Molecular exclusion electrophoresis of human serum lipoproteins: patterns in control and ischaemic heart-disease populations

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Summary

1. Electrophoresis of pre-stained lipoproteins on acrylamide-gel gradients has been carried out on serum from populations of control subjects and patients with ischaemic heart disease. The technique resolves components intermediate in position and, by inference, in size, between very-low-density and low-density lipoproteins.

2. These central band components were found in 37% of a control population but the incidence varied with age and sex, being lowest in young males and highest in elderly males.

3. The incidence of central band components in patients with ischaemic heart disease was 64% (males) and 71% (females), and the difference between these figures and those for matched control subjects was highly significant. The intensity of central band components in the group with ischaemic heart disease was significantly greater than in the control group.

4. The presence and intensity of central bands show positive correlation with serum cholesterol and triglyceride values, but many patients showing the phenomenon have normal lipid values. Of patients with ischaemic heart disease 31% showed central band components and had normal lipid values.

Key words: acrylamide-gel gradients, ischaemic heart disease, lipoproteins, molecular exclusion.

Abbreviations: VLDL, very-low-density lipoproteins; LDL, low-density lipoproteins.

Introduction

Several authors (Narayan, Narayan & Kummerow, 1965; Frings, Foster & Cohen, 1971; Naito, Wada, Ehrhardt & Lewis, 1973) have described methods of separating lipoproteins by modifying the disc-gel technique of Ornstein & Davis (1964), and Pratt & Dangerfield (1969) have adapted the concentration-gradient technique introduced by Margolis & Kenrick (1967) to obtain better resolution. For the present study, the discontinuous-gradient system described by Wright, Farrell & Roberts (1971) was adapted to suit the molecular range of very-low-density and low-density lipoproteins and used as a molecular exclusion technique (Green, 1972). This allows resolution of components intermediate in position, and by inference in size, between VLDL and LDL, and these have been studied in a control population and in patients with ischaemic heart disease. Our studies have formed the basis of a preliminary report (Green & Carney, 1975).

Methods

Selection of subjects

The control population consisted of 612 subjects: 525 came from two blood donor panels, sixty-nine from laboratory staff, and eighteen from patients admitted for minor surgery. The donors had been instructed to take no more than a light meal 2 h before blood collection. The patients with ischaemic heart disease consisted of 209 consecutive admissions to a coronary care unit, in whom the diagnosis of myocardial infarction was confirmed, the WHO
classification being used. Blood samples were taken on the morning of admission, if before 10.00 hours, otherwise on the next morning, in the fasting state.

Analytical procedures and preliminary studies

The pre-staining and electrophoretic techniques have been described (Green, 1976). With this procedure, chylomicrons remain in the top few millimetres of the gel; they are poorly stained if at all, by Sudan Black; VLDL appear as a single, or rarely as a double, diffuse band in a 3-5-4 g/100 ml gradient. LDL are resolved into one to three sharply defined bands in a 5-7 g/100 ml gradient.

The majority of sera with components migrating between VLDL and LDL show a single sharply defined band at or near the centre of a 4-5 g/100 ml gradient (Fig. 1) but a few show multiple bands. Many of the single central bands can be resolved into several components on steeper gradients, 2-5-5 or 2-5-4-7 g/100 ml: those which resolve into three or more components were classed as 'major'; others were termed 'minor'. In these steeper gradients the LDL zone is sometimes displaced and irregular.

Specimens showing single and multiple bands between VLDL and LDL were tested for reaction with β-lipoprotein antiserum (Hoechst). Longitudinally cut gel strips were embedded in agar/agarose and a trough about 7 mm on the anodal side was filled with antiserum. Electrophoresis was carried out for 16 h at 100 V, in a Tris/barbital buffer, pH 8-8 (Gelman, Cat. 51104). Transversely cut individual components were also subjected to Laurell 'rocket'-type electrophoresis. All components tested gave a positive reaction with β-lipoprotein antiserum.

Several sera which showed intense central bands were pre-stained with Sudan Black and subjected to electrophoresis on preparative agarose gels (agarose 7 g/l, Tris/glycine buffer as above). Portions of the stained β- and pre-β-lipoprotein areas were then cut out and eluted on to acrylamide gradients. When the entire β- or pre-β-lipoprotein areas were eluted, components corresponding to central bands were detected but when the area eluted was confined to the anodal front of pre-β-lipoprotein, or the cathodal tail of β-lipoprotein, only VLDL and LDL respectively were found.

