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Increased GLP-1 response to oral glucose in pre-pubertal obese children

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Abstract

Background: Gastrointestinal hormones, such as glucagon-like peptide (GLP-1), have been hypothesized to play a role in the pathogenesis of obesity-related complications. However, few data are available in youth. The objective of this study was to investigate the GLP-1 response to oral glucose load in obese pre-pubertal children and its relationship with insulin secretion.
Methods: Ten pre-pubertal obese children [five boys; 10.5±1.6 years; body mass index-standard deviation score (BMI-SDS): 2.2±0.5] and 10 controls (eight boys; 9.9±1.2 years; BMI-SDS: −0.7±0.5) underwent a modified oral glucose tolerance test (OGTT) to evaluate post-load glucose, insulin and GLP-1 responses. Insulin sensitivity [homeostasis model assessment of insulin resistance (HOMA-IR), whole body insulin sensitivity index (WBISI)] and secretion [HOMA-beta, insulinogenic index (IGI)] indexes, area under the curve (AUC) for glucose, insulin and GLP-1 were calculated.
Results: In obese children GLP-1 AUC values were higher and correlated with BMI-SDS (r=0.45; p=0.04), HOMA-IR (r=0.53; p=0.01) and fasting glucose (r=0.68; p=0.001).
Conclusions: Obese children showed an increased GLP-1 response to oral glucose. These changes might likely represent a compensatory mechanism to avoid post-prandial hyperglycemia and allow a normal glucose tolerance.

Keywords: diabetes; GLP-1; gut hormones; obesity.

Introduction

Despite being a clear tendency towards a stabilization of the obesity epidemic in children and adolescents its prevalence remains higher than ever before. Around 22 million children worldwide are obese [1–5], representing a serious public health problem [6]. In children and adolescents obesity is associated with an increased risk of developing insulin resistance (IR) and type 2 diabetes (T2D). Impaired β-cell function has been shown to be one of the key factors in defining the risk of impaired glucose metabolism. In young adults bariatric surgery approach is an effective treatment for obesity-related comorbidities such as T2D, hypertension and dyslipidemia. Interestingly, studies have shown early resolution of T2D sometimes even within days of the surgery. These effects may be documented even prior to weight loss and therefore might be related to changes in hormonal pathways achieved early after the surgery procedure. The entero-insular axis might be postulated to be one of the major factors explaining all these metabolic changes. In fact bariatric surgery restores the impaired gastrointestinal hormones secretion related to obesity, particularly glucagon-like peptide-1 (GLP-1) concentration. GLP-1 is both a gut hormone and a neuropeptide produced by post translational processing of the pre-pro-glucagon gene. It is secreted from the L-cells in the distal gastrointestinal tract in response to food ingestion in the gut. GLP-1 levels are low before a meal and increase after a meal; it enhances glucose dependent insulin secretion, suppresses glucagon secretion and inhibits gastric emptying [7, 8]. Therefore, as GLP-1 is a main inductor of postprandial insulin secretion, it makes a significant contribution to the overall post-prandial glucose metabolism.

The role of gastrointestinal hormones and especially of GLP-1, one of the most relevant incretin hormones, in childhood obesity is poorly understood [9]. Contrasting data on GLP-1 concentrations in obese subjects have been reported. Some authors documented lower fasting GLP-1 levels and attenuated GLP-1 response to oral nutrient in obese individuals. Others also found an enhanced GLP-1 response to meal ingestion in obese humans and rats [10–14]. Although IR and subtle alterations of glucose metabolism might be already detected in pre-pubertal obese children, T2D is very uncommon in this age group. Although several mechanisms might be postulated, the

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presence of a more effective gut hormone production might be one of the possible underlining mechanisms.

Therefore, the aim of this study was to investigate the GLP-1 response to oral glucose load in obese pre-pubertal children and its relationship with insulin secretion compared to healthy matched controls.

Materials and methods

Study populations

Ten Caucasian pre-pubertal obese children [five boys and five girls; mean age (±SD): 10.5±1.6 years] and 10 normal-weight age-matched pre-pubertal controls [eight boys and two girls; mean age (±SD): 9.9±1.2 years] were enrolled in the present study. Obese children, referred to the Auxo-Endocrinology Service of the Department of Pediatrics, University of Chieti, Italy, were affected by overweight or obesity [body mass index (BMI)>85° percentile for age and sex] [15]. Normal-weight controls (BMI<85° percentile) were recruited among children referred to the Auxo-Endocrinology Service of the Department of Pediatrics, University of Chieti, Italy for occasional hyperglycemia. None of the control group showed alterations in glucose metabolism after glucose load.

