Hypoleptinemia Associated with Hypermetabolism, Low Adiposity Body Composition and Overeating Behavior in Children with Jaundiced Biliary Atresia

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(Received on March 29, 2001; accepted on Sept. 13, 2001)

Abstract

Biliary atresia (BA) is one of the most common of liver disease during childhood in Japan. BA is a cicatrical obstruction of extra-hapatic biliary system in the neonatal age. This disease requires corrective surgery on the porta hepatis, following reconstruction of bile draining intestinal tube, within 2 months of age ideally. BA children develop malnourishment when their jaundice is not solved without proper drainage of bile juice and with progressive advancement of liver cirrhosis.

However they have elevated energy expenditures and have kept normal appetites. We retrospectively studied the energy balance, body composition, and serum hormone concentrations in 18 BA children (age ranged between 1–13 years). The patients were divided into two groups: the jaundiced group (group J), in whom the serum bilirubin level was ≥1.0 mg/dl; and the non-jaundiced group (group non-J), in whom the serum bilirubin level was <1.0 mg/dl. Anthropometric indices studied included height, weight, arm muscle area, and fat area as calculated by mid-upper arm circumference and triceps skin fold. An energy balance study was conducted, and serum interleukin (IL)-6 and serum leptin levels were measured. Lower adiposity, higher energy intake (overeating), higher energy expenditure, higher serum IL-6 levels, and lower serum leptin levels were found in group J compared with group non-J. To our knowledge, this is the first report correlating overeating with hypoleptinemia in pediatric BA patients with jaundice. Serum leptin levels may play a key role in eating abnormalities with persistent jaundice.

Key words: Biliary atresia; malnutrition; hypermetabolism; hypoleptinemia; overeating

Introduction

BA is a cicatrical obstruction of extra-hapatic biliary system in the neonatal age. This disease requires corrective surgery on the porta hepatis, following reconstruction of bile draining intestinal tube, within 2 months of age ideally.2 The corrective surgery ends in failure in many ways, resulting in J group patients: 1) when the operation is too late, 2) when the surgery does not give proper recovery of bile drainage, and 3) when the bile drainage is not enough to prevent ascending cholangitis. With successful operation, the post-operative cases fall into non-J group and have a good hope to have complete cure. So the conditions in non-J group is very sign to have excellent long term results. Postoperative liver dysfunction results in hyperbilirubinemia, and the Kupffer cells cannot eliminate endotoxins invading the portal vein flow through the congested intestinal mucosa of the GI tract.3–5 The endotoxinaemia stimulates the monocyte-macrophage system to produce proinflammatory and inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6.6 Jaundiced pediatric BA patients usually exhibit hypermetabolism.6 High serum IL-6 levels explain this hypermetabolism, since this cytokine suppresses appetite.7,8 While cirrhotic patients usually lose their appetites, jaundiced children with BA maintain their appetites until end-stage liver disease without encephalopathy. This maintenance of appetite is paradoxically associated with malnutrition. However, little is known about the pathogenesis of overeating in jaundiced BA patients.

Leptin, a 167-amino acid peptide hormone, is mainly expressed in mature adipocytes and/or white adipose tissue (WAT). Leptin receptor in hypothalamus is integral factor to regulate appetite, adiposity in body composition, and energy expenditure (EE).
Thus the discovery of leptin marked a major breakthrough in our understanding of body weight regulation and of the role of fat tissue as an endocrine organ. Leptin mRNA and/or peptide are produced by the placenta, fetal tissue, and hepatic stellate cells and are involved in numerous physiological functions such as fetal growth, gut-derived satiety, immune or proinflammatory responses, reproduction, intestinal absorption of nutrients, angiogenesis, and lipolysis.

