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FASTING AND POST-GLUCOSE GHRELIN LEVELS IN SGA INFANTS: RELATIONSHIPS WITH SIZE AND WEIGHT GAIN AT ONE YEAR OF AGE

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ABSTRACT Wide ranges in postnatal weight gain are seen in infants born small for gestational age (SGA); most show some catch-up growth and this may be driven by increased appetite. Ghrelin, the natural ligand of the GH secretagogue receptor, has potent orexigenic effects. In adults circulating ghrelin levels are increased in anorexia, decreased in obesity and show post prandial suppression. The aim of the present study was to test the hypothesis that rate of weight gain over the first year in SGA infants may relate to variable suppression of circulating ghrelin levels. Serum ghrelin levels were measured in 1y old infants born SGA (n=85) and in control infants born adequate for gestational age (AGA) (n=22) fasting and 10 minutes after intravenous (iv) glucose (0.5 g/Kg of 25% dextrose). Sex- and gestational age-adjusted SD scores (SDS) for body weight were calculated at birth and at 1y, and delta weight SDS between 0-1y was calculated as an index of postnatal weight gain.
In both SGA and AGA groups, ghrelin levels reduced from fasting (mean ± SE: 104.4 ± 6.4 fmol/ml) to 10 minutes post-iv glucose (82.7 ± 5.3, $p<0.0005$). There were no differences in ghrelin levels between SGA and AGA infants (fasting or post-iv glucose). However, in SGA infants ghrelin levels post-glucose, but not fasting, were positively related to current length ($r=0.28$, $p<0.05$), weight ($r=0.23$, $p<0.05$) and to change in weight SDS 0-1y ($r=0.22$, $p<0.05$). SGA infants who showed poor catch-up growth showed a larger decline in ghrelin concentrations post-iv glucose. In conclusion, circulating ghrelin levels rapidly decreased after iv glucose. Higher ghrelin levels or lower reductions in circulating levels following iv glucose were seen in SGA infants who showed greater infancy weight gain, suggesting that sustained orexigenic drive could contribute to postnatal catch-up growth.

Introduction

Ghrelin is the endogenous ligand for the growth hormone (GH) secretagogue receptor related to GH release from the pituitary (1). Ghrelin and synthetic GH secretagogues (GHRPs) act through a G-protein coupled receptor that is expressed in the hypothalamus, pituitary and pancreas (2). Ghrelin was recently isolated from the stomach, which seems to be the main source of circulating levels (3) although lower amounts have been isolated from arcuate hypothalamic neurons, pituitary, kidney, placenta, bowel and pancreas (4-7). The purified ghrelin peptide comprises 28 amino acids and has a molecular weight of 3314 KD. Structurally it is characterized by a unique post-translation addition of a straight chain octanoyl group linked to the third serine which is essential for the activity of the peptide (3).

The GHRPs were originally discovered through their effects on GH secretion (8-10) but their potential actions have recently been revised (11,12). Ghigo and co-workers provided the first evidence that ghrelin stimulates appetite (13) and a large number of animal studies have confirmed these effects are independent of GH secretion (14). In rodents, fasting and hypoglycemia increase ghrelin levels, whereas intake of food, especially carbohydrates, decreases ghrelin levels (15); these effects on appetite seem to be mediated, at least partially, through hypothalamic neuropeptide Y (16-18). In humans, circulating ghrelin levels are decreased in chronic obesity or after acute oral food intake, and are increased in cachexia and fasting (19,20).

Infants born small for gestational age (SGA) may have experienced intrauterine growth retardation due to fetal, maternal or environmental events and in response to prenatal nutritional deprivation often show postnatal GH hypersecretion (21). While the majority display postnatal rapid or "catch-up" growth, a wide range in rates of postnatal weight gain are seen and may relate to levels of satiety and food intake (22). We examined the hypothesis that variable rates of weight gain in SGA infants may be associated with variation in circulating levels or degree of suppression of ghrelin.

