Some determinants of postprandial lipaemia in Nigerian diabetic and non-diabetic subjects

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The efficiency of clearance of plasma triacylglycerols (TAG) after fatty meals in non-diabetic Caucasian subjects is believed to determine the plasma level of high-density-lipoproteins-cholesterol (HDL-C). It is unknown if this observation holds in diabetic subjects and in other racial groups. In assessing the factors that determine TAG responses to acute fat loading in a tropical African population with a low prevalence of atherosclerotic disease, twenty (nine obese) non-insulin-dependent diabetic (NIDDM) patients with optimal glycaemic control and twelve (six obese) age-matched non-diabetic subjects were given meals containing 50 g fat (in butter) and 75 g carbohydrate (in white bread) over 15 min in the morning after a 12 h overnight fast. The fasting plasma levels of glucose, TAG, total cholesterol (total-C), HDL-C, low-density-lipoprotein-cholesterol, insulin and glycosylated haemoglobin (HBA1c) were estimated; glucose and TAG levels were also measured postprandially for 8 h at 2 h intervals. Postprandial lipaemia was consistently higher in the diabetic patients (about 50–100% more than values obtained in the non-diabetic subjects, even when corrected for differences in body mass) and correlated positively with age and postprandial glycaemia. This defect in TAG clearance was even worse (by about 50%) when glucose tolerance became further impaired after ten of the diabetic patients stopped oral hypoglycaemic treatment for 1 week and the fat-tolerance test was repeated. In the obese non-diabetic subjects, but not those of normal weight, there were significant negative relationships between the postprandial lipaemia and fasting plasma levels of HDL-C and the HDL-C: total-C ratio, as reported in Caucasians. It is concluded that age and the ambient glucose concentration appear to be the important determinants of the efficiency of TAG clearance in diabetic subjects. This accords with clinical observations of increased atherogenic liability with increasing age and poorer glycaemic control. The determinants in non-diabetic subjects were less defined, indicating that postprandial lipaemia might be influenced by various factors (obesity as shown here) in different subsets of individuals.

Diabetes: Non-insulin-dependent diabetes: Atherosclerosis: Lipoproteins

Although it is well established that increased plasma total cholesterol (total-C) levels predict later development of atherosclerotic vascular disease (Department of Health, Education and Welfare, 1971), the role of circulating plasma triacylglycerols (TAG) as an independent risk factor is controversial. Supportive evidence for an important role for circulating TAG in the development of atherosclerotic vascular disease comes from the observations that: (1) the metabolism of high-density lipoproteins (HDL) is very closely related to the catabolism of TAG-rich lipoproteins (chylomicrons and very-low-density lipoproteins (VLDL); Havel et al. 1973; Redgrave & Small, 1979; Patsch et al. 1983; Patsch, 1987), (2) increased levels of TAG result in raised concentrations of chylomicron remnants, VLDL remnants and small, dense low-density lipoproteins (LDL); Garg & Grundy, 1990) and (3) hypertriacylglycerolaemia directly increases blood levels of activated factor X enhancing platelet responsiveness (Bradley & Gianturco, 1988; Brook & Aviram, 1988). Furthermore, a recent study on French diabetic patients showed that hypertri-
acylglycerolaemia most potently predicted coronary heart disease mortality (Fontbonne et al. 1989).

Plasma TAG levels in the non-fasting state depend on the amount of fat ingested as well as the uptake of TAG by extrahepatic tissues (Patsch, 1987). Since the major part of an individual’s lifetime is spent in the postprandial state (i.e. the time period between food ingestion and 6-8 h thereafter), studies in the fasting state might not always indicate the efficiency of TAG disposal mechanisms. It is, therefore, essential in investigating TAG metabolism additionally to assess the rates of TAG clearance after standard fat meals, i.e. with fat-tolerance tests.

