Regulatory Peptides 173 (2012) 21–26

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Ghrelin, leptin and insulin in healthy children: Relationship with anthropometry, gender, and age distribution

Maria Inês A. Wilasco [a,*], Helena A.S. Goldani [b], Cristina T.L. Dornelles [a], Rafael L. Maurer [a], Carlos O. Kieling [b], Marilene Porowski [c], Themis R. Silveira [a]

[a] Experimental Laboratory of Gastroenterology and Hepatology, Centro de Pesquisas do Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul—Brazil
[b] Pediatric Gastroenterology Unit, Pediatric Service—Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul—Brazil
[c] Universidade Federal de Ciências da Saúde de Porto Alegre—Brazil

ARTICLE INFO

Article history:
Received 5 May 2011
Received in revised form 17 August 2011
Accepted 23 August 2011
Available online 7 September 2011

Keywords:
Ghrelin
Leptin
Insulin
Children
Healthy

ABSTRACT

Objectives: This study aimed to establish the relationship between total ghrelin, acyl ghrelin, des-acyl ghrelin, leptin, and insulin with anthropometry, gender, and age distribution in healthy children.
Results: Data from 111 healthy children aged 4 months to 10 years were studied. All the participants underwent a pre-study screening clinical evaluation and were separated in 3 age groups. All had blood collected to assay. Anthropometric parameters were measured according to World Health Organization. In order to determine the correlation between dependent and independent variables, a multiple linear regression analysis was used. Overall median age of subjects was 60.0 months. After multiple regression analysis, correlation between total ghrelin, acyl ghrelin and des-acyl ghrelin remained significant with age. Correlation between leptin values and age, body mass index-for-age ratio, height-for-age ratio, and female gender remained significant. There was no significant correlation between insulin and ghrelin, and between insulin and leptin in all age groups. There was an inverse significant correlation between total ghrelin and des-acyl ghrelin with leptin in the whole group.
Conclusions: Ghrelin showed an inverse correlation with age and leptin showed a direct correlation with anthropometric parameters and female gender in healthy children. Insulin did not show any correlation.

1. Introduction

Ghrelin is a peptide hormone with a potent orexigenic effect and is mainly produced in the stomach where it is directly related with consumption of food. The vagally-mediated ghrelin signal reaches the hypothalamus via nuclei in the caudal brainstem, particularly the nucleus of the tractus solitarius, where it participates in neuroendocrine processes that control appetite and the secretion of growth hormone (GH) [1–5]. Effect of ghrelin on short-term regulation of food intake and long-term regulation of body weight has been described [6]. Three peptides are produced by ghrelin gene: acyl ghrelin, des-acyl ghrelin and obestatin; all may be part of a complex system with multiple elements that comprise the center of an integrated gut–brain axis that modulates appetite, digestion, gut motility, adiposity and energy partition [7,8].

Leptin, an adipocyte-derived protein, was identified as a product of the obesity gene whose autosomal recessive mutation results in profound hyperphagia and obesity [9]. It is mainly produced in white adipose tissue and circulates as the content of adipose tissue [10]. It has been shown that serum leptin concentration reflects body fat mass [10–13], and might be considered as a reliable marker of fat, body mass and energy homeostasis [14–16]. Leptin and insulin have inhibitory effects on food intake and both increase energy expenditure [9].

Insulin is an anabolic hormone produced in the pancreas and plays an important role in controlling body weight; therefore, it is one of the hormones responsible for regulating food intake and energy expenditure, and acts in the hypothalamus where it interacts with neurotransmitters involved in the mechanisms that control satiety and hunger [16].

Ghrelin, leptin and insulin are hormones related to regulation of food intake and, consequently, body weight control. The values of these hormones are subject to a great variability due to different protocols of sampling, analysis techniques and nutritional status. Therefore a standardized protocol in healthy children is highly necessary.

2. Subjects and methods

This study was approved by the Research Ethical Committee of Hospital de Clínicas de Porto Alegre. Informed and written consent was obtained from all subjects' parents.

* Corresponding author at: Laboratório Experimental de Gastroenterologia e Hepatologia do Centro de Pesquisa do Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, CEP 90035-903, Porto Alegre-RS, Brazil. Tel.: +55 51 3359 8847; fax: +55 51 3359 8183.
E-mail addresses: ineswilasco@gmail.com (M.I.A. Wilasco), hgoldani@hcpa.ufrgs.br (H.A.S. Goldani), cleal@hcpa.ufrgs.br (C.T.L. Dornelles), maurer.rafael@gmail.com (R.L. Maurer), ckieling@hcpa.ufrgs.br (C.O. Kieling), marilenegarrido@pucrs.br (M. Porowski), themis.silveira@gmail.com (T.R. Silveira).

