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Original Article

Unacylated, acylated ghrelin and obestatin levels are differently inhibited by oral glucose load in pediatric obesity: Association with insulin sensitivity and metabolic alterations☆

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SUMMARY

Background & Aims: Ghrelin levels are associated with insulin resistance, obesity and clustered abnormalities of the metabolic syndrome. Nutrients, mainly carbohydrates, influence ghrelin secretion. Obestatin is derived from the same precursor as ghrelin. Contemporary regulation of the three peptides, with respect to insulin sensitivity and metabolic syndrome, remains undefined in childhood and adolescence.
Methods: A cross-sectional study in a tertiary care center. Acylated, unacylated ghrelin, obestatin, glucose and insulin were measured at fasting and post oral glucose load in 60 pediatric obese and 22 normal weight subjects classified with respect to those alterations that cluster in metabolic syndrome.
Results: Acylated ghrelin decreased at 60 min and subsequently returned to basal levels ($p < 0.001$). Unacylated ghrelin and obestatin decreased for the entire test with a maximum inhibition at 60 and 120 min ($p < 0.0001$), respectively. Unacylated ghrelin inhibition was influenced by insulin sensitivity. The insulinogenic index was associated with the acylated ghrelin rebound ($p < 0.002$) and obestatin nadir ($p < 0.006$). Fasting unacylated ghrelin was reduced in metabolic syndrome due to insulin resistance. Obestatin variation was blunted in individuals who had alterations of the metabolic syndrome cluster ($p < 0.0001$), being predicted by lower HDL cholesterol and higher blood pressure.
Conclusions: Acylated, unacylated ghrelin and obestatin present different dynamics after glucose load in pediatric individuals. Compensatory insulin secretion to insulin resistance and insulin sensitivity are the major contributors associated with the regulation of ghrelin as well as metabolic alterations with obestatin.

1. Introduction

Ghrelin is a 28 amino acid peptide predominantly produced by the stomach but also by many other tissues including the endocrine pancreas. It circulates in bloodstream in two forms, acylated (AG) and unacylated (UAG) ghrelin.[1,2] Coexpression of the GH secretagogue receptor which binds AG, ghrelin-O-acyltransferase (GOAT), the enzyme catalyzing the addition of the octanoyl-group and ghrelin in the pancreas, suggests that this peptide is involved in glucose metabolism.[3] Epsilon cells, which mainly express and secrete ghrelin constitute a new islet population shown from fetal to adult life in both animals and humans.[4,5] AG and UAG seem to have different roles in the modulation of insulin secretion, at least in glucose stimulated conditions.[1,2,6,7] On the other hand, insulin is a physiological and dynamic modulator of plasma ghrelin concentrations[1,2,8–10] as well as glucose[1,2,11,12] in testing conditions such as an oral glucose load (OGTT). This is also apparent in chronic states of altered glucose metabolism and insulin resistance such as obesity, type 2 diabetes and the metabolic syndrome (MS).[1,2,7–10,13–15]

Recently, a new 23 amino acid peptide named obestatin (OBST), was identified in a conserved region of the pre-proghrelin sequence.[16,17]

Non-standard abbreviations: AG, acylated ghrelin; AUC, area under the curve; BMISDS, BMI standard deviation score; DI, disposition index; Ins, insulinogenic index; MS, metabolic syndrome; OBST, obestatin; OGTT, oral glucose tolerance test; UAG, unacylated ghrelin.

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Although it is expressed in the pancreas as AG and UAG, data regarding the modulation of insulin secretion by OBST are limited and controversial.[17,18] In hypothesizing a role for OBST in obesity and insulin resistance, numerous studies explored the regulation of its secretion in these conditions. However, authors showed both lower and higher OBST levels in obesity both in children and adults. OBST secretion seems to be refractory to the inhibition by insulin in insulin-resistant subjects.[17,19–23]

As previously reported, total ghrelin levels fall after administration of an OGTT in both pediatric and adult populations,[11,12] being further influenced by the grade of insulin resistance at fasting[8,9,13–15] and insulin sensitivity during OGTT.[15] Despite speculated distinct roles regarding insulin secretion, *in vivo* data for AG, UAG and OBST behavior after OGTT are scanty and controversial. A few studies in a relatively small number of subjects have shown that AG levels decrease after OGTT in several pathological populations,[24–26] while others have failed to demonstrate this in obesity.[27] Two studies showed an inhibition of OBST after OGTT in adult populations.[19,26] Moreover, no studies have evaluated if insulin sensitivity during OGTT influences the response of the three peptides, with exception of one study in Prader Willi syndrome.[25]

If insulin resistance is a player in the modulation of the ghrelin system, states of hyperinsulinism and insulin resistance, such as obesity and MS which are also characterized by other metabolic impairments, could influence the secretion of the three peptides. Indeed, MS is associated with a derangement in AG and UAG secretion in fasting conditions in both lean and obese children.[28] Ghrelin gene polymorphisms and its blood concentration seem to be both involved in HDL-cholesterol metabolism and blood pressure, two main components of the MS cluster.[10,13,29,30]

As such, the first aim of this study was to detail post-OGTT AG, UAG and OBST dynamics in obese children and adolescents. A further aim was to explore if insulin resistance and metabolic alterations clustering in MS could be related to the fasting and glucose-induced regulation of the three peptides.