Two sera which showed multiple bands between VLDL and LDL were centripuged at 30 000 g for 6 h according to Mead & Dangerfield (1974). Supernatant and infranatant were aspirated, stained with Sudan Black, and run on the usual gradients. The supernatant contained only VLDL and one or two bands closest to it in the gel, the infranatant only LDL and some of the smaller bands closer to it.

In the series from patients with ischaemic heart disease, serum cholesterol and triglycerides were originally estimated by Technicon Auto-Analyzer methods, in a propan-2-ol extract; triglycerides were later estimated by the method of Bucolo & David (1973). In the control series, cholesterol was estimated by the method of Abell, Levy, Brodie & Kendall (1952).

The upper limit of normal for cholesterol, stated by the laboratory for the method and standard (BDH) employed, was 6-7 mmol/l; inter-laboratory comparison has indicated that results are 5-10% lower than with the method of Abell et al. (1952).

The corresponding value for triglycerides was 1-86 mmol/l (Goldstein, Hazzard, Schrott, Bierman & Motulsky, 1973).

Statistical treatment

Statistical comparisons used were the unpaired Student's t-test to evaluate serum cholesterol and triglyceride data, and the chi-square test to analyse the central band frequency in the various groups. Mean values ± SEM are used throughout the paper.

![Fig. 1. Diagrammatic representation of gel gradients and lipoprotein patterns. 1, Excess Sudan Black; 2, VLDL; 3, LDL; 4, single and multiple bands. A, Gradient; B, pattern without central bands; C, pattern with single central band; D, pattern with multiple bands.](image-url)
Lipoprotein patterns in ischaemic heart disease

Results

Central band components were found in sera of 37% of 612 control subjects studied, but the incidence, and the percentage classed as 'major', varied widely with age and sex, as shown in Table 1. In the series of 209 patients with ischaemic heart disease, central bands were present in 66% of all sera, 42% being classed as 'minor' and 24% as 'major'.

Table 2 sets out the different frequencies with which these bands were detected in both sexes at various ages, in the control subjects and in those with coronary episodes. In both sexes, central bands occurred significantly more frequently in sera of the group who had experienced a coronary episode than in those of the control sample.

In all age groups in both sexes, the incidence of central bands was greater in sera of the coronary group than in those of the control sample.

In all age groups in both sexes, the incidence of central bands was greater in sera of the coronary group than in the control group, although in some groups the difference did not attain a significant level. The percentage of 'major' central bands was significantly higher in the group of patients with ischaemic heart disease as a whole (P < 0.001) and separately for males (P < 0.02) and females (P < 0.01).

In both the control and heart-disease groups, subjects with sera exhibiting central bands had a significantly higher mean serum cholesterol than those who had normal gel patterns (Fig. 2). In the control group, the mean serum cholesterol of forty randomly selected control subjects whose lipoprotein pattern did not show central bands was 4.99 ± 0.16 mmol/l, and this was significantly lower than that of 142 subjects with a 'minor' central band (P < 0.01), whose mean value was 6.23 ± 0.09 mmol/l. Twenty-seven subjects with 'major' central bands had a mean serum cholesterol of 7.02 ± 0.30 mmol/l. The difference between the mean values of subjects with 'minor' and 'major' bands was also significant (P < 0.02). In the group with ischaemic heart disease, the corresponding figures were: no central bands 5.42 ± 0.16 mmol/l, 'minor' band 5.94 ± 0.12 mmol/l, 'major' band 6.59 ± 0.18 mmol/l. The relevant mean values for serum triglyceride were 1.45 ± 0.10 mmol/l, 1.81 ± 0.14 mmol/l and 1.91 ± 0.13 mmol/l. Although the mean values for serum cholesterol and triglycerides were greater in subjects with central bands than in those with normal patterns, it is evident from Fig. 3 that not all patients with either major or minor bands had elevated serum cholesterol or triglyceride.

Twelve patients with ischaemic heart disease who exhibited central bands were studied on four more occasions at intervals up to 1 month from the episode. Eleven showed no change; the single discrepant result may have been due to faulty technique.

Some variation in central bands has been found in patients undergoing treatment, but the relative overall constancy seemed to justify the use of a single sample in population studies.