For all subjects, the absence of chronic or acute disease, malnutrition and use of medications were used as inclusion criteria.

All children underwent a detailed clinical history and family history and a complete physical examination including anthropometric parameters (height and weight). All children were pre-pubertal on the basis of breast development in girls and genital development in boys according to the criteria of Tanner [16].

All subjects performed a modified oral glucose tolerance test (OGTT). Glucose, insulin and GLP-1 concentrations after glucose load were evaluated.

The study was approved by the Ethical Committee of the University of Chieti. Written informed consent was obtained from all parents and oral consent from children.

Anthropometric measurements

Body weight was measured using a standard balance with a variability of 0.1 kg, and height was determined with Harpenden taximeter to the nearest 0.1 cm. Body mass index (BMI) was calculated as the ratio between weight in kg and height in meters squared, while standard deviation score (SDS)-BMI for age and sex as the difference between the observed value and the reference mean for age and sex, divided by the corresponding standard deviation based on published normative Italian data [15].

Laboratory procedures

Modified OGTT: Each subject was tested from 8:00 to 9:00 AM, after an overnight fast. After insertion of an intravenous catheter, blood samples were drawn for the measurement of baseline glucose and insulin levels. Thereafter subjects underwent a modified OGTT with measurement at 0, 15, 30, 60, 90, 120 min of glucose and insulin levels and with measurements at 0, 5, 10, 15, 20, 30, 60, 90, 120 min of GLP-1 levels. Blood samples were collected on ice into tubes containing dipeptidyl peptidase IV inhibitor. After centrifugation at 4 °C, plasma samples were processed into different aliquots and frozen at –70 °C until analysis.

Formula

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the equation fasting insulin (mU/L)×fasting glucose (mmol/L)/22.5 [17].

Whole body insulin sensitivity index (WBISI) was calculated as: {10/[fasting glycemia (mg/dL)×fasting insulin (μU/mL)×mean concentration of glucose (mg/dL)*mean concentration of insulin (μU/mL)]1/2} [17].

Homeostasis model of assessment-beta (HOMA-β) was calculated as: [20×fasting insulin (μU/mL)]/[fasting glucose (mmol/mL)–3.5] [18].

Insulinogenic index (IGI) was defined as: δ insulin 0–30 (μU/mL)/δ glucose 0–30 (mg/dL).

Area under the curve (AUC) values were determined using the trapezoidal rule and it was calculated for insulin, glycemia and GLP-1 during oral tolerance test [19].

Biochemical analysis

Plasma glucose level was determined by using the glucose oxidase method and plasma insulin was measured with a two side immunoenzymometric assay (AIAPACK IRI; Tosoh, Tokyo, Japan). The limit of detection was 0.5 mcU/mL with interassay coefficients of variation <7% for quality control.

Serum GLP-1 levels were determined with immunoenzymometric assay (Yanaihara Institute Inc., Fujinomiys-Shi Shizuoka, Japan: CV% interassay: 9.63–17.57).

Statistical analysis

The statistical analysis was performed using SPSS version 17.0 software for Windows (SPSS, Chicago, IL, USA). All data were analyzed with Kolmorgorov-Smirnov test and the date not normally distributed were log-transformed. All data were expressed as mean±SD. Differences between obese and control children were assessed by unpaired t-test. A linear regression analysis was used to evaluate the associations between variables of interest. A p-Value <0.05 was considered statistically significant.

Results

Anthropometric evaluation

Table 1 shows baseline anthropometric and demographic characteristics of obese and normal-weight pre-pubertal

Table 1: Anthropometric parameters and metabolic characteristics in obese and normal-weight pre-pubertal children.

Parameters	Controls (n=10)	Obese (n=10)	p-Value[a]
Sex, male/female	8/2	5/5	0.01
Age, years	9.9±1.2	10.5±1.6	0.33
Weight, kg	30.1±3.4	59.5±10.9	<0.001
SDS-weight	−0.6±0.5	1.9±0.7	<0.001
Height, cm	122±42	142.3±5.8	0.16
SDS-height	−0.2±0.2	0.1±0.9	0.23
BMI, kg/m^2	16.2±1.0	29.2±3.8	<0.001
SDS-BMI	−0.7±0.5	2.2±0.5	<0.001
Fasting glycemia, mg/dL	90.1±4.9	94.2±12.0	0.26
Fasting insulin, μU/mL	6.5±2.0	15.9±10.6	0.02
HOMA-IR	1.4±0.4	3.7±2.5	0.02
WBISI	9.3±2.7	4.7±2.5	0.002
Fasting GLP-1, ng/mL	2.9±0.4	3.5±1.4	0.21
HOMA-β	1.5±0.5	3.6±2.4	0.02
IGI	0.6±0.4	4.8±3.8	0.007

