Plasma Triglyceride and Fatty Acid Metabolism in Diabetes mellitus

Barry Lewis, Mario Mancini, Martin Mattock, Alan Chait, and T. Russell Fraser

Departments of Chemical Pathology and Medicine and the Lipid Disorders Clinic, Royal Postgraduate Medical School, London W12 OH5

Received: December 6, 1971, and in revised form: May 3, 1972

Abstract. Free fatty acid and triglyceride metabolism was studied in diet-responsive and insulin-dependent diabetics and in non-diabetic obese patients before and during treatment. Free fatty acid turnover was elevated in diabetics and in most obese patients, and was decreased by diabetic control; it showed no significant change in the obese patients during caloric restriction. Plasma triglyceride levels exceeded 180 mg/100 ml in 20 of the 34 diabetics, and gross lipoaemia occurred both in insulin-requiring and diet-responsive patients. The fractional turnover of injected triglyceride was low in 20 of 33 measurements on untreated diabetics, and was negatively correlated with endogenous triglyceride levels. The fractional turnover increased significantly during diabetic control. These findings are compatible with the view that diabetic hypertriglyceridaemia may be due in part to impaired removal of triglyceride from plasma.

Key words: Diabetes, hypertriglyceridaemia, free fatty acid, intravenous fat tolerance, free fatty acid turnover, triglyceride fractional turnover.

Hypertriglyceridaemia is a common finding in patients with diabetes mellitus, particularly in those who have vascular complications [1]. The possible role of hypertriglyceridaemia in accelerating the development of atherosclerosis is suggested by many retrospective surveys showing higher plasma triglyceride levels in patients with ischaemic heart disease [2-4], and with peripheral vascular disease [5] than in control populations; this role is also favoured by the predisposition to ischaemic heart disease seen in patients with familial hyperpre-/3 lipoproteinaemia [6, 7].

The mechanism of diabetic hypertriglyceridaemia is therefore of interest, and has recently been extensively reviewed by Nikkila [8]. As plasma free fatty acid (FFA) concentrations are commonly elevated in uncontrolled diabetics [9, 10], increased input of fatty acid into the liver is probable; its esterification to triglyceride and incorporation into very low density lipoprotein (VLDL) and secretion of the latter into plasma might therefore be enhanced. On the other hand insulin deficiency decreases triglyceride secretion by the isolated rat liver, and the resultant of these opposing influences on hepatic secretion of VLDL in diabetics is not readily predictable. However, direct observations of hepatic triglyceride secretion in diabetic dogs have recently been reported by Basso and Havel [11] who found no evidence that hypersecretion of triglyceride accounted for the demonstrably high plasma levels.

An alternative possibility is that diabetic hypertriglyceridaemia is the consequence of impaired removal of glyceride from plasma. In the present study we have attempted to assess this process with the intravenous fat tolerance test [12] and here report our findings in 34 diabetics who were studied in the uncontrolled state and reinvestigated after correction of hyperglycaemia. In addition to the fat tolerance test, plasma FFA turn-over was measured, and these kinetic studies were related to plasma lipid concentrations.

Subjects, Methods and Calculations

Thirty-four diabetic patients were investigated; twenty-two were classified as diet responsive, and the remainder were insulin-dependent. They were studied in the uncontrolled state and the majority were reinvestigated when diabetic control had been established. Satisfactory control was defined by a fasting blood glucose not exceeding 120 mg/dl and absence of glycosuria.

In the diet-responsive group there were 10 males and 12 female patients aged 48 to 70 years, mean 57.5 years. All but 5 were obese, exceeding by more than 12% their ideal body weight (Metropolitan Life Insurance Company, [13]). None was ketotic. Fasting blood glucose levels on presentation were 125 to 230, mean 188 mg/dl in this non-insulin dependent group. Treatment was by diet, without medication, comprising a daily intake in the obese subjects of 800 kcals, with 60 g carbohydrate; the lean subjects received 1800 kcals, including 150 g carbohydrate. The mean fasting blood glucose level was 102 mg/dl on the day of the repeat study.

