Adipocyte membrane phospholipids and PPAR-γ expression in obese women: relationship to hyperinsulinemia

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Adipocyte membrane phospholipids and PPAR-γ expression in obese women: relationship to hyperinsulinemia. Am J Physiol Endocrinol Metab 279: E736–E743, 2000.—We have shown that membrane sphingomyelin (SM) is an independent predictor of the variance of fasting plasma insulin (FPI) concentrations and the homeostasis model assessment (HOMA) estimate of insulin resistance in obese women. The peroxisome proliferator-activated receptor-γ (PPAR-γ) is a key component in adipocyte differentiation that may also contribute to the sensitivity of cells to insulin. PPAR-γ is activated by fatty acids, and the membrane composition may have an impact on the activity of PPAR-γ and thus on the sensitivity of adipocytes to insulin. We investigated these possible links by determining the phospholipid contents of adipocyte membranes, the mRNA expression of PPAR-γ, and the FPI and HOMA estimate of insulin resistance in obese women. The mRNA levels of tumor necrosis factor-α (TNF-α), which is suspected to play a role in insulin resistance and which downregulates PPAR-γ expression, were also quantified. FPI and HOMA were strongly positively correlated with membrane SM (P < 0.005) and cholesterol (P < 0.005). PPAR-γ mRNA levels were negatively correlated with FPI (P < 0.05) and HOMA (P < 0.05) and positively correlated with high-density lipoprotein (HDL) cholesterol (P < 0.05), membrane SM (P < 0.05), and cholesterol contents (P < 0.05). TNF-α mRNA levels were not correlated with membrane parameters. In stepwise multiple regression analysis, the variations in PPAR-γ mRNA levels were mainly explained by HDL cholesterol (31.9%) and membrane SM (17.7%). Our study shows that the expression of PPAR-γ, a major factor controlling adipocyte functions, the lipid composition of the membrane, and insulin sensitivity are probably closely associated in the adipose tissue of obese women.

Obesity; adipose tissue; plasma membrane; transcription factor; insulin resistance

# Changes in the Phospholipid composition of the Plasma Membrane

Composition can dramatically alter cell functions by modifying, for example, the interactions of extracellular ligands with their receptors. Changes in membrane composition can also alter the intracellular signal transduction pathways, because the membrane contains a pool of phospholipids available for the production of second messengers. Therefore, genetically and/or nutritionally induced changes in membrane lipid composition could be involved in several human diseases. This is particularly relevant for the insulin resistance state, which is associated with a cluster of metabolic disturbances. It has been reported that there is a connection between the fatty acid composition of phospholipids in skeletal muscle and insulin sensitivity. In other studies, insulin resistance has been associated with alterations in the physical properties of the membranes. In addition, we have shown that an altered composition of the major classes of phospholipids in erythrocyte membranes was associated with insulin resistance in obese patients. The membrane sphingomyelin content represents a major independent predictor of the variance of markers of insulin resistance in obese women.

Sphingomyelin also accumulates in the cell membranes in diseases with peroxisomal disorders, and it has been suggested that peroxisome proliferator-activated receptors (PPARs) are involved. The PPAR-γ subtype is abundantly expressed in adipose tissue, where it plays a major role in adipocyte differentiation and in maintaining the adipocyte phenotype. PPAR-γ may also play a role in the sensitivity of cells to insulin. This concept relies mainly on the discovery that PPAR-γ is a high-affinity receptor for the thiazolidinediones, a class of hypoglycemic agents that enhance insulin action in target tissues. However, the physiological regulation of PPAR-γ gene expression in vivo or its potential dysregulation in obesity and insulin resistance is not well characterized. The natural ligands of PPAR-γ include PGJ₂, a metabolite derived from arachidonic acid. In addition, certain saturated and polyunsaturated fatty acids that are major components of cell membranes also activate PPAR-γ.