This paper reviews the involvement of leptin in body weight homeostasis and obesity and in peripheral functions. It is proposed that the regulation of the endocrine system during starvation is the main physiological role of leptin. In the starved state, the serum leptin concentration decreases and the amount of food intake is increased. Because leptin expressed by adipocytes plays a key role in appetite regulation, we hypothesized that low serum leptin concentrations in malnourished children with BA and jaundice result in overeating and maintenance of appetite. Leptin hyposecretion associated with low adiposity may therefore stimulate appetite, and subsequent overeating occurs in an attempt to increase adiposity. We conducted energy balance analyses, assessed body composition, and measured serum leptin concentrations in jaundiced and nonjaundiced children with BA.

**Patients and Methods**

**Patients**

Eighteen pediatric patients (8 girls and 10 boys) with BA, ranging in age from 1 year and 8 months–13 years, who had undergone the Kasai procedure in our institution and had been followed up for more than 1 year, were enrolled in this study. Kasai procedures were done during infantile periods before 80 day-old. Informed written consent for study participation was obtained from the guardians of all the children, and the study protocol was approved by the Institutional Review Board.

The 18 patients were divided into two groups: the jaundiced group (group J, n=8), in whom serum bilirubin levels were >1.0 mg/dl; and the non-jaundiced group (group non-J, n=10), in whom serum bilirubin levels were <1.0 mg/dl.

**Methods**

All study participants underwent duplicate measurements of height and weight. Then height for age (H/A) and weight for height (W/H) were calculated and measured height and weight.

Mid-upper arm circumference (MAC) and triceps skin fold (TSF) were measured to calculate body composition. MAC was measured using a flexible, nonstretchable fiberglass tape, and TSF was measured using a Lange caliper (Cambridge Scientific Industries, Inc., Cambridge, Maryland, USA). Then the arm muscle area (AMA) and arm fat area (AFA) were calculated using the following formulae:

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AMA = \frac{(MAC - TSF) \times \pi}{2/4 \times \pi} (mm^2)
\]

\[
AFA = \text{arm area} - \text{AMA} = \left(\frac{(MAC)^2}{4 \times \pi}\right) - \text{AMA} (mm^2),
\]

where MAC and TSF are expressed in millimeters. To standardize AMA and AFA for both sexes at various ages, the calculated AMA and AFA values were divided by a standard value for the same sex and age. These standardized values were expressed as %AMA and %AFA.

Twenty-four-hour food records were taken for 3 consecutive days to evaluate the dietary history of the BA patients. A registered dietitian determined the amount of calories ingested from carbohydrate, fat, and protein. Calories from carbohydrate (CHOi), fat (Fi), and protein (Pi) were expressed as CHOi/Fi/Pi (kcal/kg/day). Their percentages in total calories ingested were expressed as %CHOi/%Fi/%Pi.

Spot measurements of EE were made using the indirect calorimetry method using the Deltatrac™ (Datex Instrument Corporation, Helsinki, Finland). EE was measured 3 hours following the evening meal to determine the timing of the postabsorptive period. The EE of 90 pediatric patients admitted to our institution for inguinal herniotomy were also measured as healthy control values.

Spot urine samples were used to analyze urinary nitrogen (UN) levels. UN and the respiratory quotient measured by indirect calorimetry were used to calculate EE from carbohydrate, fat, and protein and expressed as CHOi/Fo/Po (kcal/kg/day). In the same manner as for energy intake, the percentage of calories from each nutrient in total EE was expressed as %CHOi/%Fo/%Po.

Serum IL-6 levels were measured by radioimmunoassay as a parameter of surgical stress response. IL-6 levels were compared from aspect of body mass index (BMI) between two groups of group J and group non-J. Serum leptin levels were determined by enzyme-linked immunosorbbent assay.

**Statistical analyses**

Statistic analysis was performed using the unpaired Student-t test using Statview, version 4.5 (Abacus Concepts, Inc.). All results are expressed as mean±SD. A p value of <0.05 was considered statistically significant.

