Malnutrition and Carbohydrate and Lipid Metabolism

A. Stewart Truswell

Human Nutrition Unit, University of Sydney, Sydney, Australia 2006

This subject was last reviewed at the Chiang Mai Symposium on Protein-Calorie Malnutrition (PCM), which was held in January 1973 and published in 1975 (1). At that time, there had been nearly 20 years of exciting research since Trowell et al. (2) wrote the first comprehensive monograph on kwashiorkor in 1954. This chapter recapitulates what was known about the subject in 1973, brings it up to date with the results of recent research, and speculates on future developments.

CARBOHYDRATE METABOLISM

The Gastrointestinal Tract

Starch digestion is likely to be impaired by the reduction of pancreatic amylase reported by Barbezat and Hansen (3) in their classic study. However, there is now evidence that starch not absorbed in the small intestine is fermented in the large intestine and can give up some of its energy to the host in the form of volatile fatty acids (4). Jackson (5) suggests that carbohydrate not absorbed in the small intestine, e.g., cellulose, may, by providing a substrate for colonic bacteria, facilitate reutilization of urea through its conversion into amino acids, which may be reabsorbed from the colon. Recent work on the glycemic indexes of different starches in healthy adults (6) shows that starches in some plant foods are more readily digested than in others. Some starches can become partly "retrograded," which makes them resistant to small intestinal digestion (7). From this information, it should be possible to design clinical trials and then recommend those starchy foods that are easier for malnourished children to digest and metabolize.

Acquired lactase insufficiency is now known to be more prevalent in human adults (as in other animals) than is retention of the full infantile disaccharidase activity, which is only found in people from northern Europe and a few pastoral tribes in Africa and Arabia (8–10). One view of the history of the discovery of this genetic polymorphism around the world is that acquired lactase insufficiency was first re-
ported in protein-energy malnutrition (PEM) by Bowie et al. from Cape Town in 1963 (11). Of course, at that time, it was suggested that it was the result of protein deficiency. But a third of cases did not have lactase insufficiency and, in those that did, it often persisted for months (unlike other protein functions) after refeeding and recovery (12,13). Perhaps there was an infection; or perhaps the child’s home environment was still nutritionally inadequate.

With the realization that intestinal lactase is not inducible and that most Africans, Asians, Amerindians, and Australian Aborigines lose much of the activity of this enzyme during childhood, the question arose whether the finding of lactose intolerance in severe hospitalized PEM was a coincidental genetic trait rather than a result of protein deficiency. But in cases of severe PEM, persistent diarrhea usually responds to replacement of milk by casein plus glucose or sucrose (14,15). This research has led to an improvement in treatment.

Genetic hypolactasia usually starts to develop a year or more after the usual age of PEM in children. This has been reported from Lesotho, for example, using the breath hydrogen method (16). In Kivu, Zaire, an interaction between malnutrition and genetic hypolactasia has been noted. In the pastoral Tutsis, who normally retain lactase as adults, its loss in malnutrition is less complete, and enzyme activity is much more likely to return with recovery than in neighboring agriculturalist tribes (Shi, Havu, and Kutu), whose lactase does not persist into adult life (17).

Replacement of lactose in the diet can be useful therapy for diarrhea in children with PEM, as it can be for some tube-fed adults with hospital malnutrition (18,19). The latter treatment was a by-product of clinical research on malnourished children. Reduced levels of intestinal lactase or the finding of some breath hydrogen after lactose do not mean that all milk must be avoided. Ambulant children can usually tolerate regular moderate amounts of milk well, regardless of their ethnic origin (20–22). This is explicable in several ways, for example, persistence of some enzyme activity, reduction by lactobacilli of lactose in sour milk, and adaptation of the colon to moderate regular loads of lactose (23).

Absorption of D-xylose and of glucose has been found to be subnormal in PEM (13,24). Mehta et al. (25) report that this improves when children with marasmus are given either a high-carbohydrate diet or a high-protein diet. Alleyne et al. (26) pointed out that D-xylose is extensively metabolized by gut bacteria, so the test is of little use when there is bacterial growth in the small bowel. Following effective treatment, there is a fairly rapid return of absorptive function to normal.

In mild to moderate PEM, the major advance in management of diarrhea has been the development of oral rehydration therapy (ORT) (27,28), which is based on the facilitation by glucose of sodium and water absorption across the small intestinal mucosa. This treatment was first shown to be effective in patients with cholera, and it is now widely used for children with diarrhea in many parts of the Third World. UNICEF (1988) estimates that it may be saving the lives of 600,000 children a year (29). This is another example of a remarkably simple, effective treatment, potentially available for nearly all undernourished children, which originated in basic research. The ORT principle may be applicable in an opposite way in adult diabetics.
Salt added to test meals of starchy foods appears to increase the glycemic response in normal adults (30).

**Blood Glucose**

Fasting blood glucose may be normal or low (26,31). Severe spontaneous hypoglycemia was formerly reported as more likely in some centers than others (32). Buchanan et al. (33) found low levels of glucagon in most of a small group of kwashiorkor cases with severe hypoglycemia. Elias and Gwinup (34) have reported three very wasted adults, weighing 38, 25, and 25 kg only, who had intractable hypoglycemia that did not respond to infusion of glucose. These patients had almost complete absence of subcutaneous fat.

