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CHAPTER 2

Nutrition for children with cholestatic liver disease

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ABSTRACT

Cholestatic liver disease (CLD) in children negatively affects nutritional status, growth and development, which all lead to an increased risk of morbidity and mortality. This is illustrated by the fact that the clinical outcome of children with CLD awaiting a liver transplantation is in part predicted by their nutritional status, which is integrated in the Pediatric End-Stage Liver Disease model. Preservation of the nutritional status becomes more relevant as the number of patients waiting for liver transplantation increases and the waiting time for a donor organ becomes prolonged. Nutritional strategies are available to optimize feeding of children with CLD. Patients with CLD, however, form a heterogeneous group and the clinical manifestations of their disease vary. This makes a tailor-made approach for these children crucial. Not all aspects of nutrient metabolism and absorption in children with CLD are well understood and studied. Experiments with stable isotope-labeled triglycerides and fatty acids have provided essential information about fat absorption under physiological and cholestatic conditions in animal models and humans. We expect that in the future, tests using other isotope-labeled macronutrients, i.e. carbohydrates and proteins, can be used to further assess nutritional status of children with CLD thereby creating tailor-made nutritional therapies.

CHOLESTASIS AND NUTRITIONAL STATUS

Cholestatic liver disease (CLD) negatively affects nutritional status, growth and development, particularly in infancy, i.e. when growth rates are highest. The presence of malnutrition and growth retardation (failure to thrive) compromises the clinical outcome for children with end-stage liver disease. Cholestatic children with a poor nutritional status, who require liver transplantation have an increased risk of morbidity and mortality^{1,2}. To illustrate this, the nutritional status is an important contributor to Pediatric End-stage Liver Disease (PELD) score for children under 2 years of age. The PELD score is a reliable predictor of mortality in children with CLD on the waiting list for liver transplantation. The PELD model was implemented by the United Network of Organ Sharing (UNOS) in the United States in February 2002 as an improved algorithm for allocating livers among pediatric orthotopic liver transplant candidates, and will be adopted by the Eurotransplant society in January 2007. It has recently been shown that the PELD score also correlates with posttransplant survival³. Preservation of the nutritional status becomes more relevant as the number of patients waiting for liver transplantation increases and the waiting time for a donor organ becomes prolonged.

NUTRITION FOR CHOLESTATIC CHILDREN

Poor dietary intake is an important factor in the pathophysiological basis of malnutrition in children with CLD. Furthermore, their nutritional status may be further compromised by decreased absorption of macronutrients, including fat, carbohydrates and proteins⁴. At an early age, fat accounts for the most important dietary energy source (up to 50% of total ingested energy). Essential fatty acids (EFA) and long-chain polyunsaturated fatty acids (LCPUFA) are indispensable for proper development and function of different organs, for example the central nervous system. Micronutrient absorption may also be affected in CLD, including absorption of fat-soluble vitamins A, D, E and K.

The dietary prevention or treatment of failure to thrive during CLD involves some general principles applicable to virtually all patients and some more individual tailor-made approaches. Nutrition in infancy consists predominantly of breast milk or formula. For children with CLD, the dietary energy intake is usually increased to levels of 120-150% of recommended daily energy intake (corrected for age and gender). The adaptation of the formula diet usually involves increasing the concentration and amount ingested. In addition, up to 60% of the fat components, particularly long-chain triglycerides are substituted by medium-chain triglycerides (MCTs), whose absorption can occur relatively independently from the presence of bile components in the intestinal lumen. The carbohydrate content can be increased by supplementation of formula with maltodextrin. Breastfed children receive additional formula and MCT-rich oil, while for older children feeding with formula is often prolonged and energy-rich liquids are provided. Adequate absorption of fat-soluble vitamins during CLD can usually be obtained by considerably increasing the dosages administered daily, well above regular recommendations for the age groups. Serum levels of fat-soluble vitamins are regularly monitored, in order that dosages can be adapted. Adequate intake of EFA and LCPUFA is not frequently monitored in CLD patients, but should be reached when these fatty acids are provided in ample amounts in the diet. Nevertheless, we reported that ~70% of children with CLD requiring liver transplantation have biochemical indications of EFA and LCPUFA deficiency⁵.

Reduced gastric volume, vomiting, ascites and hypoglycemia lead to limited absorption of the required dietary nutrients when administered in regular (bolus) feedings. Under these circumstances, continuous nasogastric drip feeding may be needed to guarantee maximal uptake of nutrients.

