, the mRNA levels of both resistin and adiponectin were analyzed. Feeding both SFAs and TFAs increased lipolysis and decreased insulin-stimulated antilipolysis and glucose uptake to different magnitudes. Therefore, the mRNA levels of LPL and GLUT4 were analyzed in rat adipose tissues. It has been shown that increasing the levels of LA in diet could prevent the inhibitory effects of TFAs on arachidonic acid biosynthesis (31). Therefore, in the present study the effects of TFA were studied with two levels of LA (1 or 2 g LA/100 g diet) to understand the beneficial effects of LA on TFA-induced alteration in gene expression.

Materials and methods

Animals and diets

The procedures involved in animal experimentation were approved by the institutional animal ethical committee, National Institute of Endocrinology (NIN), Hyderabad, India. To introduce the diet-related changes in tissue metabolism in vivo it is appropriate to use weaning rats. A number of studies have used weaning rats as a model to study the effects of dietary fatty acids on biochemical and molecular aspects of insulin resistance (32–34). Since the objective of the present study was to understand the association between long-term effects of SFAs and TFAs in adipose tissue metabolism and alteration in the gene expression, weaning rats were used. Twelve male weanling Wistar/NIN rats were obtained from the National Center for Laboratory animal Sciences, NIN, and divided into four groups, and housed individually in a temperature (22 ± 2°C) and light-controlled (12 h cycle) animal facility.

The animals were fed an isocaloric (1.78 kJ/100 g diet) semi-synthetic diet containing identical dietary constituents differing only in the fatty acid composition of dietary oil for 3 months. The salt and vitamin mixtures were prepared according to American Institute of Nutrition AIN-93 (35). The composition of the experimental diet is presented in Table 1. The fatty acid composition of dietary oils (g/100 g oil) was estimated as described earlier (36) and is given in Table 2. Groundnut oil, which provides 3.8 g LA/100 g diet, was used for the preparation of control diet (CON diet). Palmolein, which provides 5 g SFA/100 g diet, was used for the preparation of the SFA diet. Vanaspati (Indian partial hydrogenated vegetable oil) was used for the preparation of TFA diets. The fatty acid composition of vanaspati was as follows: 14:0–0.9, 16:0–38.5, 18:0–5.2, 18:1 n–9 (cis)-16.2, 18:1 cis-34.0 and 18:2n – 6–5.0. 18:1 trans n – 9 is the major trans-isomer present in Indian vanaspati. Safflower oil with 75% LA was blended with vanaspati in the ratio of 93:7 (1.5 g TFA + 1 g LA/100 g diet – TFA diet 1) or 80:20 (1.3 g TFA + 2 g LA/100 g diet – TFA diet 2) to get two levels of LA. The animals had free access to water and food. The food intake and body weight gain were similar among different experimental groups.

<table>
<thead>
<tr>
<th>Table 1 Composition of the experimental dieta.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary constituent</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Starch</td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Fatb</td>
</tr>
<tr>
<td>Cellulose</td>
</tr>
<tr>
<td>Mineral mixturec</td>
</tr>
<tr>
<td>Vitamin mixturec</td>
</tr>
<tr>
<td>L-Cysteine</td>
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<tr>
<td>Choline chloride</td>
</tr>
</tbody>
</table>

a Animals were fed an isocaloric (1.78 kJ/100 g diet) semi-synthetic diet containing identical dietary constituents differing only in the composition of dietary oil for 3 months.
b The composition of dietary oil (10%) was altered among different experimental groups using either single or a mixture of two vegetable oils.
c The salt and vitamin mixtures were prepared according to the AIN-93 (35).

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**Table 2** Fatty acid composition of dietary oils (g/100 g of oil).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>CON diet</th>
<th>SFA diet</th>
<th>TFA diet 1</th>
<th>TFA diet 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>nd</td>
<td>1.4</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>16:0</td>
<td>10.3</td>
<td>45.5</td>
<td>36.3</td>
<td>32.2</td>
</tr>
<tr>
<td>18:0</td>
<td>4.3</td>
<td>3.5</td>
<td>5.0</td>
<td>4.7</td>
</tr>
<tr>
<td>22:0</td>
<td>5.7</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>18:1cis n−9</td>
<td>42.0</td>
<td>39.8</td>
<td>34.0</td>
<td>31.8</td>
</tr>
<tr>
<td>18:1trans n−9</td>
<td>nd</td>
<td>nd</td>
<td>15.0</td>
<td>13.0</td>
</tr>
<tr>
<td>18:2n−6</td>
<td>37.7</td>
<td>9.7</td>
<td>10.0</td>
<td>19.0</td>
</tr>
</tbody>
</table>

nd, not detected.