The 12 insulin-dependent diabetics (30 to 58 years, mean 40; 5 male, 7 female) comprised 8 who presented de novo with diabetes of whom 1 had mild ketosis, and 4 in whom, despite insulin treatment, control was considered to be unsatisfactory. In the latter 4 subjects, under careful observation in the ward, insulin was withdrawn for 24-48 hours while their diabetic diet was continued. One patient developed ketonuria. The investigations were made and effective insulin treatment then instituted. All had a previous history of keto-acidosis or of failure to respond to diet alone or with oral hypoglycaemic agents. Studies were re-
peated with the patients satisfactorily controlled (as defined above), either during the same admissions, 3–7 days later, or during a subsequent admission to hospital after 1–2 months. Fasting blood glucose levels in the uncontrolled state were 178–426 mg/dl, mean 271; in the controlled state, the mean level was 94 mg/dl. Of the insulin-dependent diabetics, two were obese.

Electrolyte and fluid balance was maintained by appropriate intake, orally or parenterally. During the studies on controlled insulin-dependent diabetics, insulin was administered by intravenous infusion at a rate proportional to the 24-hour requirement.

Ten obese patients, (15–32% above ideal body weight) who had normal oral glucose tolerance were also studied. They were treated with the same diet as received by the obese diabetics.

Patients were studied in the fasting state, i.e. 12–14 hours after food. The following analytical methods were used: serum triglyceride and cholesterol concentrations were measured by the semi-automated procedure of Cramp and Robertson [14] and by the Technicon N-24a method, respectively. Lipoprotein electrophoresis was performed on cellulose acetate with Oil red O staining. Preparative ultracentrifugation of lipoproteins was carried out as described by Havel, Eder and Bragdon [16].

Measurement of total FFA turnover through plasma was carried out by determining the equilibrium specific activity of FFA in plasma, during a constant infusion of albumin-complexed (1-14C) potassium palmitate; the mean specific activity at 40, 50 and 60 min. was usually employed, representing the plateau of the specific activity-time curve.

Palmitic acid, of specific activity 55.2 μCi/μM (Radiochemical Centre, Amersham) was dissolved in minimal ethanol, neutralised with potassium hydroxide, diluted in 0.9% sodium chloride and autoclaved at 15 p. s. i. for 15 min. Radiochemical homogeneity was checked by thin layer chromatography. On the morning of the study, 15 μCi of the palmitate was added slowly, with swirling, to a solution containing 0.25 g of fatty acid-poor human albumin (Lister Institute, Elstree, Herts). The complex was diluted suitably and up to 10 μCi was infused intravenously at a constant rate. At 20, 30, 40, 50 and 60 min. from the start of the infusion, blood samples were obtained from an indwelling venous cannula in the contralateral antecubital vein for FFA specific activity measurements. The cannula was occasionally flushed with isotonic saline.

Plasma was separated immediately and 4 ml were extracted by the Dole [17] procedure. Duplicate aliquots of the heptane phase were titrated with alcoholic sodium ethoxide in a one-phase system with thymolphthalein as indicator [18]. A further aliquot was fractionated by column chromatography on silicic acid-potassium hydroxide [19]. Radioactivity in the FFA fraction was counted in a liquid scintillation spectrometer, with quench correction by an internal standard of 14C-hexadecane.

FFA turnover through plasma was then calculated as follows:

Turnover (μM/min/kg) =

\[
\frac{\text{Infused dose (dpm per min.)}}{\text{FFA specific activity (dpm/μM) \times body wt. (kg)}}
\]

We have adapted the intravenous fat tolerance test [12] to nephelometric measurement [20]. Except where specified, a dose of 0.1 g triglyceride per kg body weight (Intralipid, Vitrum, Stockholm, a 10% soya bean oil emulsion, batch no. 199523) was administered intravenously as a pulse injection; eight blood samples were obtained at five minute intervals. The removal of the emulsion from plasma was in all studies exponential, at this dose level. The rate constant for the fractional disappearance of the injected triglyceride, Kp, was calculated from the computed least squares regression line. In 5 patients, a dose of 0.25–0.3 g/kg was subsequently injected; its removal from plasma was initially linear, indicating a "maximal removal rate", Km, with dimension mg/dl/min.

