Lipids and lipoproteins of malnourished children during early renutrition: apolipoprotein A-IV as a potential index of recovery\textsuperscript{1,2}

Ibtissam El Harim, Jean-Jacques Befort, Amina Balafrej, Mohamed Lahrichi, and Anik Girard-Globa

ABSTRACT Twenty-six children with marasmus and 27 with kwashiorkor were compared with 23 control children of matching ages. Kwashiorkor was characterized by increased phospholipids (NS), low (\(P < 0.01\)) apolipoprotein (apo) B-rich LDL, and near normal apo A-I and HDL-C. In children with marasmus apo B (\(P < 0.02\)) LDL-C (NS), apo A-I (\(P < 0.01\)), and HDL-C (\(P < 0.001\)) decreased. Fifteen children in each group were followed for 2 wk. Control values were progressively reached after 2 wk. In the younger children final apo B was higher than in control subjects (\(P < 0.03\)) but apo A-I was identical. Apo A-IV, assayed because it correlates with the functional state of intestine, was near normal in children with kwashiorkor and decreased with treatment. In children with marasmus apo A-IV decreased by 50\% increased with treatment in older children, but further diminished in younger children. After 2 wk apo A-IV was significantly lower in all patients than in control subjects. Apo A-IV, by remaining depressed after other variables normalized, seems a good index of nutritional status. Am J Clin Nutr 1993;58:407-11.

KEY WORDS Kwashiorkor, marasmus, renutrition, serum lipids, apolipoproteins, apo A-IV

Introduction

Protein malnutrition (kwashiorkor) and protein-energy malnutrition (marasmus) are still major pediatric problems in many parts of the world, whether they derive from food deprivation or poorly managed weaning. Lipid metabolism is severely affected in most cases. Hepatic steatosis consecutive to decreased triglyceride secretion was among the first biochemical consequences to be described (1, 2), although triglyceride concentrations seem to be variably affected, sometimes not at all (3, 4) and sometimes increased (5). The reported resistance in animal models of high-density lipoproteins (HDLs) (6) and HDL apoproteins (7) to malnutrition has led us to explore these indexes in children because of the important role played by this class of molecular complexes in the cellular balance of cholesterol and lipoproteins.

In this study we compared hospitalized children with kwashiorkor and marasmus with healthy children of similar ages from the same ethnic stock. A follow-up study allowed us to evaluate the effects of nutrition over a 2-wk period.

Special emphasis was put on the determination of apolipoprotein (apo) A-IV concentrations. This apolipoprotein is synthesized almost exclusively by the intestine in humans (8). We previously showed in rats that its concentration correlates with the state of the intestinal mucosa (9) and data obtained in human subjects under parenteral nutrition confirm that apo A-IV concentrations relate with intestinal function (10).

Subjects and methods

This work was performed in Morocco in 1989. There were no ethics committees in France or Morocco at this time.

Fifty-three children hospitalized for severe malnutrition over 2 y were included in the study. Twenty-six were diagnosed with marasmus and twenty-seven with kwashiorkor on the basis of classic clinical criterion, mainly edema (11). No attempt was made at characterizing marasmic kwashiorkor (12). Control subjects were 23 healthy children from the same region and within the same age range. Of the initial group, 15 children with marasmus and 15 with kwashiorkor were followed during 2 wk of renutrition with a balanced diet. Weight and height were recorded upon arrival and were expressed as mean \(\pm\) SD for children of the same age in the Harvard statistics (13).

Blood samples were drawn within 24 h of admission or after an overnight fast in hospitalized children. Serum was collected by centrifugation at 1800 \(\times\) g for 10 min at 4 °C after the blood had clotted, and EDTA (1 g/L) and sodium azide (0.1 g/L) were then added to it as preservatives. Triglycerides, cholesterol, and phospholipids were assayed enzymatically by using Biomérieux kits (Biomérieux, Marcy l’Etoile, France).

Apolipoprotein concentrations were determined only for those children who underwent renutrition. Apo B and apo A-I were assayed by immunoelectrophoresis by using commercially

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available plates (Sebia, Issy les Moulineaux, France). Apo A-IV was also assayed by immunoelectrohoresis according to the method of Laurell (14) with a monospecific polyclonal antibody raised in rabbits against purified human apo A-IV. The standard was a pool of 10 sera kept frozen after calibration against pure apo A-IV obtained by preparative electrophoresis. The intra assay and inter assay CVs were 6% and 8%, respectively.

HDL cholesterol was measured after precipitation of apo B-containing lipoproteins with magnesium phosphotungstate (Boehringer Mannheim, Meylan, France) and LDL cholesterol was obtained by calculation. The proportion of HDL was evaluated by selective precipitation with dextran-sulfate magnesium chloride (15).

**Statistics**

Statistical analysis was performed by using repeated-measures analysis of variance (ANOVA). Two-by-two comparisons were made by using t tests with Bonferroni’s correction to account for multiple comparisons.