Therefore, the membrane composition...
may have important consequences for the activity of PPAR-γ and thus the insulin sensitivity of adipocytes.

In addition to being an important constituent of cell membranes, sphingomyelin is a mediator in some of the actions of tumor necrosis factor-α (TNF-α; see Ref. 27), a peptide that downregulates PPAR-γ expression (54), inhibits insulin action (22, 33), and increases lipolysis (15) when added to adipose cell lines and human adipocytes. Moreover, TNF-α is produced by adipose tissue in proportion to the mass of fat (21, 26) and, at least in rodents, it has been demonstrated that adipose-derived TNF-α is a key factor in obesity-related insulin resistance (23).

Thus all of these data suggest that the expression and activity of important factors controlling adipocyte functions, the composition of the membrane, and insulin sensitivity are probably closely associated in adipose tissue. The present work was done to investigate these possible links in the subcutaneous adipose tissue of obese individuals. The distribution of cholesterol and major phospholipids in adipocyte plasma membranes and the mRNA expression of PPAR-γ and TNF-α were determined in fat biopsies taken from obese women with very different body mass indexes, fasting plasma insulin concentrations, and homeostasis model assessment (HOMA) estimates of insulin resistance. PPAR-γ and TNF-α mRNA levels were quantified by RT-competitive PCR (cPCR).

SUBJECTS AND METHODS

Selection of patients. We studied 20 obese Caucasian patients [age: 41.2 ± 2.6 yr; body mass index (BMI): 33.6 ± 0.7 kg/m²] whose body weights had been stable for several months. None of the subjects had been involved in a weight reduction program over the previous 3 mo. The glucose tolerance of the patients, based on the oral glucose tolerance test, was normal according to World Health Organization criteria. None of the subjects was treated with any drug that could influence plasma lipid concentrations or glucose tolerance. Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the Nancy University Hospital.

Biochemical determinations. Blood samples were taken from an antecubital vein in the morning after a 12-h overnight fast. Fasting plasma glucose was measured by the glucose oxidase method with a Beckman BGA II Glucose Analyzer (Beckman Instruments, Fullerton, CA). Plasma triglycerides and cholesterol were measured enzymatically (Boehringer, Mannheim, Germany). High-density lipoprotein cholesterol (HDL cholesterol) was determined in the plasma after precipitation of very low density and low-density lipoproteins (LDL) with phosphotungstic acid. LDL cholesterol was estimated using Friedewald’s formula. Plasma insulin was measured by RIA (CIS Biointernational, ORIS group, Gif-sur-Yvette, France). The sensitivity of the RIA was 1 mU/L, and the intra- and interassay coefficients of variation were 8.2 and 8.8%, respectively. The cross-reactivity with proinsulin was 14%. Fasting plasma insulin and HOMA, which is correlated with the euglycemic-hyperinsulinemic clamp (36), were chosen as insulin resistance markers. The HOMA estimate of insulin resistance was assessed by the formula [fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5].

Adipose tissue biopsies. Subcutaneous fat tissue was obtained by incisional biopsy performed under local anesthesia from the lower abdominal wall. Five of 20 fat biopsies were not large enough (<5 g) for adipocyte plasma membrane preparations. Adipose tissue was frozen in liquid nitrogen immediately and was stored at ~80°C.

RNA preparations. Total RNA from adipose tissue (~200 mg of frozen tissue) was obtained using the RNeasy total RNA kit (Qiagen, Courtaboeuf, France). The absorption ratio at 260 to 280 nm was between 1.7 and 2.0 for all preparations. RNA integrity was verified by agarose gel electrophoresis.

Quantification of the target mRNAs. The concentrations of PPAR-γ and TNF-α mRNA were measured by RT-cPCR which, after a specific RT reaction, exploits the coamplification of the target cDNA with known amounts of a specific DNA competitor molecule added in the same PCR tube (4). The reverse transcription reactions were performed on 0.2 μg of total tissue RNA in the presence of one of the specific antisense primers. The experimental conditions, the primer sequences, and the validation of the RT-PCR mRNA assays of PPAR-γ and TNF-α have been described in detail previously (3, 6).

