Impaired lipoprotein lipase activation by uraemic and post-transplant sera

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Summary

1. Lipoprotein lipase was separated from normal human post-heparin plasma by affinity chromatography and assayed with a 14C-labelled triolein emulsion. No enzyme activity was detected unless whole serum was included in the assay as a source of cofactor, apolipoprotein C-II.

2. After a 10 h fast, serum obtained from 46 normal subjects, eight patients with hypertriglyceridaemia but normal renal function, patients with chronic renal failure (24 undialysed, 20 haemodialysed) and 14 recipients of renal allografts, was added to incubation medium for the assay of lipoprotein lipase to determine the maximum activation of the enzyme.

3. When serum was obtained from normal subjects, maximum activation of the enzyme correlated positively with the concentration of triacylglycerol in the sample. Neither sex nor age had a significant effect on the maximum activation achieved by serum from control subjects.

4. The maximum lipoprotein lipase-activating capacity of serum from uraemic and transplant patients was significantly reduced when compared with serum from healthy controls or from the non-uraemic hypertriglyceridaemic patients.

5. Maximum enzyme activation correlated positively with high-density lipoprotein cholesterol in serum from undialysed patients, but did not correlate positively with total serum triacylglycerols in any of the patient groups. Only in transplant recipients was there a significant inverse relationship between serum creatinine concentrations and maximum enzyme activation.

6. Although lipoprotein lipase activation was impaired in uraemic subjects and renal transplant recipients, this appeared to be due more to the presence of an inhibitor than to cofactor deficiency.

Key words: apolipoproteins, lipoprotein lipase, renal transplantation, triacylglycerols, uraemia.

Introduction

The assay in vitro of lipoprotein lipase (EC 3.1.1.34) that has been extracted from tissues or separated from post-heparin plasma requires apolipoprotein C-II as cofactor for the hydrolysis of a laboratory-prepared triacylglycerol emulsion (Havel, Shore, Shore & Bier, 1970; La Rosa, Levy, Herbert, Lux & Fredrickson, 1970; Havel, Fielding, Olivecrona, Shore, Fielding & Egelrud, 1973). Apolipoprotein C-II is a component of both the very-low-density lipoprotein (VLDL) and the high-density lipoprotein (HDL) of plasma and can be supplied by the addition of normal serum to the incubation medium (Bier & Havel, 1970; Rogers, Barnett & Robinson, 1976).

Apolipoprotein C-II also plays an important role in triacylglycerol metabolism in vivo. Infusion of normal plasma or HDL which contains apolipoprotein C-II, into a hypertriglyceridaemic patient with a genetically determined absence of apolipoprotein C-II, caused a rapid decrease in plasma triacylglycerol concentration (Breckenridge, Little, Steiner, Chow & Poapst, 1976; Catapano, Kinnunen, Breckenridge, Gotto, Jackson, Little, Smith & Sparrow, 1979).

The ability of serum to activate lipoprotein in vitro has been used to determine apolipoprotein C-II cofactor activity (Chu, Miller & Mills, 1976;...
Rogers et al., 1976); moreover it correlates positively with VLDL apolipoprotein C-II levels measured by radioimmunoassay (Kashyap, Srivastava, Chen, Perisutti, Campbell, Lutmer & Glueck, 1977). These studies showed a positive relationship between serum triacylglycerol concentrations and apolipoprotein C-II concentrations whether measured directly in plasma or as lipoprotein lipase activation in vitro. In non-uraemic hypertriglyceridaemic subjects, total plasma apolipoprotein C-II concentrations were significantly higher than in normal control subjects and correlated positively with triacylglycerol levels (Kashyap et al., 1977; Schonfeld, George, Miller, Reilly & Witztum, 1979). Patients with chronic renal failure whether treated by dialysis or not, have elevated serum triacylglycerol concentrations (Bagdade, Porte & Bierman, 1968; Ibels, Simons, King, Williams, Neale & Stewart, 1975), impaired triacylglycerol clearance (Cattran, Steiner, Fenton & Wilson, 1974; Ibels, Reardon & Nestel, 1976; Savdie, Gibson, Crawford, Simons & Mahony, 1980) and reduced plasma lipoprotein lipase activity (Crawford, Savdie & Stewart, 1979a; Applebaum-Bowden, Goldberg, Hazzard, Sherrard, Brunzell, Huttenen, Nikkila & Ehnholm, 1979). Compared with normal control subjects, lipoprotein lipase activity from uraemic plasma in vitro was reduced and, since this occurred despite the addition of pooled normal plasma as a source of apolipoprotein C-II in the assay (Crawford et al., 1979a), a cofactor deficiency does not appear to be the sole cause of the reduced enzyme activity in renal failure.