0167-0115
doi:10.1016/j.regpep.2011.08.013

From December 2006 to March 2009, 111 healthy subjects aged 0–10 years were eligible to participate in this study. Subjects were enrolled from Pediatric Outpatient Clinics of Hospital de Clínicas de Porto Alegre, Southern Brazil. Inclusion criteria were healthy children who had been scheduled for blood sampling as routine medical checkup or as standard assessment prior to eligible minimal surgeries (such as insertion of otologic ventilation tubes, excision of benign nevus, surgery to correct inguinal hernia and hypospadia). Exclusion criteria were as follows: birth at gestational age lower than 37 weeks for children aged under 2 years; gestational age lower than 28 weeks for children aged under 3 years; current use of any restricted diet; any chronic or acute disease; use of medication except iron and oral vitamins recommended as routine practice; impossibility to anthropometry assessment; body mass index (BMI)-for-age values out of −2.0 and +2.0 standard deviation (SD) limits for children under 5 years and out of −2.0 and +1.0 SD limits for children over 5 years [17,18]; lack of appropriate fasting period prior to blood collection.

2.1. Anthropometry assessment

Anthropometric measurements have been done according to WHO training course on child growth assessment [19]. The variables were presented as standard deviation score (SDS) for height-for-age ratio (H/A), BMI-for-age ratio (BMI/A), triceps skinfold thickness-for-age ratio (TSF/A), subscapular skinfold thickness-for-age ratio (SSF/A), and mid upper arm circumference-for-age ratio (MUAC/A) according to WHO standards [17]. WHO Antho software version 3.0 was used for children under 5 years of age [20]. WHO AnthoPlus software was used for children above 5 years [21]. Frisancho's Anthropometric Standards [22] was used to calculate MUAC/A, TSF/A, and SSF/A for children above 5 years.

2.2. Blood samples and hormone assays

Peripheral blood samples of 2.0 mL were collected from all patients following a fasting period of 3–14 hours. The first group (0–24 months) had the minimum fast period (3 hours). The second group (25–60 months) had fasting period between 3 and 8 hours, depending on the biochemical analysis the physician prescribed. The third group (>60 months) had fasting period between 3 and 12 hours considering some child that had lipid profile in the medical review. Plasma and serum samples were stored at −80 °C until measurement. For ghrelin determinations the blood was taken into tubes containing EDTA-2Na (1.25 mg/mL) and aprotinin (500 U/mL). In order to obtain accurate data on ghrelin concentration, blood samples were centrifuged at latest within 30 min after collection. For the preservation of plasma ghrelin the sample was acidified with 1 mol/L HCl [23]. For leptin, insulin and glucose determinations the blood was taken into serum tubes, centrifuged and stored. Total ghrelin, acyl ghrelin and leptin concentrations were assessed by ELISA commercial kit (Linco Research, St Charles—MI, USA). Insulin was assessed using ELISA commercial kit (Diagnostics Systems Laboratories, INC Webster—TX, USA) and glucose was measured using glucose oxidase method GlicosePAP liquiform® (Labtest Diagnóstica—Lagoa Santa-MG, Brazil).

2.3. Statistical analysis

The program used for statistical analysis was SPSS version 16.0 (Chicago, IL). Continuous variables were expressed as mean ± SD or median and interquartile range (25th and 75th percentiles). Normal distribution of variables was checked by Kolmogorov–Smirnov test. Variables with normal distribution were compared by Student's *t* test and analysis of variance (ANOVA). Variables with asymmetric distribution were analyzed by Kruskal–Wallis and Mann–Whitney *U* tests. Correlation between continuous variables was assessed by Pearson or Spearman correlation coefficient test. Linearity was tested and logarithm adjustment was performed. Multiple linear regression analysis was used to associate total ghrelin with age, acyl ghrelin with age and SDS-SSF/A, des-acyl ghrelin with age, leptin with age, and SDS-BMI/A and SDS-H/A with gender. The following comparisons were excluded by stepwise model: total ghrelin with SDS-MUAC/A, acyl ghrelin with SDS-MUAC/A, des-acyl ghrelin with SDS-MUAC/A, leptin with SDS-MUAC/A, leptin with SDS-TSF/A, and leptin with SDS-SSF/A.

