Fatty Acid Synthesis in Human Adipose Tissue*

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SUMMARY

The concentration of enzymes required for fatty acid synthesis was considerably less in extracts prepared from human omental adipose tissue than from rat epididymal fat. Overall rates of synthesis as measured by the incorporation of radioactive precursors into fatty acids were also much lower in human adipose tissue. The most striking difference was found with citrate-1,5-14C, human adipose tissue incorporating the compound at a rate less than one-tenth that of rat epididymal fat. This finding was consistent with the virtual absence of the citrate cleavage enzyme in human adipose tissue. By contrast, the activities of pyruvate carboxylase and phosphoenolpyruvate carboxykinase, two enzymes necessary for the synthesis of glyceraldehyde phosphate from pyruvate, and the long chain fatty acid activating enzyme (or enzymes) were essentially similar in both human and rat adipose tissues. The results are consistent with the hypothesis that synthesis de novo of fatty acids is not an important physiological function of human adipose tissue.

The pathway and mechanism of fatty acid biosynthesis de novo have been elucidated through studies in a variety of organisms (1). In all species acetyl coenzyme A carboxylase represents an essential control point for metabolic regulation of fatty acid synthesis (2). Studies in some higher animals, particularly rats, have shown the importance of the citrate cleavage enzyme (3, 4). The function of this enzyme is to re-form acetyl-CoA in the cytosol from citrate transported through the mitochondria (5). More recently, however, the significance of the citrate cleavage enzyme in the rat has been questioned (6). Acetyl-CoA carboxylase and the citrate cleavage enzyme, together with the fatty acid synthetase complex and certain TPN-linked dehydrogenases, show increased or decreased activities which are dependent upon the over-all rate of lipogenesis (7). These enzymes, therefore, appear to be either under direct or indirect hormonal control, particularly that of insulin. The enzymes required for fatty acid biosynthesis in rat epididymal fat have been identified and their regulatory function appears to be identical with that found in liver (2, 8). Human adipose tissue which has not been so extensively investigated may have a metabolic pattern quite different from that of the rat. For the most part results with human tissue fragments and isolated fat cells show poor rates of incorporation of radioactive precursors such as glucose into fatty acids, and the response to insulin is variable and often absent (9, 10). Previous studies from this laboratory (11) have shown comparable activities of glucose 6-phosphate dehydrogenase and malic enzyme in human and rat adipose tissue. However, in human adipose tissue there was no change in enzyme concentration under conditions of fasting, refeeding, or long term insulin administration. Similar metabolic states in the rat profoundly alter the concentration of these enzymes in the direction compatible with the magnitude of over-all lipogenesis. Furthermore, it was shown that the citrate cleavage enzyme was virtually absent in human adipose tissue while significant concentrations were found in human liver. These observations raise a question concerning the pathway of fatty acid biosynthesis de novo, or indeed, whether or not it occurs at all in human adipose tissue. Further investigation as to alternative pathways or mechanism for triglyceride synthesis would seem to be necessary. The present communication gives a more detailed analysis of the concentration of enzymes required for fatty acid synthesis and the incorporation of various radioactive precursors into fatty acids in extracts of human adipose tissue.