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Subjects and Methods

Eighty-five small for gestational age (SGA: defined as birth weight <5th percentile for gestational age using the Chilean birth weight reference (23)) and 22 adequate for gestational age (AGA: birth weight >5th percentile) infants participated in this study. Subjects were recruited at birth from the neonatal units of Hospital San Borja Arriarán and Hospital Sótero del Río to a study of SGA infants and controls with a planned 3y follow-up at the pediatric endocrine unit of the Institute of Maternal and Child Research, University of Chile. All infants underwent a pre-study screening clinical evaluation to exclude those with significant medical, neurological or genetic conditions, and infants who were receiving unusual diets or any medication that could interfere with growth or appetite.

The study protocol was approved by the San Borja Arriarán Hospital Institutional Review Board. The parents or guardians gave written informed consent.

All children had weight and length measured at birth and at 1y by one nurse (A.A.). Supine length was measured using a firm box with an inflexible headboard and a movable foot-board with the feet placed perpendicular to the plane determined by the supine length of the infant. Weight was measured with an electronic scale.

At 1y of age infants had an intravenous glucose tolerance test (IVGTT) following an overnight fast. Two sites of venous access were obtained in bilateral antecubital veins. 25% dextrose 0.5 g./Kg was administered by constant infusion

over 3 mins. Blood samples were obtained at 0, 1, 3, 5 and 10 mins for glucose and insulin measurement and at –5 and 10 mins for Ghrelin. Glucose was measured immediately whereas samples for the other analyses were placed immediately on ice, centrifuged within 30 minutes, and serum was frozen at -20°C.

Ghrelin levels were measured using a commercial RIA (Phoenix Pharmaceuticals, Belmont, CA) that uses ^{125}I-labeled bioactive ghrelin as a tracer molecule and a rabbit polyclonal antibody against full-length octanoylated human ghrelin. This assay recognizes both active and inactive forms of ghrelin. The sensitivity of the assay is 10 fmol/ml, intra-assay CV was 5.5%, and the inter-assay CV was 12.1%.

Glucose was measured by the hexokinase method. Insulin levels were measured by RIA from Diagnostic System Laboratories (Webster, TX, USA). This assay has a cross reactivity of 27.5% with proinsulin and 25% with –32/33 split proinsulin. The sensitivity of the assay is 0.8 IU/ml, the intra-assay CV was 4.8% and inter-assay was CV 3.5%.

Standard deviation (SD) scores for weight and length at birth and at 1y were calculated to adjust for age and sex. We used National Center for Health Statistics growth curves, which have been found to be applicable to the Chilean population (24,25). Mid-parental height was calculated and similarly converted to SD score. Area under the curve (AUC) for glucose and insulin were calculated using the trapezoidal rule.

The data from SGA and AGA children were analyzed as two separate groups and subsequently a further analysis was performed by dividing the SGA subjects in three groups according to weight change from birth to 1y of age: "Catch-up" (SGA^+) defined as a gain of 0.67 SDS or more (n=63); "Catch-down" (SGA^-) defined as a loss of 0.67 SDS or more (n=5), and "No-change" (SGA^0) for infants who showed a change of weight between -0.67 SDS and +0.67 SDS (n=17) (26). Data are presented as mean ± SE, and were analyzed using SPSS program version 10.0 applying parametric (ANOVA) and non parametric tests (Mann-Whitney and Wilcoxon), considering $p<0.05$ as significant.

Results

SGA infants were lighter and shorter, both at birth and at 1y compared to AGA infants (Table 1), but the differences were smaller at 1y than at birth as the SGA group showed greater increases in weight and length SDS between 0-1y. Parental heights were no different in SGA versus AGA infants (Table 1).