3. Results

Overall median (25th–75th percentile) age of subjects was 60.0 (35.0–91.0) months; minimum and maximum ages were 4 and 128 months. The subjects were distributed in 3 age groups: ≤24 months (18%), 25–60 months (33.4%) and >60 months (48.6%); 53.2% male. Ten percent of children were currently on iron and vitamin medications. Median fasting time was 240 (200–605) min. Mean (±SD) hemoglobin and hematocrit values were 12.2 ± 0.7 g/dL and 36.7 ± 2.1%, respectively. Values of total ghrelin, acyl ghrelin, des-acyl ghrelin, leptin, insulin and glucose were described on Table 1.

Anthropometry values SDS-BMI/A, SDS-MUAC/A, SDS-TSF/A, and SDS-SSF/A were within −2.00 and +2.00 SD for children under 5 years and within −2.00 and +1.00 SD for children over 5 years. The SDS-H/A was within −2.00 and +2.00 SD for all ages.

There was no significant correlation between the fasting time and plasma levels of ghrelin, acyl ghrelin, des-acyl ghrelin, leptin, and insulin in the three age groups ($P>0.05$). There was no difference in total ghrelin and des-acyl ghrelin between genders. Leptin was higher in girls above 5 years when compared with girls under 2 years [2.77 (1.31–4.79) ng/mL *vs* 1.37 (0.90–2.15) ng/mL, $P=0.039$]. Insulin and glucose were not significantly different among the 3 age groups in boys and girls (Table 2).

The concentrations of total ghrelin and des-acyl ghrelin were significantly lower in boys above 60 months when compared with boys under 24 months of age ($P=0.007$ and $P=0.003$ respectively). The concentrations of total ghrelin and des-acyl ghrelin were significantly lower in girls above 60 months when compared with girls from the other 2 groups ($P=0.021$ and $P=0.013$ respectively) (Table 2). Total ghrelin, acyl ghrelin and des-acyl ghrelin values have an inverse correlation with age ($r_s=-0.475$, $P<0.001$; $r_s=-0.313$, $P=0.001$; $r_s=-0.484$, $P<0.001$ respectively). Total ghrelin and des-acyl ghrelin values did not correlate with any anthropometric parameter. Acyl ghrelin has a weak negative correlation with SDS-SSF/A ($r_s=-0.193$, $P=0.042$), but did not correlate with any other antropometric parameter. After multiple linear regression analysis, correlation between age and total ghrelin, acyl ghrelin and des-acyl ghrelin remained significant ($\beta=-0.442$, $\beta=-0.471$, $\beta=-0.442$;

Table 1
Demographic data of subjects.

Variables	$n=111$
Age (months)[a]	60.0 (35.0–91.0)
Age groups (months)	
≤24	20 (18.0)
25–60	37 (33.3)
>60	54 (48.6)
Gender	
Male/female	59 (53.2)/52 (46.8)
Iron and vitamin prophylaxis	
None	99 (89.2)
Iron	10 (9.0)
Iron + vitamin	2 (1.8)
Fasting time (min)	240 (200–605)

Data were expressed as mean (SD), median (25th–75th percentiles) or total number (%).
[a] Minimal and maximal age (4–128 months).

Table 2
Anthropometric data in standard deviation score between gender and age groups.

Variables	Gender	SDS-H/A	SDS-BMI/A	SDS-MUAC/A	SDS-TSF/A	SDS-SSF/A
Total	M ($n=59$)	0.03 ± 1.00	−0.14 ± 0.80	−0.16 ± 0.75	0.07 ± 0.62	−0.08 ± 0.60
$n=111$	F ($n=52$)	0.11 ± 1.00	−0.10 ± 0.80	0.32 ± 0.48	0.10 ± 0.66	−0.04 ± 0.64
≤24 months	M ($n=10$)	0.79 ± 0.89	−0.15 ± 0.75	0.25 ± 0.68	0.36 ± 0.81	−0.18 ± 0.93
$n=20$	F ($n=10$)	0.16 ± 1.38	−0.13 ± 0.65	0.31 ± 0.57	0.38 ± 0.83	0.49 ± 0.67
25–60 months	M ($n=22$)	−0.34 ± 0.78	0.13 ± 0.73	0.23 ± 0.56	0.19 ± 0.45	0.00 ± 0.58
$n=37$	F ($n=15$)	−0.04 ± 0.80	0.30 ± 0.72	0.56 ± 0.44	0.40 ± 0.74	−0.14 ± 0.85
>60 months	M ($n=27$)	0.05 ± 1.05	−0.35 ± 0.84	−0.63 ± 0.65	−0.12 ± 0.62	−0.12 ± 0.48
$n=54$	F ($n=27$)	0.17 ± 1.06	−0.30 ± 0.84	0.18 ± 0.43	−0.17 ± 0.40	−0.18 ± 0.33