For some time now, a special formula for infants with CLD has been available. The composition of this formula aimed to accommodate the general aspects of nutritional support needed for infants with CLD. So far, however, no data have become available to substantiate its benefit, nor its advantage over conventional dietary treatment: supplementation of MCT-rich formulas with carbohydrates, fat-soluble vitamins and EFA. Clinical data are needed to determine the role that this formula can play in the dietary treatment of infants with CLD.

BIOLOGICAL ASPECTS OF NUTRITION FOR CHOLESTATIC CHILDREN

Cholestatic diseases in children

CLD is characterized by decreased or absent hepatic secretion of bile into the intestine. The most common cause of CLD in children requiring liver transplantation is biliary atresia. Biliary atresia is a progressive disorder characterized by an inflammatory reaction towards the extrahepatic and intrahepatic bile ducts, leading to their destruction and subsequent replacement by fibrotic scar tissue. The etiology of biliary atresia remains unknown, although an inflammatory reaction to a detrimental stimulus seems to play an initiating role. Suggested initiating stimuli include specific perinatal viral infections, genetic factors, defects in immune response, as well as defects in morphogenesis. Another disease that can lead to end-stage liver disease in infancy is Alagille's syndrome, an autosomal dominantly inherited syndrome including bile duct hypoplasia, and congenital anatomical defects in other organs. Progressive Familial Intrahepatic Cholestasis (PFIC) is also a genetically transmitted disorder, but is inherited in an autosomal recessive fashion. Three phenotypic forms of PFIC have been characterized and attributed to gene defects in three different genes (PFIC1-3). Another cause of CLD is nonsyndromic paucity of the intrahepatic bile ducts, which is suggested to be the result of various infections, chromosomal disorders or metabolic disorders. Finally, inborn errors in bile acid synthesis account for part of the children with CLD. Defects have been identified in enzymes catalyzing cholesterol catabolism and bile acid synthesis⁶.

CLD in adolescents and young adults is often due to autoimmune hepatitis, primary biliary cirrhosis or primary sclerosing cholangitis^{6,7}.

Although the causes and clinical manifestations of CLD may vary, it is often accompanied by liver damage. The obstruction or absence of bile ducts leads to accumulation of bile acids in hepatocytes, which results in liver damage. Because the enterohepatic circulation of bile acids is interrupted, the resulting absence of bile acids in the intestinal lumen leads to impaired micellization and therefore to strongly reduced absorption of fats and fat-soluble nutrients. Another feature of CLD is the high serum bile acid level, which can cause secondary tissue injury.

In biliary atresia, it is often attempted to correct the enterohepatic circulation of bile acids by performing a Kasai portoenterostomy. During this procedure the liver is directly connected to the proximal small intestine to optimize bile flow into the intestine as much as possible.

However, Kasai portoenterostomy is frequently only a transient solution, due to the presence of intrahepatic bile duct damage and ongoing liver damage. Most patients with biliary atresia eventually need liver transplantation. As is pointed out above, the nutritional status of children with CLD is important for the clinical outcome of liver transplantation and for long-term survival after liver transplantation. Besides the obviously reduced absorption of fats and fat-soluble vitamins, chronic cholestasis also affects dietary intake, energy metabolism and metabolism of macronutrients as well as micronutrients.

DIETARY INTAKE AND ENERGY EXPENDITURE

Dietary intake

Reduced dietary intake is an important contributor to malnutrition in children with CLD. Fatigue, anorexia, nausea, vomiting, diarrhea, altered or reduced ability to taste, and early satiety may all contribute to decreased ingestion of food. Organomegaly and ascites can further compromise dietary intake by reducing gastric capacity. Additionally, many diet modifications, for example sodium, fluid or protein restrictions, make food even more unpalatable. These dietary restrictions are imposed on patients with relatively high risks of fluid overload and encephalopathy, which, when left untreated, can lead to serious irreversible defects⁸.

Energy metabolism

Energy expenditure is composed of the basal metabolic rate (BMR), the amount needed for growth and metabolism. Although clinical data are conflicting, some children with CLD have been shown to have an increased BMR. Shanbhogue *et al.*⁹ reported a higher BMR, when related to lean tissue in patients with end-stage liver disease. In children with biliary atresia energy expenditure was 29% higher than healthy controls¹⁰. Also Shepherd¹¹ reported higher energy expenditure per unit body cell mass in children with biliary atresia. In contrast, Muller *et al.*¹² found that patients with cirrhosis showed a variable BMR, in the range from hypometabolic to hypermetabolic. Another study showed an unchanged BMR in children with Alagille syndrome¹³. A hypermetabolic state could be an important factor in the clinical outcome for CLD, because it further aggravates nutritional status.

