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Ghrelin, visfatin and irisin in children with short bowel syndrome

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Abstract: Background: Regulation of energy balance in patients with short bowel syndrome (SBS) is disturbed due to lack of significant part of the intestine. The goal of the research was to analyse the plasma concentrations of selected regulatory peptides — ghrelin, visfatin, and irisin — in children with SBS.
Methods: To achieve this aim we recruited study group consisted of 28 children with SBS fed parenterally for at least two weeks, mean age 14 ± 5 months and mean standardised body mass index (SDS-BMI) –1.26 ± 0.84. The control group was represented 25 healthy children of matching age and SDS-BMI. The plasma concentrations of peptides (ghrelin, visfatin, and irisin) were determined using immunoassays, and liver enzymes (AST, ALT, GGT) using an auto-analyser.
Results: We observed lower visfatin and ghrelin levels in the study group as compared to controls (both $P < 0.0001$). The lowest total ghrelin concentration was observed in SBS children after ileal resection ($P = 0.0016$). Irisin concentration did not differ between the groups. Most of the SBS children showed elevated liver enzymes activities at the first measurement and during one-year follow-up.
Conclusion: Our findings showed that plasma ghrelin and visfatin themselves may play a role in the course of SBS, while a lack of disturbance in irisin might imply that it is neither playing any role nor it is affected by SBS itself.

Key words: parenteral nutrition, intestinal hormones, adipokine, myokine, infants.

Introduction

Short Bowel Syndrome (SBS) is a life-threatening condition with high mortality and morbidity [1]. The SBS prevalence depends much on the maturity of children/newborns. Despite the overall incidence of SBS of 1200/100,000 live births, the mortality rate is very high. Reported survival rates in paediatric SBS range from 73% to 89%, making it one of the most lethal conditions in infancy and childhood [2, 3]. A multidisciplinary treatment program has been associated with better survival; however, it is very expensive [1, 2]. Patient outcome depends mainly on the length of resected (or remaining) bowel, which influences directly the degree of malabsorption, and in consequence the need for specialised enteral or parenteral nutrition (PN) [3]. An increasing number of patients require long-term PN, which is accompanied by the risk of developing serious complications such as parenteral nutrition-associated liver disease (PNALD). PNALD is the most common cause of death for patients suffering from SBS [4–6]. Partial resection of intestine has an influence on the many gastrointestinal hormones [7]. It is known that disturbed gut-brain axis is one of the reasons of malnutrition in such patients [8, 9]. One of the representatives of gastrointestinal peptides is ghrelin — a physiological hunger-signal initiator, which is secreted primarily by the oxyntic mucosa of the gastric fundus [10]. Ghrelin serves multiple functions contributing to intestinal adaptation after massive small bowel resection [10–12]. On the other hand, ghrelin may induce a negative energy balance by decreasing food intake and delaying gastric emptying [10, 13]. Structural and physiological alterations in patients with SBS also generate secondary disturbances in the secretion of regulatory peptides from distal parts of the gut and even from other tissues, such as adipose tissue [3]. Variances in the post-resection release of gastrointestinal regulatory peptides may cause secondary adipokine or myokine secretion disturbances, which may also play an important role in the regulation of liver function and intestinal adaptation. Visfatin, a novel anti-inflammatory adipokine, produced primarily by visceral and subcutaneous fat and hepatocytes, is involved in multiple biological functions, including glucose and lipid metabolism, and energy homeostasis [14, 15]. Some authors have linked visfatin with the pathogenesis of liver cirrhosis and disease progression [16]. Monitoring of this parameter could be useful and easily obtainable marker of PNALD. Recently described is irisin, a molecule secreted from skeletal muscle, involved in transforming white adipose tissue to brown adipose tissue and in increasing energy expenditure [17]. Irisin improves glucose homeostasis and lipid metabolism [18]. An elevated concentration of this molecule is observed in obese patients, and may therefore be used as a potential biomarker of energy balance [19].

The aim of this study was to analyse the plasma concentrations of ghrelin, visfatin and irisin in children with SBS in relation to the postsurgical anatomy of the

gastrointestinal tract. Our findings may help to discover new biomarkers for early detection of SBS children at risk and significantly improve the quality of life and limit mortality in this group.