Earlier studies using fat-tolerance tests in non-diabetic Caucasian subjects suggested that: (1) a direct relationship exists between postprandial lipaemia (pplip; assessed by serial measurements of TAG levels after standard fatty meals) and fasting TAG levels and rates of chylomicron metabolism (Patsch, 1987), (2) peak fat absorption is observed 3-4 h after an acute fat load, and levels of plasma TAG fall to fasting values within 8 h (Patsch et al. 1983), (3) pplip is negatively correlated with plasma HDL levels (Patsch et al. 1983) suggesting that atherogenic risk is reduced with increased TAG clearance and (4) pplip may be influenced by various factors (such as diabetes) in different subsets of individuals (Arora et al. 1987; Patsch, 1987).

It is as yet unknown whether these observations hold in non-Caucasoid populations. Also, the nature of the possible influences of the diabetic state on pplip remain essentially uncharacterized. It is, therefore, proposed to examine, in Nigerian diabetic and non-diabetic subjects, the magnitude of pplip after standard fat meals, and the factors that influence its relationship with the plasma levels of the other lipoproteins and indices of glycaemic control. Nigerians, like other tropical African populations, have a relatively low prevalence of coronary heart disease, even with diabetes (Falase et al. 1973), probably related to their comparatively low plasma cholesterol levels (Agbedana & Akanji, 1988; Akanji et al. 1989), which reflects the traditional African diet (Taylor, 1971).

EXPERIMENTAL DESIGN AND METHODS

The fat-tolerance tests were performed using a modification of previous methods (Patsch et al. 1983; Arora et al. 1987; Cohen et al. 1988) to permit a simultaneous assessment of glucose tolerance.

Subjects

Study 1. Subjects, all aged 35-60 years, were recruited from the diabetic and obesity clinics at the University College Hospital, Ibadan, Nigeria. The subject groups were: (1), twenty non-insulin dependent diabetic (NIDDM) patients (eleven (nine male) of normal weight and aged 49.8 (SD 9.4) years with a body mass index (BMI) of 22.6 (SD 1.9) kg/m² and nine (six male) obese aged 51.0 (SD 9.1) years with a BMI of 32.4 (SD 6.9) kg/m²). All were ketosis-resistant and optimally controlled on small daily doses of oral glibenclamide (2.5-5 mg) with fasting plasma glucose values < 10 mmol/l and glycosylated haemoglobin (HBA1c) values < 10%; (2), twelve non-diabetic subjects closely matched for age and BMI with the diabetic subjects. Six (three male) aged 47.9 (SD 6.4) years were of normal weight (BMI 21.6 (SD 1.1) kg/m²) and six (three male) aged 48.2 (SD 5.6) years were obese (BMI 34.9 (SD 8.3) kg/m²). All had fasting plasma glucose values < 5.5 mmol/l.

Subjects with features of malabsorption syndrome or steatorrhoea, severe cardiac, renal, hepatic or cerebrovascular disease and severe diabetic microangiopathic complications were excluded, as were those taking drugs (other than anti-diabetic medications) known to
have metabolic effects. The premenopausal female volunteers were studied only during the proliferative phases of their menstrual cycles and none were on oral contraceptives. None of the subjects was a trained athlete. Additionally, all the volunteers had fasting plasma total-C (< 6.0 mmol/l) and TAG (< 1.9 mmol/l) levels in the normal range.

**Study 2.** Ten of the diabetic subjects (five obese) were subjected to repeat fat-tolerance tests after 1 week without their usual diabetic medications. In the period between these studies each subject resumed usual activity and diet with no change in body-weight.

Informed voluntary consent was obtained from each subject and the studies were approved by the College of Medicine, University of Ibadan, Nigeria, Medical Research Ethical Committee.