Table 1. Size at birth at 1y, and parental heights in small-(SGA) and appropriate-for-gestational age (AGA) infants

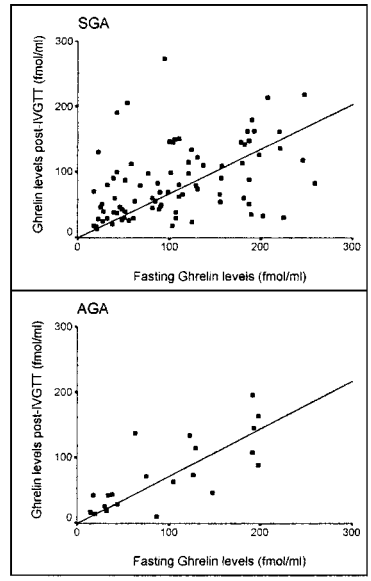
	SGA	AGA
Birth weight SDS	-2.08 ± 0.06*	0.95 ± 0.25
Birth height SDS	-1.63 ± 0.09*	0.44 ± 0.19
Gestational age (weeks)	38.6 ± 0.1	38.7 ± 0.3
One year height SDS	-0.84 ± 0.10*	-0.3 ± 0.18
One year weight SDS	-0.81 ± 0.11*	0.48 ± 0.27
Delta weight SDS 0 -1 y	1.27 ± 0.12*	-0.5 ± 0.3
Delta length SDS 0 -1 y	0.80 ± 0.10*	-0.8 ± 0.24
Paternal height SDS	-1.26 ± 0.16	-1.3 ± 0.19
Maternal height SDS	-1.37 ± 0.08	-1.1 ± 0.28

* $p<0.05$ vs AGA

No significant difference was seen between SGA and AGA infants in fasting ghrelin levels (SGA vs. AGA; 106.9 ± 7.1 fmol/ml vs. 94.8 ± 14.4, p=NS).

Ten minutes following intravenous glucose (IVGTT) a marked reduction in circulating ghrelin levels was seen in both SGA infants (fasting vs. post-IVGTT; 106.9 ± 7.1 fmol/ml vs. 85.2 ± 6.0, p=0.004), and in AGA infants (94.8 ± 14.4 vs. 76.4 ± 11.8, p=0.05). Post-IVGTT ghrelin levels were no different between SGA and AGA infants. However there was a high inter-individual variability in changes in ghrelin levels following IVGTT (Figure 1).

Figure 1. Ghrelin levels pre- and 10mins post-IVGTT in a) SGA infants (n=85) and b) AGA infants (n=22). Correlation coefficients between pre- and post-IVGTT ghrelin levels, SGA: r=0.42, p<0.0005; AGA: r=0.76, p<0.0005. Mean ± SE paired difference (pre- *minus* post-IVGTT), SGA: 21.7 ± 7.1 fmol/ml, p=0.003; AGA: 21.9 ± 9.4 fmol/ml, p=0.03.



In SGA infants, post-IVGTT ghrelin levels were positively correlated to body weight and body length at 1y (Table 2), and also positively related to gain in weight SDS between birth to 1y (Figure 2). In contrast, fasting ghrelin levels were unrelated to body size at birth or at 1y (Table 2).

Table 2. Correlations between fasting and 10 minutes post-IVGTT ghrelin levels vs. body size and infancy growth in SGA infants

	Ghrelin 0 min	Ghrelin 10 min
1 y weight SDS	0.05	0.23 *
1 y length SDS	0.1	0.28**
Delta weight SDS 0 - 1 year	0.01	0.22*
Delta length SDS 0 - 1 year	0.03	0.13

* $p < 0.05$, ** $p < 0.01$

Furthermore, change in ghrelin levels between 0-10mins showed a near significant positive correlation with gain in weight SDS (r=0.20, p=0.07), and on comparing the subgroups of SGA children, the largest reductions in ghrelin

levels post-IVGTT were seen in infants who showed the lowest postnatal weight gain (SGA^-: 59.8 ± 11.6 fmol/ml), next SGA^0 (29.9 ± 19), then SGA^+ (16.6 ± 8.0, $p<0.05$ vs. SGA^- group) (Figure 3). In SGA infants, lower ghrelin levels post-IVGTT were also related to higher AUC glucose ($r=-0.22$, $p<0.05$), but not to AUC insulin ($r=-0.09$, p=NS).

Figure 2. Change in weight SDS between 0-1y vs. ghrelin levels 10 mins post-IVGTT in SGA (• & solid line: r=0.22, p<0.05, n=85) and AGA infants (& dotted line: r=0.20, p=NS, n=22).

