Effects of the IGF-I/IGFBP-3 complex on GH and ghrelin nocturnal concentrations in low birth weight children

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Summary

Objective There is limited information regarding the effects of IGF-I and/or IGFBP-3 on circulating ghrelin concentrations. To determine the effects of IGF-I on GH and ghrelin concentrations, we examined the GH and ghrelin nocturnal profiles before and after the administration of the IGF-I/–IGFBP-3 complex (Iplex™) to low birth weight children.

Design The children were studied on two separate occasions, the first under basal conditions, and the second time after the sc administration of 1 mg/kg of Iplex™ at 2100 h. Blood samples for determination of GH and ghrelin were obtained every 20 min between 2300 h and 0700 h, while the children were sleeping. In each patient, we calculated the mean GH and ghrelin area under the curve (GH AUC and GHR AUC), both under basal conditions and after the administration of the IGF-I/IGFBP-3 complex.

Setting The study was performed at a University Research Centre located at a General Hospital in Santiago, Chile.

Patients Twenty prepubertal children (11 boys and 9 girls), born after a full-term pregnancy with a birth weight below 2·8 kg were studied at a mean ± SEM age of 7·3 ± 0·5 years (range 4–11 years). Their mean height was −1·8 ± 0·3 standard deviation score (SDS) and their mean BMI was 0·1 ± 0·2 SDS at the time of the study.

Main outcome and results Mean nocturnal GH AUC exhibited a significant decrease (2903 ± 185 *vs* 1860 ± 122 ng/ml min, $P < 0{\cdot}01$), whereas mean GHR AUC showed a significant increase after administration of the IGF-I/IGFBP-3 complex (68 ± 16 *vs* 288 ± 36 ng/ml min, $P < 0{\cdot}01$).

Conclusions These findings indicate that the IGF-I/IGFBP-3 complex appears to have opposite effects on circulating GH and ghrelin concentrations in low birth weight children, suggesting that, in addition to its known negative feed-back effect on GH, IGF-I and/or IGFBP-3 may have a positive feed-back effect on ghrelin.

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Introduction

Ghrelin is the endogenous ligand for the growth hormone (GH) secretagogue receptor and stimulates GH release from the pituitary.[1] Ghrelin and the synthetic GH secretagogues (GHRPs) act through a G-protein coupled receptor that is expressed in the hypothalamus, pituitary and pancreas.[2] Ghrelin was first isolated from the stomach, which seems to be its main source,[3] although it has been isolated from the hypothalamus, pituitary, kidney, placenta, bowel and pancreas.[4–7] The purified ghrelin peptide comprises 28 amino acids and has a molecular weight of 3·3 kD. Structurally, it is characterized by a unique post-translational addition of a straight chain octanoyl group linked to the third serine, which is essential for the activity of the peptide.[3]

Whether ghrelin secretion is regulated by GH under physiological conditions has not been clearly established. In addition, there is scant information regarding the possible effects of IGF-I on circulating ghrelin levels. In humans with active acromegaly, circulating systemic ghrelin levels are not affected by ambient GH concentrations.[8] In addition, systemic ghrelin levels are not modified by 1 year of GH replacement therapy in subjects with GH deficiency, suggesting that GH does not modulate circulating ghrelin levels.[9]

In humans, circulating ghrelin levels are decreased in chronic obesity or after acute oral food intake, and are increased in cachexia and fasting.[10,11] Girls with anorexia nervosa have higher GH concentrations, as a consequence of increased basal GH secretion and secretory burst frequency.[12] Similarly, elevated ghrelin[13,14] and GH concentrations[15,16] have been reported in adult women with anorexia nervosa. Negative feedback from low IGF-I levels in these patients has been postulated to cause increased GH secretion, but it is unclear whether IGF-I also regulates circulating ghrelin concentrations.