**Protocol**

**Test meal.** The test meal consisted of 61.7 g butter (g/kg; 810 fat, < 1 carbohydrate, 5 protein) and 152 g white bread (g/kg; 493 carbohydrate, 16 fat, 72 protein) to provide a total of 75 g carbohydrate and 50 g fat. Of the 3340 kJ (810 kcal), 6.5% was derived from protein, 37% from carbohydrate and 57% from fat. The polyunsaturated : saturated fat ratio was 0:04. The test meal was administered with 250–300 ml water, and taken over 15 min. The additional carbohydrate content improved palatability and, hence, acceptance of the fatty meal. This fat load has previously been described as well tolerated with > 95% absorption and showing high within-subject reproducibility of postprandial TAG levels (Patsch, 1987).

Each subject came as an out-patient in the morning at 08.00 hours into a metabolic ward after a 12 h overnight fast subsequent to ingestion of the usual supper and without drinking alcohol or smoking. The diabetic subjects omitted their usual drugs on the morning of study 1. Blood samples were collected fasting (twice at -15 and 0 min, the mean taken as the fasting concentration) and then at 2, 4, 6 and 8 h after starting to ingest the test meal. The test meal was generally well tolerated, and no subject reported nausea, diarrhoea, vomiting or later showed signs of gross steatorrhoea.

The subjects were recumbent throughout, except to visit the toilet and took no further meal (except water ad lib.) for the 8 h duration of the study. The blood samples were taken (into appropriate tubes placed on ice) from previously inserted antecubital venous cannulas kept patent by regular flushing with small volumes of isotonic saline (150 mmol sodium chloride/l). Plasma samples so collected were analysed for (1) TAG (all specimens; Gottfried & Rosenberg, 1973), total-C (Searcy & Bergqvist, 1960) and HDL-cholesterol (HDL-C; Burstein & Samaille, 1960) (fasting specimens only), and LDL-cholesterol (LDL-C) levels thereby calculated (Friedewald et al. 1972), (2) glucose (all specimens) (Trinder, 1969) and (3), fasting plasma insulin by specific radioimmunoassay with Amersham kits. Fasting HBA1c levels were determined by the thiobarbituric acid colorimetric method (Parker et al. 1981). These estimations were done within 1 month of specimen collection on plasma or blood haemolysates stored frozen at -20°.

**Statistical analyses.** The magnitude of the TAG and glucose responses to the test meal were quantified (Patsch et al. 1983) as: (1) area under the TAG or glucose curves (mmol/l plasma; the 8 h area glucose or TAG) calculated by the trapezoidal rule (De Sapio, 1978) normalized for the 0 h value (this is the index of pplip); (2) increase in TAG level from the 0 h value to the mean of the two highest TAG values observed postprandially calculated as:

$$\text{TAG increase (TGI (mmol))} = \frac{N_{\text{max}} + N_{\text{second}} - N_0}{2},$$

where $N_{\text{max}}$ is peak TAG value, $N_{\text{second}}$ is second highest TAG value and $N_0$ is fasting TAG value.
Table 1. *Fasting levels of plasma glucose, insulin and lipoproteins and blood glycosylated haemoglobin (HbA1c) in Nigerian diabetic and non-diabetic subjects†*  
(Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>NIDDM</th>
<th>Non-diabetic</th>
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<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose* (mmol/l)</td>
<td>5.6 ± 1.3</td>
<td>6.5 ± 2.1</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>19.8 ± 6.7</td>
<td>21.8 ± 3.5</td>
</tr>
<tr>
<td>HbA1c (%)*</td>
<td>8.6 ± 0.4</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>TAG* (mmol/l)</td>
<td>1.18 ± 0.32</td>
<td>1.30 ± 0.29</td>
</tr>
<tr>
<td>Total-C (mmol/l)</td>
<td>4.09 ± 0.98</td>
<td>4.11 ± 0.95</td>
</tr>
<tr>
<td>HDL-C* (mmol/l)</td>
<td>0.90 ± 0.26</td>
<td>0.80 ± 0.28</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.03 ± 1.07</td>
<td>3.06 ± 0.87</td>
</tr>
<tr>
<td>HDL-C: total-C*</td>
<td>0.23 ± 0.07</td>
<td>0.20 ± 0.07</td>
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</table>

NIDDM, non-insulin-dependent diabetes mellitus; TAG, triacylglycerols; total-C, total cholesterol. HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol.