As plasma apolipoprotein C-II concentrations were abnormally raised in hypertriglyceridaemia not associated with renal failure (Kashyap et al., 1977; Schonfeld et al., 1979), this study was undertaken to measure the apolipoprotein by using its lipoprotein lipase-activating ability in serum from uraemic patients, most of whom had hypertriglyceridaemia. For this investigation, enzyme derived from normal human post-heparin plasma, was assayed in the presence of four different amounts of serum from patients with chronic renal failure, renal allograft recipients or from normal or hypertriglyceridaemic control subjects. Possible relationships between maximum enzyme activation and fasting serum lipid and creatinine concentrations were sought.

Methods

Subjects

Consent was obtained from all subjects after full explanation of the procedure. Subjects with diabetes, liver disease, markedly elevated blood pressure or a history of bleeding were excluded as were those taking lipid-lowering drugs or androgenic or oestrogenic hormones. Subjects with serum triacylglycerol concentrations greater than 4·45 mmol/l were also excluded from the study as higher levels of triacylglycerol were shown to reduce the specific activity of the 14C-labelled triolein used to calculate free fatty acid release resulting in a spuriously low estimate of enzyme activity. Relative body weight was calculated from the percentage of mean ideal weight for height and sex for each patient's actual weight (Documenta Geigy, 1962).

Twenty patients with end-stage renal failure treated by haemodialysis and 24 subjects with chronic renal failure who had never received dialysis therapy were included in the study. None was nephrotic. Causes of renal failure were as described previously (Stewart, McCarthy, Storey, Roberts, Gallery & Mahony, 1975) and diet was unrestricted except for those with symptomatic uraemia or salt and water retention, in whom protein (<40 g/day) or sodium (<50 mmol/day) intake was limited.

Fourteen recipients of renal allografts were studied 5–125 months after transplantation, at a time when their graft function was stable and health was good. Diet was not restricted and immunosuppressive therapy included prednisolone (7·5–20 mg/day, mean 11·4) and azathioprine (25–200 mg/day, mean 134).

Forty-six healthy male and female subjects, none of whom was taking regular medication or was on dietary restriction, together with eight patients with normal renal function but elevated serum triacylglycerol levels were used as controls.

Collection and processing of blood samples

Venous blood was collected after a 10 h fast for determination of serum concentrations of triacylglycerol, cholesterol, creatinine (Noble & Campbell, 1970; Technicon, 1965; Technicon, 1974) and HDL cholesterol as well as lipoprotein lipase-activating capacity. Blood was drawn immediately before dialysis from patients receiving this treatment.

HDL cholesterol was measured by an enzyme assay of the serum supernatant remaining after precipitation of low-density lipoprotein (LDL) and VLDL with dextran sulphate and magnesium chloride (Savdie, Gibson, Stewart & Simons, 1979). As described previously (Crawford et al., 1979a), lipoprotein lipase was separated from blood drawn 15 min after intravenous ad-
Impaired lipase activation in renal failure

Results

As shown in Fig. 1, mean activation of lipoprotein lipase separated from the post-heparin plasma of three male and three female healthy subjects was significantly higher in healthy controls than in patients with chronic renal failure. The enzyme activity was measured by adding normal serum (control) or uraemic serum as cofactor. Total assay volume was 0.2 ml. Serum triacylglycerol concentrations in the normal and uraemic serum were 1.95 and 1.60 mmol/l, respectively. Vertical bars indicate ± 1 SE.