Kerr et al. (35) have investigated gluconeogenesis in five fasting malnourished children in Jamaica, using $^{13}$C-glucose infusion. The rate of glucose production was approximately the same in mg/kg-min while they were malnourished as it was when they had recovered. Alanine infusion produced a moderate increase in glucose production. Kerr et al. calculated that in malnutrition, amino acids account for a relatively small fraction of total gluconeogenesis; most of it appears to derive from products of glycolysis, such as lactate and pyruvate, and from glycerol.

Plasma concentrations of alanine, the major glucogenic amino acid, are reduced in severe kwashiorkor, but in pre-kwashiorkor in Uganda, it was found to be raised (36). In starvation or at the onset of anorexia in pre-kwashiorkor, alanine concentration is low (36). The plasma concentration of alanine probably reflects the balance between the opposing effects of protein deficiency and energy deficiency, which occur in severe cases of PEM (26).

The oral glucose tolerance curve may be flat but often shows a slow descent in kwashiorkor (26,31,37). Oral glucose tolerance in marasmus has been reported as normal or as impaired (31). Intravenous glucose, eliminating the intestinal factors, shows slowed disappearance in kwashiorkor but not in marasmus (38,39). The insulin response to glucose is subnormal. Histology of the islets of Langerhans has shown no consistent change (40). In Cape Town, it was reported that potassium supplementation resulted in a considerable increase in serum immunoreactive insulin after intravenous or oral glucose early in the treatment of severe cases of PEM (41,42), so that potassium deficiency may be one reason for the reduced insulin release. Improvement of glucose tolerance after an inorganic chromium supplement was reported in Jordan and Nigeria (43) and in Turkey (44) but has not been confirmed elsewhere (45). There is likely to be insulin resistance in severe PEM because of increased growth hormone (31,40,46–49) and in some cases increased functioning cortisol (26,31,40).

In mild to moderate PEM, the study of insulin and cortisol in Uganda and the Gambia by Whitehead’s group (50) is one of the major advances in this area since 1973. Ugandan children, who have low plasma albumin concentrations and are at risk of kwashiorkor, go for months with higher plasma insulin and lower plasma
cortisol than Gambian children, who, if they develop severe PEM, usually show the marasmic form. This work provides good evidence that kwashiorkor and marasmus emerge from different endocrine backgrounds. Good data from community studies are very precious in this controversial area.

Plasma glucagon rises early in fasting adults (51,52) and is also elevated in malnourished adult surgical patients (53). Secretion of this hormone is increased by trauma and sepsis. In malnourished Jamaican infants, however, Robinson and Sea-kins (54) reported that fasting glucagon was significantly lower than the values obtained after recovery. They suggest that the levels that are raised in the first days of fasting will decline if it extends to chronic malnutrition with some, but insufficient, calories and protein intake.

In 1973, it was noted (1) that there was very little information about β-OH butyrate and ketone bodies in the plasma in malnourished children except for a single report from Egypt (55). Some data have since been provided by Persson et al. (56). Ketosis is a striking feature of fasting adults, but it is still not clear how important it is in different types of malnutrition in children. It would not be difficult to study systematically now that inexpensive paper dipsticks are available. In chronically undernourished adults in the Minnesota experiment, Keys et al. (57) found no significant ketonuria.

FAT METABOLISM

The Gastrointestinal Tract

Fat malabsorption is often present in PEM (58–60). Some of the fecal fat appears to be of endogenous origin. There are four or five probable causes for the steatorrhea: insufficient pancreatic lipase (3), deconjugation of bile salts, atrophy of the small intestinal mucosa, bacterial colonization of the small intestine, and sometimes specific infections. Reduction of conjugated bile acids in aspirates from the upper small intestine and an increased proportion of free bile acids have been reported from Cape Town (61), Central America (62), Calcutta (63), and from Rohtak in India (64). Increases in the glycine/taurine ratio and in the deoxycholate/cholate ratio have been reported (62,65).

Absorption of vitamin A, as an example of fat-soluble vitamins, is impaired (66) (Fig. 1). In view of the importance of vitamin A in resistance to infection, this may be a more serious dysfunction than the reduced energy absorption. Though reduced lipid absorption is attributed and at least partly due to defective micelle formation, there is another possible mechanism. Theron et al. (67) described lipoid droplets seen on electron microscopy in the villous cells of the small intestine. The droplets stained with oil red O and there was a marked decrease in chylomicrons in the intercellular spaces. These findings are very similar to the picture in a-beta-lipoproteine-mia. It would seem that, at least in some cases of severe PEM [these finding were not confirmed in another small study by Shiner et al. (68)], there is reduced synthe-
Absorption of vitamin A in kwashiorkor. 30 mg retinol in 5 ml arachis oil was given by stomach tube after an overnight fast, and a feed of skimmed milk was given 2 hr later. (A) On admission, (B) on admission, tube in duodenum, (C) after 5 to 7 days’ feeding, (D) after full recovery. Each line is a separate patient. (From ref. 66.)
sis of intestinal apolipoprotein B48, which is essential for the formation of chylomicrons. This likelihood awaits testing in kwashiorkor.