Recently, Watanabe *et al.*¹⁴ found that bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. In this study, mice were fed a bile acid (cholic acid)-containing high fat diet. These mice showed subsequent reduction in weight gain, white adipose tissue weight and brown adipose tissue weight compared to mice on a high fat diet. In addition, animals fed a high fat diet containing cholic acid had higher CO₂ production and O₂ consumption, indicating a higher level of energy expenditure. Human skeletal muscle myocytes showed an increase in O₂ consumption after treatment with bile acids. It is presently unknown, however, whether bile acid accumulation in patients with CLD is partly responsible for increased energy expenditure.

NUTRIENT METABOLISM

Apart from reduced intraluminal bile acid concentrations, other consequences of CLD, such as gastrointestinal bleeding, impaired digestive enzyme production and secretion, mucosal congestion, villous atrophy, bacterial overgrowth or pancreatic insufficiency, can lead to

malabsorption and malabsorption of nutrients. In addition, even certain medications can aggravate malabsorption. For example, cholestyramine binds to bile acids in the intestinal lumen and thereby further reduces absorption of fat-soluble nutrients. Also, reduced availability of specific nutrients involved in digestion and/or absorption of other nutrients, specifically vitamins and minerals, affects intestinal absorption⁸. In the remaining part of this article we will focus on metabolism of fat, carbohydrates, protein and micronutrients in CLD.

Fat metabolism

CLD is characterized by malabsorption of fat. Especially long-chain triglycerides, which are digested to fatty acids and monoacylglycerols in the intestinal lumen, are poorly absorbed during cholestasis, due to their impaired micellization during bile deficiency.

The route that fat undertakes from the diet to the blood can be divided in four steps: emulsification, lipolysis (lipases), solubilization (bile) and translocation (mucosa). During lipolysis lipases catalyze the conversion of triglycerides to glycerol and fatty acids. The latter need to be solubilized by bile acids to be transported towards the vicinity of the mucosa. Here, at the unstirred water layer, the micelles disintegrate, after which fatty acids and monoacylglycerols are taken up across the apical membrane of the mucosal cells. Inside the enterocytes, the absorbed lipids are reacylated to triacylglycerols, assembled into chylomicrons, which are then secreted into lymph and subsequently appear in the circulation.

In our laboratory, Kalivianakis *et al.*¹⁵ developed a stable isotope test to quantify lipolysis and absorption of long-chain fatty acids in rats. We determined absorption and appearance in plasma of ¹³C-labeled palmitic acid in rats with malabsorption either due to chronic bile deficiency (permanent bile diversion as developed in our laboratory by Kuipers *et al.*¹⁶) or due to oral administration of the lipase inhibitor Orlistat. These models were used to discriminate between potential causes of fat malabsorption such as impaired intestinal lipolysis or reduced uptake of fatty acids. Rats were given a high fat diet (35% of total energy) compared to a low fat diet (14% of total energy) to potentiate the effect of fat malabsorption. Results were compared with the percentage absorption of ingested fat determined by fat balance. As expected, dietary fat absorption was significantly impaired in bile-deficient animals as compared to controls (Fig 1D). However, the net fat uptake (Fig 1C), defined as the difference between the amount of fat ingested (Fig 1A) and the amount of fat excreted ('lost') via the feces (Fig 1B) through fat malabsorption, was not significantly affected by the presence or absence of intestinal bile (control vs. chronic bile diversion), or the amount of fat in the diet (high fat vs. low fat). The percentage of total ingested fat absorbed in bile-deficient rats was only 87% with the low fat diet and 54% with the high fat diet (Fig 1D). Apparently, bile deficiency (without cholestasis) increases the nutrient ingestion in rats, compensating sufficiently for the increased energy and fat loss via the feces. Bile deficiency due to bile diversion in rats led to a decreased concentration of plasma ¹³C-palmitic acid, indicating impaired absorption of long-chain fatty acids. Control experiments showed that lipolysis was not affected in bile-deficient rats. Impairment of fat absorption due to Orlistat had no effect on plasma ¹³C-palmitic acid, indicating the specificity of the test¹⁵. This test can also be utilized to generate clinical data as was demonstrated by Rings *et al.*¹⁷. Rings *et al.* showed that the absorption of free fatty acids, but not fat digestion was rate-limited in neonates, and developed to adult competence within 2 months after term age. Neonates are known to have a mild 'physiological' cholestasis during the first months of life, and this may be the mechanism underlying this observation.

