Clinical Nutrition 29 (2010) 54–59

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original Article

Fasting and postprandial relationships among plasma leptin, ghrelin, and insulin in prepubertal obese children

M. Gil-Campos [a,*], C.M. Aguilera [b], M.C. Ramirez-Tortosa [b], R. Cañete [a], A. Gil [b]

[a] Unit of Paediatric Endocrinology, Reina Sofia University Hospital, Avda Menéndez Pidal s/n, 14004 Córdoba, Spain
[b] Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology, Center of Biomedical Research, University of Granada, Avda del Conocimiento, 18100 Armilla, Granada, Spain

ARTICLE INFO

Article history:
Received 16 March 2009
Accepted 15 June 2009

Keywords:
Children
Leptin
Ghrelin
Insulin
Postprandial period

SUMMARY

Background: Leptin is involved in the long-term regulation of body weight and dietary intake, while ghrelin plays an essential role in appetite control. High levels of leptin have been associated with adiposity and the suppression of ghrelin levels with increased dietary intake.

Aims: To evaluate fasting and postprandial concentrations of plasma leptin and ghrelin after intake of a standardised breakfast and to study the relationship of these hormones with adiposity and insulin resistance in obese prepubertal children.

Methods: 34 obese and 20 normal-weight prepubertal children aged 6–12 years were selected. Plasma leptin and ghrelin were measured by ELISA and radioimmunoassay, respectively. The general linear model of variance, principal-component factor, and Pearson's analyses correlation were performed.

Results: Baseline and postprandial leptin levels were higher in obese versus normal-weight children. In obese, ghrelin showed an altered pattern during the postprandial period, recovering to baseline levels at 3 h after the intake. Insulin resistance was associated with leptin and independently with ghrelin.

Conclusion: The association of ghrelin with insulin resistance provides further evidence on the regulation of ghrelin in glucose homeostasis in childhood obesity at the prepubertal age. Changes in ghrelin after dietary intake may be related to an earlier recovery of appetite in prepubertal obese children.

1. Introduction

Over the past two decades, special attention has been paid to regulators of energy homeostasis such as leptin and ghrelin in order to ascertain the pathophysiological determinants of obesity. Leptin is a well-known adipokine involved in the long-term regulation of body weight, dietary intake, and energy expenditure,[1] while ghrelin plays an essential role in appetite and meal initiation and participates in the hypothalamic regulation of energy homeostasis.[2]

Leptin inhibits both the secretion and the negative reponse mediated by feeding of gastric ghrelin, while ghrelin suppresses the secretion of leptin in the stomach. It has been proposed that this dual leptin restraint is the major regulatory arm of the feedback communication between the periphery and the hypothalamus for weight homeostasis.[3]

Plasma leptin levels are markedly elevated in obese humans and are directly associated with adiposity[1,4]. In contrast, ghrelin levels are dependent on recent food intake, increasing after fasting and decreasing after eating.[5] The postprandial suppression of ghrelin is considerably reduced in obese adults and children.[6] However, the role of meal intake on ghrelin suppression in children remains controversial.[7]

The role of ghrelin in childhood obesity, which is usually associated with insulin resistance (IR), is not fully understood.[8–10] A significant relationship between insulin and ghrelin has been reported,[10] and it has been suggested that postprandial insulin is responsible for reducing plasma ghrelin levels after food intake.[2] However, most studies have concluded that insulin is not absolutely essential for postprandial ghrelin suppression, although it facilitates persistence of this response.[11]

The objectives of this study were to evaluate fasting and postprandial concentrations of leptin and ghrelin and to study the relationships of these hormones with adiposity and IR in prepubertal obese children.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FSH, follicle stimulant hormone; HOMA, homeostasis model assessment index; IR, insulin resistance; LH, lutein hormone; OGTT, oral glucose-tolerance test; QUICKI, quantitative insulin sensitivity check index; RIA, radioimmunoassay method; SBP, systolic blood pressure; SS, subscapular skinfold; TAG, triacylglycerols; TS, tricipital skinfold.

* Corresponding author. Tel.: +34 957 010055; fax: +34 958 248960.
E-mail address: mercedes_gil_campos@yahoo.es (M. Gil-Campos).

0261-5614/$ – see front matter © 2009 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
doi:10.1016/j.clnu.2009.06.007

2. Materials and methods

2.1. Subjects

We studied 54 Caucasian children aged 6–12 years, comprising 34 obese and 20 normal-weight children. All were selected from among children referred to our Paediatric Endocrinology Unit. Inclusion criteria were absence of disease as verified by clinical examination and classification as prepubertal (Tanner I) based on Tanner criteria. We measured fasting gonadotropins and sex hormones (follicle stimulant hormone – FSH, lutein hormone – LH, estradiol, and testosterone) to validate that selected children classified as prepubertal by clinical signs were truly at this stage. Exclusion criteria were presence of pubertal development, disease, or malnutrition, being on a restrictive diet, use of medication that alters blood pressure or glucose or lipid metabolism, and history in the past year of a long rest period or consumption of hypocaloric diet. The obese and normal-weight groups were matched for age and sex. Children were classified as obese if their body-mass index (BMI) exceeded the 97th percentile for their age and sex according to Spanish standards (BMI z-score ≥ 2.0).[12] Children with a BMI z-score of 2–3 were considered moderately obese and those with a BMI z-score >3 severely obese. Children were classified as normal-weight if their BMI was between the 25th and the 75th percentile. Children with low-height were excluded from the normal-weight group. Previous reports by our group on the postprandial response of *trans* and other plasma fatty acids in obese prepubertal children were based on the same study population.[13,14]

The study was approved by the Human Investigation and Ethics Committees of the University of Granada and the Reina Sofia University Hospital of Cordoba (Spain). Written informed consent was obtained from parents and verbal approval from children.