EXPERIMENTAL PROCEDURE

Similar concentrations of a number of enzymes related to lipogenesis have been found in human omental, mesenteric, and subcutaneous adipose tissue (11). In the present study, however, only human omental fat was used since it is generally considered to be more closely related to rat epididymal fat in metabolic activity. Human omental fat obtained at surgery was rinsed in 0.25 M sucrose, blotted dry, weighed, and homogenized in a ratio of 1.0 g, wet weight, to 3.0 ml of a solution containing 0.25 M sucrose and 1.0 mM dithiothreitol. A high speed supernatant fraction was prepared by initial centrifugation of the homogenate at 32,000 × g for 10 min. After removal of the fat cake, the resulting supernatant was centrifuged at 105,000 × g for 60 min, poured over glass wool, and used directly for the various assays. The 105,000 × g precipitate representing the microsomes was rinsed and taken up in homogenizing medium. For isolation of mitochondria the homogenate was initially centrifuged at 1000 × g for 10 min to remove cell debris and nuclei. The supernatant was then centrifuged at 10,000 × g for 10 min to precipitate the mitochondria which were resuspended, washed twice, and finally taken up in homogenizing medium. Rat epididymal fat was prepared in an identical manner. Enzymatic assays and radioactive incorporation studies were carried out according to standard published procedures. The
fatty acid synthetase assay was that of Martin et al. (8); acetyl-CoA carboxylase, Martin and Vagelos (2); acetyl-CoA-1-14C incorporation into fatty acids, Martin and Vagelos (2); citrate-1,5-14C and acetate-1-14C incorporation, Sponer and Lowenstein (4); and the citrate cleavage enzyme was that of Sorensen (12). The radioactive assay for pyruvate carboxylase was that of Utter and Kees (13), and phosphoenolpyruvate carboxykinase, that of Chang and Lane (14). Fatty acid activation was studied according to the hydroxamate method as suggested by Pandol and Meade (15). The concentrations of substrates and cofactors added to give maximum rates for all reactions were identical or only slightly different from published values. In all cases preliminary protein concentration and time curves were determined, and all assays were carried out under optimum conditions at 30° which gave linear and maximum rates. Results are expressed as either micromoles or counts per min per mg of protein. Calculation on a wet weight basis gave similar values. Triglycerides and fatty acids were separated by the method of Hirsch and Ahrens (16). Properly prepared aliquots from the completed reactions were added to 20 ml of Bray's solution (17) and counted in a Packard liquid scintillation spectrometer. Oxidation and reduction of pyridine nucleotides were assayed in a Gilford recording spectrophotometer at 340 mp and 23°. Chemicals and radioactive isotopes were of the highest quality commercially available and were not purified further. Reagents were prepared in distilled deionized water. Experiments with human tissue were carried out on the same day the surgical biopsy specimen was obtained since a decrease in some of the enzyme activities has been noted on storage. Rat tissue was frequently assayed at the same time for comparison.

### Table I

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction mixture</th>
<th>Recovery</th>
<th>Fraction 1</th>
<th>Fraction 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride standard</td>
<td>-</td>
<td>91.00</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>Palmitate standard</td>
<td>-</td>
<td>0.44</td>
<td>90.00</td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>-</td>
<td>0.50</td>
<td>84.00</td>
<td></td>
</tr>
</tbody>
</table>

**RESULTS**

Initial studies on the requirements for fatty acid synthesis in human adipose tissue were carried out to determine optimal conditions for individual enzyme assays and for the incorporation of radioactive precursors into fatty acids. The results in Table I show significant decreases in activity when important substrates or cofactors are omitted from the incubation system. Since, for the most part, the methods involve measurements of radioactivity in crude cellular fractions, the results obtained lend confidence to the specificity and sensitivity of the procedures.

Radioactive fatty acids were isolated by the standard techniques of saponification with ethanolic NaOH, acidification, and extraction with petroleum ether. To be certain that the radioactivity was in the fatty acid fraction, an aliquot of the mixture to be counted was first separated by silicic acid chromatography along with radioactive palmitate and triglyceride standards. Table II presents data showing that essentially all the radioactive material recovered in the experimental reaction corresponded to the fatty acid fraction.

The effect of citrate on the activity of acetyl-CoA carboxylase in human adipose tissue is shown in Fig. 1. Activation of the enzyme by citrate appears to be similar to that produced in other organisms and tissues except that there is relatively little inhibition at higher concentrations. The concentrations of citrate in human and rat adipose tissue under basal conditions were determined (18) and found to be 10.5 and 0.9 μg per g, wet weight, respectively. These values are low when compared to rat liver (93 μg per g, wet weight) which may indicate that intracellular compartmentalization rather than actual concentration of citrate in adipose tissue plays an important role in the control of acetyl-CoA carboxylase and fatty acid biosynthesis.

Reported rates for fatty acid synthesis by tissue fragments and isolated cells from human fat are considerably lower than those obtained with rat epididymal fat (9, 10, 19). One of the reasons given to account for this discrepancy is the possible traumatization of human tissue, particularly if obtained in small amounts by needle biopsy. Also, it has been speculated that rat epididymal fat is metabolically more active than human fat, especially from subcutaneous sites. In the present experiments care was taken to use only human omental fat in amounts adequate to prevent any traumatization to the tissue. Fig. 2 compares the activities of certain important enzymes for fatty acid synthesis in human adipose tissue with those obtained with rat epididymal fat (19). The data show that the activities of acetyl-CoA carboxylase and fatty acid synthetase are considerably lower in human adipose tissue than in rat epididymal fat. The activity of citrate synthetase is also lower in human adipose tissue than in rat epididymal fat. The activity of citrate cleavage enzyme is somewhat lower in human adipose tissue than in rat epididymal fat, but the activity of fatty acid synthetase is considerably lower in human adipose tissue than in rat epididymal fat. The activity of citrate synthetase is somewhat lower in human adipose tissue than in rat epididymal fat, but the activity of fatty acid synthetase is considerably lower in human adipose tissue than in rat epididymal fat. The activity of citrate synthetase is somewhat lower in human adipose tissue than in rat epididymal fat, but the activity of fatty acid synthetase is considerably lower in human adipose tissue than in rat epididymal fat. The activity of citrate synthetase is somewhat lower in human adipose tissue than in rat epididymal fat, but the activity of fatty acid synthetase is considerably lower in human adipose tissue than in rat epididymal fat.
FIG. 1. Activation of acetyl-CoA carboxylase by citrate in human adipose tissue. Assays were run as described in Table I.