**Results**

The results of this study can be summarized as follows. The H/A and W/H values of group J did not
differ significantly from those of group non-J (HA 102.2±2.3% versus 100.8±3.5%, respectively; W/H 97.5±3.4% versus 100.0±3.3%, respectively). However, the %AMA and %AFA in group J were significantly lower than those in group non-J (%AMA 89.0±3.5% versus 96.0±4.1%, respectively; %AFA 88.0±3.1% versus 94.0±2.5%, respectively; p<0.05).

The energy intake in group J was 150.7±15.5 kcal/kg/day, which was significantly higher than that in group non-J of 95.1±11.24 kcal/kg/day (Table 1). Fi in group J was significantly higher than in group non-J (p<0.025) (Table). The EE in group J was also significantly higher than that in group non-J (98.0±12.0 kcal/kg/day versus 55.6±8.9 kcal/kg/day, respectively; p<0.05). In group J, CHOo was 48.9±3.3% and Fo was 31.9±3.5%, Po was 18.3±3.2%; in group non-J the corresponding values were 38.5±5.2% and 11.8±4.6%, 5.2±2.4%. These differences were all statistically significant (p<0.01) (Table 1).

Serum IL-6 levels were significantly higher in group J than in group non-J (8.9±1.1 pg/ml versus 2.1±0.7 pg/ml, respectively; p<0.01) (Figs. 2 and 3). Serum IL-6 levels did show linear relationship between serum bilirubin levels (Fig. 3). However, serum leptin levels were significantly lower in group J (0.50±0.20 ng/dl) than in group non-J (2.61±0.48 ng/dl) (p<0.05) (Fig. 4). And in two groups, serum leptin seems to increase according to increase of its adiposity (Fig. 4).

These results indicate that the jaundiced BA patients exhibit hypermetabolism and overeating, low carbohydrate oxidation, increased oxidation of lipids in EE, and high serum IL-6 levels. In our series of BA patients, cirrhotic children have hypoleptinemia.
Fig. 2. Serum IL-6 levels in group J and group non-J.

Fig. 3. Correlation between serum bilirubin and IL-6 level.

Fig. 4. Correlation between serum leptin level and fat volume (%AFA) in group non-J (open circles) and group J (closed circles).
Discussion

Cirrhotic patients easily develop malnutrition. Fat-related disorders, including fat maldigestion, fat malabsorption, essential fatty acid deficiency, and fat-soluble vitamin deficiency, are often observed in such patients. Weight is not an appropriate measurement for the evaluation of malnutrition in cirrhotic children with BA due to their massive ascites. As shown in the present study, indices of muscle and fat volume are clinically more useful in evaluating nutritional status and body composition than weight and height. However, these indices are calculated from only a limited body area, e.g., a single arm. More practical parameters for the assessment of body composition are therefore necessary.

The cirrhotic liver cannot eliminate endotoxins or metabolize adrenergic hormones. Elevated serum endotoxins and catecholamines lead to hypermetabolism. Studies in rats have demonstrated that serum endotoxin levels were significantly elevated in the portal vein in an ethanol-induced cirrhosis model. This may be partly caused by impaired intestinal integrity with intestinal epithelial congestion due to portal hypertension and partly caused by the inability of the cirrhotic liver to eliminate serum LPS.

We investigated serum IL-6 levels to determine whether they were correlated with the degree of liver dysfunction, malnutrition, and eating abnormalities in jaundiced patients. In our patient series, serum IL-6 levels showed a linear relationship with serum bilirubin levels. To our knowledge, this is the first paper to clarify linear correlation of serum IL-6 and bilirubin levels in BA children. Serum bilirubin level is a feasible indicator of hepatocyte metabolic function. If this function is impaired, hepatocytes cannot eliminate endotoxins, stimulating macrophages to produce proinflammatory and inflammatory cytokines. The high serum IL-6 levels in our pediatric BA patients with jaundice may be explained by IL-6 secretion from endotoxin-stimulated macrophages.