The normal range for Kp was ascertained in 32 men and 18 women. Their mean ages were 40 and 38 years respectively, range 20–64. All were ambulant, and normality was confirmed by history, physical examination, biochemical screening and resting electrocardiography.

Results

Fasting plasma triglyceride concentrations are shown in Fig. 1. Twelve of the 22 diet-responsive patients and 8 of the 12 insulin-requiring patients had levels above 160 mg/dl before treatment. All hypertriglyceridaemic patients showed increased pre β lipoprotein on plasma electrophoresis. Heavily lipemic plasma was observed in two diet-responsive and two insulin-responsive patients; inspection of stored plasma and cellulose acetate electrophoresis indicated the presence of pre β lipoprotein and chylomicra in gross excess, and this was confirmed in these 4 patients by ultracentrifugation. Both of the diet-responsive patients with gross hypertriglyceridaemia had symptoms of diabetes and diabetic retinopathy. One also had a strong family history of diabetes. In both groups, diabetic control reduced the mean triglyceride concentrations from 550 to 110 mg/dl in the diet group and from 1060 to 116 mg/dl in the insulin-treated group respectively (Fig. 1).

The turnover of plasma FFA exceeded our normal range of 6–12 μM/min./kg in all but one of the patients studied; in 17 diet-responsive patients it was 24±3 μM/min./kg, and in 5 insulin-dependent patients it was 69±27 μM/min./kg (Fig. 2); the highest rates thus occurred among insulin-requiring diabetics. Turnover was reduced towards normal in all insulin-treated patients when diabetic control was achieved, reaching 15±5 μM/min./kg (p<0.01 by paired t-test). FFA turnover was decreased in 12 of 14 diet-responsive
patients in whom repeat measurements were made, from $22 \pm 8$ to $13 \pm 3 \mu M/min./kg$ ($p<0.01$). Despite diabetic control which appeared adequate by the blood glucose measurements made, FFA turnover often remained somewhat elevated. This was true of 3 diabetic patients who were still obese when the second study was carried out, and of 7 who achieved or were initially of ideal weight.

**Fig. 2.** Plasma free fatty acid turnover in diabetes and obesity, and its response to treatment. Significance levels calculated by paired t-test

FFA turnover was somewhat elevated in 6 of 9 obese non-diabetic patients ($17 \pm 5 \mu M/min./kg$ observed weight). In 8 of these patients in whom repeat studies were made during dieting, FFA turnover showed a nonsignificant increase ($16 \pm 5$ to $18 \pm 5 \mu M/min./kg$). The interval between studies was 10–30 days and the diet received was identical with that prescribed for the obese diet-responsive diabetics. Predicted
weight loss occurred in both diabetic and non-diabetic groups.

Fig. 3 shows the relation between FFA turnover and plasma triglyceride concentration in the untreated patients; positive correlations were observed in both diabetic groups, but these were not statistically significant.

Normal ranges for the rate of removal of an intravenous pulse dose of triglyceride, K₂, in the intravenous fat tolerance test were 0.056 ± 0.016 min⁻¹ (mean ± standard deviation) in men, and 0.074 ± 0.018 min⁻¹ in women. The sex difference was statistically significant, p < 0.02. The 2-standard deviation lower limits for K₂ were 0.024 and 0.038 min⁻¹ in men and women respectively.

Fractional removal rates of exogenous triglyceride in uncontrolled diabetes is shown in Fig. 4. In two patients, no change in nephelometric reading occurred during the period of sampling, i.e. no detectable removal of exogenous triglyceride had occurred; the rate constants were recorded as zero. In all other studies, exponential disappearance of the fat emulsion was observed when the dose was 0.1 g triglyceride per kg body weight. The rate constant (K₂) varied widely.
from patient to patient, 13 of the 21 observations on diet-responsive patients lying below the 2-standard deviation lower limit for their sex, and 7 of 12 insulin-responsive patients similarly having subnormal $K_2$ rates. The rate constants increased, often strikingly, in 24 of the 27 patients when diabetic control was achieved. In the diet-responsive group, mean $K_2$ increased from 0.035 to 0.053 min.$^{-1}$ ($p<0.001$ by paired $t$-test). In the insulin group, $K_2$ increased from 0.032 to 0.062 min.$^{-1}$ ($p<0.001$).