Data are mean±standard deviation. SDS-weight, standard deviation score weight; SDS-height, standard deviation score height; BMI, body mass index. SDS-BMI, standard deviation score-body mass index; HOMA-IR, homeostasis model of assessment-insulin resistence; WBISI, whole body insulin sensitivity index; HOMA-β, homeostasis model of assessment-beta; IGI, insulinogenic index.
[a]Mann-Whitney U-test.

children. A higher prevalence of females was present in the obese group.

There were no significant differences in terms of age, height and SDS-height between the two groups. As expected, the group of obese children presented a higher weight and SDS-weight than the normal-weight group, with a higher BMI and SDS-BMI. There were no significant differences in terms of volume of glucose solution administered for the OGTT.

Modified OGTT

Table 1 shows the main metabolic parameters of study groups. Fasting glucose and insulin levels were significantly higher in obese rather than in control children. IR indexes expressed by HOMA-IR and WBISI were also significantly increased in obese children while insulin secretion indexes (HOMA-beta and IGI) were significantly lower. No differences were detected in fasting GLP-1 levels. Insulin and GLP-1 excursions during the OGTT are shown in Figure 1. Glucose excursions were not statistically different between the two groups ($p<0.05$). AUC for insulin and AUC for GLP-1 were significantly higher in obese subjects, as shown in Figure 1. Linear regression demonstrated a significant association between GLP-1 AUC values and BMI-SDS, HOMA-IR and fasting glycemia as shown in Table 2. No statistically significant association was found with age, sex, BMI, WBISI, HOMA-β.

Discussion

In this study we documented in a group of obese pre-pubertal children with normal glucose tolerance the presence of significantly increased GLP-1 AUC values after an oral glucose load compared to normal-weight healthy subjects matched for age and pubertal stage. In addition GLP-1 AUC values were significantly correlated with indexes of adiposity and IR. These data suggest a potential role of GLP-1 secretion in response to oral glucose administration in the regulation of glucose homeostasis in obese pre-pubertal subjects.

In obese children, an attenuated GLP-1 response may contribute to impaired insulin response, leading to T2D

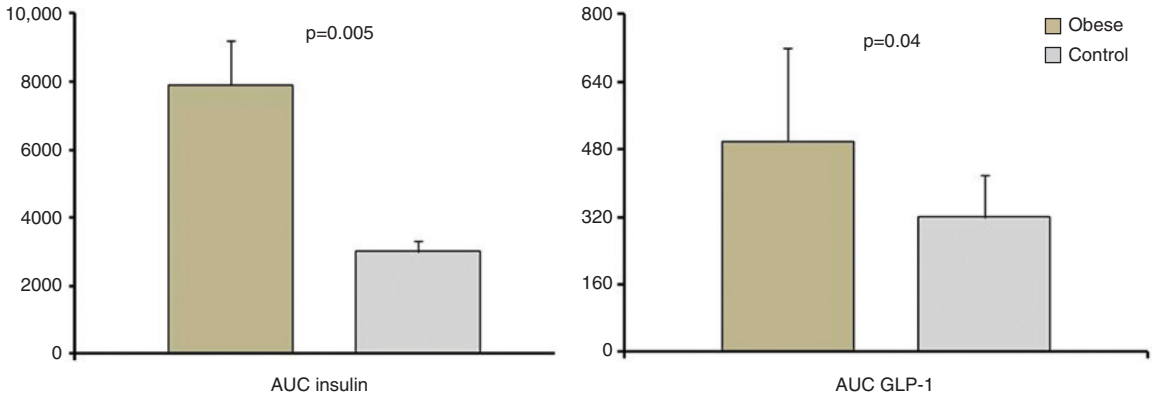

Figure 1: Insulin and GLP-1 AUC values during the OGTT.

Table 2: Factors associated with GLP-1 AUC during the OGTT.

