Intravenous fat-tolerance test in ischaemic heart disease and peripheral vascular disease

B. LEWIS, A. C. ONITIRI, I. D. P. WOOTTON, A. CHAIT, G. SIGURDSSON AND C. M. OAKLEY

Departments of Chemical Pathology and Medicine, Royal Postgraduate Medical School, London

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Summary

1. The intravenous fat-tolerance test and serum lipid and lipoprotein measurements were carried out in ninety-three normal subjects, fifty-one patients with ischaemic heart disease and thirty patients with peripheral vascular disease.

2. The fractional turnover rate of exogenous triglyceride was significantly slower in patients with ischaemic heart disease and in patients with peripheral vascular disease than in normal men. The rate was also slower in normal men than normal women.

3. Serum triglyceride and cholesterol concentrations were higher in both vascular disease groups than in control subjects.

4. The proportion of both groups of patients who had a subnormal fractional turnover rate of exogenous triglyceride was 35%, and 32% of patients had hypertriglyceridaemia in the fasting state; 27% of patients were hypercholesterolaemic.

5. Although the intravenous fat-tolerance test did not provide significantly better discrimination between cardiovascular patients and control subjects than did measurement of serum triglyceride, the results suggest that hypertriglyceridaemia in such patients may be separable into a group in which impaired triglyceride clearance may be partly responsible, and a group in which overproduction of serum triglyceride may be the major mechanism of the hyperlipidaemia.

Key words: intravenous fat-tolerance test, ischaemic heart disease, peripheral vascular disease.

Introduction

Raised plasma concentrations of very-low-density lipoprotein and of low-density lipoprotein, and of their constituent lipids, are common in ischaemic heart disease (Heinle, Levy, Fredrickson & Gorlin, 1969; Patterson & Slack, 1972; Goldstein, Hazzard, Schrott, Bierman, Motulsky, Leviniski & Campbell, 1973; Lewis, Chait, Oakley, Wootton, Krikler, Onitiri, Sigurdsson & February, 1974b; Carlson & Ericsson, 1975); the reported frequencies of 35–80% depend on the age distribution of the patients and on the criteria for selecting normal ranges for serum lipid concentrations (Lewis, 1971). Hyperlipoproteinaemia is also frequently present in patients with peripheral vascular disease (Leren & Haarbrekke, 1971; Lewis, Chait, Oakley, Krikler, Carlson, Ericsson, Boberg, Mancini, Oriente, Paggi, Micheli, Malczewski, Weisswange & Pometta, 1974a). There is an extensive overlap of serum lipid and lipoprotein concentrations between normal subjects and patients with ischaemic heart disease or peripheral vascular disease. When hyperlipoproteinaemia is classified according to the concentrations of LDL(1) cholesterol and VLDL triglyceride, together with other, qualitative data, most common types of hyperlipoproteinaemia appear to be over-represented in ischaemic heart disease (Lewis et al., 1974b). Certain of the types of hyperlipoproteinaemia delineated by these

(1) Abbreviations: LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein.
methods are probably heterogeneous, both genetically and metabolically (Lewis, 1973); they may differ in the extent to which they are associated with and predispose to atherosclerotic vascular occlusion.

It is possible that some of the disorders of lipid metabolism associated with ischaemic heart disease and peripheral vascular disease might be better defined by appropriate function tests than by simple measurements of serum lipid or lipoprotein concentrations in the steady state and under fasting conditions. A technique for use in vitro has been proposed for recognition of one form of familial hypercholesterolaemia (Khachadurian, Lipson & Kawahara, 1975). The intravenous fat-tolerance test (Boberg, Carlson & Hallberg, 1969; Lewis, Boberg, Mancini & Carlson, 1972; Rossner, Boberg, Carlson, Freyschuss & Lassers, 1974) was developed to provide a simple means of recognizing, among patients with elevated serum VLDL concentrations (endogenous hypertriglyceridaemia), those in whom the underlying mechanism was likely to be an impaired clearance of triglyceride from plasma. In this procedure, the exponential rate of removal of a fat emulsion is measured after a bolus injection. This rate showed a strong positive correlation with the fractional removal rate of VLDL triglyceride as measured by more complex procedures (Rossner et al., 1974), and with that of VLDL apolipoprotein B as measured after injection of autologous radiiodinated VLDL (Sigurdsson, 1975). The fractional removal rate of the fat emulsion seems to be largely independent of the plasma VLDL triglyceride pool size as assessed in several conditions (Carlson & Rossner, 1972; Chait, Mancini, February & Lewis, 1972), though it is conceivable that this is not the case when extreme hypertriglyceridaemia is present. It appears that the emulsion acquires apolipoprotein C by transfer from high-density lipoprotein in the circulation, rendering it a substrate for the enzyme lipoprotein lipase (Havel, Kane & Kashyap, 1973). After activation in this way, the kinetics of its metabolism in vivo and in vitro closely resembles that of chylomicrons (Hallberg, 1965).