Adipose Tissue

It is characteristic of kwashiorkor (in the pure form) that subcutaneous fat is much better retained than in marasmus, where it is always greatly diminished. This was demonstrated numerically with Harpenden skinfold callipers by Keet et al. (69). Cases of marasmus had triceps skinfold thickness that were always below the 3rd percentile for normal children of the same sex. These skinfold values in kwashiorkor cases ranged from equally low (i.e., marasmic-kwashiorkor) up to the 50th percentile. In Uganda, Rutishauser (70) recorded low triceps skinfolds in areas where marasmus was common, and the prevalence decreased with age from 6 to 48 months; in areas where kwashiorkor was common, the average triceps skinfolds were thicker. Leiter and Marliss (71) examined all the data they could find on 10 Irish Republican Army hunger strikers who starved themselves to death. This took 57 to 73 days. They calculate that at this stage most of the body fat, but only about 19% of body protein, had been used up.

Fatty Acid Patterns—Possible Mild Essential Fatty Acid (EFA) Deficiency

Earlier reports on the question of EFA deficiency gave conflicting results (72–74). Until 1975, it appeared that no one had reported elevated eicosatrienoic acid (ω9), the most characteristic change in classic EFA deficiency. Holman et al. (75) examined underweight children with PEM in La Plata, Argentina, along with comparable controls. In half the PEM cases, plasma phospholipids had low arachidonic but normal linoleic acids; in a quarter, both 20:4ω6 and 18:2ω6 were low. The mean ratio of 20:3ω9 to 20:4ω6 was 0.06 in the controls and 0.25 in the cases. Holman et al. consider that the minimum level of linoleate required to correct the effects of the mild to moderate EFA deficiency will probably be above the maintenance requirement of around 1% of calories. They suggest that 3 to 6% of calories as polyunsaturated fat may be necessary to provide for synthesis of structural lipids during rapid growth and myelination and that some of these should be ω3 polyunsaturated fats. If the fatty acid patterns are similar in other parts of the world, this is an important recommendation for treatment. It contrasts with Golden’s hypothesis of free radical damage in PEM, which leads to the conclusion that polyunsaturated fats should be avoided (76). Wolff et al. (77) collected samples from children with severe PEM in Lima, Peru, and examined them at Johns Hopkins Medical School. They contrasted marasmus and kwashiorkor cases. The latter showed a smaller reduction of red cell linoleate but a greater reduction of red cell arachidonate. Only one of 44 children had detectable 20:3ω9. Possible metabolic explanations for these findings include some form of biotin deficiency in kwashiorkor. They do not refer to Holman’s paper in their discussion.
Lipids in Skin and Brain

Strauss et al. (78) collected skin surface lipid from the forehead of children with marasmic-kwashiorkor in Medellin, Colombia. They found a reduced squalene/wax ratio, the opposite of what has been reported in starving adults who normally produce more sebum after puberty.

Yusuf et al. (79) reported on the content and composition of phospholipids in the brains of children who died of severe malnutrition in Jamaica under 2 years of age. The phospholipid/DNA ratio was higher than normal in the forebrain and cerebellum. Among the different phospholipids, sphingomyelin was found to be selectively decreased in each part of the brain in malnourished children before 1 year of age. This was thought to indicate depressed myelination.

Fat in the Liver

Fatty liver is the second major criterion (after edema) for distinguishing between kwashiorkor and marasmus. It is the most striking feature of kwashiorkor at necropsy, and it is a feature that has been looked for in animal models of kwashiorkor. In moderate cases, the fat accumulates in the periphery of the hepatic lobules. The lipid that accumulates is mainly triglyceride (80). Lipid can make up 40% of liver weight, sometimes even more, and in such cases all the parenchymal cells are distended with large droplets of lipid. Necrosis of liver cells is rare (26), a feature that argues against Hendrickse's aflatoxin theory (81,82). With refeeding, liver fat usually clears in a few days, and there is little or no chronic histologic or functional damage. Cases with gross fatty liver and hepatomegaly are more likely to die.

The fatty liver is not always palpable. Swischuk and McConnell (83) describe a characteristic radiolucency, which they propose as a radiographic sign of fatty liver in children.

A case of fatal fat embolism involving hepatic veins, right atrium, and ventricle and pulmonary artery has been reported; it was associated with a grossly fatty liver (double normal weight) in an 11-month-old black infant with kwashiorkor in Durban, Natal. There was no indication of trauma. Hruby and Wainwright (84), who described the case, could not find features of fat embolism in another 40 of their cases of kwashiorkor. Fat embolism is known in association with alcoholic fatty liver in adults.

In adults with hospital malnutrition treated with parenteral nutrition, fatty liver is likely to occur with large intakes of intravenous glucose, especially if there is associated infection (85) or burns. This does not occur with Intralipid. Serum free fatty acids are low on parenteral glucose programs. Fatty liver was not prevented if a small (requirement) amount of EFA was infused as well as the glucose (86). The liver fat presumably arises from intrahepatic fatty acid synthesis from glucose. It is not clear why it accumulates. If it were due to protein deficiency, it should not occur if mixed amino acids are given in the infusion as well, but Tulikuora and Huikuri
Plasma Lipids in PEM

In almost all reports, plasma total cholesterol has been low in kwashiorkor. There are now at least 25 groups that have reported this in children (72,73,87–116). There are only two recent papers that differ in their findings. Devi et al. (117) report no difference between total cholesterol in kwashiorkor and controls, but their control outpatients had lower levels than have been found in most control series. Wolff et al. (77) studied fatty acid patterns during treatment, and their patients had already had several days of dietary treatment (including protein) before blood was taken. Low plasma cholesterol is also reported in adult protein malnutrition (118), sometimes called adult kwashiorkor.