**Tissue preparation and total RNA extraction**

At the end of 3 months of feeding, animals were killed by CO₂ asphyxia after overnight fasting. The epididymal fat tissues were isolated and pooled from respective groups. Tissues were frozen immediately in liquid nitrogen and stored at −70°C.

Total RNA was extracted from epididymal fat tissue using TRI Reagent (Sigma) following the manufacturer’s instructions. Briefly, 500 mg of tissue were ground to fine powder in liquid nitrogen. TRI Reagent (1 ml) was added and the suspension was made uniform by passing it through an 18-gauge needle. The insoluble material and excess fat layers were removed by centrifugation at 12 000 g for 10 min at 4°C. Chloroform (0.2 ml) was added to the clear lysate and mixed vigorously. The phases were separated by centrifugation at 12 000 g for 20 min at 4°C. The top aqueous layer containing RNA was collected in a fresh tube.

RNA was precipitated by adding 0.5 ml isopropanol followed by centrifugation at 12 000 g for 20 min at 4°C. The resulting pellet was washed with 75% ethanol and re-suspended in RNase-free water. RNA was quantified spectrophotometrically and its integrity was checked on a 1.2% denaturing agarose gel.

**RT-PCR**

RT-PCR was carried out using a single tube access RT-PCR system (Promega) as per the manufacturer’s instructions. Total RNA was reverse transcribed and PCR amplified with gene-specific primers, using AMV reverse transcriptase and Tfl DNA polymerase respectively. The cycle conditions (30 cycles) were as follows: initial RT for 10 min at 25°C followed by stringent RT at 48°C for 45 min; inactivation of reverse transcriptase at 95°C for 5 min; followed by denaturation at 94°C for 30s, annealing at 50–58°C for 30s, and extension at 68°C for 30s. A final extension was carried out at 68°C for 7 min. The amount of RNA and the annealing temperature for different genes were standardized for linearity. Sequences of primers, expected fragment size and number of cycles performed for each amplification are presented in Table 3. After amplification, 5 μl of reaction mixture were electrophoresed on agarose gel (2%) in Tris–acetate EDTA buffer (pH 8.2). The ethidium bromide-stained bands were visualized by a UV-transilluminator and analyzed densitometrically using Quantity One software program (Bio-Rad, version 4.4.0). Each experiment was repeated three times for similar results.

**Results**

**Assessment of data**

The expression patterns of individual genes along with the β-actin gene (internal control) are presented in Fig. 1a, which represents RT-PCR products of one

**Table 3** RT-PCR conditions applied in the study. Primer sequences, expected amplicon size, concentration of RNA taken and annealing temperature (TM) in the RT-PCR of peroxisome proliferator-activated receptor γ (PPARγ), lipoprotein lipase (LPL), glucose transporter 4 (GLUT4), resistin, adiponectin and β-actin genes.

<table>
<thead>
<tr>
<th>Gene and Accession Number</th>
<th>Primer sequences (5’−3’)</th>
<th>Amplicon size (bp)</th>
<th>RNA Conc. (ng)</th>
<th>TM (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARγ NM_013124</td>
<td>Fwd AGGATCCATGAAGAAGAGTCTCCTTCTCCTCA</td>
<td>360</td>
<td>300</td>
<td>56</td>
</tr>
<tr>
<td>LPL NM_012598</td>
<td>Rev CTTGACATCCTCCAAAGCAT</td>
<td>520</td>
<td>300</td>
<td>58</td>
</tr>
<tr>
<td>Adiponectin NM_144744</td>
<td>Fwd AGGATCCATGCTCTAGTGGAGGAGGAGG</td>
<td>750</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>Resistin NM_144741</td>
<td>Rev GAGGATCCATGAAGAAGAGTCTCCTCCTCCTCA</td>
<td>340</td>
<td>300</td>
<td>50</td>
</tr>
<tr>
<td>GLUT4 NM_012751</td>
<td>Fwd TCTGGTGCTCTTAGTAG</td>
<td>174</td>
<td>300</td>
<td>55</td>
</tr>
<tr>
<td>β-Actin NM_031144</td>
<td>Rev CCGTTGCGCTGAGGTTTCAG G*GGG</td>
<td>250</td>
<td>30</td>
<td>58</td>
</tr>
</tbody>
</table>

* A mismatch has been introduced in the primer to polymerize β-actin gene from different species. Fwd, forward primer sequence; Rev, reverse primer sequence.
experiment. The relative mRNA expression of individual genes associated with insulin resistance in adipose tissue was calculated according to the beta-actin levels. The percentage relative expression of candidate genes in dietary SFA or TFA (TFA diet 1 and 2)-fed rats was calculated by considering gene expression observed in CON diet-fed rats as 100 and is given in Fig. 1b.