We here report a study of intravenous fat tolerance in patients with ischaemic heart disease and peripheral vascular disease, with a carefully screened normal population as control subjects.

Subjects and methods

The control population consisted of ninety-three apparently healthy working subjects. There were fifty-two men and forty-one women, roughly equal numbers being studied in each of the five decades from 20 to 69 years of age. All underwent clinical examination and resting electrocardiography; details of the selection criteria have been described (Lewis, Chait, Wootton, Oakley, Krikler, Sigurdsson, February, Maurer & Birkhead, 1974c). Most subjects were members of the technical and administrative staff of a London factory; to supplement the numbers in certain age and sex groups, a few members of the laboratory staff were also studied.

The patients with ischaemic heart disease comprised forty-five men aged 30–70 years, and six women aged 45–69 years. There were twenty-seven men with peripheral vascular disease, aged 47–70 years, and three women, aged 25–71 years. These patients had all presented with intermittent claudication and the diagnosis of lower-limb ischaemia due to atherosclerosis was confirmed angiographically; they were studied pre-operatively.

The patients with ischaemic heart disease had suffered myocardial infarction not less than 3 months previously; diagnosis required two or all of the following criteria: typical history, typical ECG, rise in cardiac enzyme plasma activities. Some patients had angina pectoris before or after myocardial infarction.

All patients and normal subjects had been consuming their normal diet and their habitual intake of alcohol for at least 3 weeks before study and none was receiving lipid-active drugs. A known change of weight exceeding 2 kg in the previous 3 months or since the onset of ischaemic symptoms, and the presence of cardiac failure, overt endocrine disease, hepatic or renal disease, were predetermined grounds for exclusion.

Blood samples were obtained, and tolerance tests were performed, in the morning after patients had fasted for 14 h. Serum lipoprotein classes were separated by preparative ultracentrifugation (Hatch & Lees, 1968), VLDL \((d < 1.006)\), LDL \((d = 1.006 - 1.063)\) and HDL \((d = 1.063 - 1.21)\) being isolated. The cholesterol and triglyceride concentrations in serum and in lipoprotein classes were measured by semi-automated procedures: samples were extracted according to the Technicon N-78 procedure employing propan-2-ol/Zeolite; cholesterol was measured by the Technicon N-24a colorimetric method, and triglyceride fluorimetrically according to the method of Cramp & Robertson (1968).
Intravenous fat tolerance was measured as described in earlier publications (Lewis et al., 1972; Carlson & Rossner, 1972). Essentially, lipid was injected in the form of Intralipid (Vitrum Laboratories, London), batch no. 193567. This is a triglyceride emulsion prepared for parenteral nutrition, containing 10% (w/v) of soya bean oil. An indwelling cannula with tap was inserted in an antecubital vein. After a base-line sample was obtained, Intralipid (1 ml/kg body weight) was injected via the cannula in a period of 1–2 min. Six timed blood samples (2 ml) were drawn over a 40 min period, for nephelometric measurement of the injected lipid emulsion. The fasting sample served as a blank. Disappearance curves were plotted semilogarithmically and were accurately exponential. The half-life was calculated from the least-squares regression line and the rate constant was calculated as $K_2 (\% / \text{min}) = 100 \times \frac{0.6931}{\text{half-life (min)}}$.

All subjects gave their informed consent to the study. No untoward reactions to the test occurred.

Results

The rate constants for the intravenous fat-tolerance test are shown in Table 1. In normal subjects a marked sex difference is evident: the fractional rate of disappearance of triglyceride was substantially faster in women ($P < 0.005$). Amongst normal subjects of either sex, mean values were slightly higher in the 20–39 years age group than in the 40–69 years age group but did not differ significantly ($P > 0.05$).

In both groups of patients and in the combined material numbering 174 subjects, those with the lowest $K_2$ values tended to have the highest serum triglyceride and VLDL triglyceride concentrations; as in previous studies (Boberg et al., 1969; Lewis et al., 1972; Rossner et al., 1974), when the fasting serum triglyceride or VLDL triglyceride concentration was plotted against $K_2$ value, the resulting curve approximated to a rectangular hyperbola, indicating that the triglyceride $\times K_2 = \text{constant}$. When $K_2$ values and VLDL triglyceride concentrations were transformed into their logarithms, the relationship became linear, with a correlation coefficient of $r = -0.67, P < 0.001$ (Fig. 1).

Mean $K_2$ values were lower in the patients than in control subjects of the same sex (Table 1). As indicated in Fig. 2, there was considerable overlap between $K_2$ values in cardiovascular disease and control groups. If the lower limit of normality for $K_2$ is set at the tenth percentile for the control subjects, i.e. 2.74%/min in men and 4.60%/min in women, nineteen of the patients with ischaemic heart disease (37%) had impaired intravenous fat tolerance, and nine of the thirty patients with peripheral vascular disease (30%) had low $K_2$ values. Sixteen of the former patients and eight of the latter patients had $K_2$ values below the fifth percentiles for their sex.

