Serum Lipoproteins in Schoolboys in Relation to Glucose-6-phosphate Dehydrogenase Deficiency and Thalassaemia Trait

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There is now evidence that an increase in the low-density serum lipoproteins plays a part in the causation of degenerative vascular disease (Gofman, Jones, Lindgren, Lyon, Elliott, and Strisower, 1950; Kannel, Dawber, Kagan, Revotskie, and Stokes, 1961). Studies of serum cholesterol in different ethnic groups has shown important differences (Adlersberg and Schaefer, 1959), but it cannot yet be decided if these differences are caused by genetic factors, by diet, or by other environmental influences (Adlersberg, Schaefer, Steinberg, and Wang, 1956).

Choremis, Kyriakides, and Papadakis (1961), studying children with Cooley's anaemia and sickle-cell anaemia, reported decreased values of serum lipids as determined by chemical analysis. Westernman, Pierce, and Jensen (1964) also found plasma total lipid, total phospholipid, and total cholesterol concentrations to be significantly lower in patients with sickle-cell anaemia than in a group of normal subjects. Fessas, Stamatoyannopoulos, and Keys (1963) described reduced levels of cholesterol and β-lipoprotein in carriers of the thalassaemia trait.

On the other hand in Negroes with glucose-6-phosphate dehydrogenase (G6PD) deficiency serum cholesterol levels are reported to be higher as compared with age-matched controls (Tarlov, Brewer, and Swanson, 1961).

In the Greek population we have a relatively large number of people with the thalassaemia trait, as well as people with G6PD deficiency. In view of this, we carried out a study of serum lipoproteins in carriers of the thalassaemia trait and in G6PD deficient subjects, in order to confirm the existence of any significant differences in the serum lipoproteins between these two groups.

**Patients and Methods**

During a previous study on the frequency of G6PD deficiency and thalassaemia in the island of Lesbos, 500 male schoolchildren were examined haematologically. For the present study we selected 17 children with thalassaemia trait, 13 with ‘partial’ G6PD deficiency, 18 with G6PD deficiency, and 30 normal boys, who served as controls. The ages of the children studied ranged from 12 to 18 years. They were all clinically healthy and, living in this restricted area of Greece, they all had the same nutritional habits (the main source of fat intake consisting of olive oil).

All boys included in our study had a venous blood specimen taken for a red and white blood count, red cell morphology, and haemoglobin estimation, all of which were done by standard procedures. Paper electrophoresis of haemoglobin was performed according to the method described by Malamos, Fessas, and Stamatoyannopoulos (1962). G6PD activity was tested by the Motulsky and Campbell-Kraut method (1961). Quantitative estimation of G6PD activity was performed in most specimens. The method used was that of Gioc and McLean (1953). The unit for this method in our laboratory represents the change of optical density per minute per mg. Hb (Δ O.D./min./mg. Hb) at a wavelength of 340 μm.

The results of these tests enabled us to classify the boys in the following four groups. (1) Normal subjects: all the tests were normal. (ii) ‘Partial’ G6PD deficiency. In this group were included children for which the Motulsky test showed a decolorization time between 60 and 180 minutes* and in which the quantitative estimation of the G6PD activity was below 6 units, with a mean value of 4·09 and a range of 5·02-1·82. We are not suggesting that this group represents a separate entity; most probably it constitutes the lower end of a normal distribution of the group with normal G6PD activity. We thought, however, that we were justified in separating them from our normal group, in which the Motulsky test showed a normal decolorization time and the quantitative estimation of G6PD activity ranged from 11·86-6·10, with a mean value of 7·02 units. If a correlation existed between G6PD activity and serum lipoprotein levels, this group ought to show levels of serum lipoproteins lying between those found in the normal group and those in

* In our laboratory a decolorization time of brilliant Cresyl Blue of up to 60 minutes represents normal G6PD activity, while a decolorization after 180 minutes is considered as showing complete deficiency of the enzyme.
Serum Lipoproteins in Relation to G6PD Deficiency and Thalassaemia

TABLE I
Age, Hb, and G6PD Activity in 78 Male Schoolchildren of the Island of Lesbos