	AUC GLP-1 p (r)
Age	0.31 (0.24)
Sex	0.08 (0.40)
BMI-SDS	**0.04 (0.45)**
Insulin AUC	**0.01 (0.59)**
HOMA-IR	**0.01 (0.53)**
WBISI	0.13 (–0.37)
HOMA B	0.14 (0.35)
Insulinogenic index	0.10 (0.38)
Glucose AUC	0.57 (0.14)
Fasting glycemia	**0.001 (0.68)**

Significant values are reported in bold.

[20–22]. GLP-1 may also reduce energy intake and enhance satiety, likely through the aforementioned delay of gastric emptying and specific GLP-1 receptors in the central nervous system. Its role in childhood obesity is poorly understood, with contradictory post-weight loss level changes reported in the literature [20–27]. In an intervention study in obese children, fasting GLP-1 concentrations were shown to be independent of age, sex and pubertal stage. In addition, although GLP-1 did not differ between lean and obese children at baseline, weight loss was associated with decreasing GLP-1 which in turn correlated with decreases in insulin levels and IR index scores [25]. Similarly to previous studies, in our study we did not find significant differences in terms of GLP-1 levels between obese and control subjects. These effects may be related to normal glucose status of the study population evaluated in our study, while changes in fasting GLP-1 levels at baseline might be better associated with the development of impaired glucose tolerance or T2D.

As recently shown by Manell et al., obese adolescents with impaired glucose tolerance had lower fasting GLP-1 levels than those with normal glucose tolerance, suggesting that this is an early-stage abnormality in obesity-related glucose dysregulation [28]. Moreover, obese adolescents showed elevated insulin and glucagon levels and that the progression to T2D was related to a further increase of these hormones as well as an early-phase hyperglucagonemic response to OGTT [28]. However, further longitudinal studies are required to confirm this hypothesis.

Data from previous studies in obese subjects who underwent bariatric surgery have postulated a potential role of gut hormones in restoring glucose metabolism. This is strongly suggested by the development of metabolic changes documented early after bariatric procedures. In fact gut hormones and especially GLP-1 concentrations have been shown to be restored after bariatric surgery, positively affecting β-cell function [29, 30]. To date, few data evaluating the role of GLP-1 concentrations in obese pre-pubertal children are available, especially evaluating this high risk population in a very early phase of the natural history of the development of glucose metabolism alteration [31]. Therefore in this study we evaluated the secretion of GLP-1 in response to a stimulus and the presence of any associations with insulin release in pre-pubertal obese children with a normal glucose tolerance during the OGTT. We documented that, although baseline levels of GLP-1 were similar between obese and normal-weight children, the obese group showed higher secretion of GLP-1 during an oral glucose load. This secretion of GLP-1 was found to be influenced by baseline IR and fasting glycemic levels and emerged to be associated with insulin secretion during OGTT. GLP-1 is known to have several beneficial effects on glucose regulation. It stimulates endogenous insulin secretion in response to oral load, suppresses glucagon secretion leading to reduced hepatic glucose production, extra-pancreatic effects, as it improves glucose elimination [7, 8]. Taking into consideration all these effects, it might be speculated that GLP-1 changes documented in obese pre-pubertal children after glucose load may represent an early adaptive response to induce a greater insulin secretion to overcome IR. The peak levels of GLP-1 preceded the response of insulin spike, suggesting a directed relationship between the increased GLP-1 release and insulin secretion by the β-cells. Therefore, we hypothesized that an increase in GLP-1 secretion might explain or at least in part contribute to higher levels of insulin in obese children, ensuring a return to blood glucose levels to normal at the end of load curve. However, the study design does not allow to differentiate whether elevated levels of GLP-1 in obese children are due to a compensatory increase in secretion or reflect a state of resistance of GLP-1. Therefore, further studies are needed to better clarify this question.

A limitation of our study is the small sample size. In addition, only GLP-1 values have been evaluated without taking into consideration the possible role of other gut secreted hormones. It needs further evaluation on larger samples and complete gut hormone patterns to confirm the results of this study. Furthermore, indirect measures of insulin secretion have been evaluated in this study, therefore studies evaluating β-cell function by the hyperglycemic clamp might be required to confirm our results.

In conclusion, in this study we showed that obese pre-pubertal children are more insulin resistant and have higher levels of insulin during an OGTT compared

to healthy normal-weight subjects. The higher levels of insulin were associated with increased levels of GLP-1. These changes might likely represent a compensatory mechanism to avoid postprandial hyperglycemia and allow a normal glucose tolerance. Further studies are required in this age group to better characterize the effect of GLP-1 concentrations on the regulation of glucose metabolism.

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