**Effects of dietary fatty acids on adipose tissue gene expression**

The data presented in Fig. 1b show that when compared with CON diet, dietary TFAs (TFA diet 1) decreased (~40%) the mRNA level of PPARgamma, while dietary SFA (SFA diet) did not alter it. The increased levels of LA in TFA diet (TFA diet 2) did not show a protective role against the effects of TFAs on mRNA levels of PPARgamma. Dietary SFA increased (~40%) the levels of LPL mRNA when compared with CON diet, while TFA decreased (~25%) the expression of LPL in epididymal fat, irrespectively of LA levels in the diet. Adiponectin mRNA levels decreased (~35%) in SFA diet when compared with CON diet, while TFA diet 1 did not alter it. Increased levels of LA (TFA diet 2) decreased (~30%) the levels of adiponectin mRNA. Both dietary SFA and TFA increased (~30%) the mRNA levels of resistin in epididymal fat. The effects were observed in TFA-fed rats irrespectively of LA levels in the diet (TFA diet 2). Dietary SFA decreased (~40%) the levels of GLUT4 mRNA, while TFA diet 1 did not alter it. Increased levels of LA in TFA-fed rats (TFA diet 2) decreased (~40%) the GLUT4 mRNA levels in epididymal fat.

**Discussion**

Insulin resistance is defined as a subnormal response to a given amount of insulin. Skeletal muscle and adipose tissue are the major target tissues for insulin-stimulated glucose uptake. Adipose tissue plays a relatively moderate role in insulin-stimulated glucose disposal when compared with skeletal muscle. However, it plays an important role in regulating insulin sensitivity of skeletal muscle and liver by secreting FFAs (37) and adipocytokines (19). Although the genetic predisposition is the major determinant of insulin resistance, diet and lifestyle also plays an important role in the etiology of insulin resistance. Among the diet, both quality and quantity of fat plays a crucial role in the development and progress of insulin resistance (38, 39). High intake of dietary SFAs is positively associated with the development of insulin resistance, whereas the intake of n-3 PUFAs is associated with increased insulin sensitivity (40). Moreover, the long-chain derivatives of PUFAs such as arachidonic acid (20:4 n-6), eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3) are identified as potent signaling molecules that regulate the expression of proteins involved in lipid and carbohydrate metabolism and thereby enhance insulin sensitivity (6).

TFAs are formed during industrial hydrogenation of vegetable oils. High intake of both SFAs and TFAs is reported as a risk factor for the development of coronary heart disease. However, when compared with dietary SFAs and TFAs are more deleterious due to the fact that TFAs increases the ratio of low-density lipoprotein (LDL) to high-density lipoprotein (HDL) towards atherogenesis, whereas dietary SFAs increase only the levels of LDL without altering the levels of HDL (41). Reports on the effects of dietary TFAs on insulin sensitivity are limited. Christiansen et al. (42) showed that both dietary SFAs and TFAs induced insulin resistance in type 2 diabetic patients. A short-term human study showed that dietary TFAs alter lipoprotein metabolism without any changes in insulin sensitivity (43).
With this background, the observations of our earlier experiment on the effects of dietary SFAs or TFAs on insulin sensitivity showed that both the dietary fatty acids decreased adipocyte insulin sensitivity in rats. However, irrespective of LA levels in the diet, TFAs decreased insulin sensitivity to a greater extent than SFAs. The greater effects of TFAs on rat adipocyte insulin sensitivity could be attributed to decreased adipocyte plasma membrane fluidity (30). In the present study both dietary SFA and TFA were found to alter the expression of genes associated with insulin sensitivity in rat adipose tissue.

It has been reported that tissue levels of PPARγ are positively associated with insulin sensitivity (10). The findings that PPARγ mRNA levels were not altered in SFA diet-fed rats suggest that the observed decrease in insulin sensitivity in SFA-fed rats may not be through the PPARγ-related mechanism. Dietary TFAs decreased insulin sensitivity to a greater extent than SFAs (30), which could be due to decreased expression of PPARγ.

