Obese subjects were compared with lean subjects to define the previously reported disturbance of plasma free fatty acid (FFA) kinetics in terms of altered net transport (lipolysis) or clearance (esterification). These measurements were made during prolonged constant infusions of $1-\text{C}^{14}$-palmitate toward the end of sustained glucose ingestion and again 6-8 hr after stopping glucose. Net transport of FFA was suppressed to equally low levels in obese and lean subjects, though at the expense of higher insulin concentrations in the obese. Whereas in the lean subjects the clearance of FFA was significantly stimulated with glucose, the obese subjects showed low clearance rates both during and after stopping glucose. When glucose was stopped, net transport rose more rapidly and to a greater extent in some obese than in the lean subjects. The increased influx of FFA led to a rise in the plasma triglyceride level only in the lean subjects. These studies suggest that clearance of plasma FFA, probably denoting esterification in tissues such as muscle and adipose tissue, is impaired in obesity and cannot be readily stimulated with glucose and insulin. Lipolysis, measured as net transport of FFA, however, is suppressible with glucose and insulin in the obese, though this might be achieved only at insulin levels that are higher than those in lean subjects.

The plasma free fatty acid (FFA) concentration is raised in obesity. This finding is thought to be primarily related to increased plasma FFA transport, which is significantly correlated with excess weight and probably reflects the greatly increased lipolytic activity within adipocytes that are increased both in number and size. However, a disturbance in the regulation of the clearance of FFA from plasma would also alter the transport and the concentration of plasma FFA. Diminished clearance of plasma FFA in obese subjects has been inferred from the decreased reduction in FFA concentration following the ingestion of sucrose or the infusion of insulin, which stimulate the uptake and reesterification of FFA within muscle and adipocytes. However, insulin also mediates the suppression of lipolysis, and this effect cannot be distinguished from the influence on reesterification by measurement of the plasma FFA level alone.

Studies of FFA kinetics during constant infusion of radiopalmitate permit quantification of transport into and from the plasma compartment. We have previously utilized the technique to define net transport of FFA as an index of lipolysis in adipose tissue, and the fractional clearance rate of FFA as a probable index of reesterification in tissues. Whereas influx of plasma FFA in the fasting state appears to occur only from adipose tissue, and is accentuated in

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obesity, efflux occurs into most organs, the liver and muscle being the two major sites of plasma FFA removal in the postabsorptive state. In obesity the removal of FFA in the liver appears to be increased, but it has been shown to be reduced in muscle. The clinical significance of this altered balance lies in the stimulus to hepatic triglyceride formation leading to possible hypertriglyceridemia and hepatic steatosis, and possibly to secondary impairment of glucose metabolism.

The present study has examined the relative importance of influx and efflux as determinants of the raised plasma FFA concentration in obesity by measuring FFA kinetics during and following sustained consumption of glucose.

MATERIALS AND METHODS

Subjects

Twelve subjects (Table 1) were studied. Five were healthy young men and women within 10% of their desirable body weight, and seven were overweight men and women who were at least 30% above their desirable body weight. Of those who were overweight, three were found to have moderate hypertension but without hypertensive cardiovascular-renal disease, and one was found to be mildly diabetic (subject 7; fasting venous glucose of 6.6 mmoles/liter). Thyroid function and plasma cortisol levels were normal in all. None was receiving drug treatment. Obesity was of long-standing duration, extending back to childhood in five of the seven obese; subjects 8 and 10 had been obese for about 10 yr.

Experimental Design

The subjects were admitted to the hospital for 3 days and ate a carbohydrate rich diet for the first 24 hr (12% protein, 10% fat, 78% carbohydrate). The food was derived from ordinary food items and supplemented with glucose drinks. The diet was changed to a liquid glucose intake over the next 30 hr; this was consumed at 3-hr intervals from 6 a.m. on the second day to 6 a.m. on the third day and then hourly until noon. No further food was taken from noon until 8 p.m. on the

