Changes in the metabolism of fatty acids in adipose tissue in obese patients with primary hypertriacylglycerolemia

Peter Arner, Peter Engfeldt, and Jan Östman
Department of Medicine and the Research Center, Huddinge Hospital, Karolinska Institute, Sweden

Abstract

Changes in the release and esterification of free fatty acids (FFA) in adipose tissue were looked for as a cause of moderate primary hypertriacylglycerolemia (HTG) in five obese subjects. Comparison was made with six obese normolipidemic subjects. The two groups were matched for body weight, tolerance of intravenous glucose, fat cell size, fasting levels of serum immunoreactive insulin, and serum insulin response to an intravenous glucose load. Subcutaneous adipose tissue was incubated in vitro with \[^{1-14}C\]palmitic acid for 30, 60, and 120 min. There was a significant, twofold increase in the rate of FFA mobilization, but no change of glycerol release in HTG patients. The adipose tissue levels of mono- and diacylglycerols were similar in the two groups of subjects and did not change during incubation. Re-esterification of FFA, calculated from the net changes in medium and in tissue FFA and glycerol release, was lower in HTG patients than in the controls (3 and 12 pmol/10⁷ cells/hr, respectively; \(P < 0.025\)). In adipose tissue of HTG patients, the amount of radioactive fatty acids incorporated into triacylglycerols (TG) was 50% lower (\(P < 0.025\)), whereas that incorporated into tissue FFA was three times higher (\(P < 0.01\)) when compared with control patients. It is concluded that, in adipose tissue of obese patients with primary hypertriacylglycerolemia, the esterification of free fatty acids to triacylglycerol is decreased. As a consequence, free fatty acids are mobilized at an increased rate.—Arner, P., P. Engfeldt, and J. Östman. Changes in the metabolism of fatty acids in adipose tissue in obese patients with primary hypertriacylglycerolemia. J. Lipid Res. 1982. 23: 422-427.

Supplementary key words
lipolysis • esterification • acylglycerols

Metabolic abnormalities in human adipose tissue may be an important component of hypertriacylglycerolemia (HTG). It has been demonstrated that inhibition of lipoprotein lipase activity in adipose and other tissues in vitro is associated with decreased removal of triacylglycerols (TG) from blood (1). It has also been shown in vitro that in HTG there is an increase in the rate of mobilization of free fatty acids (FFA) and a decrease in the ability to store FFA in the fat depots (2–5). Any of these defects may increase the level of plasma FFA and thus stimulate the synthesis of hepatic TG (6).

In the previous studies on adipose tissue FFA metabolism in HTG (2–5), no attempts were made to classify the abnormality in primary or secondary forms of HTG. The object of the present investigation was therefore to elucidate the mechanisms behind abnormal mobilization and storage of FFA by adipose tissue in primary HTG. For this purpose subcutaneous adipose tissue was obtained from five obese subjects with primary HTG of type IV according to the classification of Fredrickson, Levy, and Lees (7). The tissue was incubated in the presence of radioactive palmitic acid. Determinations were made of the incorporation of radioactivity into tissue FFA and TG, and the release of FFA and glycerol to the incubation medium. The re-esterification of FFA was calculated, taking into account changes in the tissue concentrations of FFA, monoacylglycerols (MG), and diacylglycerols (DG). A control group was composed of six subjects. The two groups were matched for degree of obesity, fat cell size, fasting levels of serum immunoreactive insulin, intravenous glucose tolerance, and serum insulin response to an intravenous glucose load.

MATERIAL AND METHODS

Patients

The series consisted of eleven patients admitted to hospital for the treatment of obesity. Five of the patients had HTG with a moderately elevated fasting plasma TG level and six had normal fasting plasma TG concentrations. Before entering the study, body-weight, fat cell size, fasting serum insulin, and intravenous glucose were determined in all subjects at the hospital’s outpatient clinic. The two groups of obese subjects were matched according to the above mentioned parameters. The fasting level of plasma TG was determined two or three times before the study. HTG was defined as a plasma TG concentration in excess of 3 mmol/l on all occasions, the presence of a clear pre-beta band on agarose lipoprotein electrophoresis, and absence of chylomicrons (8).

Abbreviations: FFA, free fatty acids; MG, monoacylglycerol; TG, triacylglycerol; DG, diacylglycerol; HTG, hypertriacylglycerolemia.
In four patients genetic analysis according to previously defined criteria (9–11) was carried out. Pure familiar form of HTG of type IV (7) was established in all these patients. The fifth patient had no family but the plasma TG concentration remained high after his body weight had been reduced by 25 kg. The patients were otherwise healthy and they did not regularly take drugs or alcohol.

