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Ghrelin, leptin and insulin in cirrhotic children and adolescents: Relationship with cirrhosis severity and nutritional status ☆

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ABSTRACT

Objective: Ghrelin, leptin, and insulin concentrations are involved in the control of food intake and they seem to be associated with anorexia–cachexia in cirrhotic patients. The present study aimed to investigate the relationship between the nutritional status and fasting ghrelin, leptin and insulin concentrations in pediatric cirrhotic patients.
Methods: Thirty-nine patients with cirrhosis and 39 healthy controls aged 0–15 years matched by sex and age were enrolled. Severity of liver disease was assessed by Child–Pugh classification, and Pediatric for End Stage Liver Disease (PELD) or Model for End-stage Liver Disease (MELD) scores. Blood samples were collected from patients and controls to assay total ghrelin, acyl ghrelin, leptin and insulin by using a commercial ELISA kit. Anthropometry parameters used were standard deviation score of height-for-age and triceps skinfold thickness-for-age ratio. A multiple linear regression analysis was used to determine the correlation between dependent and independent variables.
Results: Acyl ghrelin was significantly lower in cirrhotic patients than in controls [142 (93–278) pg/mL vs 275 (208–481) pg/mL, $P = 0.001$]. After multiple linear regression analysis, total ghrelin and acyl ghrelin showed an inverse correlation with age; acyl ghrelin was associated with the severity of cirrhosis and des-acyl ghrelin with PELD or MELD scores ≥ 15. Leptin was positively correlated with gender and anthropometric parameters. Insulin was not associated with any variable.
Conclusion: Low acyl ghrelin and high des-acyl ghrelin concentrations were associated with cirrhosis severity, whereas low leptin concentration was associated with undernourishment in children and adolescents with cirrhosis.

1. Introduction

Anorexia and undernutrition state are problems of paramount importance in children and adolescents with cirrhosis [1]. High energy and growth requirements make infants and children with cirrhosis vulnerable to the debilitating effects of undernourishment [2,3]. Severely malnourished infants with end-stage liver disease are at increased risk for pre- and post-liver transplantation morbidity and mortality [4]. Anorexia is a great problem in children and adolescents with cirrhosis, and may be an important contributing factor to undernourishment [5,6]. Reduced food intake may follow the early satiety associated with the presence of large-volume ascites or hepatosplenomegaly, leading to a clinical picture of functional gastric outlet obstruction. Poor palatability of a restricted diet may also cause anorexia [7]. The mechanisms of undernourishment and anorexia in cirrhosis are not completely understood. Both poor dietary intake and increased basal energy expenditure have been

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reported to contribute to a hypermetabolic state in patients with cirrhosis [1,2].

Ghrelin and leptin are involved in the control of food intake and energy homeostasis by gut–brain interactions [8,9], having antagonic effects and being associated with anorexia in patients with chronic diseases [10,11]. Circulating ghrelin levels rise shortly before expected food intake and fall shortly after every meal [12,13]. The orexigenic properties of acyl ghrelin in order to compensate anorexia–cachexia have been studied, regarding its potential clinical use to stimulate appetite [14]. Total fasting ghrelin and acyl ghrelin levels have been found normal or decreased and des-acyl ghrelin increased in adults with cirrhosis [15–17].

Leptin is a hormone produced from fat stores and plays an important role in appetite control [18], fat metabolism, energy expenditure and body weight regulation [19,20]. Serum leptin levels in biliary atresia and pediatric end-stage liver disease were found lower than in healthy controls [21,22].

Only few studies have assessed the leptin levels in pediatric patients with chronic liver disease and none has evaluated the ghrelin levels. The present study aimed to investigate the relationship between the nutritional status and fasting ghrelin, leptin and insulin concentrations in children and adolescents with cirrhosis, comparing with sex- and age-matched healthy controls.

2. Patients and methods

From December/2006 to December/2009 42 patients with cirrhosis aged 3 months–15 years were attended on the Pediatric Gastroenterology and Hepatology Unit, Hospital de Clinicas de Porto Alegre, a tertiary reference center for pediatric liver diseases and liver transplantation in Southern Brazil. All participants were asked to answer a questionnaire in order to identify demographic data and nutritional status. The patients were enrolled following the criteria: diagnosis of cirrhosis established either histologically as a diffuse hepatic process characterized by fibrosis and conversion of liver architecture into structurally abnormal nodules. Exclusion criteria were: patients with other chronic diseases (endocrine, renal, respiratory, cardiac or neurologic), any current acute infection, or hepatic encephalopathy at the time of enrollment for the study. From these 42 children and adolescents, 3 were excluded as 1 gave up taking part of the study, 1 developed hypothyroidism and 1 died before the enrollment. Thus, the final patients studied were 39 with cirrhosis and 39 healthy controls matched by sex and age. After the enrollment all had 2 mL of blood collected for hormones and glucose assessment.

