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Serum lipoprotein pattern as modified in G6PD-deficient children during haemolytic anaemia induced by fava bean ingestion

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Summary. In the present study, plasma lipid concentrations were determined at different times after admission in sera from G6PD-deficient children during haemolytic crisis induced by fava bean ingestion. Reductions in total, LDL and HDL cholesterol were found in association with the maximum of bone marrow hyperplasia. A return towards normal values occurred with regression of the disease. No changes in other lipid parameters were observed. These data suggest that alterations of lipoprotein pattern, other than in experimental animals, are also present in humans with non-malignant proliferative processes. These changes appear to be a consequence of the disease, probably due to an increased utilization of cholesterol by proliferating cells.

Keywords: G6PD deficiency, cell proliferation, lipid metabolism, lipoproteins

It has been previously shown that sustained processes of cell proliferation induced in liver (Dessì *et al.* 1986, 1989) and kidney (Dessì *et al.* 1988) of rats are characterized by alterations of lipoprotein metabolism mainly resulting in a marked decrease in HDL cholesterol levels.

More recently, reduced total and HDL serum cholesterol levels were observed in rats during compensatory bone marrow hyperplasia after haemolysis induced by phenylhydrazine administration (Dessì *et al.* 1990). We have suggested that the observed changes of plasma cholesterol metabolism may represent a generalized phenomenon when high rates of cell proliferation occur, irrespective of the proliferating organ or system in the body.

However, in spite of these findings in experimental models, relatively few investigations have been carried out on the possible relationship between serum cholesterol metabolism and proliferative processes in humans.

Favism is an acute red cell lysis which occurs in G6PD-deficient individuals after ingestion of fava beans (Sansone *et al.* 1958). The disease is prevalent in the Mediterranean area and, although it occurs throughout the year, its incidence is considerably higher during the spring and early summer, especially between May and June, a time

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coinciding with the ripening of the beans (Kattamis 1986). The disease generally starts some hours after bean ingestion and peaks at day 4 or 5. The recovery generally occurs at 15 days after ingestion (Kattamis 1986).

In order to verify whether changes of serum cholesterol metabolism, similar to those observed in experimental models, are also associated with bone marrow hyperplasia in humans, at different times after admission we investigated lipid metabolism in the serum from G6PD-deficient children with haemolytic anaemia induced by fresh bean consumption.

Material and methods

The study was conducted on 10 (six male and four female) G6PD-deficient children, aged between 2 and 8 years (mean 4.90±0.82) admitted to the 'Istituto di Clinica Pediatrica', University of Cagliari, between May and June 1991 for acute haemolytic anaemia induced by ingestion of beans (favism).

Patients whose conditions needed transfusions were excluded. Eighteen (11 male and seven female) non-G6PD-deficient children aged between 2 and 10 years (mean 6.44±0.58) admitted to hospital for reasons other than acute haemolytic anaemia were used as control group.

No patients were receiving drugs known to affect lipid metabolism at the time of blood samples.

Blood samples were obtained from each patient after a minimum fast period of 12 hours at days 1, 4 and 15 after admission to hospital. Haemoglobin and haematocrit levels as well as red cell and reticulocyte counts were performed by standard techniques.

HDL cholesterol was measured in serum after removal of VLDL and LDL by the magnesium phosphotungstate reagent. For LDL cholesterol determination, LDL were precipitated by adding polyvinylsulphate to the serum; its concentration was calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. Reagents for total, LDL, HDL cholesterol and for triglycerides and phospholipids were obtained commercially (Boehringer, Mannheim, FR Germany).

Results

Table 1 shows red cell and reticulocyte counts, haemoglobin levels and haematocrit values for G6PD-deficient children with haemolytic crisis at different admission dates after bean ingestion.

Haemoglobin, red cell count and haematocrit were low in all patients at the first and fourth day of admission. A recovery towards normal range values was seen at 15 days

Table 1. Haemoglobin and haematocrit levels, red cell and reticulocyte counts in G6PD-deficient children during haemolytic crisis

Day of admission	Haemoglobin (g/100 ml)	Haematocrit (%)	Red cells (millions/mm^3)	Reticulocytes (%)
1 (10)	7.99±0.30	24.39±1.24	2.88±0.18	n.d.
4 (10)	8.79±0.37	27.20±1.00	3.16±0.18	57.45±11.87
15 (10)	11.37±0.19***	35.31±0.42***	4.07±0.12***	27.45±2.43***

The values are expressed as the mean±s.e. The number of children is given in parentheses. P was evaluated by paired t test.