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Age (yr.) (mean and range)</th>
<th>Mean Hb (g./100 ml.)*</th>
<th>G6PD Activity (Δ O.D./min./mg. Hb) (mean and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Normal</td>
<td>30</td>
<td>14 10/12 (12-18)</td>
<td>14-2 (1-13)</td>
<td>7-02 (11-96-6-10)</td>
</tr>
<tr>
<td>(ii) 'Partial' G6PD deficiency</td>
<td>18</td>
<td>15 2/12 (13-18)</td>
<td>14-2 (0-93)</td>
<td>4-09 (5-92-1-52)</td>
</tr>
<tr>
<td>(iii) G6PD deficiency</td>
<td>13</td>
<td>15 4/12 (13-18)</td>
<td>13-5 (1-34)</td>
<td>0</td>
</tr>
<tr>
<td>(iv) Thalassaemia trait</td>
<td>17</td>
<td>14 7/12 (13-16)</td>
<td>12-0 (1-30)</td>
<td>Motulsky: normal decolorization time</td>
</tr>
</tbody>
</table>

* SD in parentheses.

the group with complete enzyme deficiency. (iii) G6PD deficiency with practically zero G6PD activity. (iv) Thalassaemia trait. The criteria for the diagnosis of thalassaemia trait were (a) hypochromic erythrocytosis, (b) red cell morphology showing anisocytosis, microcytosis, and the presence of oval or elongated red cells, and (c) increased Hb A² on paper electrophoresis. If all three criteria were met the child was considered as a carrier of the β-thalassaemia trait. If only the first two criteria were present, and on paper electrophoresis Hb A² was not increased, the child was considered to be a carrier of the α-thalassaemia trait.

In all children serum lipoproteins were estimated from a venous blood specimen collected between 10 a.m. and 12 a.m. The whole blood was left to clot and then the serum was separated and one drop of sodium azide 0-1%, was added as a preservative. The serum was sent to our laboratory by air and kept at 4°C. until examination which was performed within three days of the collection. Serum total lipid was estimated by the method of De la Huerga, Yesinick, and Popper (1953), total cholesterol by the method of Sackett (1925), and α- and β-lipoproteins were determined by paper electrophoresis (Salt and Wolff, 1957).

Results
Table I shows the number of children included in each group, their age, Hb, and erythrocyte G6PD activity. In the group of 17 boys with the thalassaemia trait 10 were carriers of the β-thalassaemia trait and 7 of the α. As the lipoprotein values did not differ between them we thought it justifiable to place them all in the same group. As can be seen from this Table, the mean haemoglobin level in the thalassaemia trait group was a little lower than in the remaining three groups.

All the sera studied were optically clear and on inspection of the electrophoretic paper strips there was practically no ω (chylomicron) fraction and no pre-β fraction. Table II shows the results of the total lipid, total cholesterol, α- and β-lipoprotein fractions, and β/α ratio in the four groups studied. It is clear from this Table that there are no significant differences in the lipoprotein fractions of the four groups studied. In the group with G6PD deficiency the mean of total lipids and the β-lipoprotein fraction show a tendency for higher values; these differences however, do not reach statistical significance.

Discussion
The level of the blood lipids is determined by a multitude of factors, e.g. race, heredity, age, sex, hormones, diet, physical activity (Ahrens, 1957; Carlsson, 1960; Sterky, Larsson, and Persson, 1963). The multitude of factors influencing the blood lipids makes the comparison of results obtained by different authors in different countries extremely difficult. The levels of serum total lipids and cholesterol in our normal group are somewhat lower

TABLE II
Serum Lipid and Lipoprotein Levels (means in mg./100 ml.) in Male Schoolchildren of the Island of Lesbos