In marasmus, plasma total cholesterol can be low, normal, or increased. The mean is usually lower than in recovered children but significantly higher than in kwashiorkor (101,105,113,116,119,120).

Plasma fasting triglycerides are usually low in kwashiorkor but not as consistently low as the cholesterol, while in marasmus, triglycerides are normal or increased (1,101,121). Three authors have reported variable triglyceride levels in kwashiorkor, with mean levels normal or somewhat above (56,80,122). Assuming that dehydration has been corrected and a fat-containing meal avoided, the most likely explanation for this is a day or two of protein-containing food, perhaps provided by a clinic before admission to hospital. In our study of serial changes of plasma lipids during refeeding of kwashiorkor (Fig. 2) (1), mean fasting triglycerides rose in the first 2 days from 94 to 210 mg/dl (+123%), while total cholesterol rose only from 89 to 109 mg/dl (+22%).

It may provide a useful perspective to review briefly the plasma lipid concentrations found in different forms of inadequate energy intake.

1. In experimental partial starvation of young men in the Minnesota experiment with gradual loss of 25% of body weight from moderate energy deficit over 24 weeks but with adequate dietary protein, plasma total cholesterols fell by 11% to mean levels of 151 mg/dl and triglycerides (by difference) declined by a similar proportion (123).

2. In spontaneous semistarvation and famine in adults, plasma cholesterol values have usually been moderately low but in Third World populations they may have been low before the famine (123). In semistarving Europeans in World War II, mean cholesterols were 167 mg/dl in Rotterdam and 166 mg/dl in Wuppertal, Germany (124,125).

3. In anorexia nervosa, plasma cholesterol concentration is often elevated (126–129). One possible explanation is that the patients, in avoiding carbohydrates, may eat a high percentage of calories as saturated fat (130). However, this is probably not the whole explanation (128).
4. In fasting or short-term total starvation in healthy adults (with water provided), plasma triglycerides increase by about 50%. Plasma free fatty acids (FFA) are, of course, high, and there is a smaller increase of plasma cholesterol (51,123, 131,132). A greater increase in plasma lipids has been reported in women than in men (133).

5. By contrast, in obese people on a low-energy weight-reducing diet, changes in plasma lipids have been variable, depending partly on the alcohol content of the original diet and partly on the fat content of the reducing diet and whether hyperlipidemia was present (134).

6. In total starvation for obesity, plasma triglycerides may fall considerably, accompanied by smaller reductions of total cholesterol (135).

In these different states of negative energy balance, the metabolism and clearance of the different lipoproteins await elucidation. One recent glimpse was provided by Markel et al. (132). In healthy, nonobese adult physicians on a hunger strike, there was an increase in apo-B-lipoprotein concentration of about 25%, over 9 days with a small decrease in apo-AI and a marked reduction of apo-E-lipoprotein.

Pathogenesis of the Fatty Liver in Kwashiorkor

There are four possible causes of fatty liver: (a) increased mobilization of FFA from adipose tissue, (b) increased fatty acid synthesis from glucose, (c) reduced oxidation of fatty acids in the liver, (d) reduced release of fats from the liver to plasma in lipoproteins. There is an increased plasma concentration of FFA in kwashiorkor but this is often true in marasmus as well (101,105). Increased FFAs respond to a few days' feeding of starch, but this is not effective treatment for fatty liver in PEM (101). If increased flux of FFAs (136) were the only mechanism producing fatty liver, plasma lipids would not be reduced: They would be elevated or at least normal (105).
One would not expect increased hepatic fatty acid synthesis in kwashiorkor. These children have usually been anorexic, i.e., in negative energy balance, before they present. There is a similar percentage of linoleic acid in the liver fat to that in depot fat (73,101). The former must have come from outside the liver. Fatty acid synthesis was measured by Fletcher (137) and found to be reduced. As to oxidation of fatty acids, Lewis et al. (138) found increased labeled respiratory CO₂ after giving very small doses of ¹⁴C palmitate to kwashiorkor cases—the opposite of what would be expected if fatty liver resulted from decreased fatty acid oxidation.

The principal dysfunction responsible for the fatty liver in PEM appears to be impaired synthesis of the apoprotein(s) in low-density (LDL) and/or very-low-density (VLDL) lipoproteins. This hypothesis was proposed in 1969 and 1975 (1,104), and it was demonstrated in a study (Table 1) (1) that has never been repeated that the degree of fatty liver by percutaneous biopsy in 48 cases of kwashiorkor or marasmus was related most strongly to the β-lipoprotein cholesterol concentration on admission to hospital. It was next most strongly related to total cholesterol, then to albumin, then to triglycerides, but not to α-lipoprotein cholesterol (1). Note that in this series, there were a few cases of marasmus who were found to have a moderately fatty liver and occasional cases of kwashiorkor with no liver fat seen on biopsy. We inferred that the defective apolipoprotein synthesis was because of protein deficiency in the liver.