M: male; F: female; SDS-H/A: standard deviation score for height-for-age; SDS-BMI/A: standard deviation score for body mass index-for-age; SDS-MUAC/A: standard deviation score for mid upper arm circumference-for-age; SDS-TSF/A: standard deviation score for triceps skinfold thickness-for-age; SDS-SSF/A: standard deviation score for subscapular skinfold thickness-for-age.

$P<0.001$, respectively), and correlation between acyl ghrelin and SSF/A-SDS remained significant ($\beta=-0.217$; $P=0.012$) (Table 3).

Leptin values were not significantly different among the 3 age groups in boys. Leptin was higher in girls aged above 60 months when compared with girls under 24 months ($P=0.039$). In the whole group, it was found a weak significant correlation between leptin levels with age ($r_s=0.190$; $P=0.046$) (Fig. 1). Values of leptin were more significantly lower in boys than girls ($P=0.007$) in all ages. After multiple linear regression analysis, correlation between leptin values and age, SDS-BMI/A, SDS-H/A and female gender remained significant ($\beta=0.207$, $P=0.012$; $\beta=0.415$, $P<0.001$; $\beta=0.189$, $P=0.020$; $\beta=0.271$, $P=0.001$, respectively) (Table 3).

Insulin and glucose were not significantly different among the 3 age groups in boys and girls. There was no significant correlation between insulin and ghrelin values, and between insulin and leptin values in all age groups. There was an inverse significant correlation between total ghrelin and leptin values, and between des-acyl ghrelin and leptin values in the whole group ($r_s=-0.237$, $P=0.012$; $r_s=-0.239$, $P=0.012$, respectively).

4. Discussion

This study assessed fasting concentration of total ghrelin, acyl ghrelin, leptin, insulin and glucose in 111 healthy children aged 0–10 years. The two main findings of the study were an inverse correlation between ghrelin values and age and a direct correlation between leptin values with anthropometric parameters and female gender. Insulin and glucose did not correlate with any variable of the study.

Ghrelin has already been studied throughout newborn to adulthood and except for ghrelin determinations according to pubertal stages [24], only few studies have shown a range of normal values at different ages [11,24–26]. Unlike the others, our study has some new aspects regarding ghrelin levels in children. First, it was made in a very selected healthy population, following a rigorous selection criteria which did not include overweighed and/or obese children as has been done by others [11,12,27,28]. Second, we collected blood samples with a specific fasting period whereas others did not follow or did not specify a fasting period [24–26]. The minimum fasting period of 3 hours has been chosen as it covers the length of time of 1–2 hours that plasma ghrelin levels usually rose prior to the onset of a routine meal [29]. Third, this is the first study to determine more than one ghrelin isoform simultaneously with leptin, insulin and glucose.

In our study, a significant fall in ghrelin levels with age over childhood was seen. A similar trend has been reported in a group of 121 healthy children aged 5–19 years, who had blood samples taken without fasting [25]. In regard of gender distribution, ghrelin levels have been found higher in boys than girls [11] whereas we did not find difference between genders (see Table 4). The difference between those studies and ours is likely due to the heterogeneity of

Table 3
Hormones and glucose between age groups and gender.

	0–24 months	25–60 months	>60 months	*P*
MALE	$n=10$	$n=22$	$n=27$	
Ghrelin (pg/mL)				
Total ghrelin	1950 (1576–2922)[b]	1432 (1000–2078)[a,b]	1155 (795–1551)[a]	0.007[†]
Acyl ghrelin	322 (275–735)	313 (185–617)	280 (179–421)	0.288[†]
Des-acyl ghrelin	1649 (1241–2145)[b]	1192 (792–1687)[a,b]	913 (515–1231)[a]	0.003[†]
Leptin (ng/mL)	2.15 (0.93–2.64)	1.50 (1.16–1.63)	1.33 (0.91–2.53)	0.657[†]
Insulin (μU/mL)	23.5 (13.5–33.8)	17.6 (10.4–24.1)	13.5 (5.0–20.6)	0.112[†]
Glucose (mg/dL)	87 ± 7.9	86.2 ± 14.4	92.6 ± 15.2	0.262[††]
FEMALE	$n=10$	$n=15$	$n=27$	
Ghrelin (pg/mL)				
Total ghrelin	1587 (1003–2167)[b]	1540 (1184–2160)[b]	1133 (897–1402)[a]	0.021[†]
Acyl ghrelin	304 (246–652)	392 (222–523)	272 (185–336)	0.112[†]
Des-acyl ghrelin	1343 (780–1609)[b]	1173 (983–1638)[b]	900 (645–1138)[a]	0.013[†]
Leptin (ng/mL)	1.37 (0.90–2.15)[a]	2.34 (1.21–3.32)[a,b]	2.77 (1.31–4.79)[b]	0.039[†]
Insulin (μU/mL)	16.5 (12.3–21.4)	17.7 (13.4–21.1)	14.1 (12.1–19.8)	0.359[†]
Glucose (mg/dL)	84.1 ± 11.8	90.7 ± 14.1	87.7 ± 10.7	0.411[††]