**Results**

The severity and duration of malnutrition were evidenced by the wide divergences of height and weight of patients with those of control children of matching ages (Table 1). Both groups were below 2 SDs from the means for the respective age of each child. The slightly higher values in children with kwashiorkor can be attributed to edema for weight, and to a slightly older age for height.

**Table 1**

<table>
<thead>
<tr>
<th>Characteristics of subjects before hospitalization*</th>
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<tbody>
<tr>
<td>Control (n = 23)</td>
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<tr>
<td>Ages (mo)</td>
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<td>Weight (kg)</td>
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<td>Z score</td>
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<tr>
<td>Height (m)</td>
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<tr>
<td>Plasma protein (g/L)</td>
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<td>Glucose (mmol/L)</td>
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* † ± SD.
†† Significantly different from control: †P < 0.001. †‡ Significantly different from marasmus: P < 0.05.

**Table 2**

<table>
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<th>Serum lipids before hospitalization*</th>
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<tbody>
<tr>
<td>Control (n = 23)</td>
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</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
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<tr>
<td>Phospholipids (mmol/L)</td>
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<td>Cholesterol/phospholipids</td>
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* † ± SD.
†† Significantly different from control: †P < 0.001. †‡ Significantly different from marasmus: P < 0.05.
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Plasma proteins were significantly depleted in the kwashiorkor group, with respect to both control subjects and the marasmic group. There was no significant difference between marasmus and control groups.

Triglyceride concentrations (Table 2) were elevated in both malnourished groups and total cholesterol was lowered for marasmus and kwashiorkor. Although phospholipids were unaffected in children with marasmus, they tended to be elevated in the children with kwashiorkor, but the difference was not significant because of very low concentrations in two subjects with kwashiorkor. However, the ratio of cholesterol to phospholipids was lower in this group than in either the control or marasmus group.

The decrease in cholesterol occurred mostly in LDLs (Table 3) which were lowered by 28% in the marasmus group and 38% in the kwashiorkor group. By contrast, HDL cholesterol was significantly lower only in the children with marasmus, with respect to control subjects and children with kwashiorkor. This resulted in a very different distribution of cholesterol between the two main classes of lipoproteins (Table 3). The more marked decrease in HDLs than in LDLs in the marasmus group led to an elevation of LDL:HDL whereas, on the contrary, the relative stability of HDL cholesterol in the kwashiorkor group led to a significantly lower ratio. Despite the decrease in HDL cholesterol, the proportion of HDL remained within normal ranges.

Apo B was depressed in the marasmus group by 35% (Table 4), i.e. to the same extent as LDL cholesterol. By contrast, apo B in the kwashiorkor group, although significantly lower than in control subjects, was less decreased than was LDL cholesterol, reflecting an alteration in the composition of LDL. Indeed, the ratio of LDL cholesterol to apo B was 1.1 for the kwashiorkor
group and 1.5 for the control and marasmus groups, indicating the presence of denser, protein-rich LDL.

The decrease in apo A-I concentrations was moderate in both groups, and significant only in the marasmus group. The lesser sensitivity of apo A-I than of apo B to malnutrition was illustrated by the lower ratio of apo B to apo A-I in this group.

Apo A-IV was decreased by as much as 47% in the marasmus group and not at all in the kwashiorkor group; therefore, the difference between the two malnourished groups was significant.

Recovery

This part of the study contained 15 patients from each group who were studied after 1 and 2 wk of feeding an appropriate balanced diet in adequate amounts.

Weight gain, as expected, was a poor indicator of repletion. Although marasmic children had gained an average of 1.2 ± 0.6 kg at the end of the observation period, children with kwashiorkor had gained none and weighed 7.2 ± 0.8 kg as compared with 7.4 ± 1.5 kg on arrival. Meanwhile, however, their plasma proteins had reverted to normal (Fig 1) with respect to values at admission (P < 0.001).

Triglycerides remained elevated in both groups (Fig 1) despite the fact that the children were truly fasted at the time of blood sampling. The differences from the control group were, however, not significant. In this smaller group of kwashiorkor patients, phospholipids were significantly elevated (P < 0.03) and remained high throughout the observation period (Fig 1). Total cholesterol was elevated after 1 wk of hospitalization in both groups (P < 0.05) but only in children with kwashiorkor after 2 wk (P < 0.05). LDL cholesterol (Fig 2) returned to normal values, but was significantly higher in the kwashiorkor group than in the marasmus (P < 0.001) or control groups (P < 0.01) after 1 wk. Apo B exhibited a similar pattern in the kwashiorkor group, peaking after 1 wk, but the differences between groups were not significant (Fig 3). At both times of hospitalization, concentrations of apo B were higher in kwashiorkor children 18–29 mo of age than in younger children (1 wk, P < 0.05; 2 wk, P < 0.02) (Fig 3). By contrast, in children with marasmus (Fig 3), apo B was at all times lower in older children and the difference from the younger group was significant (P < 0.03) after 2 wk of repletion. The overall increase during repletion was significant in children with marasmus (P < 0.01).