Increased resting EE in jaundiced BA patients has been reported previously. Endotoxinemia and hypercytokinemia can result in hypermetabolism in jaundiced patients. The mechanism of the development of hypermetabolism in cirrhosis is poorly understood, however. Some investigators reported that elevated plasma IL-6 levels in cirrhotic patients with ascites occurred independently of the severity of liver damage. However, other groups reported that liver damage activated the monocyte-macrophage system to produce cytokines in the liver. There have been clinical observations that plasma IL-6 and endotoxin levels increase progressively as the severity of liver dysfunction increases, as graded by the Pugh classification, in cirrhotic patients.

Serum IL-6 concentrations have been demonstrated to have a linear relation between clinical Child score and serum IL-6 and IL-6 receptor mRNA are also overexpressed in damaged biliary epithelial cells. These findings suggest that damaged biliary epithelial cells and/or the monocyte-macrophage system are stimulated to enhance IL-6 mRNA levels and produce IL-6. These IL-6 molecules then enter in the systemic bloodstream. Elevated IL-6 levels may indicate not only a proinflammatory reaction but also the degree of insult to biliary epithelial cells when jaundiced BA patients are experiencing deterioration of their condition.

In animal experiments, IL-6 administration resulted in increased EE. Several cytokines, such as TNF-α and IL-6, are thought to suppress appetite. In the present study, records of 24-hour food intake showed that jaundiced pediatric BA patients have overeating behavior. Because the coexistence of hypercytokinemia and overeating appears paradoxical, we focused on adiposity and serum leptin levels in an attempt to clarify the reason for overeating in our series of patients. Anthropometric measurements showed that jaundiced children with BA had lower adiposity. Because leptin functions as a lipostat to regulate adiposity and appetite, if adiposity increases, leptin production is enhanced to suppress appetite. Therefore the overeating in jaundiced children with BA can be explained by hyposecretion of leptin even with low adiposity. Moreover, the significantly high fat ox-
idation ratio and higher EE in group J explain the lower adiposity and lower serum leptin levels.

Generally, the energy difference between intake of calories and EE is utilized for daily activities, diet-induced thermogenesis, and developmental energy costs during childhood. In the present study, the two groups had a similar energy balance. Jaundiced pediatric BA patients easily develop maldigestion and malabsorption because of the impairment of bile production and secretion. Although the energy balance was similar between the two groups, the actual energy absorption rate in group J may be lower than that in group non-J. Therefore the calculated energy intake in group J may have been overestimated and the energy balance underestimated compared with actual values. Developmental delays in jaundiced children may result partly from resistance to growth hormone with impaired liver function, nor impaired insulin-like growth factor I production.

In the present study, jaundiced children with BA had decreased protein and fat body composition levels, high food intake, high EE, and low serum leptin levels compared with the nonjaundiced group. Theoretically, these could be corrected by altering the energy balance, adiposity, and leptin levels (Fig. 5). Leptin, like some other cytokines, suppresses appetite through the STAT3 pathway, as observed in the IL-6 family. Serum leptin secreted by WAT reaches the hypothalamus to regulate neuropeptide Y (NPY) and orexin (OX) neurons, which both stimulate appetite.

NPY-deficient mice, however, exhibit normal food intake patterns. When OX is injected into the third ventricle in mice, metabolic rates rise within 30 minutes. Therefore a redundant food intake regulation system must exist, involving leptin, NPY, OX, and melanocortins.

It has been reported that cirrhotic patients have insulin resistance that enhances fat oxidation and suppresses CHO oxidation. Another study showed that in mice fed safflower oil (SO) or fish oil (FO) for 5 months, only those fed SO developed obesity. FO-fed mice had enhanced expression of uncoupling protein 2 (UCP-2), as assessed by Northern blotting. A similar stimulation of UCP-2 expression might occur in malnourished pediatric BA patients with jaundice. The present study did not investigate NPY, OX, or UCP-2 involvement in malnutrition and overeating. Further investigations are required to clarify the mechanism of hypermetabolism, high fat, and low CHO oxidation rates, and overeating observed in our group J.

References