There has been no evidence in man to support choline (i.e., lipotropic factor) deficiency. Plasma (104,139) and red cell membrane (140) lecithins (phosphatidyl choline) are not reduced, and in any case choline requirements must be much lower in humans than in the rat because human liver contains practically no choline oxidase.

Whitehead and Lunn (50) showed that in Uganda, where the severe form of PEM is kwashiorkor, mild to moderate cases have high levels of plasma insulin and low levels of cortisol. This endocrine set promotes the movement of amino acids toward muscle and away from the liver. As Coward and Lunn (141) expressed it: “By promoting muscle protein synthesis, high insulin concentrations will deplete the plasma of essential amino acids. . . . Locking away essential amino acids in muscle when protein intake is low is likely to produce a fall in hepatic synthesis of export proteins including albumin and apo-proteins for β-lipoprotein synthesis. Hypoalbuminaemia could lead to . . . oedema, and deficiencies in β-lipoprotein synthesis to a fatty liver.”

Waterlow (142) mentioned in discussion that he found in malnourished Jamaican children with fatty livers a greatly decreased uptake of [³⁵S]methionine into VLDL. Similar results have been obtained in rats (143).

Three treatment trials give further support to this concept. Olson (114) compared the responses of a number of variables in kwashiorkor and marasmus to four diets: (I) 1 g protein and 100 kcal per kg body weight daily, (II) 1 g protein and 175 kcal per kg, (III) 4 g protein and 100 kcal per kg, or (IV) 4 g protein and 175 kcal per kg. These were given for 3 weeks, after which cure was consolidated on diet IV. Serum cholesterol rose well on both the diets of 4 g protein per kg; it stayed low or rose only a little on 1 g protein per kg (with or without the extra calories).
<table>
<thead>
<tr>
<th>Liver fat</th>
<th>$n$</th>
<th>Total cholesterol (mg)</th>
<th>β-Lipoprotein cholesterol (mg)</th>
<th>α-Lipoprotein cholesterol (mg)</th>
<th>Tri- glycerides (mg)</th>
<th>Phospholipids (mg)</th>
<th>Albumin (g)</th>
<th>Pre-β- lipoprotein band</th>
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<tr>
<td>(a) Gross (3 &amp; 4+)</td>
<td>20</td>
<td>84</td>
<td>52</td>
<td>31</td>
<td>88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129</td>
<td>1.82</td>
<td>0/15</td>
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<tr>
<td>(b) Mild to moderate (1 &amp; 2+)</td>
<td>13</td>
<td>108</td>
<td>73</td>
<td>34</td>
<td>108&lt;sup&gt;b&lt;/sup&gt;</td>
<td>160</td>
<td>2.47</td>
<td>1/8</td>
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<tr>
<td>(c) None (0)</td>
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<td>131</td>
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<td>$\frac{(c) - (a)}{\frac{1}{2}(c) + (a)}$</td>
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<td>55%</td>
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<td>35%</td>
<td>21%</td>
<td>42%</td>
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<sup>a</sup>$n = 19.$  
<sup>b</sup>$n = 10.$  
From ref. 1.
TABLE 2. Effect of day of starting dietary protein on rise of plasma cholesterol in kwashiorkor

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<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
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<th>Patient</th>
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<th>Day 4</th>
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<td>133</td>
<td>142</td>
<td>153</td>
<td>160</td>
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</table>

Persson et al. (56) in Ethiopia compared two refeeding diets in a small number of cases of kwashiorkor. One diet was Vivonex, and the other a mixture of casilan, fat, and carbohydrate. Only on the latter diet did the plasma triglycerides increase. They stayed low on the wholly "synthetic diet," which contained half the concentration of total amino acids that was in the protein in the casilan diet. "The unchanged plasma triglyceride concentration in the Vivonex group could thus suggest impaired release of triglyceride from the liver and support the hypothesis of a reduced lipoprotein synthesis" (56).

In a treatment trial in Cape Town, not hitherto published (Truswell, Hansen, and Wannenburg, 1970), children with kwashiorkor were allocated to one of two dietary groups. Both received electrolyte solutions as required, together with maize starch and glucose. Six children were started on casein (casilan) on admission; the other five were not given protein (casilan) until day 5. Fasting plasma lipids rose early in the first group but not until day 5 in the delayed protein intake group (Tables 2 and 3).

In the 1970s, there was much discussion about α-lipoproteins or high-density lipoprotein (HDL) in kwashiorkor. We found them reduced in only a minority of
TABLE 3. Effect of day of starting dietary protein on rise of plasma (fasting) triglycerides in kwashiorkor

