Effect of glucose on plasma concentrations of individual non-esterified fatty acids of non-diabetic and insulin-independent diabetic men

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Summary
The composition of plasma non-esterified fatty acids was investigated during glucose tolerance tests to determine changes in individual fatty acid concentrations after glucose administration and to determine whether differences existed between the individual fatty acid concentrations of maturity-onset diabetic and non-diabetic men. The mean fasting total non-esterified fatty acid concentration of the 9 diabetics was greater than that of the 12 non-diabetics studied. After glucose ingestion, the mean total non-esterified fatty acid concentrations of both groups decreased. Gas chromatographic analysis of the plasma non-esterified fatty acids of 6 diabetic and 6 non-diabetic men revealed that the concentrations of palmitic, stearic and oleic acids were significantly higher in the diabetic men in the fasting state. With the sole exception of stearic acid in the non-diabetic patients, the mean concentration of each of the 6 plasma non-esterified fatty acids determined decreased in both groups after glucose ingestion. At 1, 2 and 3 hr after glucose ingestion, there were no longer any significant differences between the mean concentrations of individual plasma non-esterified fatty acids of the non-diabetic and diabetic men.

Introduction
It is well established that ingestion of glucose or of a high carbohydrate meal by normal humans is followed by a decrease in plasma total non-esterified fatty acid (NEFA) concentration. However, the changes of concentrations of individual NEFA are much less studied, and information on the effect of glucose ingestion on the plasma individual NEFA of diabetic subjects, who are known to have high levels of plasma total NEFA (Schrade et al., 1963), appears to be limited to a single paper (Nakamura, Faludi and Spitzer, 1967). Therefore, the present authors have determined the composition of plasma NEFA before and after glucose ingestion in non-diabetic and diabetic subjects.

Materials and methods
Patients
The patients investigated were 21 ambulatory men who had been selected by their ward physician to have a glucose tolerance test (GTT) as part of their clinical evaluation. With but a single exception, their fasting plasma glucose values were < 7 mmol/l. Patients were classified as diabetic if the second hour plasma glucose concentration was 7.84 mmol/l or more and non-diabetic if it was < 7.84 mmol/l. All but one of the nine subjects classified as diabetic also had a first hour glucose exceeding 10.36 mmol/l, thus meeting both the first hour and second hour criteria of Fajans and Conn (1959) adjusted for plasma glucose concentration. Both criteria were met by each of the 6 diabetic patients whose plasma total NEFA were separated into individual NEFA. The mean age of the 9 diabetic patients was 54 (ranging from 23 to 73) years and that of the 12 non-diabetic patients was 49 (ranging from 22 to 78) years. The weight: height ratios of the 9 diabetics and the 12 non-diabetics were 2.33 ± 0.35 and 2.34 ± 0.43 (mean ± s.d.) respectively. The diabetic patients did not require insulin therapy. The patients had a variety of diseases with atherosclerotic heart disease being the most common. Medications were not restricted. No patient had had recent surgery, severe renal or hepatic failure, or severe anaemia.

Procedure
Patients fasted for approximately 14 hr and did not smoke for at least 8 hr before blood was drawn.
and until the last sample was taken. Thirty ml of blood was drawn into plastic syringes from ante-cubital veins at 8 a.m. immediately before the ingestion of 100 g of glucose in 250 ml of solution (dextrose solution for glucose tolerance test (GTT), Unitech Co., Sun Valley, California). Blood samples were obtained again at one, 2 and 3 hr after glucose ingestion. One portion of each blood sample was transferred to a glass tube containing heparin. After gently mixing, it was kept in melting ice for 10 min until it was centrifuged at 4°C at 3000 r.p.m. (2000 g at bottom of tube) for 15 min in an International Centrifuge Model PR-1 using rotor #269. The plasma so obtained was frozen for later use for NEFA and triglyceride analysis. Another portion of each blood sample was processed for plasma glucose determination by the ferricyanide technique using a Technicon AutoAnalyzer (1963). The method of Trout, Estes and Friedberg (1960) was used for the determination of total plasma NEFA concentration. The separation and estimation of individual fatty acids by gas chromatography have been described elsewhere (Davis et al., 1974). The method used for the determination of plasma triglyceride was essentially the same as that described by Van Handel and Zilversmit (1957) except that tripalmitin was used as a standard instead of corn oil.