Negative correlations between rate constant and plasma triglyceride level were found in both groups of untreated diabetics (Fig. 5), and in the obese non-diabetics. These correlations were highly significant.

In Table 1, the standard fat tolerance test is compared with the results of administering a larger dose of triglyceride, in this case 0.25–0.3 g/kg body weight. In the first 60 min. after injection, this dose disappeared linearly, by zero order kinetics, suggesting saturation of the mechanisms for peripheral removal of triglyceride. As the fractional turnover of intravenously injected triglyceride was negatively correlated with the plasma triglyceride concentration, it was of interest to assess also this "maximal" removal rate ($K_1$) of the injected triglyceride. The rate $K_1$ was increased in 4 out of 5 patients by control of their diabetes. In these 4 patients the plasma triglyceride concentration was reduced during diabetic control, while it was unchanged in the remaining patient. The zero-time plasma triglyceride concentration was calculated to be at least 1000 mg/dl after the injection.

In Table 2, the rate constant $K_2$ in 3 groups of hypertriglyceridaemic patients is shown, together with the normal range. Lowest $K_2$ values occurred in 11 men with primary, familial hypertriglyceridaemia (elevated pre $\beta$-lipoprotein, Type IV in the classification of Fredrickson [6]). Similar but slightly higher $K_2$ values were observed in the patients with hypertriglyceridaemia due to uncontrolled diabetes. The patients with alcoholic hypertriglyceridaemia [28] had a mean $K_2$ value.
Normal men
Normal women
Familial hyper-triglyceridaemia (men)
Alcoholic hyper-triglyceridaemia (men)
Diabetic hyper-triglyceridaemia (men)
Diabetic hyper-triglyceridaemia (women)

<table>
<thead>
<tr>
<th>Number</th>
<th>$K_t$ (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>0.056 ± 0.016</td>
</tr>
<tr>
<td>18</td>
<td>0.074 ± 0.018</td>
</tr>
<tr>
<td>11</td>
<td>0.015 ± 0.0090</td>
</tr>
<tr>
<td>5</td>
<td>0.035 ± 0.012</td>
</tr>
<tr>
<td>7</td>
<td>0.020 ± 0.012</td>
</tr>
<tr>
<td>11</td>
<td>0.031 ± 0.0053</td>
</tr>
</tbody>
</table>

which was about twice as great as that in the familial and the diabetic groups.

Discussion

The data presented indicate that diabetics, whether insulin-dependent or diet-responsive, commonly have raised plasma triglyceride levels, slow fractional turnover in the intravenous fat tolerance test and increased plasma FFA turnover rates.

Findings were similar in both groups of diabetics; and there was no tendency for lean and obese diabetics to differ in triglyceride levels or clearance. There was a tendency for the highest turnover rates of FFA to occur in insulin-requiring diabetics; this is in conformity with earlier work on high FFA concentrations in plasma, showing striking changes in insulin-dependent patients [10] and more normal levels in obese diabetics [21]. None of our diabetics was severely ketotic.

While some studies have shown increased FFA turnover in diabetics [10, 22], normal rates have also been reported [23]. In the latter report, the fasting blood glucose of the diabetic patients was 167 ± 49 mg/dl, suggesting that in some of them, the diabetic state was relatively mild at the time of study.

Control of the diabetes significantly decreased plasma triglyceride concentrations, all but five falling to below 160 mg/dl. This is true of control by caloric restriction, or by isocaloric carbohydrate restriction, with or without insulin. By contrast, FFA turnover, though initially high in all but one patient, did not fall to normal in the majority of patients, though treatment reduced the turnover rate in 17 out of 19 studies. The positive correlation between FFA turnover and plasma triglyceride concentration was associated with several exceptions and was not statistically significant.

The exponential rate of disappearance from plasma ($K_t$) of the parenteral fat emulsion Intralipid has been proposed as a measure of the fractional turnover of endogenous plasma triglyceride [12]. Using a modification of this technique [20], we have observed subnormal $K_t$ values in about half of our untreated diabetics. The rate constants were lowest in the most hypertriglyceridaemic patients, there being a highly significant negative correlation between triglyceride level and $K_t$. In almost all patients the $K_t$ value increased and the plasma triglyceride concentration fell when diabetic control was established.