Infants born small for gestational age may experience intrauterine growth retardation due to fetal, maternal or environmental adverse events, and in response to prenatal nutritional deprivation they often show postnatal GH hypersecretion and low IGF-I levels.[17] Relatively little information is available, however, regarding serum ghrelin concentrations in low birth weight children. We recently described similar fasting ghrelin levels at 1 year between small for gestational age and adequate for gestational age infants.[18]

To determine the effects of IGF-I on GH and ghrelin concentrations in low birth weight children, we examined the GH and ghrelin nocturnal profiles before and after the administration of a sc bolus the IGF-I/IGFBP-3 complex (Iplex™). This protocol was performed

in a cohort of short, low birth weight children who were undergoing a study of IGF-I sensitivity.[19] We studied nocturnal GH and ghrelin concentrations in view of the fact that both GH and ghrelin are actively secreted during the night, and appear to be regulated by sleep.[20] We hypothesized that the increase in serum IGF-I levels which is observed after the administration of the IGF-I/IGFBP-3 complex, would induce changes in the nocturnal profile of GH and ghrelin that would provide information regarding the potential effects of IGF-I on ghrelin.

Patients and methods

The children participating in this study were recruited from the paediatric endocrinology clinic and from a follow-up clinic for low birth weight children at the San Borja Arriarán Hospital in Santiago, Chile. The study protocol was approved by the San Borja Arriaran Hospital Institutional Review Board, and the parents gave written informed consent.

Twenty children (11 boys and 9 girls) with low birth weight, defined as a birth weight < 10th percentile for gestational age using local birth weight standards, participated in this study.[21] This cohort is part of a group of low birth weight children who are being studied for markers of IGF-1 sensitivity at our Institute. The children were born after a full term pregnancy, and at the time of the study they were 4–11 years old. All subjects were prepubertal, and had a normal body mass index for age. The children's clinical characteristics are shown in Table 1. We were careful to exclude children who were overweight, in order to limit the potential metabolic effects of excess weight in our patient sample. Ethnically, the Chilean population is considered to be an admixture of 30% Amerindian and 70% European, mainly Spanish.[22] Children with any identifiable chronic or genetic illness were excluded. Specifically, the children had normal body proportions, determined by measuring their arm span and upper to lower segment ratio, normal blood cell count, blood chemistry, plasma electrolytes, renal and liver function tests, urinalysis, thyroid function, and karyotype (girls).

The clinical and anthropometric evaluation of all children was performed by a paediatric endocrinologist and by a genetics specialist. One nurse (A.A.) determined the children's height with a Harpenden stadiometer, and the weight with a manual scale (Seca, Germany).

Study protocol

The children were admitted to our clinical research unit at approximately 1700 h to become acclimatized to the hospital environment. The ghrelin and GH nocturnal profiles were determined twice, separated by an interval of 2–4 weeks. On the first night, nocturnal serum GH and ghrelin concentrations were measured under basal conditions, and on the second night, they were determined after IGF-I/IGFBP-3 (Iplex™) administration, which was kindly donated by Insmed Inc. (Glen Allen, VA, USA). After admission, a short iv catheter was placed in a forearm vein, and at 1900 h the children received a standard meal which provided 600 calories (10% protein, 25% fat and 65% carbohydrate), and they were allowed to play and watch TV. At 2100 h the children were placed in bed, and they fell asleep shortly thereafter. Blood samples (0·8 cc) were obtained from the catheter every 20 min from 2300 h to 0700 h, while the children were asleep. On the second night, the same procedure was followed, except for the fact that we administered Iplex™ at a dose of 1 mg/kg of body weight as a sc. bolus at 2100 h. In addition, serum IGF-1 and IGFBP-3 levels were determined basally at 2100 h, and after Iplex™ administration at 2400 h on the second night. Blood glucose was measured with an Accu Sensor device manufactured by Roche at hourly intervals between 2100 and 2400 h.