FIG. 2. Comparison of enzymes for fatty acid synthesis in rat and human adipose tissue. Assay methods are as described in Table I or "Experimental Procedure." The values represent an average of at least six animals or human surgical specimens with the standard deviation shown above each bar.

FIG. 3. Comparison of rates of fatty acid synthesis from various radioactive precursors in rat and human adipose tissue. Assays are as described in Table I and results expressed as in Fig. 2.

acid synthesis in rat epididymal and human omental adipose tissue. The concentration of citrate cleavage enzyme in the rat is considerably higher than either the acetyl-CoA carboxylase or fatty acid synthetase and thus would not appear to be rate-limiting. Of importance is the extreme difference between the citrate cleavage enzyme in rat and human adipose tissue, an observation which has been previously reported (11). The activity of the citrate cleavage enzyme in human adipose tissue is barely detectable by spectrophotometric assay and is, in order of magnitude, some 25 times lower than in the rat, while the acetyl-CoA carboxylase and fatty acid synthetase are about 5-fold less than comparable activities in rat epididymal fat.

The relationship between the incorporation of important radioactive precursors into fatty acids in rat and human adipose tissue are compared in Fig. 3. In the rat the rates of incorporation of acetyl-CoA-1-14C and citrate-1,5-14C into fatty acids are similar and less than when malonyl-CoA is added to the reaction mixture. It would appear from these results that acetyl-CoA carboxylase rather than the citrate cleavage enzyme is the rate-limiting step, which agrees with the results in Fig. 2. The pattern in human adipose tissue is both qualitatively and quantitatively different. The rate of acetyl-CoA incorporation with or without malonyl-CoA is approximately 3- to 5-fold less than in the rat. In order to compare directly all the substrates tested, this incorporation of acetyl-CoA-1-14C with malonyl-CoA was multiplied by 8 in order to account for acetate per mole of palmitate. Incorporation of citrate into fatty acid is 5-fold less than that from acetyl-CoA and 16-fold less than its rate of incorporation in the rat. The low incorporation of citrate is consistent with the virtual absence of the citrate cleavage enzyme in human adipose tissue. When expressed as counts per min, the
comparative values for citrate incorporation are even lower in human adipose tissue. Since the carrier citrate added is both an activator as well as a substrate, the value of 10 μmoles used in converting the counts per min to micromoles incorporated may be too high. To account for the fact that only the carbon atom in position 1 of citrate is readily incorporated into fatty acids, the amount of citrate converted was calculated by dividing the radioactivity of the isolated fatty acids by one-half the specific activity of the citrate-1,5-14C (4).

Somewhat surprising was the finding of a low rate of incorporation of acetate into fatty acids by rat epididymal fat. This is in contrast to its active incorporation in rat liver (20) and lactating mammary gland (21). In fact, as is shown in Fig. 3, acetate is a better substrate for fatty acid synthesis in human than in rat adipose tissue. Since acetate appeared to be a much better precursor for fatty acid synthesis than citrate (Table I and Fig. 3) in human adipose tissue, the characteristics of its incorporation were studied in somewhat more detail. Fig. 4 illustrates the activation by various tricarboxylic acids of the rate of acetate incorporation into fatty acids. Citrate is slightly more effective than isocitrate, while fumarate and malate give considerably less activation. Succinate and oxalacetate are essentially ineffective.

It has been observed that rat epididymal fat incubated in the absence of glucose can convert pyruvate to glycerol by way of a dicarboxylic intermediate (22, 23), and it was reasoned that the metabolic pathway involved may have some significance during periods of glucose deprivation. The basis for this proposal was the observation that rat adipose tissue contains significant amounts of the gluconeogenic enzymes, pyruvate carboxylase (24) and phosphoenolpyruvate carboxykinase (23). Inasmuch as the enzymes for fatty acid synthesis de novo in human adipose tissue appeared to be considerably less than those found in the rat, it seemed also appropriate to study the key enzymes for the synthesis of glyceride glycerol from non-glucose precursors. Table III compares the activities of pyruvate carboxylase and phosphoenolpyruvate carboxykinase in rat and human adipose tissue. In contrast to the dissimilar concentrations of the fatty acid synthesizing enzymes in the two tissues, there appeared to be approximately equal amounts of pyruvate carboxylase and phosphoenolpyruvate carboxykinase, indicating the “shuttle pathway” is operative in human as well as in rat adipose tissue.