When the proportion of vascular disease patients with low $K_2$ values (<tenth percentile) was compared with the proportion showing hyperlipidaemia (>ninetieth percentile), the percentages were not

<table>
<thead>
<tr>
<th>Table 1. Rate constants ($K_2$) for intravenous fat-tolerance test, serum and VLDL triglyceride concentrations and serum cholesterol concentration in normal subjects and in patients with ischaemic heart disease (IHD) or peripheral vascular disease (PVD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results are shown as mean values±SEM. Significance of results (comparison with sex-matched subjects): * $P&lt;0.05$; ** $P&lt;0.01$; *** $P&lt;0.001$.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>$K_2$ (%/min)</th>
<th>Serum triglyceride$^{(1)}$ (mmol/l)</th>
<th>VLDL triglyceride$^{(1)}$ (mmol/l)</th>
<th>Serum cholesterol (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>52</td>
<td>4.61±0.21</td>
<td>1.27±0.077</td>
<td>0.69±0.076</td>
<td>5.81±0.14</td>
</tr>
<tr>
<td>Women</td>
<td>41</td>
<td>7.85±0.51</td>
<td>0.99±0.054</td>
<td>0.34±0.045</td>
<td>5.81±0.15</td>
</tr>
<tr>
<td>IHD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>45</td>
<td>3.08±0.18***</td>
<td>2.20±0.28</td>
<td>1.34±0.19</td>
<td>6.87±0.26</td>
</tr>
<tr>
<td>Women</td>
<td>6</td>
<td>4.21±0.43***</td>
<td>1.92±0.68</td>
<td>1.32±0.18</td>
<td>6.66±0.52</td>
</tr>
<tr>
<td>PVD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>27</td>
<td>3.62±0.29**</td>
<td>1.62±0.21</td>
<td>0.85±0.18</td>
<td>6.71±0.16</td>
</tr>
<tr>
<td>Women</td>
<td>3</td>
<td>4.70±1.01*</td>
<td>1.76±1.15</td>
<td>0.93±0.99</td>
<td>8.06±1.37</td>
</tr>
</tbody>
</table>

$^{(1)}$ Because of skewness of distribution these values were derived as the antilogarithms of the mean values of the logarithmically transformed data.
Fig. 1. Relation between $K_2$ value and VLDL triglyceride concentration in normal subjects (○), patients with ischaemic heart disease (□) and patients with peripheral vascular disease (▲). Both variables are plotted on a logarithmic scale.

Fig. 2. Fractional turnover rates ($K_2$) of exogenous triglyceride in the two groups of patients (IHD and PVD, see Table 1) and in normal subjects (N).
significantly different: 35% of patients had low $K_2$ values, and 32% had elevated serum triglyceride concentrations, and 33% had elevated VLDL triglyceride concentrations; 27% of the patients were hypercholesterolaemic. However, the patients with low $K_2$ values were not always hypertriglyceridaemic, and vice versa. Of twenty-eight who had the present study was based on the premise that the Discussion
The present study was based on the premise that the intravenous tolerance test provides an index of the fractional turnover rate of endogenous plasma triglyceride (VLDL triglyceride), as suggested by the studies of Rosner et al. (1974) and of Sigurdsson (1975). As in previous studies (Boberg et al., 1969; Lewis et al., 1972; Rossner et al., 1974), a negative correlation was demonstrable between the rate constant ($K_2$) for the fractional disappearance of triglyceride and the concentration of VLDL triglyceride in serum. Although a highly significant negative linear correlation was obtained between the logarithmically transformed data, Fig. 1 shows that there is a very considerable scatter. Evidently other variables than the fractional removal rate (as assessed by the $K_2$ value) are important in determining VLDL triglyceride concentration in serum; of these the rate of VLDL secretion may well be the major one (Nicoll, Sigurdsson & Lewis, 1975).

The mean $K_2$ value was significantly lower in patients with cardiac or peripheral ischaemia, particularly ischaemic heart disease, than in control subjects. However, the intravenous fat-tolerance test was not in general a more sensitive discriminator between patients and control subjects than the concentration of VLDL triglyceride or of total serum triglyceride. By the former test, 37% of patients with heart disease were abnormal, and the same percentage had elevated VLDL triglyceride concentration.

About 60% of the cardiovascular disease patients with elevated VLDL triglyceride concentrations had subnormal $K_2$ values. The test may therefore identify a group of patients, among those with ischaemic heart disease or peripheral vascular disease, in whom hypertriglyceridaemia is due in part to decreased fractional turnover of triglyceride, whereas in a substantial number of patients hypertriglyceridaemia occurs without evidence of a clearance defect.

It is possible, therefore, that the intravenous fat-tolerance test provides a test of function by which a sub-group of patients with ischaemic heart disease or peripheral vascular disease may be recognized in which there is a metabolic defect leading to impaired clearance of triglyceride from plasma.

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References