Table 1. Clinical characteristics and growth factor concentrations in low birth weight children

	All ($n = 20$)	Girls ($n = 9$)	Boys ($n = 11$)
Gestational age (weeks)	38·8 ± 0·3	38·4 ± 0·3	39·1 ± 0·3
Birth weight (SDS)	−2·19 ± 0·18	−2·23 ± 0·18	−2·08 ± 0·24
Birth length (SDS)	−2·05 ± 0·32	−2·20 ± 0·46	−1·93 ± 0·46
Age at study (years)	7·3 ± 0·5	6·6 ± 0·5	7·8 ± 0·5
Weight at study (SDS)	−1·16 ± 0·30	−0·56 ± 0·35	−1·65 ± 0·42
Height at study (SDS)	−1·84 ± 0·31	−1·51 ± 0·54	−2·12 ± 0·36
BMI at study (SDS)	0·03 ± 0·19	0·33 ± 0·27	−0·21 ± 0·26
IGF-I (basal) (ng/ml)	213·8 ± 17·9	251·2 ± 30·0	183·1 ± 18·0
IGF-I (post IP) (ng/ml)	461·9 ± 27·1*	508·2 ± 41·4*	423·9 ± 33·2*
IGFBP-3 (basal) (mg/l)	2·7 ± 0·2	2·9 ± 0·3	2·5 ± 0·3
IGFBP-3 (post IP) (mg/l)	3·4 ± 0·2*	3·6 ± 0·4*	3·2 ± 0·3

*$P < 0·05$ (Basal vs. Iplex™).

In each patient, we calculated the mean hormone concentration, mean number of peaks, mean peak amplitude, and mean hormone area under the curve (AUC) for both GH and ghrelin under basal conditions, and after administration of the IGF-I/IGFBP-3 complex.

Assays

Serum GH was measured by a double antibody RIA with a sensitivity of 0·8 ng/ml and an intra-assay and interassay coefficient of variation (CV) of 10% and 6·5%, respectively. All reagents for the GH RIA were donated by the National Hormone and Pituitary Program (human GH-I-3, antihuman GH-2 antisera, human GH-RP).

Serum IGF-I levels were determined using a locally developed RIA[23] requiring sample extraction as a first step. The sensitivity of this assay is 5 ng/ml. Intra- and interassay CVs were 8·6% and 10·2%, respectively.

Serum IGFBP-3 concentrations were determined using a commercial IRMA (Diagnostic System Laboratories, Webster, TX, USA). The sensitivity of the assay is 0·1 mg/l, intra-assay CV was 1·1%, and the interassay CV was 1·8%.

Serum ghrelin was measured using a commercial RIA (LINCO Research, St. Charles, MO, USA) that uses ^{125}I-labelled ghrelin as a tracer, and a rabbit polyclonal antibody against full-length octanoylated human ghrelin. The sensitivity of the assay is 93 pg/ml, and its intra-assay CV 6·5%, and the interassay CV 12·1%.

Statistical analysis

The standard deviation score (SDS) for BMI, weight and height were based on the National Centre for Health Statistics (NCHS). Results

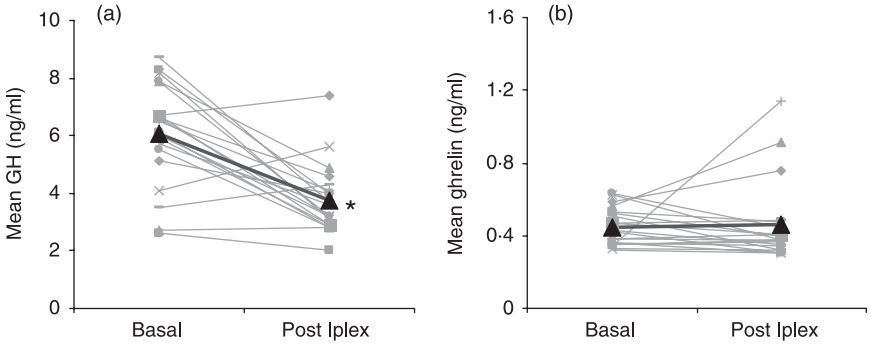

Fig. 1 Individual and mean basal and post Iplex™ serum GH and ghrelin nocturnal levels in low birth weight children. (a) mean GH and (b) mean ghrelin nocturnal concentrations. After Iplex™ administration, there is a significant decrease in mean GH levels, but there is no significant change in mean nocturnal ghrelin levels. Individual patients are shown by the grey lines and the mean by the dark line. $^*P < 0{\cdot}01$.

