Diabetologia (2005) 48: 309–316
DOI 10.1007/s00125-004-1635-9

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S. M. Pöykkö · O. Ukkola · H. Kauma · E. Kellokoski · S. Hörkkö · Y. A. Kesäniemi

The negative association between plasma ghrelin and IGF-I is modified by obesity, insulin resistance and type 2 diabetes

Received: 14 June 2004 / Accepted: 15 September 2004 / Published online: 2 February 2005

Abstract *Aims/hypothesis:* Ghrelin is a natural growth hormone-releasing peptide thought to be involved in the regulation of energy metabolism. The recent studies concerning the association between ghrelin and insulin-like growth factor-I (IGF-I) concentrations have shown either negative correlation or no correlation at all. The aims of this study were to clarify the association between ghrelin and IGF-I concentrations in a large cohort and to characterize whether obesity, insulin resistance and type 2 diabetes affect this association. *Methods:* We analysed fasting plasma ghrelin and IGF-I concentrations of 1,004 middle-aged subjects of the population-based OPERA study. Insulin resistance was estimated using QUICKI. *Results:* IGF-I concentrations were negatively associated with ghrelin concentrations in the analysis of all subjects before (β=−0.32, p<0.001) and after adjustments for BMI, insulin levels, sex and age (β=−0.40, p<0.001). The association was particularly strong in males and in the higher BMI tertiles. The degree of association varied in relation to the glycaemic status: no insulin resistance: r^2=6.5% (p<0.001), insulin resistance without type 2 diabetes: r^2=21.0% (p<0.001), type 2 diabetes: r^2=25.4 (p<0.001). IGF-I levels explained larger proportion (r^2=9.8%) of the variation in ghrelin concentrations compared to fasting insulin concentration (r^2=3.0%) and BMI (r^2=1.5%). *Conclusions/interpretation:* There is a negative and independent association between ghrelin and IGF-I concentrations in middle-aged subjects. The interaction between IGF-I and ghrelin is modified by obesity, IR and type 2 diabetes. Further studies are warranted to elucidate the role of ghrelin in the development of these states.

S. M. Pöykkö (✉) · O. Ukkola · H. Kauma · E. Kellokoski · S. Hörkkö · Y. A. Kesäniemi
Department of Internal Medicine, University of Oulu,
P.O. Box 5000, 90014 Oulu, Finland
e-mail: seppo.poykko@ppshp.fi
Tel.: +358-8-3154445
Fax: +358-8-3154543

Keywords Ghrelin · Hormone · Insulin-like growth factor I · Insulin-like growth factor binding protein 1 · Insulin resistance · Metabolism · Obesity · Type 2 diabetes

Abbreviations GH: Growth hormone · IGF-I: Insulin-like growth factor-I · IGFBP-1: Insulin-like growth factor binding protein 1 · IR: Insulin resistance

Ghrelin is a recently discovered peptide hormone, which is mainly secreted from the stomach [1]. The effects of ghrelin are mediated through the growth hormone secretagogue receptor (GHSR), which is widely distributed in the body [2]. In addition to its marked growth hormone (GH)-releasing activity [1], ghrelin acts as a powerful orexigenic hormone [3, 4] and this effect appears to be independent of changes in GH [5]. Recent studies have shown that ghrelin also modulates insulin and glucose metabolism [6], and it has further been shown to have beneficial hemodynamic properties [7, 8]. It is a somatotrophic, orexigenic and adipogenic hormone, which may link the regulatory systems for growth and energy balance [9].

The determinants and the regulation of ghrelin concentrations in physiological conditions are poorly known at the moment. Fasting state and meal ingestion probably have the most significant effect on the variation of ghrelin concentration. Food intake and hyperglycaemia suppress ghrelin secretion [10, 11] but it is controversial whether changes in glucose or insulin mediate this effect [10, 12]. Several studies have shown ghrelin to be negatively associated with BMI [13–15] and insulin [13, 15, 16].

Despite the fact that ghrelin has a powerful, rapid, and dose-dependent effect on GH release in experimental studies [1, 17, 18], the functional role of ghrelin in the long-term regulation of GH concentrations in physiological conditions has not yet been clarified. The recent studies are either for [19–22] or against [23–26] the role of ghrelin in the regulation of GH concentrations. If ghrelin has a significant role in the long-term regulation of GH concentration, there should be a detectable association between ghrelin and IGF-I, the most important peripheral effector protein of GH ac-

tion. However, the results of the recent studies concerning the association between ghrelin and IGF-I are somewhat conflicting showing negative correlation in children and adolescents [27–30] but no correlation at all in adult subjects [31–33]. Sample sizes of these studies have been relatively small.