Results

Twenty-one men (12 non-diabetic and 9 diabetic) had a 3-hr GTT. Figure 1 shows the mean concentrations of total NEFA and of glucose for each group. The diabetic group had a higher mean initial plasma total NEFA than the non-diabetic group (t=2.46, P < 0.025). The differences between the mean fasting total NEFA concentration and the mean hourly concentrations after glucose ingestion (upper portion of Fig. 1) were greater in diabetics than in non-diabetics at the levels of significance indicated in the figure. However, the per cent. changes in concentration of total NEFA from the fasting level (not shown in Fig. 1) were nearly the same for diabetics and non-diabetics at the first and second hour after glucose ingestion. The differences between the mean fasting glucose concentration and the mean hourly concentrations after glucose ingestion (lower portion of Fig. 1) were significantly greater in diabetics than in non-diabetics as expected.

In comparing the individual NEFA of 6 non-diabetic and 6 diabetic patients, it was found that the concentrations of palmitic, stearic and oleic acids were significantly higher in the plasma of the fasting

![Figure 1. Glucose tolerance test of 12 non-diabetic (broken lines) and 9 diabetic patients (unbroken lines). P-values (comparing diabetic and non-diabetic subjects) indicate significance of the differences between the means from fasting to subsequent hourly levels as determined by the Wilcoxon rank sum test. For example, the change in NEFA from fasting concentration to first hr (1 hr) concentration in diabetic patients is significantly different from the change in NEFA during the same interval in non-diabetic patients at a 5% level of significance. Traditional to s.i. units for fasting plasma glucose 55 mg/dl – 3·0 mmol/l (factor, 0.056).](image)

**Table 1. Plasma fatty acid concentrations of fasting non-diabetic and diabetic subjects (µEq/l plasma, mean (± s.d.))**

<table>
<thead>
<tr>
<th></th>
<th>C14 (Myristic)</th>
<th>C16 (Palmitic)</th>
<th>C16:1 (Palmitoleic)</th>
<th>C18 (Stearic)</th>
<th>C18:1 (Oleic)</th>
<th>C18:2 (Linoleic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic*</td>
<td>6</td>
<td>51.2 (50.1)</td>
<td>92.7 (32.0)</td>
<td>23.6 (8.7)</td>
<td>41.8 (8.5)</td>
<td>14.9 (14.9)</td>
</tr>
<tr>
<td>Diabetic*</td>
<td>6</td>
<td>17.6 (20.8)</td>
<td>137.7 (32.1)</td>
<td>21.3 (12.0)</td>
<td>81.6 (19.3)</td>
<td>25.0 (85.2)</td>
</tr>
<tr>
<td>Significance of differences**</td>
<td>NS</td>
<td>p &lt; 0.025</td>
<td>NS</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

*The weight-height ratios of 6 non-diabetics and 6 diabetics were 2.33 ± 0.37 (mean ± s.d.) and 2.38 ± 0.33 respectively. Their mean ages were 44.3 ± 18.7 and 50.7 ± 17.1 years respectively.

**By Student's t-test.

NS = Not significant (P > 0.05).
diabetics than in that of the fasting non-diabetics (Table 1). There was no significant difference between non-diabetics and diabetics in any of the 6 individual NEFA concentrations in the subsequent hour after glucose intake.

The mean concentrations of 6 major individual plasma NEFA during GTT of 6 non-diabetic and 6 diabetic patients are shown in Fig. 2. In the non-diabetic group, the mean concentration of each fatty acid except stearic acid decreased after glucose ingestion. None of the 6 non-diabetic patients tested had a sharp fall in the plasma stearic acid concentration after glucose ingestion, and the mean plasma stearic acid concentration was actually slightly higher at each hour after glucose ingestion in contrast to the 6 diabetic patients whose mean plasma stearic acid concentration fell precipitously after glucose ingestion. In the diabetic group, the mean concentration of each fatty acid at each time after glucose ingestion was lower than the mean fasting concentration. However, for myristic and palmitoleic acids, the differences from the fasting levels were never significant at the 5% level.

Pearson correlation coefficients between the fasting concentrations of total NEFA and each of the 6 individual NEFA of the 12 patients showed statistically significant relationships between total NEFA and palmitic ($r=0.86$, $P<0.001$), stearic ($r=0.62$, $P<0.01$), oleic ($r=0.94$, $P<0.001$) and linoleic ($r=0.80$, $P<0.005$) acids. Glucose concentration was positively related to that of palmitic ($r=0.70$, $P<0.01$), stearic ($r=0.77$, $P<0.005$) and linoleic ($r=0.60$, $P<0.005$) acids. Neither age of the subjects nor the concentration of triglyceride was significantly correlated with any of the fasting plasma individual NEFA concentrations.