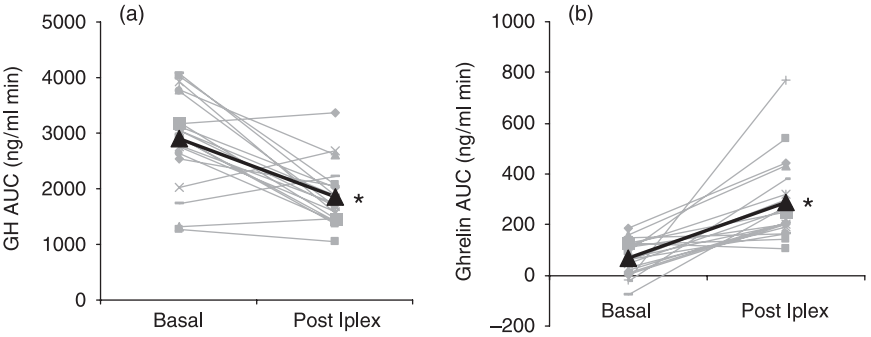

Fig. 2 Individual and mean basal and post Iplex™ serum GH and ghrelin AUC in low birth weight children. (a) mean GH AUC and (b) mean ghrelin AUC. After Iplex™ administration, there is a significant decrease in mean GH AUC, and an increase in mean ghrelin AUC. Individual patients are shown by the grey lines and the mean by the dark line. $^*P < 0{\cdot}01$.

are shown as mean ± SEM. Differences between groups were assessed by the Student *t*-test for parametric variables, and by the Mann–Whitney test for nonparametric variables. To compare the nocturnal GH and ghrelin nocturnal profiles for each patient before and after Iplex™ administration, differences were assessed by the Wilcoxon test. Analysis of the pulsatile characteristics of the nocturnal GH and ghrelin profiles was performed using the computer program Pulsar.[24] Calculations for GH and ghrelin AUC were performed with the trapezoidal method. Statistical analysis was performed with the SPSS program v11·5.

Results

The clinical characteristics and the growth factor concentrations of the children are shown in Table 1. As expected, the children had a low birth weight and birth length, and a low height and weight at the time of the study. Their BMI, however, was normal for age. As shown in Table 1, serum IGF-I and IGFBP-3 levels were within the normal range basally, and increased significantly after the administration of Iplex™. The magnitude of the increase in circulating concentrations, however, was greater for IGF-I than for IGFBP-3. The haematocrit did not change significantly after nocturnal blood sampling in any patient. In addition, serum glucose did not change significantly between 2100 h and 2400 h after the administration of the IGF-1/IGFBP-3 complex.

Mean nocturnal GH concentrations in low birth weight children before and after Iplex™ administration are shown in Fig. 1a. The Figure shows that the low birth weight children exhibited significant decrease in mean GH from 6·1 ± 0·4 to 3·8 ± 0·3 ng/ml after administration of the IGF-I/IGFBP-3 complex ($P < 0{\cdot}001$). However, no differences were observed in mean ghrelin concentrations after administration of the complex (Fig. 1b).

No difference in mean hormone peak number was observed after the administration of the complex (4·3 ± 0·2 peaks/8 h *vs* 4·5 ± 0·2 peaks/8 h for GH, and 6·3 ± 0·3 peaks/8 h and 5·8 ± 0·4 peaks/8 h for ghrelin). Mean GH peak amplitude, however, showed a significant decrease after Iplex™ administration (10·0 ± 0·8 *vs* 6·9 ± 0·7 ng/ml, $P < 0{\cdot}05$), whereas mean ghrelin peak amplitude did not show any difference following administration of the complex (0·29 ± 0·03 *vs* 0·31 ± 0·05 ng/ml).

Mean GH AUC decreased significantly after the administration of the IGF-I/IGFBP-3 complex (2903 ± 185 *vs* 1860 ± 122 ng/ml/8 h, $P < 0{\cdot}01$, Wilcoxon), as shown in Fig. 2a. In contrast, mean ghrelin AUC increased significantly after Iplex™ administration (68·4 ± 15·6 *vs* 288·2 ± 35·6 ng/ml/8 h, $P < 0{\cdot}01$, Wilcoxon), as shown in Fig. 2b. In addition, a very similar proportion of patients classified by sex (eight of nine girls and nine of 11 boys), increased their nocturnal ghrelin concentrations in response to Iplex™. We did not document any consistent temporal pattern for the changes in GH and ghrelin following the administration of IGF-I/IGFBP-3 complex to these children.