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Desirable* Weight (kg)</th>
<th>Plasma Triglyceride (mmoles/liter)</th>
<th>Clinical Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>22</td>
<td>55</td>
<td>55</td>
<td>0.47</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>22</td>
<td>67</td>
<td>62</td>
<td>0.71</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>23</td>
<td>70</td>
<td>71</td>
<td>0.46</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>20</td>
<td>69</td>
<td>68</td>
<td>0.41</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>38</td>
<td>68</td>
<td>67</td>
<td>0.51</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>18</td>
<td>107</td>
<td>69</td>
<td>1.22</td>
<td>Normal, overweight</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>42</td>
<td>101</td>
<td>73</td>
<td>4.32</td>
<td>Diabetes mellitus, overweight</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>46</td>
<td>116</td>
<td>80</td>
<td>2.00</td>
<td>Hypertension, overweight</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>27</td>
<td>112</td>
<td>58</td>
<td>1.51</td>
<td>Normal, overweight</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>41</td>
<td>110</td>
<td>71</td>
<td>2.46</td>
<td>Hypertension, overweight</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>46</td>
<td>105</td>
<td>58</td>
<td>1.60</td>
<td>Hypertension, overweight</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>38</td>
<td>101</td>
<td>71</td>
<td>2.21</td>
<td>Normal, overweight</td>
</tr>
</tbody>
</table>

third day. The calorie intake was calculated to be eucaloric on the basis of surface area and minimal physical activity; in fact, body weight did not change by more than 400 g over the 3 days in the lean subjects, but losses of 1 kg or more (maximum of 2 kg in subject 8) occurred in the obese. These losses have been attributed largely to changes in fluid balance.

FFA kinetics were measured on the third day from 9 a.m. until 8 p.m., essentially as described before. Radiopalmitate (the sodium salt complexed to human serum albumin) was infused intravenously at a constant rate (0.1 ml/min) from 9 a.m. till noon and again from 2 p.m. till 8 p.m. Four samples of venous blood were collected from an indwelling needle in the opposite arm (kept patent with 0.15 M NaCl) between 10:30 a.m. and noon during the final phase of glucose consumption, when glucose was being administered hourly, and further samples were taken at 3, 6, 7, and 8 p.m. during the period of fasting.

Informed consent was obtained from each subject.

Laboratory Procedures

The radiopalmitate used was 1,14C-palmitic acid obtained from Radiochemical Centre, Amersham, England. A maximum of 30 μCi was infused. Radiochemical purity of 99% was established by chromatography; sterility was checked after Millipore (0.22 μm) filtration.

The specific radioactivity of plasma FFA was measured after the extracted plasma lipids had been separated by thin-layer silicic acid chromatography; the mass of FFA was quantified by complexing the FFA to radiolabeled nickel chloride and measuring the radioactivity in a scintillation counter. This method gave highly reproducible values at low concentrations of FFA. Plasma glycerol was measured enzymatically, plasma triglycerides in a Technicon Mark II Auto-Analyzer, plasma insulin by radioimmunoassay, and blood glucose enzymatically.

Plasma volume was estimated as 4.5% of total body weight.

Calculations

Net transport of plasma FFA. At constant plasma FFA specific radioactivity the following formula was used:

\[
\text{Net transport (μmole/min)} = \frac{\text{Infusion rate (dpm/min)}}{\text{Specific radioactivity (dpm/μmole)}}.
\]

A constant specific radioactivity (variability between values less than 10%) was achieved during the period of glucose ingestion. Steady-state conditions did not prevail during the postglucose period; the specific activity fell as the influx of FFA into the plasma rose. However, considering the fast turnover of FFA, it was thought that the constant-infusion technique, coupled with frequent sampling, would nevertheless provide a reasonable estimate of the prevailing net FFA transport.

Fractional turnover rate of plasma FFA. This represents the fraction of the plasma FFA pool cleared per minute; it was calculated as

\[
\text{Clearance (min}^{-1}) = \frac{\text{Plasma FFA net transport (μmole/minute)}}{\text{Plasma FFA pool size (μmole)}}.
\]

Differences during and after glucose consumption were analyzed for statistical significance using the grouped or paired t test.