The six control subjects had a plasma TG concentration below 2.1 mmol/l on all occasions. Electrophoresis disclosed only a light staining pre-beta band. Except for obesity, there were no metabolic disturbances. All HTG and reference subjects were in a caloric steady state as judged from histories of diet and body weight, and there were no apparent differences in eating habits between the two groups. Clinical characteristics of the two groups are given in Table 1.

The study was approved by the Ethical Committee of the Karolinska Institute. Details of the study were explained to each patient and his or her consent was obtained.

**Table 1. Clinical features of obese patients with hypertriacylglycerolemia (HTG) and obese control**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Relative Body Weight</th>
<th>Plasma TG</th>
<th>Plasma Total Cholesterol</th>
<th>Plasma HDL Cholesterol</th>
<th>Fasting Blood Glucose</th>
<th>Fasting Serum Insulin</th>
<th>k-Value</th>
<th>EIR</th>
<th>Fat Cell Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTG</td>
<td>F:3 M:2 47 ± 3</td>
<td>156 ± 7</td>
<td>3.8 ± 0.1</td>
<td>6.2 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>0.2</td>
<td>1287 ± 151</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>F:4 M:2 47 ± 5</td>
<td>155 ± 9</td>
<td>1.6 ± 0.1</td>
<td>6.0 ± 0.2</td>
<td>4.5 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>0.2</td>
<td>1295 ± 144</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS NS NS 0.001 NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The relative body weight was obtained from tables computed by Documenta Geigy (44). TG, triacylglycerol; HDL, high density lipoprotein; EIR, early insulin response to intravenous glucose injection (17); NS, not significant.

**Experimental procedures**

The patients were fed the general hospital diet for 3 or 4 days. After an overnight fast, subcutaneous adipose tissue (6–8 g) was obtained surgically from the femoral region under local anaesthesia (12). Just before the specimens were taken, venous blood samples were drawn for the determination of glucose (13), and the levels of TG (14), total cholesterol, high density lipoprotein cholesterol (15), and immunoreactive insulin (16). Immediately after the specimens had been taken, 25 g of glucose was injected intravenously over a period of 2.5 min, and the k-value for the glucose disappearance and the early serum insulin response to the injected glucose were determined (17).

Adipose tissue was divided into segments weighing 0.2–0.3 g and was pre-incubated for 30 min in Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°C) with dialyzed bovine serum albumin (40 mg/ml) (Fraction V, Armour Pharmaceutical Co., England; fatty acid:albumin molar ratio 0.77) and glucose (2 mg/ml). About 0.6 g of tissue was then incubated for 30, 60, and 120 min in fresh medium (6 ml) of the above type to which had been added [1-14C]palmitic acid (approximately 10⁶ cpm/ml; specific activity 27 Ci/mole; The Radiochemical Centre, Amersham, England). Each incubation was performed in triplicate with air as the gas phase. After incubation, aliquots of the medium were removed for enzymatic determination of glycerol (18) and extraction and titrimetric estimation of FFA (19). The pre-incubation and incubation procedures have been described in detail elsewhere (20). There was no change in pH of the medium during incubation. The tissue levels of MG, DG, and FFA, and the radioactivity in these lipids were determined after separation by combined column and thin-layer chromatography, as described in detail elsewhere (21). FFA was estimated by titration and the amounts of MG and DG present were determined from their glycerol content after hydrolysis (22). Tracer amounts of 3H-labeled internal standards were always added for recovery purposes as described earlier (21). The radioactivity in TG was calculated as: radioactivity in total lipids minus radioactivity in (DG + MG + FFA). Insignificant incorporation of labeled palmitic acid into adipose tissue cholesterol esters, phosphatidic acid, and other phospholipids was observed, in both the control and the HTG patients. No radioactivity was found in the glycerol moiety of the acylglycerols. All the radioactive samples were counted in a Tri-Carb-Liquid system. Quenching was corrected for by the use of internal standards. The composition of the scintillation fluid has been described elsewhere (21). We have previously observed that, in adipose tissue obtained from obese subjects and incubated in vitro under basal conditions, less than 0.1% of mobilized glycerol is re-utilized by the tissue (23).

The fat cell diameter was determined by the method of Sjöström Björntorp, and Vrina (24). The mean fat cell volume and the fat cell number were calculated by the method of Hirsch and Gallian (25).