2.2. Anthropometric and blood pressure measurements

Anthropometric measurements: Children were barefooted and in their underwear. Body weight (kg) was measured using a standard beam balance (Seca), precision 100 g, range 0–150 kg. Height (cm): using a precision stadiometer (Seca), precision 0.2 cm, range 70–200 cm. Waist circumference (WC) was measured with an unelastic tape, precision 0.1 cm, range 0–150 cm, the subject in a standing position; the tape was applied horizontally midway between the lowest rib margin and the iliac crest about the level of the umbilicus, at the end of gentle expiration. Skinfold thicknesses (mm) were measured at the left side of the body to the nearest 0.1 mm with a skinfold caliper (Holtain, UK, range 0–40 mm).

Blood pressure was measured with a random-zero sphygmomanometer. The cuff size (bladder-size) was sufficiently long to surround at least two thirds of the upper arm. The centre of the inflatable part of the cuff (bladder) was positioned over the brachial artery of the inner side of the upper arm. The blood pressure was measured after resting with no change of position for at least 5 min, in a sitting position and using the right arm unless there was a deformity.

2.3. Dietary assessment and intervention

Children and parents were individually interviewed to obtain information on lifestyle and dietary habits. Dietary intake was estimated by means of a previously validated food-frequency questionnaire and 72-h dietary survey using a database for the composition of Spanish foods.[15] Both obese and normal-weight children received a standardised breakfast at 9 a.m., which included 200 ml of whole milk, 10 g of sugar, 18 g of cocoa powder, 10 g of butter, 30 g of toasted bread, and 20 g of marmalade, providing 438 kcal, 9.8 g of proteins, 15.5 g of fat, and 64.8 g of carbohydrates.

2.4. Sampling

Baseline blood samples were obtained from children while they were fasting, using an indwelling venous line to measure levels of glucose, insulin, sex hormones (FSH, LH, estradiol, and testosterone), lipids, leptin, and ghrelin. Blood samples were also drawn at 1, 2, and 3 h after intake of the breakfast for determination of leptin and ghrelin. All samples were processed within 2 h of sampling and divided into aliquots for immediate analysis or long-term storage at −80 °C until their analysis.

2.5. Biochemical analysis

Plasma glucose, insulin, sex hormones, and lipids were analysed as previously reported.[13] HOMA index was calculated by the equation HOMA = fasting glucose (G_0) (mM) × fasting insulin (I_0) (μU/mL)/22.5; and the quantitative insulin sensitivity check index (QUICKI) by the equation QUICKI $= 1/(\log I_0 + \log G_0)$.

Plasma leptin was measured with a solid phase ELISA kit using specific monoclonal antibodies from Biosource Europe SA, Nivelles, Belgium. Plasma ghrelin levels were assessed by means of a radioimmunoassay method (RIA) from Linco Research Inc., St. Charles, MO, USA.

2.6. Statistical analysis

Data are expressed as means ± SEM. Variables that did not follow a normal distribution (plasma triacylglycerols-TAG-, insulin, and HOMA) were log-transformed before analysis. Comparisons of sociodemographic and clinical variables between obese and control children, adjusted for age and sex, were assessed with general linear models of variance. The general linear model of variance for repeated measurements was used to evaluate the effects of the two sources of variation in the study, obesity, and postprandial time. Post hoc comparisons using Bonferroni tests were performed to evaluate mean differences among the different postprandial times. $P < 0.05$ was considered significant. Correlations of leptin or ghrelin with other variables were assessed by means of Pearson's correlation coefficients.

Principal-component factor analysis was used to investigate the potential contribution (factor loading of leptin and ghrelin) to IR. Extraction of the initial set of uncorrelated components was accomplished with the principal-factor method, followed by orthogonal rotation of components using Varimax with Kaiser normalisation to facilitate interpretation. Seven variables related to IR syndrome (BMI z-score, HOMA index, systolic blood pressure (SBP) and plasma TAG, HDL-cholesterol, uric acid, and glucose levels) and plasma leptin and ghrelin levels were included in the factor analysis. Factor loading, i.e., the product-moment correlation (a measure of linear association) between an observed variable and an underlying factor, was used to interpret the factor structure. Loadings are equivalent to Pearson correlation coefficients, with a higher loading indicating a stronger relationship between a factor and a variable. Analyses were performed using the software program SPSS version 15.1 (Statistical Package for Social Sciences, SPSS Inc. Chicago, IL, USA).