Relation to WHO classification

The incidence of the different lipoprotein patterns in the present series of ischaemic heart disease, together with the varieties of hyperlipaemia, are shown in Table 3. Hyperlipaemia was found in 101 of 209 patients (48.4%).
### Table 2. Comparison of central bands in control subjects and in patients with ischaemic heart disease

*P* shows significance of difference between values in the two groups of subjects.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Control subjects</th>
<th>Ischaemic heart disease</th>
<th>Total</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean age (years)</td>
<td>Central bands</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Minor</td>
<td>Major</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-44</td>
<td>69</td>
<td>40</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>45-54</td>
<td>90</td>
<td>50</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>55-64</td>
<td>57</td>
<td>58</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>65-74</td>
<td>21</td>
<td>70</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
<td>52</td>
<td>88</td>
<td>20</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-54</td>
<td>58</td>
<td>50</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>55-64</td>
<td>36</td>
<td>58</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>65-74</td>
<td>30</td>
<td>70</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>58</td>
<td>45</td>
<td>7</td>
</tr>
</tbody>
</table>


Lipoprotein patterns in ischaemic heart disease

Cholesterol alone was elevated in twenty-nine (13.9%), triglycerides alone in forty-two (20.1%) and both values were elevated in thirty (14.4%).

In eighteen cases of hypertriglyceridaemia, where increase in VLDL was present and central bands were absent, the average serum cholesterol was 5.52 mmol/l and triglycerides 3.05 mmol/l. VLDL was grossly abnormal in only two, and in one of these the serum cholesterol was also high (10.36 mmol/l). The thirty-four cases in which increase in VLDL co-existed with a central band or bands showed wide variation. In twenty-three cases the central band was assessed as relatively minor; the average serum cholesterol was 6.73 mmol/l and serum triglycerides 3.64 mmol/l; nine had cholesterol values over 6.7 mmol/l. These twenty-three cases may correspond to the fifteen cases described by Lewis, Chait, Oakley, Wootton, Krikler, Onitri, Sigurdsson & February (1974), with high VLDL cholesterol and triglyceride values, and assigned by them to WHO type IV.

One subject in a donor panel of 254, and...
Table 3. Lipoprotein patterns in 209 patients with ischaemic heart disease

<table>
<thead>
<tr>
<th>Lipoprotein pattern</th>
<th>n (%)</th>
<th>Elevated cholesterol (6·7 mmol/l)</th>
<th>Elevated triglycerides (1·85 mmol/l)</th>
<th>Elevated cholesterol and triglycerides</th>
<th>Possible WHO type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>43 (20·6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased VLDL</td>
<td>18 (8·6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased LDL*</td>
<td>16 (7·7)</td>
<td>16</td>
<td></td>
<td>1</td>
<td>IIA</td>
</tr>
<tr>
<td>Increased VLDL and central band</td>
<td>34 (16·3)</td>
<td></td>
<td>17</td>
<td>17</td>
<td>IIB, IV-V, ? III</td>
</tr>
<tr>
<td>Increased VLDL and LDL</td>
<td>2 (1·4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central band alone</td>
<td>95 (45·5)</td>
<td>13</td>
<td>8</td>
<td>9</td>
<td>IIB</td>
</tr>
<tr>
<td>Total</td>
<td>209 (100·0)</td>
<td>13·9%</td>
<td>20·1%</td>
<td>14·4%</td>
<td></td>
</tr>
</tbody>
</table>

* Eight cases with the pattern also showed central bands, six being minor.

fifteen of the patients with ischaemic heart disease, exhibited multiple bands between the middle of the 4–5 g/100 ml gradient and the normal LDL position; two of these showed an additional band below VLDL. This pattern of multiple bands was provisionally thought to correspond to WHO type III (Doyle, Pinkus & Green, 1974), since the sera show a 'broad β' lipoprotein band on cellulose acetate electrophoresis, and a comparable pattern was found by Naito et al. (1973) on acrylamide disc electrophoresis in WHO type III, which they confirmed by ultracentrifugation. However, in the present study, greater resolution has been achieved by the use of a molecular exclusion or pore-limit technique, gel entry of all components, and the use of tubes rather than flat-bed gels. Since the effect of charge on migration is cancelled, gradients offer a convenient way of displaying a multi-component system in order of molecular size, assuming that VLDL and LDL behave as spherical particles (Scanu & Wisdom, 1972).