Adipocyte plasma membrane isolation. At first, a total membrane fraction was prepared as previously described (7), with minor modifications. Ten milliliters of a HEPES-sucrose buffer [buffer 1: 20 mmol/l HEPES, 255 mmol/l sucrose, and 1 mmol/l EDTA, pH 7.4, containing protease inhibitors (5 μg/ml leupentin, 5 μg/ml pepstatin, and 5 μg/ml aprotinin)] were added to adipose tissue (5–7 g), and the tissue was homogenized at 4°C using an Ultra-Turrax (TP18) homogenizer. The homogenate was then centrifuged at 500 g for 10 min at 4°C. The supernatant was separated from the pellet, and fat cake and then was centrifuged at 150,000 g for 2 h at 4°C. The pellet (total membrane fraction) was then treated as previously described (34). It was suspended in buffer 1 and layered carefully on a sucrose cushion (41% in buffer 1) that was then centrifuged for 1 h at 95,000 g. The plasma membrane fraction that settled at the interface was collected, diluted in buffer 1, and washed at 4°C by centrifugation at 200,000 g for 20 min. The resulting pellet was suspended in Tris-EDTA buffer [buffer 2: 1 mmol/l Tris, 1 mmol/l EDTA, and 10 mmol/l NaCl], and the protein content was estimated by the Lowry method. The membrane suspensions obtained with this method have been characterized by use of marker enzyme activities. The plasma membrane fractions were enriched four- to sevenfold in 5′-nucleotidase activity compared with homogenized tissue. The plasma membrane fractions were stored at ~80°C until further analysis.

Membrane lipid determination. Lipids were extracted from adipocyte membranes with methanol and chloroform (16) containing 50 mg/l 2,6-di-t-butyl-p-cresol to prevent lipid peroxidation. The organic phase was evaporated to dryness under a stream of nitrogen at room temperature. The lipid residue was dissolved in chloroform, and its components were separated by HPLC (51). Lyso phosphatidylcholine (10 μg/100 μg proteins) was added as internal standard before extraction. Phospholipids were detected with an evaporative light scattering detector. Phospholipid data were expressed as a weight concentration calculated from calibration curves established for each phospholipid. Membrane cholesterol was determined by the method of Zlatkis and Zak (55).

Statistical analysis. Data are presented as means ± SE or medians with 25th and 75th percentiles. Variables were assessed for normality by the skewness and kurtosis test. To improve the skewness and kurtosis of the distributions, waist-to-hip ratio (WHR), fasting blood glucose, phosphati-
Table 1. Clinical characteristics of study population

<table>
<thead>
<tr>
<th></th>
<th>Obese Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>41.2 ± 2.6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>33.6 ± 0.7</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.84(0.79–0.93)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>5.40 ± 0.16</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.30 ± 0.15</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.18 ± 0.06</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>3.56 ± 0.20</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/l</td>
<td>4.77(4.49–4.93)</td>
</tr>
<tr>
<td>Fasting plasma insulin, mU/l</td>
<td>19.5 ± 2.2</td>
</tr>
<tr>
<td>HOMA</td>
<td>4.30 ± 0.58</td>
</tr>
</tbody>
</table>

Data are means ± SE or medians with 25th and 75th percentiles (data that follow a log normal distribution); n = 20 patients. HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA, homeostasis model assessment.

Table 2. Cholesterol and phospholipid content of adipocyte membranes

<table>
<thead>
<tr>
<th></th>
<th>Obese Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>256.9 ± 38.9</td>
</tr>
<tr>
<td>Phosphatidycholine</td>
<td>387.0(253.6–562.1)</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>410.0 ± 49.6</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>106.5 ± 12.8</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>85.0(63.3–93.3)</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>54.2 ± 6.2</td>
</tr>
</tbody>
</table>

Data in µg/mg proteins are presented as means ± SE or medians with 25th and 75th percentiles (data that follow a log normal distribution); n = 15 patients.