Serum lipid concentrations (Table 1)

In healthy control subjects total serum and HDL concentrations of cholesterol were significantly higher in females than in males. There was no sex difference in body weights (percentage of ideal) or serum triacylglycerol concentrations.

Male and female patients with chronic renal failure not treated by dialysis, had elevated serum triacylglycerol and reduced HDL cholesterol concentrations but body weights and total cholesterol concentrations which were similar to...
**TABLE 1. Comparison of relevant clinical and biochemical parameters in chronic renal failure, transplant recipients and normal control subjects**

Results are means ± 1 SE. Significance of differences: compared with control subjects of the same sex, (1) \( P < 0.001 \); (2) \( P < 0.01 \); (3) \( P < 0.05 \) and compared with the opposite sex in the same subject group, (4) \( P < 0.001 \); (5) \( P < 0.02 \); (6) \( P < 0.05 \).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Age (years)</th>
<th>Serum creatinine (mmol/l)</th>
<th>Serum triacylglycerols (mmol/l)</th>
<th>Serum cholesterol (mmol/l)</th>
<th>HDL cholesterol (mmol/l)</th>
<th>Weight (% of ideal)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>18</td>
<td>41 ± 3</td>
<td>0.09 ± 0.003</td>
<td>0.94 ± 0.10</td>
<td>5.32 ± 0.24</td>
<td>1.07 ± 0.04</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>Chronic renal failure, undialysed</td>
<td>10</td>
<td>46 ± 5</td>
<td>0.88 ± 0.12</td>
<td>1.98 ± 0.14</td>
<td>5.15 ± 0.39</td>
<td>0.78 ± 0.07</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>Chronic renal failure, dialysed</td>
<td>7</td>
<td>38 ± 5</td>
<td>1.13 ± 0.07</td>
<td>2.79 ± 0.43</td>
<td>6.29 ± 0.45</td>
<td>0.86 ± 0.07</td>
<td>99 ± 10</td>
</tr>
<tr>
<td>Transplant recipients</td>
<td>9</td>
<td>44 ± 3</td>
<td>0.14 ± 0.01</td>
<td>1.83 ± 0.24</td>
<td>7.01 ± 0.50</td>
<td>1.26 ± 0.11</td>
<td>101 ± 4</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>28</td>
<td>50 ± 2</td>
<td>0.07 ± 0.003</td>
<td>0.96 ± 0.09</td>
<td>6.04 ± 0.25</td>
<td>1.37 ± 0.05</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>Chronic renal failure, undialysed</td>
<td>14</td>
<td>51 ± 4</td>
<td>0.84 ± 0.08</td>
<td>2.35 ± 0.30</td>
<td>6.57 ± 0.40</td>
<td>0.91 ± 0.09</td>
<td>106 ± 5</td>
</tr>
<tr>
<td>Chronic renal failure, dialysed</td>
<td>13</td>
<td>50 ± 4</td>
<td>0.94 ± 0.06</td>
<td>1.90 ± 0.19</td>
<td>6.09 ± 0.39</td>
<td>0.95 ± 0.05</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>Transplant recipients</td>
<td>5</td>
<td>48 ± 6</td>
<td>0.12 ± 0.008</td>
<td>2.07 ± 0.36</td>
<td>8.54 ± 1.06</td>
<td>1.69 ± 0.21</td>
<td>116 ± 7</td>
</tr>
</tbody>
</table>

**FIG. 2.** Comparison of the activation of normal lipoprotein lipase with increasing amounts of serum from 24 undialysed uraemic patients (●——●) and from normal control subjects matched for age and sex (●——●). Significance of differences: * \( P < 0.001 \); ** \( P < 0.05 \). Results are means ± 1 SE.

those of control subjects. The same results were obtained from dialysed uraemic patients.