Components intermediate in position between VLDL (or pre-β-lipoprotein) and LDL (or β-lipoprotein) have been described in cellulose acetate (Dahlen & Ramberg, 1974), agarose gel (Papadopoulos & Bedynek, 1973), acrylamide disc (Frings et al., 1971; Naito et al., 1973; Mead & Dangerfield, 1974) and acrylamide-gel gradient electrophoresis (Pratt & Dangerfield, 1969; Melish & Waterhouse, 1972), and in density-gradient ultracentrifugation (Hinton, Kowalski & Cohen, 1973).

Discussion

The technique of separating pre-stained lipoproteins on acrylamide-gel gradients was first described by Pratt & Dangerfield (1969), and its general validity established by them. The locations of VLDL and LDL were confirmed by Melish & Waterhouse (1972). In the present study, greater resolution has been achieved by the use of a molecular exclusion or pore-limit technique, gel entry of all components, and the use of tubes rather than flat-bed gels. Since the effect of charge on migration is cancelled, gradients offer a convenient way of displaying a multi-component system in order of molecular size, assuming that VLDL and LDL behave as spherical particles (Scanu & Wisdom, 1972).

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Dahlen & Ramberg (1974) associated pre-β-lipoprotein with the Lp(a) antigen of Berg (1963), and Mead & Dangerfield (1974) tentatively identified one of their mid-bands with the 'sinking pre-β-lipoprotein' phenomenon (Rider, Levy & Frederickson, 1970), which may reflect a relatively high concentration of Lp(a) (Morrisett, Jackson & Gotto, 1974).

Many authors have indicated some association between these or similar components and...
ischaemic heart disease, but there is a wide variation in the reported frequency in normal subjects and patients, and age and sex variation have not been fully taken into account (Table 4).

The main result of our study is the demonstration of components intermediate in position and, by inference, in size between VLDL and LDL, in an overall 37% of a control population, with age and sex variation and the presence in patients with ischaemic heart disease matched for age and sex, of a significantly higher proportion, both in number and intensity. The greater resolution of acrylamide gradients allows a distinct serum pattern to be demonstrated in 30% of patients with ischaemic heart disease who were not hyperlipaemic at the time. Whether they have been hyperlipaemic in the past, or whether specific hyperlipoproteinaemia is significant, is a matter of conjecture. We have many examples of the variability of VLDL in serial studies to emphasize the hazard of assessment on a single sample, in agreement with Mishkel, Nazir & Crowther (1975); in one striking example, the patient in whom the highest serum cholesterol (10.7 mmol/l) and triglyceride (11.7 mmol/l) was recorded in our series was re-admitted 1 month later with both values normal but with a persisting central band.

The figure for hyperlipaemia, 48-4%, is within the range of 43-57% in several published series (Lewis et al., 1974). But major differences are apparent in the incidence of WHO type II A, and of mixed hyperlipaemias, type IV with high cholesterol values, and type II B. Our figures for type II A in ischaemic heart disease are 3.8% or 6.8% if six cases with an additional minor central band are included. This compares with 14% (ischaemic heart disease) and 10% (control) in the Hammersmith series. Other authors (van Melson, de Greve, Vanderveiken, Vastesaeger, Blaton & Peeters, 1975) have reported figures up to 33%. The difference may lie in the thirteen cases in our series of hypercholesterolaemia in which central bands were the predominant feature, suggesting that some at least of these bands are included with β-lipoprotein or LDL in other systems.

On the basis of the data of Lewis et al. (1974), one would expect about 8% in ischaemic heart disease cases of WHO type II B. Central bands were present in all fourteen cases in our series which might correspond to this type, and were the main feature in ten; LDL was noted as increased in only three. No case in our series met one WHO requirement for type III—a 'broad β' band on zone electrophoresis and absence of LDL on acrylamide gel.

It is clear that the greater resolution of acrylamide gradients does not assist in assigning mixed hyperlipaemias to the four possible WHO categories. Of thirty cases of mixed hyperlipaemias in the present series, central bands were present in twenty-nine; they were the sole
pattern anomaly in nine, and the main feature in a further ten cases. Although our data and techniques differ markedly from those of Goldstein & Brown (1975), the possible analogy between this common feature of gel patterns, and their proposition of a single-gene basis for 'combined hyperlipaemia', merits further investigation.

Acknowledgments

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References