Materials and Methods

Participants

We recruited 28 consecutive infants with a clinical diagnosis of SBS to a prospective cohort study. SBS was defined as loss of at least 50% of the small intestinal length from surgical resection or congenital defect. The study was limited to patients ≤6 weeks of age at the time of SBS onset, totally parenterally fed for at least two weeks. Detailed clinical data are presented in Table 1. Patients were excluded from the trial if they had a termination of PN, other feeding protocols, or systemic or severe chronic disease (besides SBS). All nutrition requirement was covered by PN and the prescription followed a standard protocol. Amino acids were given in the amount to cover daily requirements. Per 1 g of amino acid nitrogen, 175 other calories were administered in the proportions of 70% carbohydrates to 30% lipids. The control group recruited in a day-care centre was represented by 25 healthy infants (Table 1). These infants (36% girls) were breastfed or fed with regular age-appropriate formula. In all individuals, we evaluated standardised body mass index (SDS-BMI) after adjustment for gestational age and gender.

Table 1. Demographic and Clinical Data.

Variable	SBS children (n = 28)	Control (n = 25)
age, months	14 ± 5	16 ± 7
female, %	9 (32)	9 (36)
SDS-BMI, kg/m^{2*}	–1.26 ± 0.84	–0.81 ± 0.55
TPN time, days	22 (17–31)	
last surgery, days	24 (16–34)	
cause of SBS		
necrotizing enterocolitis, %	9 (32)	
gastroschisis, %	5 (18)	
intestinal atresia, %	5 (18)	
meconium ileus, %	5 (18)	
cystic fibrosis, %	2 (7)	
other, %	2 (7)	

Table 1. Cont.

Variable	SBS children (n = 28)	Control (n = 25)
part of resected bowel		
jejunum resection, %	5 (18)	
jejuno-ileum resection, %	4 (14)	
ileum resection, %	9 (32)	
jejunocolic resection, %	0	
ileocolic resection, %	2 (7)	
colon resection, %	6 (22)	
jejuno-ileum and colon resection, %	2 (7)	
cause of SBS in isolated ileum resection subgroup		
necrotizing enterocolitis, %	4 (45)	
gastroschisis, %	1 (11)	
intestinal atresia, %	2 (22)	
meconium ileus, %	2 (22)	

Continuous variables are presented as a median (interquartile range) or a mean ± standard deviation and categorical variables are presented as numbers (percentages).

Abbreviations: SDS-BMI, body mass index sd score; SBS, short bowel syndrome.

The protocol of the study was approved by the Jagiellonian University Bioethical Committee (opinion no. 122.6120.154.2015). All subjects' parents or legal guardians provided written informed consent prior to inclusion in the study.

Assays

All infants underwent a venipuncture in the morning, before feeding. Plasma was separated by centrifugation and stored in 200 µl aliquots at –80°C until further analysis. Plasma concentrations of total ghrelin were measured using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits supplied by Merck Millipore (Darmstadt, Germany). For the quantitative determination of visfatin and irisin, we used the commercial ELISA kit from BioVendor (Brno, Czech Republic). The measurements were conducted according to the manufacturers' guidelines. We also measured the activity of liver enzymes: serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) using

a VITROS 5.1 auto-analyser (Ortho Clinical Diagnostics, USA) during one-year follow-up.

Statistical analysis

Categorical variables were presented as counts and percentages; continuous variables were expressed as a mean ± standard deviation or as a median and interquartile range (IQR). Normality was assessed by the Shapiro–Wilk test in each group. The differences between the groups were compared by the Student's t-test for normal distribution variables or U-Mann-Whitney test for continuous variables with a distribution different from normal. A probability of <0.05 was considered statistically significant. All calculations were carried out with STATISTICA v.13.5 software (StatSoft Inc., USA).