Table 3. mRNA levels of PPAR-γ and TNF-α in subcutaneous adipose tissue

<table>
<thead>
<tr>
<th></th>
<th>Obese Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR-γ</td>
<td>33.2 ± 3.7</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.52 ± 0.07</td>
</tr>
</tbody>
</table>

Data in amol/µg total RNA are presented as means ± SE; n = 20 patients. PPAR-γ, peroxisome proliferator-activated receptor-γ; TNF-α, tumor necrosis factor-α.

The mean values of PPAR-γ and TNF-α mRNA levels are shown in Table 3. For PPAR-γ, only the total (γ₁ + γ₂) mRNA amount of the nuclear receptor was analyzed. PPAR-γ mRNA concentrations ranged from 7.3 to 68.5 amol/µg total RNA. TNF-α mRNA was very low (0.52 ± 0.07 amol/µg total RNA).

Table 4 shows the Pearson correlation coefficients between the insulin resistance markers (fasting plasma insulin levels and HOMA values), the clinical and metabolic characteristics of the subjects, and adipocyte membrane parameters. Fasting plasma insulin correlated positively with BMI (P < 0.05), WHR (P < 0.01), fasting blood glucose (P < 0.05), membrane sphingomyelin (P < 0.005), phosphatidylcholine (P < 0.05), and cholesterol (P < 0.005) contents. The most important correlations were with sphingomyelin (r = 0.698, P < 0.005) and cholesterol (r = 0.723, P < 0.005) membrane contents (Fig. 1, A and B). The correlations with the HOMA estimates of insulin resistance were similar to those observed with fasting plasma insulin, except for a positive correlation with phosphatidylylcholine and TNF-α that does not exist with fasting plasma insulin. Because membrane sphingomyelin and cholesterol contents were strongly correlated with the insulin resistance markers, these two membrane components were also analyzed with regard to the anthropometric variables that characterized obesity in this study. There was no correlation with BMI or WHR.

Next, we analyzed the possible relationships with the amounts of the target mRNAs (Table 4). PPAR-γ mRNA levels were negatively correlated with the fasting plasma insulin concentrations (r = −0.470, P < 0.05), and HOMA (r = −0.482, P < 0.05) and positively correlated with HDL cholesterol (r = 0.467, P < 0.05). Figure 2, A and B, shows the correlations between PPAR-γ mRNA levels and fasting plasma insulin and HDL cholesterol concentrations. There was also a negative correlation between PPAR-γ mRNA levels and the amounts of sphingomyelin (r = −0.553, P < 0.05) and cholesterol (r = −0.512, P < 0.05) in the membrane (Fig. 3, A and B). The concentrations of TNF-α mRNA were not correlated with any other measured parameter, except with WHR (r = 0.535, P < 0.05).

Stepwise multiple regression analyses were performed to evaluate the relative contribution of biological parameters and membrane lipid concentrations to the relationships with PPAR-γ mRNA. In models with BMI or WHR, plasma lipids, parameters, membrane lipid concentrations, fasting plasma insulin, or HOMA values, the variations in PPAR-γ mRNA concentra-
Table 4. Pearson correlation coefficients for the relationships between insulin resistance markers and clinical characteristics, plasma lipid parameters, lipid membrane contents, and mRNA levels of PPAR-γ and TNF-α.