RESULTS

FFA Flux

Measurements were made during the final 90 min of glucose consumption, when glucose was being administered hourly. In each individual, the four blood
Table 2. Plasma FFA Kinetics and Glycerol Concentration During and After Sustained Glucose Consumption

<table>
<thead>
<tr>
<th>Period</th>
<th>Lean Subjects</th>
<th>Obese Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 Mean ± SD</td>
<td>6 7 8 9 10 11 12 Mean ± SD</td>
</tr>
<tr>
<td>Glucose effect*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma FFA concentration (µmoles/liter)</td>
<td>61 ± 6.2 35 ± 24 55 ± 58 47 ± 17</td>
<td>44 ± 57 110 ± 165 39 ± 60 75 ± 79 ± 45</td>
</tr>
<tr>
<td>FFA net transport (µmoles/min)</td>
<td>125 ± 119 80 ± 76 105 ± 101 ± 22</td>
<td>96 ± 98 124 ± 217 65 ± 97 105 ± 114 ± 48</td>
</tr>
<tr>
<td>FFA clearance (min⁻¹)</td>
<td>0.88 ± 0.65 0.56 ± 0.67 0.61 ± 0.67 ± 0.12</td>
<td>0.44 ± 0.37 0.20 ± 0.27 0.32 ± 0.31 0.30 ± 0.37 ± 0.07</td>
</tr>
<tr>
<td>Glycerol concentration (µM/liter)</td>
<td>33 ± 34 11 ± 30 29 ± 27 ± 9</td>
<td>42 ± 34 50 ± 129 45 ± 60 ± 75 ± 89 ± 60 ± 39</td>
</tr>
<tr>
<td>Postglucose effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma FFA concentration (µmoles/liter)</td>
<td>460 ± 312 139 ± 374 295 ± 317 ± 119</td>
<td>740 ± 208 413 ± 594 ± 312 ± 403 ± 560 ± 461 ± 181</td>
</tr>
<tr>
<td>FFA net transport (µmoles/min)</td>
<td>511 ± 385 181 ± 507 405 ± 398 ± 134</td>
<td>798 ± 300 446 ± 1290 425 ± 460 ± 585 ± 614 ± 336</td>
</tr>
<tr>
<td>FFA clearance (min⁻¹)</td>
<td>0.45 ± 0.44 0.42 ± 0.44 ± 0.40 ± 0.40 ± 0.43 ± 0.02</td>
<td>0.24 ± 0.31 0.23 ± 0.28 ± 0.28 ± 0.27 ± 0.27 ± 0.03</td>
</tr>
<tr>
<td>Glycerol concentration (µM/liter)</td>
<td>94 ± 78 97 ± 105 96 ± 94 ± 10</td>
<td>154 ± 75 100 ± 152 ± 114 ± 119 ± 34</td>
</tr>
</tbody>
</table>

*The value for each subject is the mean of four measurements taken during the final 90 min of glucose consumption (glucose was administered hourly).
†The value for each subject is the mean of three measurements taken 6-8 hr after stopping glucose.

With glucose consumption, plasma FFA concentrations fell to very low levels: the means (± SD) for the lean and obese subjects were 47 ± 17 and 79 ± 45 µmoles/liter, respectively, but the difference was not statistically significant. Plasma glycerol levels were also higher in the obese subjects, but the difference...
did not reach a significant level. Plasma FFA fluxes were similar in the two
groups (101 ± 22 and 114 ± 48 μmoles/min, respectively).

The major difference between the two groups was in the fractional turnover
rate or clearance of FFA: in every obese subject the clearance was lower than in
the lean subjects (difference between the groups, $p < 0.001$).

Stopping glucose led to sharp increases in the plasma FFA and glycerol con-
centrations; these increases were statistically not dissimilar in the lean and obese
subjects. Differences in FFA transport (μmole/min) also were not statistically
significant.

Clearance of FFA (min⁻¹) fell significantly only in the lean subjects ($p < 0.01$
on paired t test). In the obese subjects, the mean clearance of FFA changed
little, with only one obese subject (No. 6) showing the kind of fall that was seen
in each of the lean subjects. Nevertheless, the average clearance for the obese
subjects (0.27 ± 0.03/min) remained significantly lower than in the lean sub-
jects (0.43 ± 0.02/min, $p < 0.001$).

Plasma FFA transport was therefore almost equally suppressed in lean and
obese subjects by prolonged glucose consumption. By contrast, clearance of
plasma FFA was significantly reduced in the obese subjects and could not be
significantly stimulated with glucose. Lean subjects responded to glucose with a
significant increase in FFA clearance.