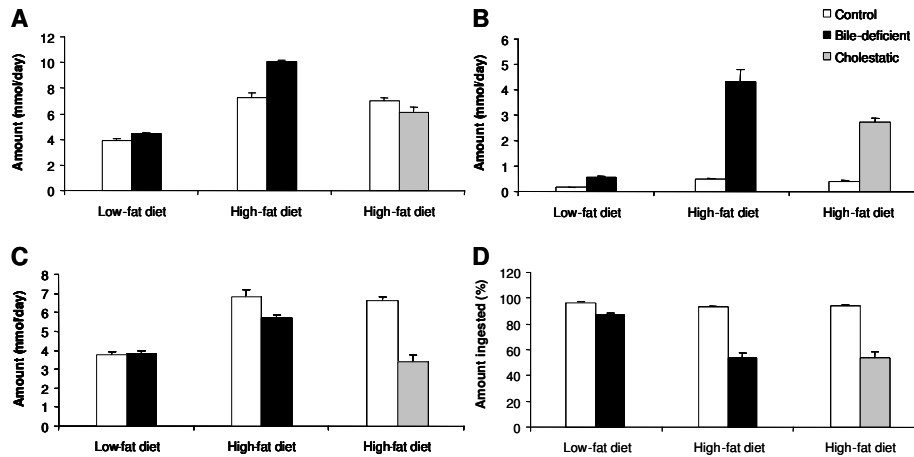


Figure 1 Fat absorption in bile-deficient and cholestatic rats on low fat and high fat diet, with (A) fat intake, (B) fecal fat excretion, (C) net fat uptake, and (D) dietary fat absorption as % of amount ingested. [adapted from 15 and 18].

EFA and LCPUFA are crucial for normal development and function. They cannot be synthesized endogenously and therefore must be provided by the diet. As is reviewed by Sealy *et al.*⁵, the percentage of n-3 and n-6 fatty acids is reduced in pediatric cholestasis. This observation reflects the inability of CLD patients to absorb sufficient amounts of EFA and LCPUFA, due to absence of bile in the intestinal lumen in combination with frequently compromised dietary intake. An important consequence of this inability to acquire sufficient amounts of LCPUFA is EFA deficiency. The quantitatively most important EFAs are linoleic acid (LA) and α -LA, members of the n-6 and n-3 family of fatty acids. Linoleic and linolenic acid are precursors for LCPUFAs, including arachidonic acid (C20:4n-6), eicosapentaenoic acid (C22:5n-3), and docosahexaenoic acid (C22:6n-3), respectively.

Minich *et al.*¹⁸ investigated fat malabsorption as a possible cause of EFA deficiency in rats that were intestinally bile deficient due to either permanent bile diversion or to bile duct ligation (cholestasis). Absorption of the EFA LA was quantified by fat balance and by measuring plasma concentrations of [¹³C]-LA after its intraduodenal administration. Plasma concentration of [¹³C]-LA was decreased in bile-diverted rats, while net absorption of LA from the intestine was unaffected. The fact that net absorption of fat and LA was not affected in bile-deficient rats corresponded with increased food intake (see above) in addition to relative preservation of EFA absorption under bile-deficient conditions, in comparison with nonessential saturated fatty acids, such as palmitic acid (C16:0) and stearic acid (C18:0). In cholestatic rats, however, both plasma concentration and net absorption of unlabeled or [¹³C]-labeled LA were decreased. Metabolism of LA into arachidonic acid was not affected, indicating that LA deficiency in these rats is due to decreased net absorption. The compensatory increase in nutrient ingestion during intestinal bile deficiency on the basis of permanent bile diversion (see above) did apparently not occur in rats with intestinal bile deficiency on the basis of bile duct ligation. It is tempting to speculate that the accumulation of bile products during the latter cholestatic condition abolishes the compensatory mechanism of increased nutrient ingestion. This observation is in accordance with clinical experience that nutrient intake and appetite are compromised in children with CLD.

Children with CLD are sometimes given a fat-restricted diet, since effects such as steatorrhea/diarrhea are expected. According to the study of Kalivianakis *et al.*¹⁵ the amount and fraction of dietary fat lost via the feces is indeed significantly less on a low fat than on a high fat diet (Fig 1B). However, net fat uptake from a high fat diet was almost 2-fold higher than that from a low fat diet (Fig 1C). The net absorption reflects the amount of nutrients (fat) that actually becomes available for energy and growth needs of CLD patients. This observation underlines a clinical strategy to increase, rather than to restrict the amount of fat intake in patients with CLD, even at the expense of steatorrhea, in order to maximize their nutritional condition.