We did not observe any correlation between birth weight SDS and the GH or ghrelin responses to the administration of Iplex™. We did observe, however, a direct correlation between birth weight SDS and baseline nocturnal ghrelin concentrations ($r = 0{\cdot}457$, $P = 0{\cdot}043$).

Discussion

The release of GH from the pituitary is mainly regulated by two hypothalamic hormones: growth hormone-releasing hormone (GHRH) and somatostatin.[25] In addition, an endogenous peptide

ligand for the growth hormone secretagogue receptor (GHS-R), which has been named ghrelin, has been purified from rat stomach and subsequently cloned.[3] Ghrelin can stimulate GH release from the pituitary, but the physiological role of ghrelin in the regulation of GH secretion has not been clearly established. Evidence in adolescents and adults indicates that ghrelin is secreted in a pulsatile fashion in humans, suggesting either that ghrelin participates in the pulsatile secretion of GH, or that the two hormones may be cosecreted.[26,27] However, there is scant information regarding the possible regulation of circulating ghrelin levels by IGF-I.

In order to determine the effects of IGF-I on ghrelin concentrations, we examined the GH and ghrelin nocturnal profiles before and after the administration of the IGF-I/IGFBP-3 complex to healthy, low birth weight children. Our results demonstrate an increase in mean ghrelin AUC after Iplex™ administration, which suggests that IGF-I and/or IGFBP-3 may regulate systemic ghrelin concentrations. We should note that we achieved a very significant increase in mean IGF-I concentrations after the administration of 1 mg/kg of Iplex, which indicates that we may have achieved supraphysiological levels of IGF-I for prepubertal children with this dose of the IGF-I/IGFBP-3 complex. It is unclear whether we would have achieved a similar effect on the GH and ghrelin nocturnal profiles in these children if we had administered a lower dose of the drug. We should also mention that we observed a less marked increase in serum IGFBP-3 concentrations compared to IGF-I after the administration of the IGF-I/IGFBP-3 complex. Similar results were observed by Camacho-Hubner *et al.*[28] after administering this drug to children with GH insensitivity. This suggests that the effects observed in these low birth weight children were mainly caused by the administration of IGF-I, rather than by IGFBP-3.

An alternative explanation for our findings is that the increase in mean nocturnal ghrelin AUC observed in these patients after the administration of the IGF-I/IGFBP-3 complex, was caused by the decrease in mean nocturnal GH AUC. However, we did not document that the temporal pattern of GH and ghrelin release was compatible with this model. In addition, in patients with acromegaly, circulating ghrelin concentrations are not affected by ambient GH concentrations[8] and systemic ghrelin concentrations are not modified by 1 year of GH replacement therapy in patients with GH deficiency.[9] These observations suggest that GH does not appear to regulate circulating ghrelin levels. Likewise, we did not observe any significant changes in serum glucose levels after the administration of Iplex™, suggesting that the increase in ghrelin levels was not caused by a reduction in serum glucose levels.

In support for an effect of IGF-I on ghrelin, studies in women with severe undernutrition caused by anorexia nervosa have demonstrated that circulating concentrations of ghrelin increase after treatment with IGF-I, oestrogen or the combination of both hormones.[29] In addition, studies in mice have indicated that ghrelin gene expression is age-dependent, and influenced by gender and the level of circulating IGF-I.[30] In this study, IGF-I was not administered to the animals, but the authors documented that ghrelin gene expression is increased during the periods when higher concentrations of IGF-I are observed in this species. These studies are consistent with the findings of our study, and suggest that IGF-I may have a positive feed-back effect on ghrelin.