Data were expressed as mean (±SD) or median (P25–P75).
[a,b] Equal letters mean no significant difference by Mann–Whitney *U* test.
[†] Kruskal–Wallis test.
[††] ANOVA-one way.

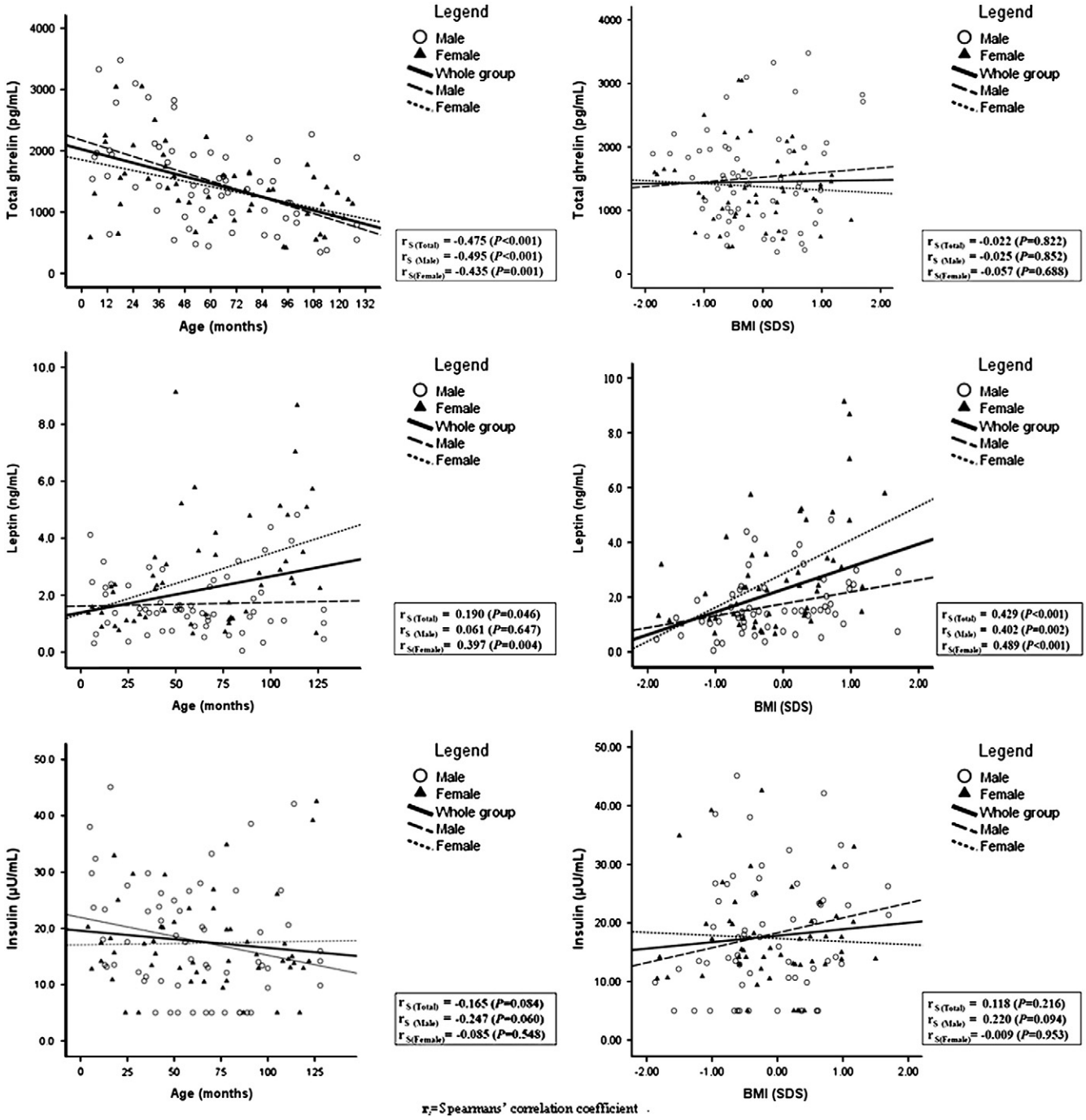

Fig. 1. Correlations of total ghrelin, leptin and insulin with age and body mass index.

the subjects and the non-fasted condition their blood samples were taken. It is known that ghrelin may have a role on the control of GH and insulin growth factor 1 (IGF-1) hormones; however it is not yet fully clarified the mechanisms that regulate the secretion of ghrelin. One of the most important factors that regulate its secretion is believed to be related with feeding [2].

Ghrelin plays a role in the regulation of long-term energy balance and body weight control. Our observation of a negative correlation between ghrelin concentration and age could sustain the hypothesis that in early infancy, ghrelin may exert a strong growth hormone releasing action to promote body growth. It has already been found that there is a significant ghrelin increase after birth peaking during the first 2 years of life and a further decrease until the end of puberty [24].

A recent study showed that during periods of energy insufficiency, exposure to acyl ghrelin may limit energy utilization in specific white adipose tissue depots by lipid retention dependent on GH secretagogue receptors [30]. In accordance with this finding, ghrelin concentration has been found low in obese patients [31,32]. We have not found significant correlation between ghrelin and BMI; however, this is controversial as a negative correlation has been found in another study that included

Table 4
Percentile distribution of total ghrelin, acyl ghrelin, leptin and insulin in boys and girls.

		P3	P25	P50	P75	P97
Total ghrelin (pg/mL)						
≤24 months	M	638	1576	1950	2922	1576
	F	585	1003	1587	2167	1003
25–60 months	M	445	1000	1432	2078	1000
	F	669	1184	1540	2160	1184
>60 months	M	346	795	1155	1551	795
	F	418	897	1135	1402	897
Acyl ghrelin (pg/mL)						
≤24 months	M	108	275	322	735	792