* Mean values for non-diabetic subjects were significantly different from corresponding values for the subjects with NIDDM (*P* < 0.05).
† For details, see pp. 154–155.

All the results are expressed as means and standard deviations. Differences between and within subject groups were computed by Wilcoxon’s matched-pairs signed rank tests for paired observations and Mann–Whitney U tests for unpaired observations, as appropriate. The regression analyses were done using Spearman’s rank correlation coefficients (r); Armitage & Berry, 1987). The level of statistical significance was *P* < 0.05.

**RESULTS**

**General**

As expected (Table 1), the diabetic subjects had higher fasting plasma levels of glucose (*P* < 0.05), TAG (*P* < 0.005) and blood HbA1c (*P* < 0.001). Plasma insulin levels did not differ significantly between the various groups. Postprandial glycaemia (ppg), representing the area under the 8 h glucose v. time curve, was also expectedly higher in the diabetics (*P* < 0.001) (Table 2, Fig. 1). There were no differences in fasting plasma total-C and LDL-C levels between the diabetic and non-diabetic subjects, although the latter had higher levels of HDL-C (*P* < 0.02) and the HDL-C: total-C ratio (*P* < 0.05; Table 1). The trends of these observations were similar between the obese and normal-weight subgroups of the diabetic and non-diabetic subjects (Tables 1 and 2).

**Pplip in diabetic and non-diabetic subjects**

The magnitude of postprandial increases in plasma TAG levels expressed either as pplip or TAG increase (TGI) was greater in the diabetic subjects (*P* < 0.005) (Fig. 2, Table 2). This difference persisted when either variable was expressed per unit BMI (*P* < 0.001) (Table 2) and exhibited a similar trend in the obese and normal-weight subjects between whom there were no significant differences for each group (diabetic or non-diabetic).
Table 2. Changes in plasma glucose and triacylglycerol levels after the fat meals in Nigerian diabetic and non-diabetic subjects†

<table>
<thead>
<tr>
<th>Subjects...</th>
<th>NIDDM</th>
<th>Non-diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>n...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (20)</td>
<td>4.05</td>
<td>1.59</td>
</tr>
<tr>
<td>Obese (9)</td>
<td>4.05</td>
<td>1.59</td>
</tr>
<tr>
<td>Non-obese (11)</td>
<td>4.05</td>
<td>1.59</td>
</tr>
</tbody>
</table>

pplip, postprandial lipaemia (mmol/l per l); ppg, postprandial glycaemia (mmol/l per l); TGI, triacylglycerol increase; BMI, body mass index; NIDDM, non-insulin-dependent diabetes mellitus.

* Mean values for non-diabetic subjects were significantly different from corresponding values for subjects with NIDDM (P < 0.05).
† For details, see pp. 154–155.

Fig. 1. Postprandial plasma glucose levels in the Nigerian non-insulin-dependent diabetic (■) and non-diabetic (▲) subjects (for details of subjects, see pp. 154–155). Points are means and standard deviations, represented by vertical bars, for twenty diabetic subjects and twelve non-diabetic subjects. At each time-point mean values for diabetic subjects were significantly different from those for non-diabetic subjects (P < 0.05). For details of procedures, see p. 155.

Correlations of pplip

In all the subject groups, as expected, significant positive correlations were established between the two indices of pplip and TGI (diabetics \( r_s: \) all 0.68, obese 0.83, normal weight 0.68; non-diabetic \( r_s: \) all 0.94, obese 0.92, normal weight 0.90, all \( P < 0.05 \)). As there were some differences in the pattern of correlations between the diabetic and non-diabetic subjects, they are probably best considered separately.