<table>
<thead>
<tr>
<th>Patient</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>96</td>
<td>90</td>
<td>92</td>
<td>113</td>
<td>100</td>
<td>152</td>
<td>150</td>
<td>131</td>
</tr>
<tr>
<td>JBM</td>
<td>100</td>
<td>92</td>
<td>71</td>
<td>73</td>
<td>108</td>
<td>177</td>
<td>129</td>
<td>93</td>
</tr>
<tr>
<td>KB</td>
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<td>83</td>
<td>52</td>
<td>94</td>
<td>92</td>
<td>94</td>
</tr>
<tr>
<td>PDL</td>
<td>77</td>
<td>73</td>
<td>103</td>
<td>94</td>
<td>114</td>
<td>167</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>85</td>
<td>70</td>
<td>73</td>
<td>126</td>
<td>315</td>
<td>305</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>Mean</td>
<td>82</td>
<td>78</td>
<td>82</td>
<td>98</td>
<td>138</td>
<td>179</td>
<td>180</td>
<td>(105)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Recovered</th>
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</thead>
<tbody>
<tr>
<td>JL</td>
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<td>106</td>
<td>179</td>
<td>158</td>
<td>144</td>
<td>148</td>
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<td>133</td>
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<tr>
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<td>100</td>
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<td>79</td>
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<td>75</td>
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</tr>
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<td>125</td>
<td>133</td>
<td>146</td>
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<td>144</td>
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<td></td>
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<tr>
<td>CM</td>
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<td>247</td>
<td>261</td>
<td>269</td>
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<td>200</td>
<td>150</td>
<td>60</td>
</tr>
<tr>
<td>PS</td>
<td>63</td>
<td>68</td>
<td>83</td>
<td>118</td>
<td>110</td>
<td>93</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>106</td>
<td>116</td>
<td>136</td>
<td>142</td>
<td>135</td>
<td>134</td>
<td>119</td>
<td>(91)</td>
</tr>
</tbody>
</table>

cases (Table 4) (104). A report from Jamaica agreed (109), but three other reports, from South America (99), India (100), and Uganda (111), found that reduction of α-lipoproteins was more marked than of β-lipoprotein. The early methodology available on paper electrophoresis was not very quantitative, especially for the fainter α-band, and we used an improved electrophoretic system. We were initially surprised that β-lipoproteins were more consistently reduced, because α-lipoproteins contain a higher proportion of protein.

Since 1975, however, HDLs (formerly α-lipoproteins) have been viewed differently. They emerged in 1975 as negative risk factors for coronary heart disease (144–146). By 1978, they were "center-stage" (147,148). HDLs are sometimes (simplistically) visualized as taking cholesterol from the periphery back to the center of the body, e.g., the liver. Their concentration tends to be low in children in the Third World (149). They are not synthesized primarily in the liver; they contain seven apolipoproteins—apo A-I, A-11, A-IV, apo C-I, C-11, C-111, and apo E, some of which originate in the intestine, some in the liver, and others are acquired by transfer from LDL in the blood (150). HDLs may or may not be low in PEM
TABLE 4. Number of cases with α- and β-lipoprotein cholesterol greatly reduced (half mean concentration of recovered cases)

<table>
<thead>
<tr>
<th></th>
<th>Gross fatty liver</th>
<th>Mild to moderate fatty liver</th>
<th>No fatty liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Lipoprotein cholesterol ≤ 20 mg/dl</td>
<td>0/20</td>
<td>0/13</td>
<td>0/15</td>
</tr>
<tr>
<td>β-Lipoprotein cholesterol ≤ 55 mg/dl</td>
<td>14/20</td>
<td>5/13</td>
<td>2/15</td>
</tr>
</tbody>
</table>

Same cases as in Table 1.

(151), but this is not related to the mechanism that produces fatty liver. With treatment and recovery, it is β-lipoprotein cholesterol that increases in the plasma—presumably reflecting clearance of lipid from the liver; α-lipoprotein cholesterol does not change significantly (Fig. 3).

We now know—but from research in adults, not from work on PEM—that apolipoprotein B-100 (apo B-100) is an absolute prerequisite for secretion of the triglyceride-rich lipoproteins from the liver into the blood plasma (150,152). This apolipoprotein is assembled, like albumin, by ribosomes bound to the endoplasmic reticulum in the liver. It is a very large protein, with a molecular weight of 549,000 and it contains 4,536 amino acid residues per molecule. It is little wonder that its synthesis is very susceptible to lack of amino acids in the liver. Furthermore, apo B-100 is rich in leucine. There are 505 leucine residues (by my count) in the amino acid sequence reported by Yang et al. (153), which is 11% of all the amino acids. Plasma leucine is much reduced in kwashiorkor (154). Apolipoprotein B passes from the endoplasmic reticulum to the Golgi apparatus and from there is secreted into the space of Disse and then into the blood, where it picks up apolipoprotein C and more cholesterol ester from HDL by the lecithin-cholesterol acyltransferase (LCAT) reaction. In the periphery, lipoprotein lipase takes away most of the triglyceride from VLDL, and it is transformed [via intermediate density lipoprotein (IDL)] to cholesterol-rich LDL (formerly β-lipoprotein). Since the turnover of VLDL is more rapid than that of LDL, this may explain why the more stable LDL (e.g., β-lipoprotein cholesterol) correlates more reliably than VLDL or plasma triglycerides with the degree of fatty liver in PEM. It also explains why plasma triglyceride concentration goes up much faster than plasma cholesterol when protein is provided in the diet. Apo B-100 is over 95% of total lipoproteins in plasma LDL but only 35% of total lipoproteins in VLDL (150).