	F	71	246	304	652	756
25–60 months	M	107	185	313	617	1053
	F	117	222	392	523	1098
>60 months	M	90	179	280	421	583
	F	98	185	272	336	529
Leptin (ng/mL)						
≤24 months	M	0.31	0.93	2.15	2.64	4.12
	F	0.76	0.90	1.37	2.14	2.36
25–60 months	M	0.37	1.17	1.49	1.63	2.98
	F	0.65	1.21	2.34	3.32	9.13
>60 months	M	0.05	0.91	1.33	2.53	4.82
	F	0.66	1.31	2.77	4.79	8.67
Insulin (μU/mL)						
≤24 months	M	13.1	13.5	23.5	33.8	45.1
	F	5.0	12.3	16.5	21.4	32.9
25–60 months	M	5.0	10.4	17.6	24.1	29.8
	F	5.0	13.4	17.6	21.1	29.7
>60 months	M	5.0	5.0	13.5	20.6	42.1
	F	5.0	12.1	14.1	19.8	42.6

P: percentile; M: male; F: female.

overweighed adolescents with BMI-SD between +2.00 and +1.00 [24]. Nevertheless, we selected only subjects within the scores that exclude overweighed and obese subjects.

In our sample there was no association between fasting time and hormones in any age group ($P>0.005$); however studies with bigger samples and the same rigorous criteria of selecting children should be conduct to confirm these findings.

Leptin is affected by many variables including gender, pubertal stage, weight, diet and the analysis technique [33]. Short variations are expected in the values assayed by different methodology, but great variability should be due to different pubertal stages and BMI included in the studies [11,12,24–28]. Some studies included overweighed and/or obese children [11,12,27,28], and older children with advanced pubertal stage [11,24–28]. Higher leptin values than ours have been described in children; however it may indicate inclusion of advanced pubertal stage in subjects until 15 years [26].

It has been described that deficient leptin patients usually eat all types of food, even those which are usually not appreciated by children at the same age. The administration of leptin in those patients changed their behavior making them more selective to food [34]. Those findings may indicate that leptin is involved in the reward properties of food; thus this may mean that appetite has individual variation and might be associated with a higher susceptibility to obesity.