**Plasma Triglyceride, Plasma Insulin, and Blood Glucose Concentrations**

The plasma triglyceride levels were significantly higher in the obese subjects
at the end of the glucose period (Table 3). Six to 8 hr after stopping glucose, the
triglyceride concentration rose significantly only in the lean subjects ($p < 0.01$);
there were only inconstant and small changes in the obese.

The blood glucose concentration was not significantly higher in the obese
subjects, even during glucose consumption (when subject 7 was excluded); the
mean values of lean and obese subjects 6–8 hr after glucose were not signifi-
cantly different. The plasma insulin levels were significantly higher in the obese
subjects only in the postglucose period ($p < 0.05$).

**Intercorrelations**

No significant correlations were observed between the insulin concentra-
tions (or the postglucose changes in insulin) and other parameters that might
be influenced by insulin—i.e., the fall in plasma FFA clearance in the lean sub-
jects, the rise in plasma triglyceride concentration in the lean subjects, the rise
in plasma FFA transport in the whole group. The increase in FFA transport
and the increase in triglyceride concentration in lean subjects were also not
significantly correlated.

**DISCUSSION**

The main purpose of the study was to determine whether the raised plasma
FFA concentration in obesity reflects primarily an increased influx or dimin-
ished clearance of FFA. Previous studies had established that both factors are
probably important, in that net transport of plasma FFA is closely correlated
with excess body weight²³ and plasma FFA levels are not as readily suppressed
by sucrose ingestion\textsuperscript{5} or insulin\textsuperscript{6} infusion as in lean subjects. The interpretation of the latter findings is less clear since increased availability of glucose or insulin might simultaneously suppress lipolysis and stimulate esterification of FFA.\textsuperscript{7} We have attempted to distinguish between these possibilities by making independent calculations of the in vivo influx and clearance rate of plasma FFA in circumstances that would be expected to suppress the former and stimulate the latter.

The lean subjects showed both responses during sustained glucose consumption: (1) plasma FFA and glycerol concentrations were markedly suppressed, implying inhibition of triglyceride lipolysis in adipose tissue; (2) plasma FFA net transport was similarly reduced, confirming the inhibition of lipolysis; and (3) clearance of plasma FFA was significantly stimulated, indicating increased reesterification of FFA, presumably within tissues such as muscle and adipose tissue. In contrast, the obese subjects scarcely increased the rate of clearance of plasma FFA, although net transport, and hence the influx of FFA into plasma, was suppressed to the very low values seen in the lean subjects. The plasma FFA and glycerol concentrations remained higher (though not significantly so) in the obese than in the lean subjects, suggesting that clearance as well as influx determine the plasma FFA level. Though a high-carbohydrate diet stimulates the utilization of glucose in enlarged adipocytes in vitro,\textsuperscript{16} it apparently failed to stimulate FFA clearance in vivo. When glucose consumption ceased, the increase in net transport appeared to occur more rapidly and to a greater extent in some obese subjects (e.g., Nos. 6, 9, and 12) than in lean subjects.

The failure of FFA clearance to rise in the obese subjects suggests a diminished capacity to reesterify FFA within tissues or a reduced responsiveness of those tissues to the increase in the supply of glucose and insulin. The blood glucose concentrations reached similar levels in the lean and obese subjects (excluding subject 7, who proved to have diabetes), but the plasma insulin levels were higher in the obese, both during glucose consumption and postglucose. The higher insulin:glucose ratio in obese than in lean subjects is well recognized;\textsuperscript{17} it demonstrates a relative resistance of some glucose-utilizing tissues to the action of insulin, due possibly to reduced numbers of receptor sites for insulin in cellular membranes.\textsuperscript{18} The present data would be consistent with a relative failure of cells to respond to insulin as measured by the reesterification of FFA.

Our results do not define the reasons for diminished FFA clearance and reduced responsiveness to insulin in obesity. Though the liver in obesity shows reduced sensitivity to the actions of insulin on hepatic glucose metabolism,\textsuperscript{19} there is no evidence that the handling of FFA is adversely influenced. There is, in fact, contrary evidence that the extraction of FFA from the splanchnic circulation is increased in obese subjects,\textsuperscript{10} probably in direct proportion to the increased FFA transport.