Discussion

The results of these experiments on GTT of 12 non-diabetic and 9 diabetic patients agree with established patterns of glucose and total NEFA change. It should be noted that the mean plasma total NEFA concentration of the diabetics decreased continuously and more rapidly (Fig. 1) than that of the non-diabetics. This greater response to glucose intake has been observed by others (Nakamura et al., 1967; Shafrir and Gutman, 1965; Goto et al., 1973; Hills, Marks and Kien, 1962; Berson and Yalow, 1965). However, much less decrease in plasma total NEFA during GTT in diabetics than in non-diabetics also has been reported (Bierman, Dole and Roberts, 1957; Reitman, 1967).

The 6 fasting diabetic men, whose plasma NEFA was separated, had significantly higher mean concentrations of palmitic, stearic and oleic acids than 6 fasting non-diabetic men (Table 1). Although the mean of the sum of the 6 individual NEFA concentrations of the diabetics was only approximately one-third higher, their mean stearic acid concentration was approximately twice that of the non-diabetics. After glucose ingestion there were greater decreases in the mean plasma concentrations of palmitic, stearic and oleic acids in the diabetics so that statistically significant differences in concentration between the diabetic and non-diabetic patients no longer existed. By the third hour after glucose ingestion, the mean plasma concentration of each individual NEFA (Fig. 2), as well as of total

![Fig. 2. Mean concentrations ± 1 s.d. are shown for each of the 6 individual NEFA separated (a) non-diabetic, (b) diabetic patients. $P$ values (obtained by paired $t$-tests) indicate the statistical significance of the differences between the fasting and subsequent values. Absence of $P$ values indicates $P>0.05$. , fasting; , 1 hr; , 2 hr; , 3 hr.](http://pmj.bmj.com/)
NEFA (Fig. 1) of the diabetics, was similar to that of the non-diabetics. Nakamura et al. (1967) found that the mean concentration of each of 6 major detected individual plasma NEFA was higher in 5 fasting diabetic subjects than in 3 controls (no statistical analysis presented). Although the mean plasma myristic acid concentration of the 5 diabetic subjects of Nakamura et al. (1967) was approximately twice that of the 3 controls, the 6 non-diabetic patients (whose myristic acid concentration varied considerably) in the present study had a mean value approximately 3 times that of the 6 diabetics (0.05 < P < 0.10). The reason for the difference is not clear; perhaps the state of health, severity of diabetes, composition of dietary fat and the fatty acid composition of the adipose tissue of the patients may have played some roles leading to differences in fatty acid mobilization during an overnight fast.

With the sole exception of stearic acid in the non-diabetic patients, the mean concentration of each of the 6 individual NEFA determined was lower in both groups of patients at each of the 3 hr tested after glucose ingestion than in the fasting state (Fig. 2). The reason that the mean stearic acid concentration of the non-diabetic patients did not decrease at any hour after glucose ingestion while that of the diabetic patients was significantly lower by the first hour is not apparent. The authors are aware of only one previous report of the plasma concentrations of these 6 NEFAs after glucose ingestion. The mean plasma concentration of each NEFA of the 3 controls and of the 5 diabetics of Nakamura et al. (1967) was lower one hr after glucose ingestion than it was in the fasting state with the exception of myristic acid in the controls. Hamlin, Delp and Rowe (1969) reported only the concentration of oleic acid which decreased after glucose ingestion.

The decrease of total NEFAs in the present studies is a reflection of the changes of individual NEFA, primarily of oleic and palmitic acids which have the highest concentrations among the major NEFA in human plasma (Farstad, 1967; Hagenfeldt et al., 1972; Hagenfeldt and Weser, 1973; Nordøy, Strøm and Berntsen, 1974). Insulin is known to lower plasma NEFA concentrations by inhibiting the release of fatty acids from adipose tissue and not by removal of fatty acids from plasma (Bierman, Schwartz and Dole, 1957). The anti-lipolytic effect of insulin requires a smaller concentration than that required to promote glucose utilization. Although the authors did not determine insulin concentrations, the rapid initial decline of NEFA in their diabetic patients could be interpreted as an indication that these patients were not insulin-deficient diabetics. Shafrir and Gutman (1965) also found that some diabetics had a rapid decrease in plasma NEFA concentration after glucose ingestion and interpreted this as an indication of normally functioning adipose tissue.

There was a significant positive correlation between the fasting plasma concentration of palmitic acid and glucose and of stearic acid and glucose. These correlations may be a reflection that the concentrations of these fatty acids as well as the concentrations of glucose are elevated in diabetic subjects. The lack of correlation between fasting plasma individual NEFA and plasma triglyceride is not surprising since in the fasting state the delivery of NEFA from adipose tissue is much accelerated while the release from endogenous (plasma) triglyceride is minimal (Nikkilä, 1971).

Acknowledgment

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References


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