Increased hepatic lipogenesis but decreased expression of lipogenic gene in adipose tissue in human obesity

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Received 7 March 2001; accepted in final form 30 August 2001

Although it is clear that both genetic (6) and environmental factors, such as decreased levels of physical activity and increased energy intake, play a role in the development of obesity, the mechanisms leading to the excessive depot of triglycerides (TG) in adipose tissue are still poorly defined. Both decreased lipolytic activity, through genetic variations or abnormal regulation of adrenoceptors (26) or hormone sensitive lipase (25, 27), and enhanced expression and activity of the TG synthesis and storage pathways can play a role. A decreased capacity of muscles for fatty acid oxidation could promote this storage by diverting fatty acid metabolism toward reesterification (22). Indeed, oxidation of an oral long-chain TG load was lower in obese than in lean subjects (4). Most of the TG appearing in the organism each day are provided by the diet. Endogenous synthesis of fatty acids (de novo lipogenesis), which takes place mainly in the liver and in adipose tissue, is considered to be a minor contributor (5). This was based, for adipose tissue, on in vitro studies examining the activity of lipogenic enzymes and incorporation of glucose carbons into fatty acids (summarized in Ref. 5) and on in vivo studies of the metabolic fate of orally ingested or intravenously infused (32, 33) glucose. These studies concluded that, contrary to what is observed in rats, the lipogenic capacity of adipose tissue is negligible in normal humans and, albeit increased, remains low in obesity (5). This view was recently challenged on the following grounds: 1) reexamination of the respective lipogenic capacity of rat and human adipose tissue suggested that human adipose tissue is an important site of fatty acid synthesis (41); 2) fatty acid synthase (FAS) gene transcription and FAS activity are increased by insulin in cultured human adipocytes (8, 38); and 3) the body weight gain of normal subjects overfed with carbohydrates could not be explained by the increase in liver lipogenesis, suggesting that significant de novo lipogenesis occurred in another place, probably adipose tissue (1). These observations supported the hypothesis that excessive expression and activity of the lipogenic pathway in adipocytes could play a role in the development of human obesity. In addition, the development of inhibitors of FAS activity (24) could provide a valuable approach to the treatment of obesity (30) if this hypothesis was demonstrated to be true. On the other hand, it has been shown recently that the levels of sterol regulatory element-binding protein (SREBP)-1c mRNA, the main transcription factor that controls the expression of the lipogenic pathway (16, 17), were decreased in the adipose tissue of obese mice (39). Therefore, to determine whether increased lipogenic capacity, not only of liver but also of adipose tissue, could play or not play a role in the pathogenesis of obesity, we measured in the...
present report hepatic lipogenesis and the expression of FAS and SREBP-1c in adipose tissue of lean and obese subjects. These measurements were repeated in some obese subjects consuming a hypocaloric diet.

**METHODS**

*Subjects.* Written informed consent was obtained from 10 normal subjects and 8 obese patients after full explanation of the nature, purpose, and possible risks of the study. The control group consisted of six women and four men [age 20–51 yr, body mass index (BMI) 19–25]. No control subject had a personal or familial history of diabetes or obesity or was taking any medication; all had normal physical examination and normal plasma glucose and lipid concentrations (Table 1). All had a stable body weight during the year before the study. Subjects with unusual dietary habits were excluded. The obese group consisted of six women and two men (age 22–50 yr, BMI 31–50) with normal physical examination, except for the enlarged fat mass, and taking no medication. They had gained between 2 and 4 kg of body weight during the previous year. Except for one subject who had slightly increased TG (2.17 mM) and cholesterol (6.80 mM) concentrations, all had plasma glucose and lipid levels within normal values (Table 1).