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Total Lipid</th>
<th>Total Cholesterol</th>
<th>Lipoprotein Lipid*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>β</td>
</tr>
<tr>
<td>(i) Normal</td>
<td>30</td>
<td>389± (97)</td>
<td>155 (24)</td>
<td>249± (71)</td>
</tr>
<tr>
<td>(ii) 'Partial' G6PD deficiency</td>
<td>18</td>
<td>371 (102)</td>
<td>154 (23)</td>
<td>230 (71)</td>
</tr>
<tr>
<td>(iii) G6PD deficiency</td>
<td>13</td>
<td>441 (75)</td>
<td>157 (15)</td>
<td>292 (50)</td>
</tr>
<tr>
<td>(iv) Thalassaemia trait</td>
<td>17</td>
<td>383 (75)</td>
<td>151 (23)</td>
<td>248 (53)</td>
</tr>
</tbody>
</table>

* SD in parentheses.

The α- and β-lipoprotein lipid values do not add up exactly to the total lipid. This small difference represents the faint ω band present in a few paper strips.

† t = 1·72; p > 0·05. ‡ t = 1·99; p > 0·05. § t = 1·51; > 0·10.
than those reported by others (Hodges, Sperry, and Andersen, 1943; Dine and Jackson, 1953; Rafstedt, 1955; Hard and Esselbaugh, 1960; Salt, Wolff, Nestadt, and Lloyd, 1960; Sterky et al., 1963). This difference could perhaps be explained by the method of selection of the children used as normal controls. Some authors have studied children admitted to hospital for conditions thought to be unrelated to lipid disturbances. Our material, however, consisted of healthy boys attending school. On the other hand, the schoolchildren we studied had different eating habits, and for all of them the main source of fat intake consisted of unsaturated fatty acids (olive oil).

We designed our study in such a way as to limit as many as possible influencing factors. The children were all of the same sex and age-group, and they were living in a small area of Greece. The main difference between the four groups we studied was their different genetic constitution concerning the presence of the thalassaemia trait and the absence of G6PD activity. As we have mentioned already, we do not believe that group 2 with 'partial' enzyme deficiency constitutes a genetically separate entity. In fact the lipoprotein findings in this group are similar to the findings in our control group, and only in the G6PD deficient boys is there a tendency for somewhat higher values. Our findings in the schoolboys with G6PD deficiency are not statistically different from those in the control group.

Choremis et al. (1961), studying the blood lipids and lipoproteins in thalassaemia and sickle-cell anaemia, reported lower serum lipids and a characteristic lipoprotein distribution on paper electrophoresis. We cannot, however, compare our results with their findings, as we were not dealing with patients with the disease, and because their studies were done in both sexes, in a much wider age range, most probably with much lower Hb levels (the Hb levels of their patients are not mentioned).

Fessas et al. (1963), studying the serum cholesterol in adult men carriers of the thalassaemia trait, found that the carriers of thalassaemia trait had consistently lower cholesterol and \( \beta \)-lipoprotein values than the non-thalassaemia controls. These authors have also selected only male subjects of a restricted area, and the only fundamental difference between their material and ours is the different age of the subjects studied. If the different lipoprotein findings at different ages in thalassaemia trait carriers are confirmed, then one could speculate that, whatever the cause of lower serum cholesterol and \( \beta \)-lipoprotein fraction in adult carriers of the thalassaemia trait, it does not operate at a younger age.

Further studies will be needed to confirm these findings and to elucidate the mechanisms involved. We believe that longitudinal studies with similar groups to the ones we studied could give useful information, showing perhaps a different pattern of lipoprotein changes in each group 10 or 20 years later.

**Summary**

A total of 78 clinically healthy schoolboys aged 12 to 18 from a homogeneous population of the island of Lesbos were examined for thalassaemia trait, G6PD deficiency, and serum lipoproteins. They were separated into four groups, (i) normal subjects, (ii) those with 'partial' G6PD deficiency, (iii) those with G6PD deficiency, and (iv) those with the thalassaemia trait. Serum total lipid, total cholesterol, \( \alpha \)- and \( \beta \)-lipoprotein fractions, and \( \beta/\alpha \) ratio did not differ significantly in the four groups. Only in the group with G6PD deficiency did the serum total lipid and \( \beta \)-lipoprotein fraction show a tendency for somewhat higher values, but this slight difference did not reach statistical significance.

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