Before interpreting low $K_t$ values to signify reduced fractional turnover of endogenous plasma triglyceride, it is necessary to discuss an alternative explanation, namely that the slow disappearance of the fat emulsion from plasma is a consequence of an expanded plasma triglyceride pool. Boberg [12] has argued against the latter interpretation on the basis of theoretical considerations and particularly Hallberg's demonstration that $K_t$ is independent of the amount of exogenous triglyceride [24]. Recently, Boberg has found, in normal subjects and patients with primary hypertriglyceridaemia, a close positive correlation between $K_t$ in the intravenous fat tolerance test and the fractional turnover of endogenous VLDL [25]; the latter was derived from estimates of VLDL secretion rate, based on measurement of arterio-venous differences in triglyceride concentration across the liver [26].

In some hypertriglyceridaemic states, $K_t$ and plasma triglyceride levels are reciprocally related; this is the case in diabetes (Fig. 5) and in many patients with primary hyperlipoproteinaemia (Table 2), [12, 20]. However, there are two situations in which high plasma VLDL levels (i.e. expanded endogenous triglyceride pool size) are not correlatable with $K_t$ values: this is seen when plasma triglyceride levels rise during alcohol withdrawal without a significant change in $K_t$ [27], and also in patients with alcoholic hypertriglyceridaemia (Table 2) in whom triglyceride levels fall during alcohol withdrawal without a significant change in $K_t$ [28]. It is therefore clear that $K_t$ can be independent of the size of the endogenous triglyceride pool.

In diabetes, we have also attempted to test the significance of the $K_t$ value by carrying out the fat tolerance test at a higher dose level ($K_t$ of Boberg et al. [12]); by raising the plasma triglyceride sufficiently, the emulsion disappears at a linear rate, by zero order kinetics, implying saturation of removal mechanisms. In four of five such studies the $K_t$ value, a measure of this linear rate of removal of injected triglyceride, was found to increase when the diabetes was controlled. These observations suggest that the enhanced rate of disappearance of the fat emulsion, when diabetes is controlled, is not a consequence of difference in pool size, i.e. of substrate availability. Nikkila [8] has affirmed that the theoretical source of error, arising from an expanded endogenous triglyceride pool size, falls away when the injected triglyceride load is substantially larger than the endogenous pool.

The data obtained with the intravenous fat tolerance test, therefore, are compatible with the possibility that diabetic hypertriglyceridaemia in man is commonly associated with decreased removal of triglyceride from
plasma. In alloxan-diabetic rats, clearance of lipoprotein obtained from other rats is impaired [29]; and the removal of an injected triglyceride emulsion from plasma is impaired in the diabetic dog but rendered normal by insulin [30].

The rate of removal of triglyceride from plasma may be limited by lipoprotein lipase activity, as in familial chylomicronaemia [31, 32]; i.e. the Type I hyperlipoproteinaemia of Fredrickson, Levy and Lees [6]. As assessed by measurement of post-heparin lipolytic activity, no impairment of activity of this enzyme was found in most diabetics, whether hypertriglyceridaemia was present or not [33, 34]; Bagdade, Porte and Bierman [35] have, however, found reversible subnormal post-heparin lipolytic activity in a group of diabetics with the relatively uncommon pattern of gross hyperlipidaemia due to chylomicronaemia. In diabetic rats, adipose tissue shows low lipoprotein lipase activity but that in heart muscle may be increased [36, 37]. However, other stages in triglyceride uptake from plasma could be rate limiting, e.g., re-esterification of fatty acids, a process which may be defective in diabetes [21, 38].

The effects of insulin and the diabetic state on triglyceride production have been studied in a number of experimental systems. Insulin deficiency depresses fatty acid synthesis by rat liver slices [39] and by the isolated perfused rat liver [40]; the liver of the diabetic rat has also a decreased ability to esterify fatty acid to triglyceride, which is partly restored by insulin [41]. Using the same system, this group has also shown impaired incorporation of labelled amino-acids into apolipoprotein [42]. Only in a cell-free supernatant from diabetic liver has increased fatty acid esterification been reported [43]. Topping and Mayes [44] have recently reported in abstract that insulin enhances VLDL secretion by the isolated perfused rat liver, and analogous results have been obtained in the "perfusion" system [45].