*Protocols.* The protocol of the study was approved by the local ethical committee and by the Institut National de la Santé et de la Recherche Médicale, and the study was conducted according to the Hurriet law. All tests were performed in the Human Nutrition Research Center in Lyon. All subjects were studied while consuming their usual diet and a 7- to 10-kg reduction in body weight. For women, the test was performed during the first 10 days of the menstrual cycle to take into account the known variations of lipogenesis during the menstrual cycle (there are no menstrual variations for cholesterol synthesis; see Ref. 13). All subjects abstained from alcohol and heavy physical activity except for the enlarged fat mass, and taking no medication. They had gained between 2 and 4 kg of body weight during the previous year. Except for one subject who had slightly increased TG (2.17 mM) and cholesterol (6.80 mM) concentrations, all had plasma glucose and lipid levels within normal values (Table 1).

Table 1. **BMI, hormonal, and metabolic parameters measured in the postabsorptive state**

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>Obese Subjects</th>
<th>Obese Subjects After Weight Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>22.4 ± 0.7</td>
<td>38.1 ± 2.2†</td>
<td>30.6 ± 1.8‡</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>13.2 ± 1.7</td>
<td>48.5 ± 6.5†</td>
<td>33.0 ± 3.5†</td>
</tr>
<tr>
<td>Fat mass, %body wt</td>
<td>20.9 ± 2.4</td>
<td>47.2 ± 2.3*</td>
<td>39.8 ± 3.1†</td>
</tr>
<tr>
<td>NEFA, µM</td>
<td>479 ± 53</td>
<td>541 ± 57</td>
<td>577 ± 41</td>
</tr>
<tr>
<td>TG, mM</td>
<td>0.55 ± 0.05</td>
<td>1.45 ± 0.26*</td>
<td>1.18 ± 0.50‡</td>
</tr>
<tr>
<td>Cholesterol, mM</td>
<td>4.16 ± 0.25</td>
<td>5.35 ± 0.24†</td>
<td>4.99 ± 0.41‡</td>
</tr>
<tr>
<td>Insulin, mU/l</td>
<td>8.1 ± 1.0</td>
<td>19.6 ± 2.1*</td>
<td>11.2 ± 1.4‡</td>
</tr>
<tr>
<td>Leptin, µg/l</td>
<td>6.9 ± 1.5</td>
<td>28.5 ± 4.1†</td>
<td>13.3 ± 3.3†</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; NEFA, nonesterified fatty acid; TG, triglyceride. *P < 0.01 and †P < 0.05 vs. control group. ‡P < 0.05 vs. obese subjects before weight loss.
mRNA (0.1–25 amol added to the RT medium) were quantified to perform a dose-response curve, as recommended (2). For the assays of mRNAs in the tissue samples, serial dilutions of the competitor plasmids were performed (10⁻¹⁰ - 5 amol/µl) in 10 mM Tris-HCl (pH 8.3) - 1 mM EDTA buffer.

For each target mRNA, a reverse transcription reaction was performed from 0.1 µg of total RNA with 2.5 units of thermostable reverse transcriptase (Tth DNA polymerase; Promega, Charbonniere, France) in 10 mM Tris-HCl, pH 8.3, 90 mM KCl, 1 mM MnCl₂, 0.2 mM deoxynucleoside triphosphates, and 15 pmol of the specific antisense primer, in a final volume of 20 µl. The reaction lasted 3 min at 60°C followed by 15 min at 72°C and then 5 min at 99°C. After being chilled, 4 µl of water were added to the RT medium, from which 20 µl were used for competitive PCR reaction. Sense primers labeled in the 5’-position with Cy-5 fluorescent dye (Eurogentec, Seraing, Belgium) were used during the PCR. For this reaction, the 20 µl of RT medium were added to 80 µl of PCR mix (10 µl of 10 mM Tris-HCl, pH 8.3, 100 mM KCl, 0.75 mM EGTA, and 5% glycerol) containing 0.2 mM deoxynucleosidetriphosphates, 5 units of Taq polymerase (Life Technologies, Cergy Pontoise, France), 45 pmol of the corresponding sense primer, and 30 pmol of the antisense primer. Four 20-µl aliquots were then transferred in PCR tubes containing 5 µl of defined working solutions of the competitor cDNA. The PCR conditions were as follows: 2 min at 94°C followed by 40 cycles (40 s at 94°C, 60 s at 55°C, and 40 s at 72°C) and finally 10 min at 72°C. The PCR products were analyzed with an automated laser fluorescence DNA sequencer (ALFexpress; Pharmacia, Uppsala, Sweden) in 4% denaturating polyacrylamide gels. The amounts of PCR products (competitor and target) were calculated by integrating peak areas with the use of the Fragment Manager software from Pharmacia. The initial concentration of target mRNA was determined at the competition equivalence point as previously described (2). To accurately compare the results of RT-competitive PCR, the target mRNAs of different samples were always measured in the same run of RT and PCR using the same mix of medium.