Recently, a clinical study of peripubertal boys indicated that short-term testosterone priming for GH testing induced an increase in IGF-I levels, which led to a significant decline in ghrelin serum levels,[31] suggesting that sex steroids may also modulate ghrelin secretion. This effect was not observed in peripubertal girls treated with oestrogen. These authors suggest that the decline in serum ghrelin levels may not be attributable solely to the administration of testosterone, but that concomitant changes in IGF-I may have influenced ghrelin levels. The effects of IGF-I on ghrelin, however, were not investigated directly in this study.

It is known that IGF-I exerts a negative feedback effect on GH secretion. IGF-I suppresses GH secretion *in vivo*[32] and reduces basal and GH-releasing hormone-stimulated GH release *in vitro* in rat pituitary cells.[33,34] In addition to its effect on GH release, IGF-I has been shown to inhibit GH synthesis.[35] IGF-I pretreatment may decrease the GH response to synthetic GHS by inhibiting GHS-R gene expression in the rat pituitary.[36] These effects were achieved using IGF-I concentrations within the physiological range, suggesting that endogenous IGF-I may play an important role in the regulation of GH secretion by regulating GHS-R expression.

In summary, our study shows that the administration of the IGF-I/IGFBP-3 complex to low birth weight children decreased mean nocturnal GH AUC concentrations, confirming that IGF-I has a negative feed-back effect on the circulating concentrations of GH. In addition, we documented that the administration of the complex led to an increase in mean nocturnal ghrelin AUC, suggesting that IGF-I and/or IGFBP-3 may regulate systemic ghrelin levels in healthy, low birth weight children. The effects of Iplex™ on nocturnal ghrelin levels may be due to either direct effects of IGF-I and/or IGFBP-3 on ghrelin, or to indirect effects of the lower GH concentrations observed after administration the IGF-I/IGFBP-3 complex.

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References

1 Kojima, M., Hosoda, H., Matsuo, H. & Kangawa, K. (2001) Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. *Trends in Endocrinology and Metabolism*, **12**, 118–122.

2 Papotti, M., Ghè, C., Cassoni, P., Catapano, F., Deghenghi, R., Ghigo, E. & Muccioli, G. (2000) Growth hormone secretagogue binding sites in peripheral human tissues. *Journal of Clinical Endocrinology and Metabolism*, **85**, 3803–3807.

3 Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. & Kangawa, K. (1999) Ghrelin is a growth hormone-releasing acylated peptide from stomach. *Nature*, **402**, 655–660.

4 Gualillo, O., Caminos, J., Blanco, M., Garcia-Caballero, T., Kojima, M., Kangawa, K., Dieguez, C. & Casanueva, F. (2001) Ghrelin, a novel placental-derived hormone. *Endocrinology*, **142**, 788–794.

5 Volante, M., Allia, E., Gugliotta, P., Funaro, A., Broglio, F., Deghenghi, R., Muccioli, G., Ghigo, E. & Papotti, M. (2002) Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet

cells and related endocrine tumors. *Journal of Clinical Endocrinology and Metabolism*, **87**, 1300–1308.
6 Mori, K., Yoshimoto, A., Takaya, K., Hosoda, K., Ariyasu, H., Yahata, K., Mukoyama, M., Sugawara, A., Hosoda, H., Kojima, M., Kangawa, K. & Nakao, K. (2000) Kidney produces a novel acylated peptide; ghrelin. *FEBS Letters*, **486**, 213–216.
7 Gnanapavan, S., Kola, B., Bustin, S.A., Morris, D.G., McGee, P., Fairclough, P., Bhattacharya, S., Carpente, R.R., Grossman, A.B. & Korbonits, M. (2002) The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *Journal of Clinical Endocrinology and Metabolism*, **87**, 2988–2991.
8 Barkan, A.L., Dimaraki, E.V., Jessup, S.K., Symons, K.V., Ermolenko, M. & Jaffe, C.A. (2003) Ghrelin secretion in humans is sexually dimorphic, suppressed by somatostatin, and not affected by the ambient growth hormone levels. *Journal of Clinical Endocrinology and Metabolism*, **88**, 2180–2184.
9 Janssen, J.A.M., van der Toorn, F.M., Hofland, L.J., van Koetsveld, P., Broglio, F., Ghigo, E., Lamberts, S.W.J. & van der Lely, A.J. (2001) Systemic ghrelin levels in subjects with growth hormone deficiency are not modified by one year of growth hormone replacement therapy. *European Journal of Endocrinology*, **145**, 711–716.
10 Tschop, M., Weye, R.C., Tataranni, P.A., Devanarayan, V., Ravussin, E. & Heiman, M.L. (2001) Circulating ghrelin levels are decreased in human obesity. *Diabetes*, **50**, 707–709.
11 Nagaya, N., Uematsu, M., Kojima, M., Date, Y., Nakazato, M., Okumura, H., Hosoda, H., Shimizu, W., Yamagishi, M., Oya, H., Koh, H., Yutani, C. & Kangawa, K. (2001) Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation*, **104**, 2034–2038.
12 Misra, M., Miller, K., Bjornson, J., Hackman, A., Aggarwal, A., Chung, J., Ott, M., Herzog, D., Johnson, M. & Klibanski, A. (2003) Alterations in growth hormone secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. *Journal of Clinical Endocrinology and Metabolism*, **88**, 5615–5623.
13 Hotta, M., Ohwada, R., Katakami, H., Shibasaki, T., Hizuka, N. & Takano, K. (2004) Plasma levels of intact and degraded ghrelin and their responses to glucose infusion in anorexia nervosa. *Journal of Clinical Endocrinology and Metabolism*, **89**, 5707–5712.
14 Nakai, Y., Hosoda, H., Nin, K., Ooya, C., Hayashi, H., Akamizu, T. & Kangawa, K. (2003) Plasma levels of active form of ghrelin during oral glucose tolerance test in patients with anorexia nervosa. *European Journal of Endocrinology*, **149**, R1–R3.
15 Scacchi, M., Pincelli, A., Caumo, A., Tomasi, P., Delitala, G., Baldi, G. & Cavagnini, F. (1997) Spontaneous nocturnal growth hormone secretion in anorexia nervosa. *Journal of Clinical Endocrinology and Metabolism*, **82**, 3225–3229.
16 Stoving, R., Veldhuis, J., Flyvbjerg, A., Vinten, J., Hangaard, J., Koldkjaer, O.G., Kristiansen, J. & Hagen, C. (1999) Jointly amplified basal and pulsatile growth hormone secretion and increased process irregularity in women with anorexia nervosa: indirect evidence for disruption of feedback regulating within the growth hormone-insulin like growth factor-I axis. *Journal of Clinical Endocrinology and Metabolism*, **84**, 2056–2063.
17 Deiber, M., Chatelain, P., Naville, D., Putet, G. & Salle, B. (1989) Functional hypersomatotropism in small for gestational age (SGA) newborn infants. *Journal of Clinical Endocrinology and Metabolism*, **68**, 232–234.
18 Iniguez, G., Ong, K., Pena, V., Avila, A., Dunger, D. & Mericq, V. (2002) Fasting and post-glucose ghrelin levels in SGA infants: relationships with size and weight gain at one year of age. *Journal of Clinical Endocrinology and Metabolism*, **87**, 5830–5833.
19 Román, R., Iñiguez, G., Salazar, T., Avila, A., Barrera, A., Fuentes, A., Mericq, V., Gunn, R.D. & Cassorla, F. (2006) Sensibilidad a IGF-I en niños pequeños para la edad gestacional. *Revista Medica de Chile*, **134**, 21–30.