Carbohydrate metabolism

In children with CLD, carbohydrate homeostasis can be affected by hepatic failure itself, for example by a decreased capacity of gluconeogenesis. Frequently also peripheral utilization of glucose is reduced, which may decrease the risks of hypoglycemia. In CLD, hepatic degradation of insulin may also be decreased, which may be one of the causes for the 2-fold higher insulin response in CLD compared to control patients. Elevated plasma levels of insulin in combination with glucose tolerance imply insulin resistance, which could be further aggravated by increased circulating free fatty acids as seen in CLD⁸.

Apart from the hepatic effects on glucose homeostasis, the intestinal carbohydrate absorption could also be affected by CLD. However, no data are yet available about carbohydrate absorption under cholestatic conditions. Recently our group has started to investigate intestinal carbohydrate absorption under cholestatic conditions *in vitro* and *in vivo*. The underlying hypothesis for this research is that cholestasis, i.e. high serum bile acid concentrations could alter intestinal function by affecting proliferation, differentiation and/or apoptotic cell death of the absorptive intestinal epithelium¹⁹. These effects of cholestatic bile acid concentrations on other cell types have been well delineated. For example, relatively low concentrations of bile acids induce apoptosis in hepatocytes²⁰.

Protein metabolism

The catabolic reduction in total body protein as seen in CLD is mainly due to extensive liver damage. Tavill²¹ and McCullough and Glamour²² found no significantly changed protein turnover in CLD patients. Amino acid oxidation is normal or reduced in these patients, consistent with appropriate adaptation to reduced nutrient supplies. Due to hepatic insufficiency occurring in later stages of CLD, oxidation of aromatic amino acids is reduced as is the metabolism of branched-chain amino acids. Mager *et al.*²³ reported an increased dietary need for branched-chain amino acids in children with mild-to-moderate chronic CLD, due to increased postabsorptive leucine oxidation. In general, amino acids seem to be conserved in CLD, probably due to the body's ability to increase protein synthesis and reduce amino acid oxidation. This increased protein synthesis is, however, at the cost of muscle proteolysis^{21,22}. Increased protein oxidation resulted in a virtually zero nitrogen balance in children with biliary atresia and even in oxidation of endogenous proteins¹⁰. Stable experimental conditions do not necessarily reflect the spectrum of clinical conditions frequently encountered, such as episodes of metabolic stress or infections that can increase protein turnover and catabolism⁸. Interestingly, Sokal *et al.*²⁴ found that branched chain amino acids improve body composition and nitrogen balance in a rat model of extrahepatic cholestasis.

Overall, addition of proteins, and especially specific amino acids such as branched chain amino acids, could improve nutritional status of children with CLD. However, care must be taken, because an excess of protein can negatively influence encephalopathy.

Micronutrient metabolism

The absence of bile acids in the intestinal lumen as observed in cholestasis reduces absorption of fat-soluble vitamins A, D, E and K, as briefly described above. Calcium uptake is at risk as a result of formation of nonsoluble calcium fatty acid soaps during fat malabsorption. Hypovitaminosis D may increase renal loss of phosphate and hypovitaminosis A may induce zinc deficiency. Zinc deficiency has a negative impact on cognitive function, appetite and taste, immune function, wound healing and protein metabolism. In addition, zinc deficiency has frequently been associated with EFA deficiency²⁵. Finally, uptake of selenium can be disturbed due to EFA deficiency and iron depletion is seen as a result of gastrointestinal bleeding, insufficient uptake, transport and handling of iron. In addition, liver dysfunction strongly reduces storage capacity of vitamins such as folate, riboflavin, nicotinamide, pantothenic acid, pyroxidine, vitamin B₁₂, thiamine and vitamin A. Hepatocellular injury in CLD also results in defects in vitamin activation, conversion, release and transport⁸.

As described above, children with CLD receive higher dosages of fat-soluble vitamins. Furthermore, addition of zinc to the diet could counteract a part of the poor dietary intake.

CONCLUDING REMARKS

Influencing nutritional intake and metabolism is a critical aspect of the management of children with CLD. Patients with CLD form a heterogeneous group and the clinical manifestations of their disease vary. This makes a tailor-made approach for these children crucial. Not all aspects of nutrient metabolism and absorption in children with CLD are well understood and studied. Experiments with stable isotope-labeled triglycerides and fatty acids have provided essential information about fat absorption under physiological and cholestatic conditions in animal models and humans. We expect that in the future, tests using other isotope-labeled macronutrients, i.e. carbohydrates and proteins, can be used to further assess nutritional status of children with CLD, thereby creating tailor-made nutritional therapies.

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