Calculations. The fractional contributions of cholesterol synthesis and hepatic lipogenesis to plasma free cholesterol and to plasma TG pools, respectively, were calculated from the deuterium enrichments in free cholesterol, palmitate of TG, and in plasma water, as previously described (10, 11). In short, the deuterium enrichments that would have been obtained if endogenous synthesis were the only source of plasma cholesterol or palmitate of TG were calculated from plasma water enrichment. The comparison of the actual enrichments observed with these theoretical values gives the contributions, expressed as the fractional synthesis rate, of endogenous synthesis to the pools of rapidly exchangeable free cholesterol and of plasma TG during the time between the ingestion of the loading dose of deuterated water and blood sampling (12 h). An important assumption (21), and possible limitation, in these calculations of lipids synthesis is that the number of incorporation sites of deuterium in the molecules synthesized is not significantly modified by diet. Fat-free mass (FFM) was calculated from the volume of the loading dose (LD) of deuterated water ingested and the deuterium enrichment in plasma water (IEw) as FFM = (LD/IEw)/0.732 (42). All results are shown as means ± SE. Comparisons were performed with two-tailed Student’s t-test for non-paired values or paired values as appropriate.

RESULTS

Lipid synthesis. The concentrations of metabolites and hormones measured in the postabsorptive state are shown in Table 1. Compared with control subjects, the obese group had higher concentrations of glucose, TG, cholesterol, insulin, and leptin (P < 0.01 for all). In the obese group studied after weight loss, plasma TG, cholesterol, insulin, and leptin were decreased (P < 0.05 for all) compared with the values observed before restriction of energy intake. Table 2 shows the diet consumed by the control and obese subjects. During the week before the study, obese and control subjects had comparable total energy intake, expressed as kilocalories per day or relative to FFM. Despite a tendency for higher lipid intake, the proportions in the diet of carbohydrates, fat, and proteins were similar. The daily intake of cholesterol and the proportion of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids were also comparable. Energy restriction in obese subjects was accompanied by a decrease in the contribution of fat to energy intake; the proportions of SFA, MUFA, and PUFA were unchanged.

The fractional contribution of endogenous synthesis to plasma free cholesterol and TG pools in the postabsorptive state is shown in Fig. 1. The contribution of hepatic lipogenesis was largely enhanced in obese patients (14.5 ± 1.9 vs. 7.6 ± 1.3%, P < 0.01), whereas the moderate increase in the contribution of cholesterol synthesis (5.2 ± 0.9 vs. 3.3 ± 0.5%) failed to reach significance (P = 0.10). Reduction of energy intake in four obese subjects lowered hepatic lipogenesis (P < 0.05) to values (4.1 ± 1.8%) comparable to those observed in control subjects. The moderate decrease in cholesterol synthesis was not significant (P < 0.30).

mRNA concentrations in adipose tissue. Compared with control subjects, obese subjects had a large decrease in FAS (P < 0.01) mRNA concentrations. The decrease of SREBP-1c mRNA levels in obese subjects was of borderline significance (P = 0.06). After caloric restriction, FAS mRNA concentrations decreased slightly but not significantly in obese subjects; SREBP-1c mRNA levels, on the contrary, were in-