Results

Clinical and demographic data of studied and control groups are presented in Table 1. We observed lower total ghrelin and visfatin concentrations in the study group when compared to control individuals (97.78 ± 19.65 pg/ml vs. 182.17 ± 65.44 pg/ml; P <0.0001, and 1.05 ± 0.96 ng/ml vs. 6.42 ± 3.27 ng/ml; P <0.0001 respectively, Fig. 1A and 1B). We found that children after isolated ileum resection (32%) had significantly lower total ghrelin concentration when compared to the other SBS children (69.28 pg/ml ± 17.05 vs. 126.99 ± 24.57 pg/ml; P = 0.006). We did not find any differences in the irisin concentration between healthy and SBS children (2.67 ± 0.72 mg/l vs. 2.76 ± 1.41 mg/l; P = 0.69, Fig. 1C). At the first measurement in the study an elevated activity of ALT was observed in 84% of patients (mean multiplicity of the upper limit of reference range was 2.1 ± 0.9) and during one year we did not note any significant changes. AST activity during initial determination was increased in 56% of the study group (mean multiplicity of the upper limit of reference range was 1.7 ± 0.2) and did not change significantly during the study time. Elevated activity of GGT was found in 76% of SBS patients (mean multiplicity of the upper limit of reference range was 2.9 ± 0.7) with no significant alterations during one-year follow-up. We also observed a negative correlation between visfatin concentration and ALT activity in the SBS children at the first measurement (P = 0.0012; R = –0.41). No correlations between ghrelin or irisin with liver enzyme activity were found at all time points.

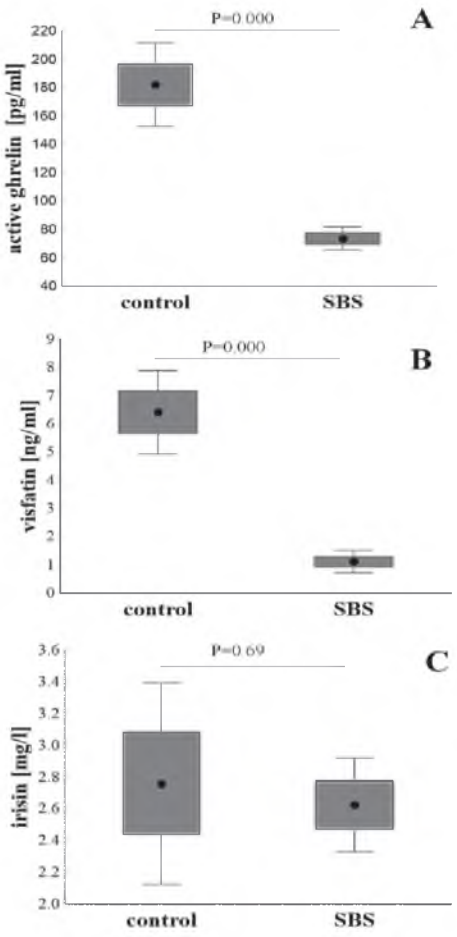

Fig. 1. Difference between the ghrelin, visfatin and irisin concentration between control and SBS patients. **A.** Mean active ghrelin concentration in the study (n = 28) and control group (n = 25). **B.** Mean concentration of visfatin in the study and control group. **C.** Mean concentration of irisin in the study group and control group. Middle point = mean value; box = standard error of the mean; bars = standard deviation.

Discussion

To our knowledge, this study is the first to demonstrate a decrease in plasma ghrelin and visfatin concentrations in children suffering from SBS treated with PN. This area of research is important as SBS is a life-threatening condition.

Ghrelin has a vast range of physiological functions, including orexigenic, metabolic and hormonal functions [10]. The data about levels of this peptide plasma after partial resection of the gut are contradictory. In animal studies, it was indicated

that ghrelin secretion in the stomach increases immediately after massive small-bowel resection. It was concluded to be a compensation mechanism protecting from malnutrition [20]. This observation was confirmed in the clinical study in nine adult SBS patients with jejuno-colonic anastomosis [20]. On the other hand, Kršek *et al.* observed a lower concentration of ghrelin in 24 SBS patients when compared to healthy individuals [21]. Although the highest concentration of ghrelin is found in the stomach, the authors noted that a significant amount of ghrelin is likewise produced by intestinal neuroendocrine cells in the whole intestine [21]. The authors hypothesised that plasma ghrelin concentration decreases in SBS patients due to a massive resection of tissue mass that is able to secret ghrelin in substantial amounts [21]. It was justified by observations by Date *et al.*, that ghrelin is also secreted by endocrine cells in the small intestine [22]. Similarly, in our study, ghrelin levels were diminished in SBS children as compared to healthy children. Lower ghrelin levels in paediatric SBS patients may result not only from the loss of intestinal neuroendocrine cells secreting ghrelin, but from disturbances in feedback regulation between different parts of the gut [7]. We found that children following exclusively ileal resection had significantly lower ghrelin concentration than did other SBS children. This is surprising because Gillard *et al.* suggested that jejuno-colonic anastomosis creates a specific environment that increases hunger signals and may be associated with higher ghrelin secretion [20]. This discrepancy could result from a different age of studied groups, and therefore differences in regulatory mechanisms. Recently, Yamada *et al.* have indicated that the administration of ghrelin diminishes PN-associated intestinal mucosal atrophy due to an increased villus height and crypt depth [12]. They highlighted the clinical significance of ghrelin application, which is likely to show promise in the treatment of malnutrition, particularly in paediatric patients with SBS [12]. Monitoring of ghrelin concentration seems to be a marker of intestinal adaptation in paediatric SBS patients.