Alterations of serum leptin due overnutrition or undernutrition in perinatal period may determinate metabolic changes during adolescence and adulthood [35]. The impact of nutritional programming represents a relevant public health problem [36]. In this context, we hypothesize that the higher values obtained for some subjects from our experimental group, which was constituted exclusively by normal weight healthy children, might be a consequence of nutritional programming through maternal obesity or undernutrition.

We identified a positive correlation between serum leptin with gender and anthropometric parameters (SDS-BMI/A, SDS-H/A, SDS-MUAC/A, SDS-TSF/A and SDS-SSF/A). This is in accordance with other studies where leptin values showed parallel changes in nutritional status and energy storage across a broad range of nutritional status, from starvation to obesity [14,37]. Furthermore, a significant correlation between all anthropometric parameters is consistent with the literature, since these parameters reflect higher adipose tissue. In the multivariate analysis leptin was significantly associated with age, female gender, SDS-BMI/A and SDS-H/A.

The most important limitation of our study is the small amount of patients. We had only 20 patients aged 0–2 years and in this group it was not feasible to evaluate insulin and glucose. We think this issue is not a major limitation to the results we found, because our sample had rigorous criteria of selection.

In conclusion, we have shown that plasma total ghrelin, acyl ghrelin and des-acyl ghrelin were negatively correlated with age in healthy children. Acyl ghrelin had a weak positive correlation with SDS-SSF/A. Leptin was positively correlated with gender and SDS-BMI/A. Insulin did not show any correlation statistically significant. This study may be useful to provide data for standardization of ghrelin, leptin and insulin in healthy children. Moreover, this study might be helpful for further comparison with children with disorders that affect appetite and feeding behavior. Further multicentric studies focusing the building of percentile curves for these peptides in the pediatric population are needed.

Conflict of interest

The authors declare no conflict of interest.

Specific author contributions

Maria Ines Wilasco conducted the study, analyzed the data and drafted the paper; Helena A S Goldani helped design the study and revised the manuscript. Cristina Dorneles helped collect and analyze the data; Rafael L Maurer and Marilene P Garrido helped laboratory assays; Carlos O Kieling helped statistical analysis; Themis R Silveira designed and conducted the study, and revised the final draft of the paper.

Acknowledgements

This study was supported by grant from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil. We would like to thank Post-graduate Program in Health Science in Children and Adolescents, FAMED/UFRGS and FIPE, Grupo de Pós-Graduação e Pesquisa, Hospital de Clínicas de Porto Alegre, for their help and financial support.

References

[1] Kojima M, Hosoda H, Date Y. Ghrelin is a growth hormone-releasing acylated peptide from stomach. Nature 1999;402:656–60.
[2] Kojima M, Kangawa K. Structure and function of ghrelin. Results Probl Cell Differ 2008;46:89–115.
[3] Kojima M, Kangawa K. Ghrelin: from gene to physiological function. Results Probl Cell Differ 2010;50:185–205.
[4] Vivenza D, Rapa A, Castellino N, Bellone S, Petri A, Vacca G, Aimaretti G, Broglio F, Bona G. Ghrelin gene polymorphisms and ghrelin, insulin, IGF-I, leptin and anthropometric data in children and adolescents. Eur J Endocrinol 2004;151:127–33.
[5] Blanks WA. The blood–brain barrier: connecting the gut and the brain. Regul Pept 2008;149:11–4.
[6] De Vriese C, Perret J, Delporte C. Focus on the short- and long-term effects of ghrelin on energy homeostasis. Nutrition 2010;26:579–84.
[7] Chen CY, Asakawa A, Fujimiya M, Lee SD, Inui A. Ghrelin gene products and the regulation of food intake and gut motility. Pharmacol Rev 2009;61:430–81.
[8] Abizaid A, Horvath TL. Brain circuits regulating energy homeostasis. Regul Pept 2008;149:3–10.
[9] Zhang YY, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional of the mouse obese gene and its human homolog. Nature 1994;372:425–32.
[10] Considine R, Sinha M, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF. Serum immunoreactive leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292–5.