### Table 2. Dietary intake of lean and obese subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>Obese Patients Before Energy Restriction</th>
<th>Obese Patients After Energy Restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake, kcal/day</td>
<td>1,825 ± 132</td>
<td>1,860 ± 124</td>
<td>1,400 ± 137</td>
</tr>
<tr>
<td>Energy intake, kcal·kg⁻¹·day⁻¹</td>
<td>35.8 ± 3.2</td>
<td>34.9 ± 2.3</td>
<td>28.0 ± 1.8§</td>
</tr>
<tr>
<td>Proteins, %</td>
<td>16.1 ± 0.7</td>
<td>17.9 ± 1.2</td>
<td>23.8 ± 2.1</td>
</tr>
<tr>
<td>Carbohydrates, %</td>
<td>45.4 ± 1.8</td>
<td>39.6 ± 3.1</td>
<td>45.8 ± 3.2</td>
</tr>
<tr>
<td>Lipids, %</td>
<td>38.6 ± 1.3</td>
<td>42.5 ± 2.8</td>
<td>30.2 ± 1.1</td>
</tr>
<tr>
<td>SFA, %</td>
<td>43.4 ± 4.5</td>
<td>40.1 ± 4.3</td>
<td>37.1 ± 3.9</td>
</tr>
<tr>
<td>MUFA, %</td>
<td>42.1 ± 3.2</td>
<td>43.7 ± 2.4</td>
<td>43.8 ± 4.9</td>
</tr>
<tr>
<td>PUFA, %</td>
<td>14.5 ± 2.1</td>
<td>16.2 ± 1.8</td>
<td>19.0 ± 3.3</td>
</tr>
<tr>
<td>Cholesterol, mg/day</td>
<td>235 ± 29</td>
<td>240 ± 19</td>
<td>198 ± 32</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFM, fat-free mass; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.


**DISCUSSION**

We searched in the present report for evidence that enhanced lipogenesis may contribute to the development and/or maintenance of the excessive fat mass of obese subjects. We found indeed an increase in postabsorptive hepatic lipogenesis that was normalized by a moderate restriction of energy intake. The lipogenic capacity of adipose tissue, as appreciated by the measurement of FAS mRNA concentrations, was, on the contrary, found to be decreased in obese subjects and was, if anything, further decreased during energy restriction. Moreover, SREBP-1c mRNA levels were also lower in the adipose tissue of obese subjects. Although we could not measure, because of the limited amount of adipose tissue sampled, FAS protein levels or the active, nuclear form of SREBP-1c protein, these findings strongly suggest that neither FAS expression nor the activity of the main transcription factor stimulating the expression of lipogenic genes was increased in obese patients. These modifications in obese subjects of hepatic lipogenesis and of expression in adipose tissue of lipogenic genes before energy restriction were observed in the absence of significant differences in the contribution of fat and carbohydrates to total energy intake or in the repartition of ingested SFA, MUFA, and PUFA.

The increase in hepatic lipogenesis agrees with previous reports (13). In the absence of liver biopsies, not performed for obvious ethical reasons, we cannot determine the role of enhanced expression of lipogenic genes and of increased substrate availability for lipid synthesis in this stimulation of hepatic lipogenesis. However, the lack of a simultaneous increase in cholesterol synthesis would rather support a role for enhanced expression of lipogenic genes. This would be consistent with the effects of high insulin levels on liver lipogenic genes demonstrated in vitro (14, 18, 19). Whatever the exact mechanism, this increase in hepatic lipogenesis appears to be moderate at first glance.

Assuming a basal postabsorptive secretion rate of TG by the liver of ~0.15 μmol·kg⁻¹·min⁻¹ (20, 33), this lipogenesis would represent a net production of 3.0 μmol/day instead of 1.0 in control subjects, i.e., a daily excess of ~1.5–2 g. This is a minimal estimate since it is probable that this excess in lipogenesis is more marked in the postprandial situation. If we assume an excess in liver TG secretion of 5 g/day, this still seems low compared with a daily oral intake of ~100 g/day. However, if we consider the possible contribution of excess hepatic lipogenesis to body fat stores on a yearly basis, this would represent 0.7–1.8 kg. Thus a seemingly moderate metabolic disturbance could be not trivial on a long-term basis.