These effects of insulin deficiency on the isolated liver would tend to decrease triglyceride secretion in the diabetic state; but in the diabetic animal there is also an increase of free fatty acid turnover and presumably of hepatic FFA uptake [46]. The net effect of these conflicting influences on hepatic triglyceride secretion has recently been assessed by Basso and Havel [11], who observed that in pancreatectomized dogs, splanchnic triglyceride secretion is decreased.

An unresolved difficulty is the fact that little more than half of our uncontrolled diabetics had hypertriglyceridaemia. It is by no means clear what determines the presence or absence of this abnormality. Bagdade [35] has reported that extreme lipaemia tends to occur in severe but non-ketotic diabetics of long duration; our 4 patients conform entirely with this description. The plasma triglyceride pool is determined by input as well as output rates. There is little evidence to suggest increased input of triglyceride into plasma in diabetics; but it remains possible that differences of secretion rate within the normal range, or varying degrees of impairment of secretion may interact with reduced uptake from plasma to produce the wide range of triglyceride levels seen in diabetics. The degree of insulin deficiency may be critical; at its severest extent, ketosis would be expected and would lead to early diagnosis and treatment. Insulin deficiency of lesser severity could be present in Bagdade's and our intensely lipaemic patients, permitting a more normal rate of triglyceride secretion than in the patients with more complete failure of insulin production.

Despite these multiple and often antagonistic actions, the acute effect of insulin on plasma triglyceride levels is relatively clear-cut. In man, insulin (with glucose) reduces triglyceride concentrations [47, 48]; and our data on insulin treatment of diabetics show a reduction of elevated or normal triglyceride levels in all but one patient (the exception remaining substantially obese at the time of the repeat study). In the fasted rat, glucose refeeding decreases the plasma triglyceride concentration, by a process which may involve increased peripheral uptake of this lipid [49].

What is less clear, as Nikkila [8] has pointed out, is the chronic effect of hyperinsulinaemia. In a normal population, a striking positive correlation has been shown between triglyceride levels and insulin responsiveness to an oral glucose load [50]. Although hyperinsulinaemia has been observed in patients with endogenous hypertriglyceridaemia [51], Glueck, Levy and Fredrickson [52] found insulin responses to be high, normal or low in roughly equal proportions of patients with hypertriglyceridaemic states. It is conceivable that a sub-group of hypertriglyceridaemic patients may exist in whom insulin plays a pathogenetic role; but such a role appears not to be universal.

It was of interest that FFA turnover, though reduced by diabetic control, often remained somewhat elevated. The criteria for adequate control were the absence of glycosuria and reduction of fasting glucose levels to below 120 mg/dl. While these are commonly accepted criteria they do not necessarily imply normalisation of glucose metabolism throughout the day. The residual elevation of FFA turnover occurred both in obese and lean diet-responsive patients (Fig. 2) and in the insulin-dependent patients who were all of ideal body weight at the time of the repeat study. The degree to which FFA turnover was increased in the uncontrolled diabetics was not related to the presence of obesity. Highest turnovers occurred in lean, insulin-dependent patients, though only two were mildly ketotic at the time of study.

The effect of caloric restriction on FFA turnover in obese patients was noteworthy. In the obese diabetics, turnover decreased substantially or slightly; however, in 4 of the 5 non-diabetic obese patients who were studied on a second occasion, this decrease did not occur. Their FFA turnovers usually increased slightly, as would be expected of normal subjects during caloric
restriction. Similar observations have been reported in fasted obese patients [53], and Jackson [54] found a rise in plasma FFA concentrations in the majority of obese patients treated by fasting. The reason for this apparent difference in behaviour of obese diabetics and non-diabetics is not clear.

Acknowledgements. We thank Dr. Q. J. Hobson, Dr. Clara Lowy and Dr. Dennis M. Krilker for permission to study their patients. Financial support from Glaxo Laboratories is gratefully acknowledged.

References
45. Letarte, J., Fraser, T. R.: Stimulation by insulin of the incorporation of U14 C-glucose into lipids released by the liver. Diabetologia 5, 368 (1969).