Serum triacylglycerol and total cholesterol concentrations were elevated in male and female transplant recipients, whose serum creatinine concentrations were slightly above normal levels. HDL cholesterol concentrations and body weights were similar to those of the control subjects.

**FIG. 3.** Comparison of the activation of normal lipoprotein lipase with increasing amounts of serum from 20 dialysed uraemic patients (●——●) and from normal control subjects matched for age and sex (●——●). Significance of differences: * \( P < 0.001 \). N.S., Not significant. Results are presented as means ± 1 SE.

**Activation of lipoprotein lipase from normal plasma**

Neither in patients nor in control subjects was there any significant difference between the maximum lipoprotein lipase activation by serum from male or female subjects.
Impaired lipase activation in renal failure

Normal subjects. Lipoprotein lipase was maximally activated when a 20 μl portion of serum from healthy subjects was added to the assay medium (Figs. 2, 3 and 4).

Hypertriglyceridaemic subjects. The activation of lipoprotein lipase by serum from eight subjects with normal renal function and elevated serum triacylglycerol levels (2·45–4·45 mmol/l, mean 3·38) was not significantly different to the activation obtained with control serum (Table 2).

Patients with chronic renal failure. Maximum activation of lipoprotein lipase occurred with 10 μl of uraemic serum and this was significantly less than with normal control subjects when 10 μl, or more, of serum was added to the incubation medium (Figs. 2 and 3). The pattern was the same for serum from undialysed and dialysed uraemic patients.

Maximum lipoprotein lipase activation by serum from dialysed or undialysed patients was significantly less \( (P < 0·001) \) than that obtained with serum from matched normal control subjects (Table 2).

Transplant recipients. When greater than 10 μl of serum from transplant recipients was included in the incubation medium, lipoprotein lipase activation was less than that obtained with similar amounts of normal serum (Fig. 4). Maximum enzyme activation was lower \( (P < 0·001) \) with serum from these patients when compared with matched normal controls (Table 2).

Correlations

A positive correlation between maximum lipoprotein lipase activation and triacylglycerols was shown in serum from normal subjects \( (r = 0·49, P < 0·001) \) (Fig. 5) but not in serum from any of the patient groups, whereas in undialysed males with chronic renal failure there was a negative correlation between serum triacylglycerols and maximum enzyme activation \( (r = -0·65, P < 0·05) \). Maximum enzyme activity was related to serum HDL cholesterol in undialysed uraemic patients \( (r = 0·50, P < 0·05) \) whereas in transplant recipients maximum lipase activation correlated with serum creatinine concentrations \( (r = -0·73, P < 0·01) \) as well as the daily prednisolone dose \( (r = -0·64, P < 0·02) \).

No association was found when maximum lipoprotein lipase activation was correlated with age, body weight or total serum cholesterol concentrations in patients or control subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Maximum lipoprotein lipase activity (μmol of fatty acids h⁻¹ ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic renal failure, undialysed</td>
<td>24</td>
<td>5·0 ± 0·2 (^{(1)})</td>
</tr>
<tr>
<td>Chronic renal failure, dialysed</td>
<td>20</td>
<td>5·3 ± 0·2 (^{(1)})</td>
</tr>
<tr>
<td>Transplant recipients</td>
<td>14</td>
<td>5·6 ± 0·3 (^{(1)})</td>
</tr>
<tr>
<td>Hypertriglyceridaemia with normal renal function</td>
<td>8</td>
<td>7·0 ± 0·5</td>
</tr>
</tbody>
</table>

\(^{(1)}P < 0·001\)
Discussion

Lipoprotein lipase, when separated from normal post-heparin plasma, was only partially activated if serum from uraemic patients or transplant recipients was used as a source of cofactor in the assay. The ability of normal serum to activate lipoprotein lipase in vitro has been attributed to apolipoprotein C-II activity (Chu et al., 1976; Rogers et al., 1976; Cryer & Jones, 1978).