<table>
<thead>
<tr>
<th></th>
<th>Fasting Insulin</th>
<th>HOMA Values</th>
<th>PPAR-γ</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r    P</td>
<td>r    P</td>
<td>r    P</td>
<td>r    P</td>
</tr>
<tr>
<td>Age</td>
<td>-0.154 0.517</td>
<td>-0.092 0.700</td>
<td>-0.131 0.582</td>
<td>-0.119 0.677</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.483 &lt;0.05</td>
<td>0.562 &lt;0.01</td>
<td>-0.216 0.361</td>
<td>-0.078 0.750</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.564 &lt;0.01</td>
<td>0.477 &lt;0.05</td>
<td>-0.148 0.534</td>
<td>0.535 &lt;0.05</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>0.509 &lt;0.05</td>
<td></td>
<td>-0.443 0.051</td>
<td>0.103 0.675</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.261 0.266</td>
<td>0.268 0.254</td>
<td>-0.276 0.239</td>
<td>0.018 0.949</td>
</tr>
<tr>
<td>Total plasma cholesterol</td>
<td>0.414 0.069</td>
<td>0.418 0.067</td>
<td>-0.096 0.688</td>
<td>-0.294 0.222</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.114 0.632</td>
<td>0.162 0.495</td>
<td>-0.231 0.326</td>
<td>-0.250 0.302</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.314 0.177</td>
<td>-0.362 0.117</td>
<td>0.467 &lt;0.05</td>
<td>-0.208 0.394</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>0.698 &lt;0.005</td>
<td>0.637 &lt;0.01</td>
<td>-0.553 &lt;0.05</td>
<td>-0.356 0.211</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>0.531 &lt;0.05</td>
<td>0.462 0.083</td>
<td>-0.319 0.246</td>
<td>-0.158 0.589</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>0.440 0.101</td>
<td>0.514 &lt;0.05</td>
<td>-0.380 0.162</td>
<td>0.003 0.991</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>-0.274 0.322</td>
<td>-0.198 0.479</td>
<td>-0.441 0.099</td>
<td>-0.127 0.664</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>-0.300 0.305</td>
<td>-0.334 0.265</td>
<td>-0.097 0.753</td>
<td>0.115 0.722</td>
</tr>
<tr>
<td>Membrane cholesterol</td>
<td>0.723 &lt;0.005</td>
<td>0.734 &lt;0.005</td>
<td>-0.512 &lt;0.05</td>
<td>0.069 0.815</td>
</tr>
</tbody>
</table>

Fig. 1. Relationship between fasting plasma insulin concentrations and the sphingomyelin (A) and cholesterol (B) content of adipocyte plasma membranes.

Fig. 2. Relationship between peroxisome proliferator-activated receptor-γ (PPAR-γ) mRNA levels and fasting plasma insulin (A) and high-density lipoprotein (HDL) cholesterol (B) concentration.
tions were mainly explained by HDL cholesterol (31.9%) and membrane sphingomyelin (17.7%; Table 5).

DISCUSSION

We have investigated the relationship between the cholesterol and phospholipid composition of the adipocyte membranes, insulin resistance markers, and the expression of the adipose-specific nuclear receptor PPAR-γ in subcutaneous adipose tissue in obese women.

The main phospholipids of the adipocyte plasma membrane are phosphatidylcholine and phosphatidylethanolamine, with sphingomyelin, phosphatidylserine, and phosphatidylinositol being less abundant. This phospholipid profile is compatible with data for rat adipocytes (19). The mean values of total phospholipids-to-protein (1.10 ± 0.10), phosphatidylcholine-to-protein (0.45 ± 0.06), and cholesterol-to-protein (0.26 ± 0.04) mass ratios are close to those reported for the adipocyte plasma membranes of massively obese patients (24).

Obese subjects that had a higher sphingomyelin and cholesterol content in their adipocyte membrane are less sensitive to insulin, as assessed by HOMA. These results confirm our recent observations that insulin resistance is associated with an increased sphingomyelin content in erythrocyte membranes (9). Twenty percent of the women in the obese group studied were menopausal, and none was on hormone replacement therapy. The hormonal status may influence the phospholipid composition of the adipocyte plasma membrane. It has been documented that several hormones may influence phospholipid synthesis (35, 39), but there was no correlation between age and membrane sphingomyelin content in the obese group studied, indicating that the observed differences are not likely related to the hormonal status of the patients.