All patients underwent laboratory investigation, ultrasound liver scan or gastrointestinal endoscopy as required by the routine clinical management. Most cirrhotic patients were on a regular treatment based on individual clinical requirement, such as: vitamins A, D, E, and K, ursodesoxicolic acid, antacids or proton pump inhibitors, diuretics, and none were on steroid treatment. Three patients were on enteral nutrition with extensively hydrolised protein, and none was using amino acid-based feeding formula. Cirrhotic patients had blood sampling for biochemical assessment done as part of a routine clinical follow-up, according to local specific protocols. The severity of liver disease was assessed according to Child–Pugh classification [23,24], and Pediatric for End Stage Liver Disease (PELD) score for patients under 12 years or Model for End-stage Liver Disease (MELD) score [25] for those aged older than 12 years. Higher values indicate more severe disease, and the score 15 was the cut-off used for these criteria.

Healthy controls were enrolled from pediatric outpatient clinic from Pediatric Department who had been scheduled for blood sampling as routine medical checkup or as standard assessment prior to eligible minimal surgeries as described elsewhere [26].

This study was approved by the Ethics and Research Committee of Hospital de Clinicas de Porto Alegre. Informed and written consent was taken from all participants and caretakers.

2.1. Biochemical analysis

Blood samples for ghrelin and leptin concentrations were collected between 08:00 and 12:00 h in the morning, following a period of at least 3-hour fasting. Plasma and serum were immediately separated by centrifugation for 10 min at 3000 rpm (4 °C) and then stored at −80 °C until subsequent analysis. For the ghrelin determinations the blood was taken into tubes containing EDTA-2Na (1.25 mg/mL) and aprotinin (500 U/mL). The tubes were centrifuged immediately, acidified and stored. Plasma total ghrelin and acyl ghrelin, as well as serum leptin concentrations, were measured in duplicate using a commercial ELISA kit (Linco Research, St Charles, MI, USA). Des-acyl grelin concentration was calculated by subtracting acyl ghrelin from the total ghrelin. For the leptin determinations the blood was taken into serum tubes, centrifuged and stored. Insulin was assessed using ELISA commercial kit (Diagnostics Systems Laboratories, Inc., Webster, TX, USA) and glucose were measured using glucose oxidase method GlicosePAP liquiform® (Labtest Diagnóstica, Lagoa Santa, MG, Brazil).

2.2. Dietary assessment

A 24-hour dietary recall was administered to the mothers or guardians of each child to evaluate daily amount of food ingestion, taken as reference the Dietary Reference Intakes (DRI) [27] and Recommended Dietary Allowances (RDA) [28]. Previous history of anorexia was defined by a decreased daily food intake less than 80% of RDI for age at least over the last 30 days.

2.3. Antropometry assessment

Anthropometry was measured according to WHO training course on child growth assessment [29] by the same researcher. The variables used were: body mass index-for-age ratio (BMI/A), weight-for-age ratio (W/A), height-for-age ratio (H/A) [30], mid upper arm circumference-for-age ratio (MUAC/A), triceps skinfold thickness-for-age ratio (TSF/A), and subscapular skinfold thickness-for-age ratio (SSF/A) [31]. The Standard Deviation Score (SDS) was used according to WHO standards and reference [30]. WHO *Anthro* software version 3.0 was used for children under 5 years of age [32]. WHO *AnthroPlus* software was used for children above 5 years [33]. Frisancho Anthropometric Standards [34] was used to calculate MUAC/A, TSF/A, and SSF/A for children above 5 years. In cirrhotic patients with ascites we did not consider weight-for-age ratio (W/A) and body mass index-for-age ratio (BMI/A) [35] since these parameters may underestimate the diagnosis of undernourishment.

In cirrhotic patients undernourishment was defined as SDS-H/A, SDS-MUAC/A and/or SDS-TSF/A below −2.00 [30,31]. Nutritional status of cirrhotic patients, including TSF/A, SSF/A, and H/A, were determined and classified according to WHO and Frisancho Anthropometric Standards references [31,34]. Undernourishment was defined based on the z-score under −2.00 for H/A and MUAC/A; and/or TSF/A z-score below −2.00 [30,34].

All controls were healthy and well-nourished children according to the WHO standard reference [30,31]. The cut-off used was SDS-H/A −2.00 and +2.00 for all ages. For children under 5 years the same cut-off for all anthropometric parameters was used. For children and adolescents above 5 years the cut-off −2.00 and +1.00 for all anthropometric parameters was used, except SDS-H/A.

2.4. Statistical analysis

Data were processed and analyzed using SPSS 17.0 *version*. Patients were grouped according to categorical variables. An exploratory analysis was performed with comparisons between groups by using non-parametric analysis (Mann–Whitney *U* test) and Chi-square test was used whenever appropriate to compare prevalence. Spearman's rank