*$P<0.05$ *vs* day 1.

**$P<0.05$ *vs* day 4.

Table 2. Total, HDL and LDL cholesterol levels in G6PD-deficient children during haemolytic crisis

Day of admission	Total cholesterol (mg/dl)	HDL-C (mg/dl)	HDL-C (%)	LDL-C (mg/dl)
1 (10)	117.32±9.81	36.09±4.17	30.71±2.12	59.31±7.17
4 (10)	101.03±8.02**	25.33±3.73**	24.53±2.81**	46.71±5.93**
15 (10)	122.00±10.40*	34.08±3.23*	29.79±3.56*	69.61±8.81*
Controls (18)	142.01±8.01	42.26±2.96	32.10±2.02	74.77±8.82

The values are expressed as the mean±s.e. The number of the children is given in parentheses.

*$P<0.05$ *vs* 4 days evaluated by paired *t* test.

**$P<0.05$ *vs* controls evaluated by *t* test.

Table 3. Triglyceride and phospholipid levels in G6PD deficient children during haemolytic crisis

Day of admission	Triglycerides (mg/dl)	Phospholipids (mg/dl)
1 (10)	60.21±10.96	216.74±14.32
4 (10)	55.33±9.87	183.27±12.09
15 (10)	61.98±9.49	198.30±7.20
Controls (18)	66.88±7.64	214.48±11.61

The values are expressed as the mean±s.e. The number of children is given in parentheses.

after admission. High levels of reticulocytes were also observed during haemolytic crisis (day 4) with a drop at day 15.

Low levels of haemoglobin, hematocrit and red cells and high levels of reticulocytes were associated with significant reductions in total, LDL and HDL cholesterol concentrations (Table 2). These lipid parameters significantly increased at day 15 after admission, a time coinciding with recovery from the disease (Table 2).

No significant changes in triglyceride or phospholipid levels were observed at any time point (Table 3).

Discussion

Cell proliferation is a biological condition that requires large amounts of cholesterol for cell growth and division, this molecule being essential for membrane biogenesis. Indeed, proliferating cells show increased capacity to synthesize cholesterol and to accumulate cholesterol esters (Dessì *et al.* 1984, 1986). It is therefore conceivable that during processes of sustained cell proliferation, intracellular cholesterol metabolism affects cholesterol distribution in the plasma.

Results consistent with this hypothesis were previously found in our laboratory in various experimental models of cell proliferation involving liver (Dessì *et al.* 1986, 1989) and kidney (Dessì *et al.* 1988): a constant finding of these studies was a marked decrease of HDL cholesterol levels in the plasma compartment. More recently alterations of plasma cholesterol metabolism, mainly consisting in a decrease of total and HDL cholesterol levels, were also observed during compensatory hyperplasia of bone marrow after haemolysis induced by phenylhydrazine administration in rats (Dessì *et al.* 1990). It has been suggested that the decreased content of HDL cholesterol could be related to the extent of cell proliferation for a greater utilization of cholesterol by proliferating cells, thus resulting in a lesser availability of this molecule for transport into the plasma compartment.

In humans, alterations of plasma cholesterol metabolism have been reported in patients with different types of haematologic neoplasms (Ginsberg *et al.* 1986; Dessì *et al.* 1991). In addition, several epidemiological

studies have shown an association between low serum cholesterol levels and cancer incidence and mortality (Kark *et al.* 1980; Williams *et al.* 1981; Garcia-Palmieri *et al.* 1981). However, the question of whether changes in cholesterol metabolism are predisposing factors for cancer or a result of malignant process has not been resolved.

In the present study, reductions in total, LDL and HDL cholesterol were found in sera from G6PD-deficient children during acute haemolytic anaemia induced by ingestion of fava beans. From our data, the changes in cholesterol metabolism appear to be related to bone marrow hyperplasia since they occur together with the increased activity of the haematopoietic system, as indicated by the very high reticulocyte levels found at this time. A return towards normal values was also seen 15 days after admission, a time coinciding with recovery from the disease. These results clearly demonstrate that alterations of plasma cholesterol metabolism other than in experimental animals and in cancer patients also occur in subjects with non-malignant proliferative processes. These findings indirectly support the hypothesis that the changes of cholesterol metabolism observed in plasma of neoplastic patients (Ginsberg *et al.* 1986; Dessì *et al.* 1991; Kark *et al.* 1980; Garcia-Palmieri *et al.* 1981) may be a consequence of the proliferative process, even if tumoral, rather than a predisposing factor for cancer.

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