As a nuclear receptor, PPAR-γ participates in the regulation of genes involved in the differentiation of adipocytes and also in lipid metabolism (47). Its activation by the thiazolidinediones leads to the improvement of insulin action in vivo and in vitro (45). PPAR-γ is therefore considered as playing a pivotal role in insulin sensitization and glucose metabolism (5). We find large individual variations in the amounts of PPAR-γ mRNA in subcutaneous adipose tissue. This is in keeping with our previous data (3, 43). The variability in the amount of PPAR-γ mRNA is not linked to the BMI of the subjects, as previously reported (43). The concentration of PPAR-γ mRNA is positively correlated with the plasma concentration of HDL cholesterol and negatively with some markers of insulin resistance, membrane cholesterol, and sphingomyelin contents. Interestingly, in another recent study, we found a similar association between HDL cholesterol and the amounts of mRNA for PPAR-γ subcutaneous adipose tissue (6). Nearly similar correlations in the intraperitoneal adipose tissue were also recently reported (30). The authors reported that PPAR-γ mRNA levels were negatively correlated with fasting plasma insulin in obese nondiabetic subjects and positively with HDL cholesterol in postobese subjects. A high PPAR-γ expression and activity may therefore be associated with a high sensitivity to insulin in the adipose tissue of obese subjects. Its is, however, important to note that this relationship between insulin resistance and adipose tissue PPAR-γ expression has not been found when lean controls, obese nondiabetic subjects, and type 2 diabetics patients have been investigated together (43). It seems, therefore, that PPAR-γ gene expression may be associated with insulin sensitivity in obese subjects characterized by large differences in fasting plasma insulin, sphingomyelin, and cholesterol contents of their adipocyte membranes.

Table 5. Stepwise multiple linear regression model for PPAR-γ mRNA levels

<table>
<thead>
<tr>
<th>Variables</th>
<th>β-Coefficient</th>
<th>Partial $r^2$</th>
<th>$F$</th>
<th>Partial $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>10.374</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.382</td>
<td>0.319</td>
<td>4.503</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>-0.169</td>
<td>0.177</td>
<td>4.209</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Adjusted $r^2 = 41.2\%, F = 5.895, P < 0.025$. 

Fig. 3. Relationship between PPAR-γ mRNA levels and the sphingomyelin (A) and cholesterol (B) membrane contents.
There may be several links between PPAR-γ gene expression, plasma metabolic parameters, adipocyte membrane composition, and impaired insulin action. In our study, stepwise multiple regression analysis indicated that plasma HDL cholesterol concentration could explain ≈30% of the variance in PPAR-γ mRNA levels. This relationship is supported by recent data showing that adding purified HDL particles to the culture medium stimulates human preadipocyte differentiation in vitro (50). It is possible that the induction of the expression of PPAR-γ, a key molecular factor of this process, could be involved in the adipogenic effect of HDL. However, the mechanisms whereby plasma HDL cholesterol concentrations are related to the amounts of PPAR-γ mRNA in adipose tissue are not yet clear. Perhaps the changes in the bidirectional transfer of cholesterol between cells and the HDL particles can participate in the regulation of the expression of the PPAR-γ gene. In fact, HDL delivers cholesterol in an esterified form to adipocytes and removes free cholesterol from the cells. Adipocytes are enriched in the transcription factor ADD1/SREBP1c (adipocyte determination differentiation-dependent factor/sterol regulatory element-binding protein), which is activated by proteolytic cleavage after cholesterol depletion of the cell (44). Interestingly, ADD1/SREBP1c has recently been shown to regulate PPAR-γ gene expression in cultured adipocytes, and response elements have been found in the promoter sequence of the human PPAR-γ gene (14). Moreover, the expression of the activated form of ADD1/SREBP1c in cultured adipocytes leads to the production of ligands for PPAR-γ (28). Therefore, the intracellular amounts of free cholesterol may be a link between plasma HDL cholesterol concentrations and PPAR-γ gene expression in adipocytes.