Diabetic subjects. There were consistent positive correlations between pplip and age \( (r_s: \) all 0.60, obese 0.70, normal weight 0.62, all \( P < 0.05 \)) and ppg \( (r_s: \) all 0.58, obese 0.88, normal weight 0.65, all \( P < 0.05 \)) which persisted even when pplip was corrected for body
Fig. 2. Postprandial plasma triacylglycerol (TAG) levels in Nigerian non-insulin-dependent diabetic (▲) and non-diabetic (●) subjects (for details of subjects; see pp. 154–155). Points are means and standard deviations, represented by vertical bars, for twenty diabetic subjects, and twelve non-diabetic subjects. *P < 0.05; **P < 0.01. For details of procedures, see p. 153.

Table 3. Effect of 1 week cessation of drug treatment on fasting plasma glucose and lipoprotein levels and postprandial lipaemia (pplip) and glycaemia (ppg) in Nigerian diabetic subjects†

(Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>With drug treatment</th>
<th>Without drug treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1.11</td>
<td>0.25</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>7.7</td>
<td>4.3</td>
</tr>
<tr>
<td>Total-C (mmol/l)</td>
<td>4.21</td>
<td>0.88</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>0.94</td>
<td>0.27</td>
</tr>
<tr>
<td>pplip (mmol/8 h per l)</td>
<td>2.19</td>
<td>0.90</td>
</tr>
<tr>
<td>ppg (mmol/8 h per l)</td>
<td>14.5</td>
<td>4.1</td>
</tr>
<tr>
<td>TGI (mmol/l)</td>
<td>0.42</td>
<td>0.20</td>
</tr>
</tbody>
</table>

TAG, triacylglycerols; total-C, total cholesterol; HDL-C, high-density-lipoprotein-cholesterol; TGI, triacylglycerol increase.
* Mean values were significantly different from those obtained with drug treatment (P < 0.05).
† For details, see pp. 154–155.

mass. No significant relationships were established with the fasting levels of blood HBA1c and plasma insulin, LDL-C, HDL-C, total-C and TAG. In the normal-weight diabetics, but not in the obese, however, pplip was positively correlated with the fasting plasma glucose level ($r_s = 0.74$, $P < 0.001$). These relationships were also seen for TGI with and without correction for the BMI.

Non-diabetic subjects. In all non-diabetic subjects as a whole no significant relationships between pplip and any of the variables studied could be established. Specifically, the relationship between pplip and age was not significant, even when pplip was expressed on
Fig. 3. Plasma triacylglycerol (TAG) levels after fat meals in Nigerian non-insulin-dependent diabetic subjects receiving drug treatment (■) and after 1 week without drug treatment (▲). For details of subjects and treatment, see pp. 154–155. Points are mean values and standard deviations, represented by vertical bars, for ten subjects. For details of procedures, see p. 155.

Effect of cessation of drug treatment
The results obtained when the daily sulphonylurea treatment was stopped for 1 week in ten diabetic (five obese) subjects and the fat-load test repeated are indicated on Table 3 and Fig. 3. There were no significant changes in fasting plasma levels of TAG and total-C, but plasma glucose increased ($P < 0.05$) while HDL-C levels decreased slightly, albeit significantly ($P < 0.05$). Without drug treatment pplip and TGI increased by about 50% ($P < 0.005$), and at this time correlated positively only with postprandial glycaemia (ppg) ($r_s = 0.68, P < 0.05$) which also increased with drug cessation. No other relationships could be established.

DISCUSSION
As there were differences in the pattern of responses between the diabetic and non-diabetic subjects, they are separately considered.

Changes in diabetic subjects
We have demonstrated that pplip is increased in Nigerian diabetics, and that it showed significant positive correlations with ppg and, less consistently, with fasting plasma glucose (although not HBA1c) levels. Also, when the diabetic subjects stopped their usual oral hypoglycaemic drugs for 1 week, during which the only significant changes observed in
fasting plasma were increased glucose and reduced HDL-C levels with the levels of TAG and the other lipoproteins remaining essentially unchanged, pplip increased quite significantly and maintained its positive correlation with ppg.