The balance method described by Vaughan (26) was used to calculate re-esterification of FFA. This method is based on the assumption that triacylglycerols are completely hydrolyzed so that 1 mole of glycerol formed by
hydrolysis of TG corresponds to 3 mole of FFA. Thus, re-esterification of FFA can be calculated as net glycerol release \( \times 3 - (\text{net FFA release} + \text{net change in the tissue FFA concentration}) \).

When the absolute amount of fatty acids taken up from the medium by the adipose tissue was calculated, it was necessary to take into consideration the fact that the specific radioactivity of medium fatty acids decreases throughout the incubation period because of release of unlabeled FFA from adipose tissue. Therefore the method of Dole (27) was used to take into account the continuous dilution of the specific radioactivity. From the initial and final concentrations of FFA and radioactivity, the mean specific radioactivity of medium fatty acids in each incubation tube was calculated using equations evolved by Dole (27).

Statistical analyses were performed using Student's unpaired t-test. The values given are the mean \( \pm \) standard error of the mean (SE).

**RESULTS**

The tissue concentrations of FFA, MG, and DG were approximately the same in the patient and control groups and did not change significantly during incubation (Fig. 1). The rate of glycerol release was also similar in the two groups but the rate of FFA release was almost twice as rapid in the HTG group (\( P < 0.025 \) at 120 min). Since the concentration of partial acylglycerols was constant during the incubation period, the non-isotopic "balance method" of Vaughan (26) gives an accurate estimation of FFA re-esterification. During the last hour of incubation, \( 3.1 \mu \text{mol of FFA/10}^7 \text{ cells was re-esterified in the HTG group against 11.7 in the control group; the difference is statistically significant (} P < 0.025 \). The absolute values for the fatty acid uptake by adipose tissue are shown in Fig 2. There was a linear increase in the rate of fatty acid incorporation into TG in both groups, but the rate was twice as rapid in the control group (\( 2 \mu \text{mol/10}^7 \text{ cells/hr} \) than in the HTG group (\( 1 \mu \text{mol/10}^7 \text{ cells/hr} \)), and the difference was significant (\( P < 0.025 \)). In both groups the incorporation of fatty acids into tissue FFA increased during the first 30 min of incubation and was then rather constant. However, the incorporation of fatty acids in tissue FFA was three times greater for the HTG patients than for the controls at 30 min (\( P < 0.05 \)), 60 min (\( P < 0.05 \)), and 120 min (\( P < 0.01 \)), respectively. In both groups the incorporation of fatty acids from the medium in DG plus MG was less than 20% of the total radioactive uptake throughout the

![Fig. 1](https://example.com/fig1.png) Lipolysis and re-esterification of free fatty acids (FFA) in adipose tissue in obese HTG patients (solid lines, filled bar) and obese control subjects (broken lines, open bar). Adipose tissue was incubated and the release of FFA and glycerol to the medium and the tissue levels of FFA, monoacylglycerol (MG) and diacylglycerols (DG) were determined. The re-esterification of FFA between 60 and 120 min of incubation was calculated. The statistical symbols are \( \times = P < 0.05 \), \( \times \times = P < 0.025 \), and \( \times \times \times = P < 0.01 \).
incubation period (data not shown). The total fatty acid incorporation increased successively during the incubation and at 2 hr was similar in adipose tissue of control subjects and HTG patients (5.7 ± 1.1 and 6.5 ± 0.9 μmol/10^7 cells, respectively). The initial and final specific radioactivities of the incubation medium were in the same order of magnitude in both groups (Table 2). The increase in the medium FFA concentration of 2 hr of incubation was about 70% higher for the HTG patients than for the controls, but the difference in isotopic dilution was only about 25% between the two groups (Table 2). At 1 hr of incubation, however, isotopic dilution was 65% greater in HTG than in the control state (data not shown).

There were no differences in adipose tissue metabolism between males and females in controls or HTG patients (data not shown).

### DISCUSSION

Adipose tissue of the obese patients with primary HTG in this study displayed enhanced mobilization of FFA and decreased esterification of FFA to TG, in comparison with tissue from obese normolipidemic subjects. The conclusion that HTG is associated with inhibited esterification is supported by two independent findings in this study, namely, decreased re-esterification of FFA when measured with a non-isotopic "balance method" (26) and decreased incorporation of fatty acids into TG as measured by an isotope technique. It is not likely that the latter findings are influenced by hydrolysis of newly synthesized radioactive acylglycerols since we, in a previous study (21), found no evidence of recirculation of radioactivity when human adipose tissue was incubated in vitro. Although media specific activity was similar in both groups, the findings may have been influenced by differing dilutions of specific activity of FFA entering the tissue. A more likely explanation, however, for the observation in HTG patients that the incorporation of labeled palmitic acid was increased in tissue FFA, is that HTG is associated with decreased FFA esterification. The latter may be due to inhibition at one or several steps in the synthesis of TG. However, in order to localize an esterification defect, it is necessary to take into account the existence of several FFA andDG pools in fat cells that are involved in either synthesis or hydrolysis of acylglycerols (27-30). There are, however, no methods for isolating and determining all these pools.