[11] Park HS, Lee KU, Kim YS, Park CY. Relationships between fasting plasma ghrelin levels and metabolic parameters in children and adolescents. Metabolism 2005;54: 925–9.
[12] Viso González ME, Solano L, Sánchez A, Portillo Z, Llovera D. Serum leptin in eutrophic and overweight Venezuelan children and adolescents. Arch Latinoam Nutr 2005;55:47–54.
[13] Nagy TR, Gower BA, Trowbridge CA, Dezenberg C, Shewchuk RM, Goran MI. Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children. J Clin Endocrinol 1997;82:2148–52.
[14] Dhillon SS, Belsham DD. Leptin differentially regulates NPY secretion in hypothalamic cell lines through distinct intracellular signal transduction pathways. Regul Pept 2011;167:192–200.
[15] Schwartz MW, Morton GJ. Obesity: keeping hunger at bay. Nature 2002;418: 595–7.
[16] Schwartz MW, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404:661–71.
[17] WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva: World Health Organization; 2006.
[18] WHO Multicentre Growth Reference Study Group. WHO child growth standards: head circumference-for-age, arm circumference-for-age, triceps skinfold-for-age and subscapular skinfold-for-age: methods and development. Geneva: World Health Organization; 2007.
[19] World Health Organization. Training course on child growth assessment. Geneva: WHO; 2008.
[20] WHO Anthro for personal computers. Version 3, 2009: software for assessing growth and development of the world's children. Geneva: WHO; 2009 (http://www.who.int/childgrowth/software/en/).
[21] WHO. AnthroPlus for personal computers manual: software for assessing growth of the world's children and adolescents. Geneva: WHO; 2009 http://www.who.int/growthref/tools/en/.
[22] Frisancho AR. Anthropometric standards. An interactive nutritional reference of body size and body composition for children and adults. Ann Arbor: The University of Michigan Press; 2008. p. 149–55.
[23] Hosoda H, Doi K, Nagaya N, Okumura H, Nakagawa E, Enomoto M, Ono F, Kangawa K. Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. Clin Chem 2004;50:1077–80.
[24] Soriano-Guillén L, Barrios V, Chowen JA, Sánchez I, Vila S, Quero J, Argente J. Ghrelin levels from fetal life through early adulthood: relationship with endocrine and metabolic and anthropometric measures. J Pediatr 2004;144:30–5.
[25] Whatmore AJ, Hall CM, Jones J, Westwood M, Clayton PE. Ghrelin concentrations in healthy children and adolescents. Clin Endocrinol 2003;59:649–54.
[26] Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF. Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. J Clin Endocrinol 1997;82:2849–55.
[27] Bjarnason R, Boguszewski M, Dahlgren J, Gelander L, Kriström B, Rosberg S, Carlsson B, Albertsson-Wikland K, Carlsson LM. Leptin levels are strongly correlated with those of GH-binding protein in prepubertal children. Eur J Endocrinol 1997;137:68–73.
[28] Coutant R, Lahlou N, Bouvattier C, Bougnères P. Circulating leptin level and growth hormone response to stimulation tests in obese and normal children. Eur J Endocrinol 1998;139:591–7.
[29] Davies JS, Kotokorpi P, Eccles SR, Barnes SK, Tokarczuk PF, Allen SK, Whitworth HS, Guschina IA, Evans BA, Mode A, Zigman JM, Wells T. Ghrelin induces abdominal obesity via GHS-R-dependent lipid retention. Mol Endocrinol 2009;23:914–24.
[30] Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001;50:1714–9.
[31] Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001;50:707–9.
[32] Haqq AM, Lien LF, Boan J, Arlotto M, Slentz CA, Muehlbauer MJ, Rochon J, Gallup D, McMahon RL, Bain JR, Stevens R, Millington D, Butler MD, Newgard CB, Svetkey LP. The Study of the Effects of Diet on Metabolism and Nutrition (STEDMAN) weight loss project: rationale and design. Contemp Clin Trials 2005;26:616–25.
[33] Venner AA, Doyle-Baker PK, Lyon ME, Fung TS. A meta-analysis of leptin reference ranges in the healthy paediatric prepubertal population. Ann Clin Biochem 2009;46: 65–72.
[34] Farooqi IS, O'Rahilly S. Leptin: a pivotal regulator of human energy homeostasis. Am J Clin Nutr 2009;89:980S–4S.
[35] Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A, Breier BH, Harris M. Neonatal leptin treatment reverses developmental programming. Endocrinology 2005;146:4211–6.
[36] Guilloteau P, Zabielski R, Hammon HM, Metges CC. Adverse effects of nutritional programming during prenatal and early postnatal life, some aspects of regulation and potential prevention and treatments. J Physiol Biochem 2009;60:17–35.
[37] Büyükgebiz B, Öztürk Y, Yılmaz S, Arslan N. Serum leptin concentration in children with mild-to-moderate protein energy malnutrition. Pediatr Int 2003;45:550–4.