These observations confirm that pplip varies acutely with fluctuations in the ambient glucose concentration. This may be because both glucose utilization (glycolysis and glycogenesis) and TAG deposition into adipose tissue and muscle are impaired in states of insulin resistance, as is likely in the diabetic subjects studied here who had mild fasting hyperinsulinaemia (Reaven & Greenfield, 1981). One other important determinant of pplip in the diabetic subjects was age, with which positive correlations were established. This accords with previous observations in non-diabetic Caucasian subjects (Angervall, 1964) and probably accounts, at least in part, for the increase in prevalence of atherosclerotic vascular disease with increasing age.

The diabetic subjects studied here had a mild form of the disease, at least as judged by lack of severe complications, and only slightly elevated fasting plasma glucose and insulin and blood HBA1c levels. The finding of differences in postprandial TAG responses compared with non-diabetic subjects in whom glucose levels are only slightly lower and insulin levels similar would, therefore, suggest that the observations in the diabetics are peculiar to the diabetic state. It remains uncertain whether these observations would hold with more severe NIDDM or even in insulin-dependent diabetes (IDDM) where, although pplip is likely to be increased, the underlying basis would be insulin deficiency with reduced plasma and adipose tissue lipoprotein lipase (EC 3.1.1.34) activity (Reaven & Greenfield, 1981).

Changes in non-diabetic subjects
Here, age did not appear to influence pplip significantly although, as indicated earlier, it may have proved important had greater numbers of subjects been studied. The important relationships were found in the obese subjects in whom negative links could be demonstrated between the degree of pplip and fasting plasma levels of HDL-C and HDL-C: total-C ratio. This supports the earlier report that the breakdown of TAG-rich particles in circulation contributes to levels of HDL. However, these observations were not seen in the normal-weight subjects which may detract from their usefulness in drawing any valid conclusions.

Comparison with reports in Caucasians
In normolipidaemic non-diabetic Caucasian subjects the important determinants of pplip are HDL and its HDL sub-fraction (negative correlation), fasting TAG levels (positive relationship), rate of chylomicron metabolism (inverse correlation), post-heparin plasma lipolytic activity (inverse relationship) and fasting levels of apolipoproteins B and E (strong positive correlation) (Patsch et al. 1983; Patsch, 1987). No detailed study has, to our knowledge, been attempted in diabetic subjects, and information in non-diabetics has simply been extrapolated to diabetics. This is likely to give an erroneous impression especially as it has been documented that pplip shows very wide inter-individual variability and is influenced by numerous factors in different subsets of individuals (Patsch, 1987).

The determinants in Caucasians could only be reproduced in the obese non-diabetic subjects studied here although, due to lack of requisite facilities, apolipoprotein levels were not measured. The findings, however, suggest, as is known in clinical practice, that obesity increases liability to coronary heart disease even in a population with a low prevalence of such disorders (Williams, 1971; Shaper, 1972; Falase et al. 1973). The development of obesity might expose the Nigerian subject to the atherogenic risk factors by overwhelming the protection conferred by the natural diet.
Conclusion

Our results indicate that a fat load is inefficiently metabolized in diabetic patients, even when the disease is mild. The degree of inefficiency in handling a fat load is worse with acute hyperglycaemic episodes and increasing age. These observations, therefore, provide further evidence, albeit based mainly on statistical correlations, for a role for acute hyperglycaemia in the pathogenesis of diabetic vascular disease. In non-diabetic subjects obesity appears to confer the liability to atherosclerosis described in Caucasian subjects to Nigerians, despite the protective effect of the traditional diet. The study also confirms earlier observations that different factors influence pplip in different subset of individuals, especially as the pattern of correlations in Nigerian subjects differed somewhat from the described pattern in Caucasians.

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