Human adipocytes synthesize little cholesterol de novo: intracellular and membrane cholesterol is derived primarily from lipoproteins (2). The binding capacity of HDL to the membrane receptor (48) of adipocyte is affected by membrane lipid composition, particularly by the type of fatty acids in membrane sphingomyelin (56). In addition, the phospholipid contents of HDL particles and the cell membranes play a major role in the distribution of free cholesterol between the cell membrane and HDL (25). We have found in this study that PPAR-γ mRNA levels are negatively correlated with the cholesterol and sphingomyelin content in the adipocyte membranes. Therefore, changes in intracellular free cholesterol could be favored by changes in the distribution of phospholipid classes, especially sphingomyelin and also cholesterol contents in the adipocyte membrane.

Differences in sphingomyelin concentration appear to explain ~17% of the variability of PPAR-γ mRNA in the stepwise multiple regression analysis. An increased sphingomyelin content in the adipocyte membrane might also participate in PPAR-γ gene expression through an indirect effect on the transcriptional activity of ADD1/SREBP1c. The accumulation of sphingomyelin in adipocyte plasma membranes may be due to a decreased sphingomyelinase activity, as previously reported for other cell types (13). Recently, sphingomyelinase has been reported to induce the activation of ADD1/SREBP1 through a sterol-independent mechanism (32). As discussed above, the activation of ADD1/SREBP1c could then produce ligands for PPAR-γ and increase PPAR-γ expression. A high sphingomyelin content in the adipocyte membrane may therefore be related to a decreased PPAR-γ gene expression and activity through this mechanism.

TNF-α was another candidate for the link between insulin resistance and lipid composition of the membrane. In fact, the intracellular action of TNF-α involves, at least in part, plasma membrane sphingomyelin to generate ceramide (27). In addition, TNF-α in cultured adipose cells leads to a decreased expression of PPAR-γ in cultured adipose cells (54). Some data have also suggested that increased expression of TNF-α in adipose tissue might be associated with insulin resistance during obesity (23, 26). In agreement with recent works (6, 38), we found extremely low concentrations of adipose TNF-α mRNA, even in fat samples from extremely obese women. There was no correlation between subcutaneous adipose tissue TNF-α mRNA levels and the phospholipid composition of the adipocyte membrane, insulin resistance markers, or the BMI in the studied obese women. This extremely low level of expression of TNF-α was consistent with the lack of significant in vivo production of the cytokine by human adipose tissue in obese subjects (37). These results do not, however, exclude the possibility that TNF-α plays a role in insulin resistance in humans. In fact, elevated concentrations of circulating TNF-α have been found in insulin-resistant obese patients with and without type 2 diabetes mellitus (11, 40, 41). However, it is likely that plasma TNF-α does not originate from adipose tissue in these situations.

In conclusion, our findings in the adipocytes of insulin-resistant obese patients pointed to a potentially important role for sphingomyelin and cholesterol in the membrane. Because strong interactions exist between the cholesterol and sphingomyelin contents of the adipocyte membranes, those that are enriched in cholesterol are also enriched in sphingomyelin (18). It is likely that cholesterol levels in the membrane reflect the intracellular cholesterol concentration, which may in turn affect the regulation of the expression of several important genes, including the gene for the nuclear receptor PPAR-γ. Differences in membrane cholesterol content between obese individuals may then affect both the structural membrane properties and the function of the adipocytes, two effects that may contribute to modifications of insulin action. It is now important to discover what determines the difference in cholesterol content of the adipocyte membrane between obese individuals. The altered metabolism of lipoproteins and the interaction of HDL with cell membranes are presently being investigated.

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REFERENCES


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