The relationship between LPL action and insulin resistance is well established (28). The hydrolytic release of lipid metabolites from lipoproteins by LPL essentially increases insulin insensitivity. Moreover, studies suggest that overexpression of LPL gene increases triglyceride level and insulin resistance in rat skeletal muscle and liver (29). Our previous observations showed that feeding both SFAs and TFAs increased triglyceride levels in plasma, suggesting increased lipolysis due to decreased adipocyte insulin sensitivity or decreased clearance of triglyceride from plasma (30). In the present study the increased levels of LPL mRNA in SFA diet-fed group could be extrapolated with increased adipocyte lipolysis and insulin resistance observed in our earlier study. Whereas, in TFA diet-fed groups (TFA diet 1 and 2), the observed decrease in the levels of LPL mRNA did not agree with the increased adipocyte lipolysis observed in the same. Moreover, TFAs have been shown to decrease adipocyte insulin sensitivity by decreasing the levels of arachidonic acid and fluidity in adipocyte plasma membrane (30). Therefore, dietary TFA-induced alterations in adipocyte insulin sensitivity may not be through alteration of LPL gene expression.

It has been shown that both mRNA and protein levels of adiponectin are directly associated with insulin sensitivity (14–19). The suggested mechanisms of enhanced insulin sensitivity are increased triglyceride clearance (fatty acid oxidation) and decreased hepatic glucose production (19). In the present study, the observed decrease in the level of adiponectin mRNA in SFA-fed rats could be correlated with decreased insulin sensitivity. In the previous observation, the plasma triglyceride levels are increased in SFA-fed groups, suggesting increased lipolysis and decreased clearance of triglyceride (30). In the present study the decreased mRNA levels of adiponectin in SFA-fed rats could be associated with decreased insulin sensitivity, possibly due to decreased clearance of triglycerides. Reports suggested that PPARγ-dependent synthetic insulin sensitizers upregulate adiponectin mRNA (11). In the present study, although the mRNA level of PPARγ was not altered in SFA-fed rats, there was a decrease in the level of adiponectin mRNA, suggesting a possible impairment in ligand-dependent activity of PPARγ. It is not clear why, despite the decrease in PPARγ expression in TFA diet-fed rats, the adiponectin expression was not altered. However, the decrease in adipocyte insulin sensitivity in TFA-fed rats suggests that the effects of dietary TFAs on insulin sensitivity may not be mediated through adiponectin gene expression.

Increased levels of adipose tissue resistin gene and plasma protein were correlated with insulin resistance in a murine model (19). In the present study, the increase in the levels of resistin mRNA in both SFA- and TFA-fed groups (TFA diet 1 and 2) suggests that both dietary SFAs and TFAs decrease adipose tissue insulin sensitivity, possibly by upregulating resistin gene expression.

It is known that dietary SFAs are the major source for formation of ceramide. Long & Pekala (44) have reported that ceramide can downregulate GLUT4 mRNA in 3T3-L1 adipocytes. It is well established that SFAs induce insulin resistance through the membrane fluidity plays a major role in insulin-stimulated translocation of GLUT4 and glucose uptake in adipocytes (48). Therefore, the decreased insulin sensitivity in TFA-fed rats could be due to decreased adipocyte plasma membrane fluidity (30).

It has been shown that the undesirable effects of dietary TFAs on mitochondrial function could be prevented by increasing LA in the diet to a level of 2% total energy (en%) in rats (31). In the present study, at unaltered dietary levels of TFAs (3 en%), the increased levels of LA (4 en%) did not show a protective role against the effects of TFAs on PPARγ and resistin gene expression. It has been shown that dietary fatty acids compete for the activities of Δ5 and Δ6 fatty acid desaturases; moreover TFAs decrease PUFA biosynthesis by inhibiting Δ5 and Δ6 fatty acid desaturase (49, 50). Zevenbergen et al. (31) further showed that the effects of dietary TFAs on liver microsomal membrane arachidonic acid level could be diminished by increasing the levels of LA in the diet to 5 en%, suggesting that the beneficial effects of LA against TFAs is purely based on the amount of LA.
in the diet. Therefore increasing the level of LA to an amount which can withstand the competition from TFAs for fatty acid desaturase activities would be beneficial in preventing the effects of TFAs on adipose tissue metabolism. Moreover, the effects of increased LA on adiponectin and GLUT4 mRNA in TFA 2-fed rats are unexplainable.

In conclusion, dietary SFAs decrease adipocyte insulin sensitivity, possibly through upregulation of mRNA levels of resistin and LPL and downregulation of adiponectin and GLUT4, in addition to its effects on adipocyte plasma membrane phospholipid fatty acid composition. In TFA-fed rats the decrease in adipocyte insulin sensitivity could be mainly due to its effects on adipocyte plasma membrane phospholipid fatty acid composition and adipocyte plasma membrane fluidity. The downregulation of PPARγ and upregulation of resistin gene expression could be added effects of dietary SFAs on insulin resistance.

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