As far as is known, none of the modern technology of lipoproteins has been applied to an investigation of changes of lipoprotein metabolism in human PEM with or without fatty liver. As well as changes in lipoprotein production, there may be changes in peripheral lipid uptake. Agbedana et al. (155,156) report reduced activity of hepatic triglyceride lipase in kwashiorkor but not in marasmus.
In a recent letter, Dr. Dhansay of Tygerberg Hospital near Cape Town informs me that they have been diagnosing fatty liver in children with kwashiorkor noninvasively by $^{133}$Xenon lung scan. They are measuring plasma apolipoproteins on admission and at day 10. Apo A-1 and A-2 (in HDL) were low on day 1 and normal at day 10. Apo B was also low and rose by day 10, more in VLDL and IDL than in LDL.

Meghelli-Bouchenak et al. (157) in Dijon, France, have recently reported the changes in apolipoproteins in growing male rats on 2% casein or 5% gluten diets, compared with a control diet (15% casein). Apolipoproteins were separated by ultracentrifugation, delipidation, then electrophoresis on different gels for the medium- and high-molecular-weight apolipoproteins. Rats on both very-low-protein diets stopped growing; serum triglycerides fell strikingly, and liver triglycerides rose. The high-density apolipoproteins, A-1, A-11 and A-IV (which are more than 50% synthesized in the intestine), remained essentially unchanged, and so did apo E, while the apolipoproteins of VLDL, apo B and apo C (which mainly originate from the liver), were significantly lower in the protein-depleted animals than in the controls. Reduction of apo B-48 was more striking than the reduction of apo B-100, but rats differ from humans in that they secrete both apo B-48 and apo B-100 from the liver; humans secrete apo B-100 from liver and apo B-48 from the intestine.

There are two practical conclusions from the research on plasma lipids and their relation to fatty liver. One is that a generous protein intake gives quicker clearing of fatty liver in children with kwashiorkor than a low intake, e.g., 1 g protein per kg body weight. This role of protein in treatment might also be applicable in prevention. In communities where kwashiorkor is common, it would be worth carrying out controlled preventive trials with protein-rich supplements.

Second, plasma total cholesterol, a routine clinical biochemistry estimation, is a useful test to indicate the likelihood of fatty liver. We first suggested its use for classifying PEM in 1967 (158). Alleyne et al. (26), in their monograph, state that “serum β-lipoprotein levels are also low in kwashiorkor, but not so in marasmus, and this is probably associated with the development of a fatty liver. Theoretically, therefore, this estimation might be of interest, although Coward and Whitehead (1972) have pointed out that serum β-lipoprotein levels do not fall until albumin lev-
els have dropped below 3 g/dl.” In our work, low total cholesterol was as good a predictor of fatty liver as low β- (or LDL) cholesterol and it is a simpler and more reproducible method. Cholesterol determinations have been neglected in the battery of biochemical tests recommended by Blackburn and followers (159) for adult hospital malnutrition. Plasma cholesterol or LDL should be investigated to see if they are as sensitive to trauma as albumin is (160). Plasma cholesterol might also have some value as a test for following the response of kwashiorkor patients to treatment. Thirty years ago, Schendel and Hansen (95) reported that “serum cholesterol is low prior to therapy and rises significantly as recovery occurs. An arrested rise or fall during the early stages of treatment coincides with the onset of complications or with inadequate therapy. Serial serum cholesterol estimations provide a sensitive biochemical index of success or failure of therapy.”

ACKNOWLEDGMENTS

My main practical experience with PEM was 15 years of stimulating collaboration in Cape Town with my friend Professor John Hansen (now in Johannesburg). He has done more than anyone else to improve the nutrition and health of black children in South Africa.

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DISCUSSION

Dr. Bates: Is there anything known about the effect of protein-energy malnutrition (PEM) on the 7-oxhydroxylase of cholesterol in the liver? The 7-oxhydroxylase is an enzyme that is
affected by marginal vitamin C deficiency and is the first, and rate-limiting, step in cholesterol oxidation (1). Second, studies done in the 1960s on the effect of ethionine (an analog of methionine), which was potent in producing fatty liver in rats, helped to clarify the role of β-lipoproteins in this response (2).

Dr. Truswell: I think that the ethionine fatty liver model in rats is another indication of the role of lipoprotein synthesis in the development of the fatty liver (3). Ethionine is an antagonist to methionine, so that proteins cannot be made in the liver. I know nothing about the activity of cholesterol 7-α-hydroxylase in malnutrition, but I doubt that its reduction could explain the overall picture in which the liver fat is mostly triglyceride. There is definitely a massive rise in the plasma triglycerides when dietary treatment contains protein.

Dr. Jackson: I understand that some of the apolipoproteins have an unusual amino acid profile with a particularly high requirement for leucine. Do you know if it is possible to promote the synthesis of these proteins by any particular dietary protein with an appropriate amino acid pattern?

Dr. Truswell: From the published sequence, 11% of the 4,536 amino acid residues of apolipoprotein B-100 are leucine. I do not know if this is exceptionally high among plasma proteins, but it must be one of the most abundant amino acids in this lipoprotein.

Dr. Pudjiadi: We studied 74 liver biopsies from children with kwashiorkor. There was fatty infiltration in nearly all specimens. Fibrosis was found in 45 cases and necrosis in 12. One boy with fibrosis was followed until the age of 10. While the fibrosis did not disappear, cirrhosis did not develop either (4).