Visfatin is expressed at highest levels in adipose tissue, liver, muscles, and bone marrow [14, 15]. A recent study performed by de Boer *et al.* showed that concentration of plasma visfatin was significantly lower in cirrhotic patients (children and adults) compared to healthy controls [16]. They also noted that visfatin levels decreased together with the worsening stage of disease [16]. The authors hypothesised that the main reason for the reduction of circulating visfatin levels in those patients is decreased hepatic synthetic function as a result of hepatocyte damage [16]. Similarly, conclusions were reported by Kukla *et al.* who evaluated visfatin expression in liver biopsy samples using immunohistochemical assay [23]. The authors described the negative association between circulating visfatin concentrations and the fibrosis stage in non-alcoholic fatty liver disease (NAFLD) and suggested a potential role of this molecule in the pathogenesis of NAFLD [22]. In our study, visfatin levels were lower in SBS patients compared to the healthy subjects and negatively correlated with serum

ALT activity. These observations suggest that diminished concentration of plasma visfatin could be recognised as a predictive factor of PN-associated liver fibrosis. In our samples, the elevated activity of liver enzymes was detected and therefore suggested early/minor liver disease of SBS children. The clinical utility of visfatin as a marker of PNALD requires further elucidation.

Irisin is a recently identified novel myokine [17]. Some authors reported increased irisin concentration in obese patients, and its reduction after weight loss [24, 25]. Moreover, Crujeiras *et al.* observed that irisin concentration increased when weight loss was reversed [24]. On the other hand, Sanchis-Gomar *et al.* reported lack of correlation between irisin level and BMI [26]. These contrasting observations could be the results of the measurement differences because there is still no consensus concerning the identification of the circulating, soluble irisin molecules, the varying presence of target epitopes among manufacturers, and finally differing mechanisms of secretion [27, 28]. In spite of the existing controversy, most authors indicated correlations between irisin and different markers of adiposity, suggesting that plasma irisin levels reflect body adiposity. Recently the associations between irisin and liver diseases have been shown [27–29]. Choi *et al.* noted higher levels of irisin in patients with NAFLD, suggesting a potential role of irisin in the pathogenesis of this disease [29]. Furthermore, Rizk *et al.* found increased irisin levels in metabolic syndrome patients with elevated levels of liver enzymes [28]. They indicated a relationship between circulating irisin concentration and lipid as well as glucose metabolism [28]. However, in our study, we did not observe any changes in this peptide concentration in SBS children. Primarily, the lack of the alterations in irisin concentrations could be due to the similarity between the study and control groups according to SDS-BMI, which may exclude the possible variances in the secretion of the analysed peptides depending on differences in adipose and muscle tissue volume. Secondly, it may suggest no functional connection between gut peptides and the peptides secreted from skeletal muscles.

Study limitations

The limitation of the study lies in the small number of members of the study groups. However, SBS children with TPN are a unique group, and 25 patients could be recognised as a sufficient sample size. It is a consequence of the rarity of SBS and underlying pathology. However, due to statistical rules, this may lead to underestimation of the possible association between variables (type II error). This and other possible similar limitations could be overcome by increasing the sample size, thus improving the statistical power of the study.

Conclusion

Our research indicates that energy misbalance in children with SBS is associated with secretion of peptides from the gastrointestinal tract as well as with the secretion of peptides from adipose tissue and liver, while myokines pathways are not disturbed.

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Conflict of interest

None declared.

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