By contrast, skeletal muscle develops profound resistance to insulin, at least in experimental obesity as defined by the incorporation of glucose into glyco-gen.\textsuperscript{7} Insulin-mediated uptake of glucose by the human forearm is also diminished in obesity.\textsuperscript{11}
The evidence in adipose tissue is less clear. On the one hand, enlarged human adipocytes have reduced numbers of receptor sites for insulin binding and adipose tissue from obese animals shows a reduced capacity to incorporate glucose into CO₂. In contrast, enlarged human fat cells are metabolically more active than smaller cells obtained from lean subjects. The rate of utilization of glucose for esterification of FFA is increased both in vivo and in vitro in direct proportion to the enlargement of the cell. Since the incorporation of glucose into α-glycerophosphate is crucial to the process of fatty acid reesterification, the capacity of adipose tissue to clear FFA from the circulation should not be diminished. Furthermore, the activity of glycerokinase, which mediates the reutilization of glycerol for fatty acid esterification, is also increased in adipocytes of obese people and can be further enhanced with insulin, though this mechanism is probably of minor importance. The overall diminution in FFA clearance in obesity may therefore reflect reduced reesterification in muscle rather than in adipose tissue.

The ability of the obese subjects to suppress FFA transport to the very low levels seen in lean subjects also suggests that the antilipolytic action of glucose and insulin remains highly effective in enlarged adipocytes, though possibly only in the presence of higher concentrations of insulin. Though insulin alone may not inhibit basal rates of lipolysis in vitro, the infusion of insulin into man rapidly suppresses FFA transport, which is probably largely due to diminished influx.

The high-carbohydrate diet led to a rise in the plasma triglyceride concentration in most individuals. This effect was less marked in the lean subjects during the actual consumption of glucose, which has been shown to attenuate the triglyceride-raising effect of high-carbohydrate diets. The rapid and significant rise in the triglyceride levels in the lean subjects when glucose was ceased has been previously shown, as in the present study, to reflect the increased availability of substrate FFA. Removal of circulating triglyceride might also have become progressively reduced, since glucose and insulin stimulate the activity of tissue lipoprotein lipase. In the obese subjects the plasma triglyceride levels were higher than in the lean during carbohydrate feeding, partly due to their higher initial values. However, the obese subjects did not show the rise in triglyceride concentration when glucose was stopped; this may reflect the more complex origin of circulating triglycerides in obesity. Stored hepatic triglyceride is a major source of plasma triglyceride in obese subjects, and this is likely to have been accentuated by the high intake of glucose, which stimulates lipogenesis in the liver even in lean subjects.

It should be noted that several of the obese subjects were also mildly hypertriglyceridemic and that the obese group as a whole had a significantly higher mean plasma triglyceride concentration than the lean group. This finding may be relevant as Larsson et al. have reported reduced incorporation of glucose into glyceride-glycerol in adipocytes of overweight hypertriglyceridemic individuals. Furthermore, enlarged adipocytes from the hypertriglyceridemic subjects were metabolically less responsive to insulin than adipocytes of similar size obtained from overweight normolipidemic subjects; the cells were particularly resistant to the antilipolytic action of insulin studied in vitro. In the latter respect our obese subjects, whose average plasma triglyceride level was
similar to that of Larsson’s group of hypertriglyceremic individuals, showed a similar degree of responsiveness to the antilipolytic effect of glucose feeding to that of our lean subjects, though the insulin levels were on average higher in the obese. In an earlier study we had shown that norepinephrine-stimulated FFA influx into plasma was greater in hypertriglyceridemic than in normotriglyceridemic subjects, which is consistent both with heightened lipolysis and resistance to the antilipolytic effect of insulin.

Finally, it is unlikely that obesity can be represented metabolically as a homogeneous entity. Albrink and Meigs were among the first to demonstrate the variability in the distribution of excess fat in middle-aged subjects and the influence of preadulthood obesity. Of our 7 obese subjects, 5 were known to have been obese in childhood; the remaining two (Nos. 8 and 10), who were athletes in their youth, had been overweight for about 10 yr. While these two, together with subjects 11 and 7, who were, respectively, hypertensive and mildly diabetic, did not show striking differences in FFA metabolism from the remaining three obese subjects who were otherwise healthy, the latter three (Nos. 6, 9, and 12) showed the highest “rebound” increments in FFA turnover after glucose was ceased.

REFERENCES