Dr. Truswell: Because fibrosis is not a usual feature in kwashiorkor, one questions whether it might have existed before the onset of kwashiorkor or was caused by an additional toxin or virus (5–8).

Dr. Durie: Can you explain this process of fibrosis and fatty liver in terms of obesity?

Dr. Truswell: I would attribute the fatty liver to the excessive production of fatty acids in the liver, associated with some degree of hypertriglyceridemia. It does not seem to be the result of a block keeping the fat from getting out, but from too much energy coming into the liver.

Dr. Warrier: In several children with PEM, lecithin was found to be high in the red blood cell (RBC) membrane at a time when the osmotic fragility was increased. After protein supplementation, however, the RBC lecithin level decreased and the osmotic fragility returned to normal. What is the mechanism for these changes?

Dr. Truswell: Studies in Uganda also reported increased lecithin in red cell phospholipids (9). However, in our study, the percentage of phosphatidylcholine in plasma phospholipids was unchanged from the beginning to the end of treatment. One thing seems clear: There is no evidence, in humans, that the fatty liver in kwashiorkor is related to choline deficiency.

Dr. Ballabriga: I believe that the sequence of the loss of fatty acids during the process of starvation is dependent on the previous composition of the stores. The pattern of an infant who had been on a diet of coconut oil will definitely differ from one fed another vegetable oil. Do you agree?

Dr. Truswell: I agree. Two additional points. First, in the fatty liver of kwashiorkor, the percentage of essential linoleic acid (which has to come from outside the liver) is the same as that found in the adipose tissue triglycerides (10,11). In other words, the fat in the liver is not being synthesized in the liver; it is coming from the adipose tissue. Second, there is a sharp rise in plasma fasting triglycerides during treatment of the child with kwashiorkor with a low-fat diet. This initial rise is due to the transport of triglycerides from the liver to the periphery. When dietary fat is added, then the plasma lipids are somewhat higher (12).
Dr. Ballabriga: Is it also possible that the decreased rate of oxidation will depend on the quantity of carnitine that is available for transporting free fatty acids to the mitochondria? It would be interesting to study the index free fatty acid/3-hydroxybutyrate. A high index ratio would show that oxidation is reduced. The reduced ability of carnitine to offer fatty acids to the liver might also be considered a factor in premature newborns who are carnitine-deficient.

Dr. Truswell: My review of the literature did not reveal much on β-hydroxybutyrate or ketone bodies in PEM.

I do not consider carnitine deficiency a likely cause of the fatty liver in kwashiorkor. In systemic carnitine deficiency, there is fatty change not only in the liver but also in the voluntary muscles and in the myocardium (13, 14). That combination has never been reported in PEM. In addition, although careful electron-microscopy has been done, the expected morphologically abnormal mitochondria have never been reported in kwashiorkor.

Dr. Ballabriga: Premature and small-for-date infants are in approximately the same metabolic state, relative to the Whitehead index of amino acids (serine + glycine + glutamine + taurine/leucine + isoleucine + valine + methionine), as those with PEM. Contrary to malnutrition, however, values of transferrin are normal in prematures. It is important to be aware in prematures and small-for-dates, especially while on total parenteral nutrition (TPN), of the quantity of available carnitine required for fatty acid transportation.

Dr. Truswell: I would feel more secure if there were more good studies on carnitine in relation to the fatty liver.

Dr. Jackson: As the endogenous synthesis of carnitine requires the involvement of two essential amino acids, it has been suggested as a possible cause of the fatty liver of kwashiorkor. However, from the limited data available, it is difficult to draw a specific association.

Dr. Suskind: The mobilization of vitamins A and E from the livers of children with kwashiorkor is an important factor in the recovery syndrome of malnourished children with fatty, infiltrated livers. I am interested in the distribution of fat in the perportal versus the central lobular area of the liver. Is there a difference in the metabolism of each type of cell, or are the differences due to the different physical location? I also wonder about the role of essential fatty acid deficiency in the skin manifestations of children with marasmus-kwashiorkor and kwashiorkor.

Dr. Truswell: I visualize vitamin E's coming out of the liver being carried on low-density lipoproteins [very-low-density lipoprotein (VLDL) and LDL]. The vitamin A that comes out at the same time seems to be carried on retinol-binding proteins rather than on lipoproteins.

I believe the most prominent skin lesions in PEM are similar to those of pellagra. When we measured urinary n’methylnicotinamide and its 2-pyridine, we found lower excretions in cases with more severe skin lesions (15). The reported essential fatty acid (EFA) deficiency does not appear to be very dramatic or constant. However, it tends to worsen during treatment, provided the treatment did not include polyunsaturated fat.

Dr. Tanner: In reference to carnitine, most cases labeled systemic carnitine deficiency have quite a different pattern of fat deposition, microvesicular rather than one large globule in the hepatocyte. I believe most of these cases are probably inborn errors of metabolism, of which the most common is medium chain acyl dehydrogenase deficiency.

Dr. Truswell: I agree, but the possibility of carnitine deficiency is theoretically possible with the deficiency of the contributory essential amino acids lysine and methionine. While the fatty liver does not look morphologically deficient, we need more studies measuring carnitine accurately in fatty versus nonfatty livers. One way to start would be to compare plasma free and esterified carnitine on admission and during recovery in children with kwashiorkor versus those with marasmus.
REFERENCES