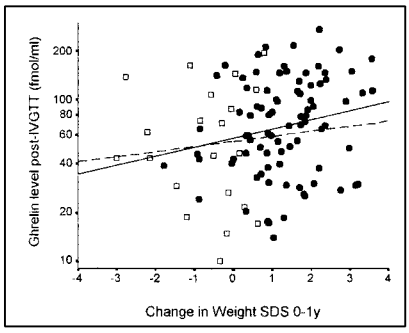
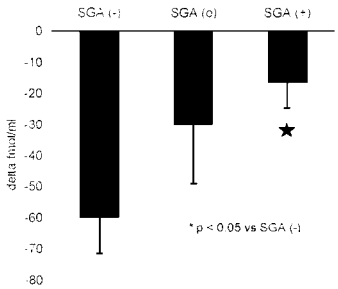


Figure 3. Change in circulating ghrelin levels from baseline to 10 minutes following an intravenous glucose load in SGA infants, grouped by postnatal weight gain.



Discussion

In this study ghrelin levels in one year old small- (SGA) or adequate-for-gestational age (AGA) infants fell rapidly by around 20% following intravenous (iv) glucose administration. A recent study in adults showed that ghrelin levels suppressed by 20-30% with enteral feeding but not following the co-administration of glucose and insulin to maintain euglycemia (27). The authors suggested that the decrease in ghrelin on feeding may be due to local gastric effects rather than a response to metabolic changes. However other studies have reported rapid decreases in ghrelin levels after simple glucose infusion in rodents (28) and in normal human adults (29). Our study confirms that this regulation is present during infancy, appeared to relate to the level of plasma glucose excursion but not to insulin levels, and furthermore may influence rate of weight gain during the first year of life.

There is good evidence that ghrelin has an important role in appetite and satiety regulation both in rodents and humans through the activation of hypothalamic neuropeptide Y (15,18). In a recent randomised double-blind cross-over study, ghrelin infusion in human volunteers resulted in increased appetite and a 28% increase in energy consumption from a free-choice buffet (30). In addition, mutations in the prepro-ghrelin/Ghrelin gene have recently been reported in an obese Swedish cohort, although the functional significance of these genetic variations remains to be elucidated (31).

We hypothesized that if circulating ghrelin levels promote orexigenic drive in humans then these levels might relate to rate of weight gain in infancy which has been linked to variations in satiety (22). We observed that among SGA infants both the degree of ghrelin suppression and the actual ghrelin levels 10 minutes after iv glucose were related to infancy weight gain. This is the first described relation between higher circulating ghrelin levels and greater rate of weight gain, and is consistent with the potential stimulatory effects of ghrelin on food intake (30).

SGA is defined on the basis of low birthweight and infers prenatal growth restraint. Such infants usually show postnatal compensatory catch-up growth and long before the elucidation of mechanisms that regulate appetite, low birthweight infants with rapid weight gain were demonstrated to have reduced satiety (22). In a large population-based birth cohort we previously reported association between more rapid infancy weight gain and lower leptin levels at birth (32). Leptin resistance has been demonstrated to appear with age and weight gain (33) while low circulating ghrelin levels in obese adults suggest that weight gain in these subjects is not driven by increased appetite due to ghrelin (19). While some obese adults lack significant meal-related suppression of ghrelin (34), it is possible that sensitivity to the effects of circulating satiety and appetite regulatory peptides is highest at younger ages.

In our study, the absence of correlation between weight gain and fasting ghrelin levels might suggest that in infants ghrelin may have a greater influence on post-meal satiety rather than pre-meal stimulation of appetite. Similarly in the study by Ounsted and Sleigh (22) more rapid weight gain in SGA infants was related to larger volumes of milk consumed per feed. The lack of correlation between ghrelin levels and rate of weight gain in our AGA infants may reflect the smaller number of subjects in this group, or could also relate to the absence of postnatal catch-up growth in these infants.

In conclusion, we propose that reduced ghrelin suppression and higher post-prandial ghrelin levels could result in sustained orexigenic drive and could contribute to postnatal catch-up growth in SGA infants. Further studies are needed to determine whether fasting or post-meal ghrelin levels may prospectively predict weight gain in infancy.

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