During hospitalization, the concentrations of HDL cholesterol (Fig 2) and of apo A-I were also restored (Fig 4). Return to control values was slower in the marasmus than in the kwashiorkor group. There was no difference between age groups. After 2 wk, apo A-I increased by 20% in the kwashiorkor group (NS) and by 32% in the marasmus group (P < 0.03).

Apo A-IV concentrations were strikingly different at the beginning of treatment: very low in the marasmus group (P < 0.001 vs control subjects) but near normal in the kwashiorkor group (Fig 5). During repletion, apo A-IV decreased in the kwashiorkor group and increased in the marasmus group to reach similar concentrations, which were only ≈60% of control values.
(P < 0.001 for marasmus and 0.005 for kwashiorkor). Discrimination on the basis of age showed that apo A-IV decreased in kwashiorkor children of all ages except one who had 60 g/L plasma proteins and may have had marasmic kwashiorkor. By contrast, in the marasmus group, concentrations of apo A-IV increased steadily in older children (P = 0.03), whereas they underwent a further decrease in younger children (P < 0.03) as in those with kwashiorkor.

Thus, by the end of the renutrition period, apo A-IV was the only index that remained significantly altered in both groups.

Discussion

All the children in this study were < 75% of expected weight for their age, i.e. clinically malnourished. Although it is difficult to make a clear-cut distinction between kwashiorkor and marasmus, it is generally agreed that children with edema and ≥ 60% of expected weight are considered to have kwashiorkor whereas those without edema and ≤ 60% of expected weight should be considered marasmic (1). In this study clinical diagnosis yielded a kwashiorkor group with an average 69% of expected weight and a marasmus group with 59%. The classification was further justified by a significantly decreased concentration of proteins in the kwashiorkor group but not in the marasmus group. The efficiency of renutrition was demonstrated by return of plasma proteins to normal values by the end of the study period.

Triglycerides, reportedly very low in kwashiorkor (2), were moderately elevated. They remained so during refeeding, but with great individual variations, which preclude speculation. In both groups, phospholipid concentrations were maintained at control values. This may have contributed to the relative stability of HDL because it appears that phospholipid hydrolysis is a prerequisite to the uptake of HDL cholesteryl ester by cells (16) and to its transfer to apo B-containing lipoproteins by the cholesteryl ester transfer protein (CETP). Such a mechanism would also account for the deficit of LDL cholesterol and for the fact that the cholesteryl ester–rich HDL fraction remained as large as in the control group. The overall result of these combined alterations is a sparing of phospholipids and cholesterol. Moreover, the higher ratio of apo B to apo A-1 in kwashiorkor than in marasmus suggests that uptake of LDL through the apo B/apo E receptor is not stimulated as it is in starved animals (17).

During hospitalization, triglycerides were elevated in many children of both groups, as is often reported (2). The hypothesis of a kwashiorkor-induced repression of the apo B/apo E receptor was reinforced by elevated total cholesterol and apo B, particularly in children aged 18–29 mo (Fig 3).

Elevated concentrations of phospholipid persisted in children with kwashiorkor even after 2 wk of renutrition, when most other indexes had reverted to normal. It is therefore likely that some of the key factors of lipoprotein metabolism such as lecithin:cholesterol:acyl transferase (LCAT), CETP, and perhaps hepatic lipase, remained altered after the more easily measured indexes had normalized.

Apo A-I concentrations were not much affected. In experimentally malnourished rats, apo A-I is also reported to remain near normal (7) and a rebound has been observed upon renutrition, similar to that evidenced in children with kwashiorkor. Down-regulation of the binding sites responsible for apo A-I catabolism is unlikely to occur (18), but the alterations in surface HDL composition may suffice to reduce their binding and uptake.

In the older group of marasmic children, apo A-IV rose progressively from very low values, as expected from our previous observations, linking its concentration to the functional state of the intestinal mucosa (9). In the younger marasmic children as well as in all but one child with kwashiorkor, apo A-IV concentrations, which were initially less depressed, declined with recovery. We have no explanation for what appears to be a highly selective preservation of apo A-IV during kwashiorkor. Surprisingly little alteration of the intestinal mucosa occurs during total fasting in adults (19) or even severe protein-energy malnutrition in children (20). Because concentrations decrease with recovery, some sort of preservation is more likely than persistent synthesis.

Lymph is known to contain appreciable amounts of apo A-IV (21, 22). Edematous infiltration could therefore play a role in this paradoxical evolution of apo A-IV. At the end of the study period, concentrations were not different in either group and were still significantly lower than in control subjects. All other indexes were normalized, although the children had clearly not completely recovered after such a short treatment. We believe our findings warrant further exploration of apo A-IV concentrations as a particularly sensitive index of nutritional status and of digestive recovery after severe malnutrition. Further investigation is needed to ascertain its value in longer-term experiments and to determine whether it can be used as an early sign of incipient marasmus or of kwashiorkor before the onset of edema.

References