Both total and VLDL levels of plasma apolipoprotein C-II have been shown to rise with serum triacylglycerol concentrations in normal subjects (Kashyap et al., 1977; Schönfeld et al., 1979), and in the present study a similar positive correlation between maximum enzyme activation reflecting apolipoprotein C-II levels and triacylglycerol concentrations was obtained in the control group. However, in uraemic subjects no such relationship was demonstrated, possibly because measurement of enzyme activation by sera from these patients reflects factors additional to apolipoprotein C-II activity. One such factor could be the reduction by hypertriglyceridaemic sera of the specific radioactivity of the $^{14}$C-labelled triolein used to calculate free fatty acid release. However, serum triacylglycerol levels as high as 4.45 mmol/l did not reduce the measured enzyme activity as shown in tests with hypertriglyceridaemic patients with normal renal function, and uraemic patients and transplant recipients with serum triacylglycerol levels above this value were excluded from this study.

The ability to activate normal lipoprotein lipase was reduced in uraemic sera, and this reduction was more pronounced the greater the quantity of serum added to the assay medium. This suggests that in uraemic serum there is an inhibitor which competes with apolipoprotein C-II, and that this competition is concentration dependent.

The observation that the activity of lipoprotein lipase which has been obtained from uraemic plasma is reduced, despite the presence of normal serum as cofactor in the assay (Crawford et al., 1979a), indicates that impaired lipolysis is not due solely, or even mainly, to lack of apolipoprotein C-II. In a study of seven uraemic patients receiving haemodialysis treatment, directly measured apolipoprotein C-II levels were reduced in plasma VLDL and HDL (Rapoport, Aviram, Chaimovitz & Brook, 1978). However, the validity of the methods used for measuring apolipoprotein C-II has been challenged (Herbert, 1979; Schrecker & Greten, 1979) and further investigation is required to confirm or refute these observations.

The pathogenesis of uraemic hypertriglyceridaemia is not known. Several investigations have shown that lipoprotein lipase activity is reduced in post-heparin plasma, but have failed to demonstrate any relationship between serum triacylglycerol concentrations and enzyme activity (Huttunen, Pasternack, Vanttinen, Ehnholm & Nikkila, 1978; Applebaum-Bowden et al., 1979; Crawford et al., 1979a). Serum from uraemic patients has been shown to inhibit the activity of lipoprotein lipase isolated from rat adipose tissue (Murase, Cattrfan, Rubenstein & Steiner, 1975) and normal human plasma (Crawford, Savdie, Stewart & Mahony, 1979b). These and the present results, which show impaired enzyme activation, indicate that a serum inhibitor could be an important factor in the reduced lipoprotein lipase activity and elevated serum triacylglycerol concentrations demonstrated in chronic renal failure.

HDL cholesterol was reduced in uraemic patients as shown previously (Ibels et al., 1975; Bagdade, Casaretto & Albers, 1976; Brunzell, Albers, Haas, Goldberg, Agadoa & Sherard, 1977) and, as in the study by Savdie et al. (1979),
transplantation restored the concentrations to within control values. Thus the nature of the hyperlipidaemia changes when the patient with chronic renal failure receives a functioning transplant. The lipoprotein pattern shown in transplant recipients is variable, with either VLDL or LDL, or both, elevated (Ibels et al., 1975). Total serum triacylglycerol and cholesterol concentrations are elevated although plasma lipoprotein lipase activity is not reduced (Mordasinì, Frey, Flury, Klose & Greten, 1977; Crawford et al., 1979a). Thus the finding of impaired enzyme activation in the transplant group in the present study was unexpected. The negative correlation between maximum lipoprotein lipase activation and either serum creatinine concentrations or prednisolone dose, must be interpreted in the light of results in uraemic patients in whom creatinine levels, which were both higher and spread over a greater range, did not correlate either positively or negatively with lipoprotein lipase activation. Therefore, it seems likely that immunosuppressive therapy is the cause of impaired lipoprotein lipase activation in transplant recipients.

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References


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