It is well established that obese subjects have an accelerated rate of lipolysis in vivo and in vitro (31), and increased rate of lipolysis in adipose tissue is one reason for elevated plasma FFA levels. The latter may cause HTG since an increased plasma FFA level can stimulate the hepatic TG production (32). However, previous studies of the rate of lipolysis in vitro in patients with HTG have yielded conflicting results, both increased (2, 3) and normal rates (4) having been reported. When, in the present study, account was taken for changes in partial acylglycerols of human adipose tissue in obesity (23), there was no evidence of any further increase in the rate of lipolysis.

### TABLE 2. Specific radioactivity of [1-14C]palmitic acid and FFA concentration in the incubation medium

<table>
<thead>
<tr>
<th></th>
<th>Specific Radioactivity</th>
<th>Medium FFA Concentration</th>
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<tbody>
<tr>
<td></td>
<td>0 min of incubation</td>
<td>120 min of incubation</td>
</tr>
<tr>
<td></td>
<td>cpm/μmol FFA</td>
<td>μmol/ml</td>
</tr>
<tr>
<td>HTG</td>
<td>3,090,000 ± 300,000</td>
<td>2,070,000 ± 240,000</td>
</tr>
<tr>
<td>Control</td>
<td>2,620,000 ± 240,000</td>
<td>1,780,000 ± 100,000</td>
</tr>
<tr>
<td>F</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Aliquots of the incubation medium containing [1-14C]palmitic acid were taken before and after 120 min incubation for the determination of radioactivity and FFA concentration. Specific radioactivity = the concentration of radioactivity divided by the concentration of FFA. Values for HTG patients were statistically compared with those for control subjects.
of lipolysis in vitro when the obese HTG patients were compared with the obese control subjects. The observed twofold increase in the rate of FFA release cannot, therefore, have been due to abnormal lipolysis. It may, however, be attributed to the impaired capacity of adipose tissue to re-esterify FFA in HTG. For several reasons, the presently used “balance method” (26) for the calculation of FFA re-esterification can be considered to give an accurate estimation of re-esterification. First, there was no evidence of partial hydrolysis of TG. Second, we have previously shown that insignificant re-utilization of glycerol takes place under the incubation conditions used here (23). In contrast to our findings, Carlson and Walldius (4) found no significant alteration of the FFA release in HTG, but they did not measure the tissue FFA level and FFA release was not related to fat cell size; both factors may be of importance for the tissue FFA level and FFA release was not related to reduced conditions used here (23). In contrast to our findings, Carlson and Walldius (4) found no significant alteration of the FFA release in HTG. For several reasons, it may, however, be attributed to the impaired capacity of adipose tissue to re-esterify FFA in HTG. For several reasons, we have previously shown that insignificant re-utilization of glycerol takes place under the incubation conditions used here (23). In contrast to our findings, Carlson and Walldius (4) found no significant alteration of the FFA release in HTG, but they did not measure the tissue FFA level and FFA release was not related to fat cell size; both factors may be of importance for FFA mobilization.

It was necessary to include only obese subjects in the present investigation because the methods required large amounts of adipose tissue. It is therefore not known if our findings also hold true for non-obese subjects. It may, however, that Walldius (33) found similar low rates of fatty acid incorporation in adipose tissue of obese and lean persons with HTG.

It is not known how our in vitro results relate to the in vivo state since plasma FFA was not determined in the present study. However, caution must be exercised in translating in vitro studies to the in vivo state where the hormonal and substrate milieu are so different. Previous studies of the relationship between plasma FFA and HTG have yielded conflicting results (34–42). While some investigators have found increased FFA levels or turnover in HTG (34–37), others have not (38–42). Furthermore, Boberg and his colleagues (42) found no correlation between FFA and TG turnover rates in vivo when they compared normal and HTG subjects. It must be kept in mind, however, that steady state conditions are difficult to obtain when FFA metabolism is to be studied in vivo, since there are marked diurnal variations in plasma FFA (43). It has been shown (43) that these variations are most prominent during the morning when most in vivo studies are performed.

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REFERENCES