2. Materials and methods

From November 2008 to December 2010, 30 prepubertal and 40 pubertal pediatric subjects with primary obesity and a body mass index (BMI) equal or higher than 97th percentile were consecutively enrolled. They were sedentary (engaging in less than 1 or 2 h per week of mild physical activity at school). Obesity linked to genetic syndromes or organic dysfunctions like craniopharingiomas were excluded. Exclusion criteria also included the presence of type 1 and 2 diabetes, renal dysfunction, liver steatosis and other conditions known to influence body composition and energy balance (insulin and glucocorticoid treatments, endocrine diseases including sleep apnea syndrome). A group of 22 age-matched lean controls were also recruited. They engaged 6 h per week of moderate or vigorous physical activity. Physical activity was recorded by a register and was not instrumentally measured. All subjects regularly went to school and their socio-cultural environment was similar with a medium-high social extraction.

Subjects underwent a complete clinical and auxological evaluation by a trainee research team using the Italian growth charts.[31] The pubertal stages were determined by inspection by physicians, using the criteria and definitions described by Marshall and Tanner. Patients were divided into prepubertal (stage 1) and pubertal (stages 2–5) subjects. Height was measured by the Harpenden stadiometer and weight with light clothing by using electronic scale. BMI was calculated as body weight divided by squared height (kg/m^2). BMI standard deviation score (BMISDS) was calculated with LMS method.[31] Waist circumference was measured at the high point of the iliac crest around the abdomen and was recorded to 0.1 cm. The waist-to-height ratio was calculated by dividing waist circumference (cm) by height (cm) and used as another surrogate measure of central fat distribution. Systolic and diastolic blood pressure were measured three times on the left arm and after 15 min at rest in the supine position by using a standard mercury sphygmomanometer; the average was recorded and stratified according to pediatric percentiles of National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents.[32]

Children and adolescents underwent an evaluation of metabolic alterations clustering in MS by using the modified NCEP-ATP III criteria of Cruz and Goran.[33] Impaired fasting glucose and impaired glucose tolerance were defined according to MS and American Diabetes Association classifications. Accordingly, MS was defined by the presence of 3 or more of the following 5 criteria: 1) waist circumference $\geq$ 90th percentile for age and gender; 2) triglycerides (TG) $\geq$ 90th percentile for age and gender; 3) HDL-cholesterol $\leq$ 10th percentile for age and gender; 4) impaired fasting glucose or glucose tolerance; 5) blood pressure $\geq$ 90th percentile for age and gender. Waist circumference percentiles were defined according to sex and age.[34] Triglycerides and HDL-cholesterol percentiles were considered in accordance to distribution based on American Academy of Pediatrics cut-off values.[35]

After a 12-h overnight fast, blood samples for AG, UAG, OBST, total cholesterol, HDL-cholesterol, triglycerides, GH and IGF-I were measured. LDL-cholesterol was determined using the Friedwald formula. All subjects underwent an oral glucose tolerance test (OGTT, 1.75 g of glucose solution per kg, maximum 75 g). Blood samples were drawn for the determination of glucose and insulin every 30 min and of AG, UAG, OBST every 60 min from 0′ to 120′ min. The energy intake and food requirements were defined for each subject starting from breakfast and ending at bedtime with direct questions to both children and parents and using validated food frequency questionnaires before performing tests. To assess food consumption, foods were divided according to the classic basic food groups of the Italian food pyramid elaborated by the Italian Institute of Research on Food and Nutrition. A balanced diet (50–60% of carbohydrates; 15–20% of proteins; 30% of total fats of which saturated less than 7%) was suggested in the two weeks before the study; the daily dietary intake was calculated mirroring that registered at the moment of recruitment to avoid weight and hormonal changes.

The area under the curve (AUC) for parameters after OGTT was calculated according to the trapezoidal rule. The stimulus for insulin secretion related to the increment of plasma glucose as insulinogenic index was calculated as the change in insulin concentration from 0 to 30 min (Ins30) and from 0 to 120 min (Ins120). Insulin resistance was calculated using the formula of HOMA-IR = (fasting glucose $\times$ fasting insulin/22.5). Beta cell function at fasting was calculated using the formula of HOMA-B =(20 $\times$ fasting insulin)/(fasting glucose-3.5). Insulin sensitivity during OGTT was calculated from the Matsuda index. The disposition index, which reflects the capacity of pancreatic islets to compensate for lower insulin sensitivity, was defined as the product of the Matsuda Index and Ins 30 (DI30) or Ins120 (DI120).[36] All beta-cell function measures yielded similar results.

The study protocol was approved by the Local Ethical Committee and informed consent was obtained by all infant's parents before the evaluations.

Human AG and UAG (pg/ml) were measured by ELISA kits (BioVendor - Laboratori Medicina GmbH, Heidelberg Germany) AG: sensitivity: 0.2–0.6 pg/ml. Intra and inter-assay coefficient of variation ranges: 11.8–13.2%. UAG: sensitivity 0.3–0.8 pg/ml. Intra and inter-assay coefficient of variation ranges: 10.3–10.9%. OBST (ng/ml) was measured by EIA kit (Peninsula Laboratories, LLC, CA-