One alternative to fatty acid synthesis de novo in human adipose tissue would be the uptake of fatty acids from serum, particularly after hydrolysis of triglycerides at the fat cell membrane. An important enzymatic reaction would then be the activation of the fatty acids to CoA derivatives. Table IV compares the rates of activation of fatty acids in rat and human adipose tissue. As reported for rat liver (15), almost the entire activity was obtained in the microsomal fraction of adipose tissue, and the enzyme concentrations were similar. There was a decrease in hydroxamate formation with decrease in chain length of saturated fatty acids, and unsaturated oleic acid was acylated almost as rapidly as palmitic acid. It should be noted that in the rat the
concentration of the long chain fatty acid activating enzyme (or enzymes) is approximately equal to that of the fatty acid synthesizing enzymes. By contrast, in the human, the activating enzyme (or enzymes) is from 15 to 30 times higher.

**DISCUSSION**

The present results show that fortified high speed supernatants from human adipose tissue synthesize fatty acids from citrate at a rate less than one-tenth that of the same preparation from rat epididymal fat. The concentrations of the enzymes related to fatty acid synthesis in human adipose tissue are significantly less than those in rat adipose tissue. The greatest difference which appears to account for these findings in human fat is the fact that citric cleavage enzyme activity is minimal or absent. The present results confirm and extend previous studies which led to the suggestion that fatty acid synthesis de novo was not a physiological function of human adipose tissue (11). Galton (19) has shown that in isolated adipose tissue cells approximately 0.6% of glucose-14C was recovered in fatty acids and the rest in glyceride glycerol, whereas palmate-14C was readily incorporated into neutral lipid. These studies are consistent with the results presented and the proposed hypothesis for lipogenesis in human adipose tissue. Recently it has been shown that under normal conditions and then re-esterified in the fat cells (11, 26).

Pyruvate carboxylase was first identified in rat epididymal fat by Wise and Ball (24) who suggested that it might function in the formation of precursors for fatty acid synthesis. More recently phosphoenolpyruvate carboxykinase has also been observed in rat adipose tissue (23). Ballard et al. (23) as well as Chakrabarty and Leveille (30) have shown that pyruvate carboxylase and phosphoenolpyruvate carboxykinase are active in the synthesis of the glyceride glycerol moiety from non-glucose precursors. In contrast to the lack of fatty acid synthesis in human adipose tissue, there would appear to be no impairment in the synthesis of glyceride glycerol from pyruvate. It was hypothesized that this pathway may be of metabolic significance in the rat during periods of fasting (23). Whether this may also be the case in human adipose tissue cannot be ascertained from the present work.

In this study no attempt was made to examine the effects of metabolic factors such as fasting, refeeding, and insulin administration on fatty acid synthesizing enzymes. However, previous work (11) has shown that related lipogenic enzymes, including glucose 6-phosphate dehydrogenase and the malic enzyme, in human adipose tissue are not sensitive to these stimuli, and it may tentatively be inferred that similar results would be obtained with acetyl-CoA carboxylase and the fatty acid synthetase complex. Gries and Steinke (10) have shown an effect of insulin on glucose-14C incorporation into 14CO2 and long chain fatty acids in human adipose tissue. It should be noted, however, that they obtained 55% of the radioactivity incorporated into total lipids as glyceride glycerol, whereas the fatty acid labeling was minimal. Insulin had no effect on the glyceride glycerol fraction. It may also be significant that the most pronounced effect of insulin was in younger subjects. The present studies were carried out with adult adipose tissue and it may be that the metabolic pathway and regulation of fatty acid synthesis is different in children than it is in adults.

Of some interest was the observation that acetate incorporation was at least 2-fold greater than that of citrate in human adipose tissue, and that it was almost negligible in rat adipose tissue. Acetic acid levels in human blood as determined by gas-liquid chromatography were found to be in the range of 1 mg/100 ml. While this is some 5- to 10-fold less than levels found in ruminants that actively convert acetate to fatty acids, it may be of physiological importance in humans if the turnover of this compound is found to be significantly high. Further studies related to this possibility are now under investigation.

**REFERENCES**


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