We found, on the other hand, no evidence for an increased lipogenic capacity of adipose tissue in obese subjects. However, we sampled only subcutaneous adipose tissue, and results could be different in visceral adipose tissue, as shown for other mRNA levels (29). It should be stressed also that we studied subjects with a large and long-lasting excess in body mass. Their picture is overall comparable to the one described recently in ob/ob mice with established obesity (39, 40). This picture could be different in recent-onset, dynamic obesity. Indeed Zucker rats have during their period of dynamic obesity with rapidly expanding fat stores a large increase in adipose tissue lipogenic capacity (20).

Therefore, the possibility that the expression of lipogenic genes is also increased in adipose tissue of humans with dynamic obesity remains. The decreased expression of lipogenic genes that we observed in the present study could be a late and adaptive process aimed at limiting or preventing a further development of fat mass.

The decrease of FAS gene expression in adipose tissue contrasts anyway with the enhanced hepatic lipogenesis, and the mechanisms behind this discrepancy are unclear. Although there are some differences, as for example in the way PUFA suppress the transcription of lipogenic genes (34–36), the basic mechanisms responsible for the regulation of the expression of the lipogenic pathway are considered to be similar in hepatocytes and adipocytes (14, 19). Insulin stimulates the transcription of lipogenic genes in rat hepatocytes and adipocytes, and this action has been confirmed in human adipocytes (8, 38). It is possible that the difference in insulin concentration between portal and peripheral plasma plays a role in the in vivo difference we

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**Table 3. mRNA concentrations in subcutaneous adipose tissue**

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>Obese Subjects</th>
<th>Obese Subjects After Weight Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 8)</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>FAS</td>
<td>47.5 ± 5.6</td>
<td>13.4 ± 3.2*</td>
<td>8.7 ± 3.1*</td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>7.7 ± 2.0</td>
<td>3.1 ± 0.6</td>
<td>7.6 ± 1.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects; FAS, fatty acid synthase; SREBP, sterol regulatory element-binding protein. Units are amol/μg total RNA. *P < 0.01 vs. the control group. †P < 0.05 vs. the obese group before weight loss.
observed between liver and adipose tissue lipogenic capacity in obese patients. The raised leptin levels of obese subjects could also intervene. There are data supporting a suppressive action of leptin on the transcription of FAS (3) and SREBP-1c (40) and on in vivo lipogenesis (7, 28). This action is present in both liver and adipose tissue, but a direct, paracrine effect in adipose tissue could explain a more marked action on adipocytes in vivo. The rise in SREBP-1c mRNA levels in the presence of decreased leptin concentrations during energy restriction in obese subjects would also support a role for leptin; however, the trend for lower FAS mRNA concentrations after energy restriction cannot be explained by these modifications of plasma leptin and SREBP-1c mRNA levels. It remains possible that the active, nuclear form of SREBP-1c was decreased during energy restriction despite the increase in mRNA levels. Tumor necrosis factor-α, whose expression and secretion by adipocytes is increased in obesity (23), could also explain the decrease in FAS mRNA levels, since it reduces the expression of several genes, including FAS, in adipocytes (12). Last, although it is clear that the transcription factor SREBP-1c plays a major role in the regulation of lipogenic gene expression (16, 17), it should be kept in mind that SREBP-1 alone is a weak activator of transcription and requires for its full action the presence of other transcription factors such as nuclear factor-κ and specificity protein-1 (31, 43). Studies of the relative expression and interactions of SREBP-1 and its cofactors in liver and adipose tissue could help to better understand the decreased expression of lipogenic genes we observed in the adipose tissue of obese patients and the discrepancy between the evolution of SREBP-1c and FAS mRNA levels during energy restriction.

In conclusion, the present results are compatible with a role for enhanced hepatic lipogenesis in the development and/or maintenance of increased fat stores in obesity but do not support a role for increased lipogenic capacity of adipose tissue, at least for the maintenance of excess fat mass. This does not preclude the possible usefulness of inhibitors of FAS in the prevention or treatment of obesity, since such compounds have been shown in mice to act also through a decrease in appetite and food intake (30).

We thank J. Peyrat for help in performing the tests and R. Cohen for the measurements of plasma leptin and insulin concentrations. This work was supported by Fisheries and Agriculture Industrial Research Grant CT97–3011 from the European Economic Community.

REFERENCES