20 Dzaja, A., Dalal, M.A., Himmerich, H., Uhr, M., Pollmacher, T. & Schuld, A. (2004) Sleep enhances nocturnal plasma ghrelin levels in healthy subjects. *American Journal of Physiological Endocrinology and Metabolism*, **286**, E963–E967.
21 Juez, G., Lucero, E., Ventura-Juncá, P., Tapia, J., González, H. & Winter, A. (1989) Estudio neonatal del crecimiento intrauterino en 11543 recién nacidos chilenos de clase media 1978–87. *Revista Chilena de Pediatria*, **60**, 198–202.
22 Cifuentes, L., Valenzuela, C.Y., Cruz-Coke, R., Armanet, L., Lyng, C. & Harb, Z. (1988) Genetic characterization of the hospital population of Santiago. *Revista Medica de Chile*, **116**, 28–33.
23 Iniguez, G., Villavicencio, A., Gabler, F., Palomino, A. & Vega, M. (2001) Effect of nitric oxide on the expression of insulin-like growth factors and the insulin-like growth factor binding proteins throughout the lifespan of the human corpus luteum. *Reproduction*, **122**, 865–873.
24 Merriam, G.R. & Watchter, K.W. (1982) Algorithms for the study of episodic hormone secretion. *American Journal of Physiology*, **243**, E310–E318.
25 Frohman, L.A., Downs, T.R. & Chomoczynski, P. (1992) Regulation of growth hormone secretion. *Frontiers in Neuroendocrinology*, **13**, 344–405.
26 Misra, M., Miller, K.K., Kuo, K., Griffin, K., Stewart, V., Hunte, R.E., Herzog, D.B. & Klibanski, A. (2005) Secretory dynamics of ghrelin in adolescent girls with anorexia nervosa and healthy adolescents. *American Journal of Physiological Endocrinology and Metabolism*, **289**, E347–E356.
27 Koutkia, P., Canavan, B., Breu, J., Johnson, M.L. & Grinspoon, S.K. (2004) Nocturnal ghrelin pulsatility and response to growth hormone secretagogues in healthy men. *American Journal of Physiological Endocrinology and Metabolism*, **287**, E506–E512.
28 Camacho-Hubner, C., Rose, S., Preece, M.A., Sleevi, M., Storr, H.L., Miraki-Moud, F., Minuto, F., Frystyk, J., Rogol, A., Allan, G., Sommer, A. & Savage, M.O. (2006) Pharmacokinetic studies of recombinant human insulin-like growth factor I (rhIGF-I) /rhIGF-binding protein-3 complex administered to patients with growth hormone insensitivity syndrome. *Journal of Clinical Endocrinology and Metabolism*, **91**, 1246–1253.
29 Grinspoon, S., Miller, K.K., Herzog, D.B., Grieco, K.A. & Klibanski, A. (2004) Effects of estrogen and recombinant human insulin-like growth factor-I on ghrelin secretion in severe undernutrition. *Journal of Clinical Endocrinology and Metabolism*, **89**, 3988–3993.
30 Liu, Y.L., Yakar, S., Otero-Corchon, V., Low, M.J. & Liu, J.L. (2002) Ghrelin gene expression is age-dependent and influenced by gender and the level of circulating IGF-I. *Molecular Cell Endocrinology*, **189**, 97–103.
31 Lebenthal, Y., Gat-Yablonski, G., Shtaif, B., Padoa, A., Phillip, M. & Lazar, L. (2006) Effect of sex hormone administration on circulating ghrelin levels in peripubertal children. *Journal of Clinical Endocrinology and Metabolism*, **91**, 328–331.
32 Bermann, M., Jaffe, C.A., Tsai, W., Demott-Friberg, W.R. & Barkan, A.L. (1994) Negative feedback regulation of pulsatile growth hormone secretion by insulin-like growth factor I. *Journal of Clinical Investigations*, **94**, 138–145.
33 Yamashita, S. & Melmed, S. (1986) Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone

secretion and messenger ribonucleic acid levels. *Endocrinology*, **118**, 176–182.

34 Yamasaki, H., Prager, D., Gebremedhin, S., Moise, L. & Melmed, S. (1991) Binding and action of insulin-like growth factor I in pituitary tumor cells. *Endocrinology*, **128**, 857–862.

35 Yamashita, S. & Melmed, S. (1987) Insulin-like growth factor I regulation of growth hormone gene transcription in primary rat pituitary cells. *Journal of Clinical Investigations*, **79**, 449–452.

36 Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H. & Oikawa, S. (2005) Insulin-like growth factor-I down-regulates ghrelin receptor (growth hormone secretagogue receptor) expression in the rat pituitary. *Regulatory Peptides*, **127**, 203–206.