Based on the strong GH-releasing effect of ghrelin, it can be hypothesised that there is a positive correlation between ghrelin and IGF-I concentrations. On the other hand, based on the recent studies demonstrating negative correlation between ghrelin and IGF-I concentrations in children and adolescents [27–30], a negative correlation can be hypothesised in adults as well. In order to test these competing hypotheses, we analysed the fasting plasma IGF-I and ghrelin concentrations in a large, randomly selected, population-based sample of middle-aged subjects. Since we have previously shown ghrelin to be associated with type 2 diabetes and insulin resistance (IR) [15], our further aim was to analyse whether these metabolic disturbances affect the association between ghrelin and IGF-I. In addition, we wanted to test whether IGFBP-1, the only acute regulator of IGF-I bio-availability [34], is associated with ghrelin concentrations.

Subjects and methods

Subjects OPERA (Oulu Project Elucidating the Risk of Atherosclerosis) is a population-based epidemiological study addressing the risk factors and disease endpoints of atherosclerotic cardiovascular diseases. The detailed study design has been previously described [35]. The control cohort and hypertensive cohort were randomly selected from Finnish national population registers by age stratification. The hypertensive cohort consists of 600 unrelated subjects (300 men and 300 women aged 40–59 at the time of recruitment) from the city of Oulu entitled to the reimbursement of the costs of antihypertensive medication. For each hypertensive subject, an age- and sex-matched control subject was randomly selected from the same register, excluding the subjects entitled to reimbursement for antihypertensive medication. The overall participation rate of the hypertensive cohort was 86.5% and that of the controls 87.7%. In this study, a total of 1,004 subjects with IGF-I and ghrelin measurements available were screened. An informed consent was obtained from each participant. The study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethical Committee of the Faculty of Medicine, University of Oulu.

Biomedical factors and laboratory analyses Blood glucose concentration was measured with the glucose dehydrogenase method and plasma insulin concentration with the double RIA method (AIA-PACK IRI, Tosoh Corp., Tokyo, Japan). Insulin sensitivity was assessed using fasting plasma insulin concentrations and a quantitative insulin sensitivity check index (QUICKI=1/[log (fasting insulin)+log (fasting glucose)]) [36]. Type 2 diabetes and IR were determined according to the WHO criteria. The subjects with insulin sensitivity below the lowest quartile of the control cohort (QUICKI<0.563) were regarded as insulin-resistant. The questionnaire presented to all participants elicited detailed information about their smoking habits, alcohol consumption, physical activity, use of medication and medical history.

To obtain a measure of overall ghrelin concentrations, we analysed fasting plasma ghrelin concentrations, which have been shown to correlate strongly with the 24-h integrated AUC values [37]. We used a commercial RIA kit (Phoenix Pharmaceuticals, Belmont, CA, USA), which recognizes both acylated and desacylated ghrelin [15]. The sensitivity of the assay was 12 pg/ml (ED_{80}), and the inter- and intra-assay coefficients of variation (CV), as given by the manufacturer, were 7.5 and 4.0%, respectively. Inter-assay CV in our analyses was 11.2%. Due to the low intra-assay CV of the method [38], only a single measurement for each sample was performed.

IGF-I assay (DSL-10-2800 ACTIVE Non-Extraction IGF-I ELISA; Diagnostic Systems Laboratories, Webster, TX, USA) uses a modified version of the standard acid–ethanol extraction procedure with intra- and interassay CVs of 4.5–8.6 and 3.3–6.8%, respectively. IGFBP-1 was measured with an immunoenzymometric assay (IGFBP-1 IEMA test; Oy Medix Biochemica, Kauniainen, Finland) with an intra-assay CV of 2.4–3.4% and an interassay CV of 4.9–7.4%.

Statistical methods The association between ghrelin and the variables studied was assessed using analysis of covariance (ANCOVA), linear regression analysis, and partial correlation analysis. The following variables were entered into the multivariate model: BMI, fasting insulin, IGF-I, IGFBP-1, age, sex and study group. To compare the means of the variables measured, Student's *t*-test and ANCOVA with Bonferroni corrections were used. Chi square test was carried out to assess the frequency differences in the sex ratio or the ratio of the study cohorts between the ghrelin quartiles.

The interaction between the explanatory variables was tested using ANCOVA. Linear regression analysis was used to adjust the individual ghrelin concentrations for inter-assay variation. Three subjects with very high ghrelin concentrations (>3 SD over the mean) and three subjects with very low IGF-I concentrations (>3 SD below the mean) were excluded from the analyses as outliers. Furthermore, the three subjects found to have type 1 diabetes were excluded from this study. Altogether, the data of 995 subjects were analysed.

Log-transformed values of IGF-I, IGFBP-1, insulin and QUICKI were used to normalise the skewed distributions. All calculations were made with the SPSS (version 9.0; SPSS, Inc.) statistical package. A p value of less than 0.05 was regarded as statistically significant.

Table 1 Main characteristics of the study subjects by ghrelin quartiles

	First quartile (n=247)	Second quartile (n=248)	Third quartile (n=248)	Fourth quartile (n=252)	p*	p**
Ghrelin (pg/ml)	368 (357–378)	572 (561–582)	743 (733–754)	984 (974–995)	<0.001	<0.001
IGF-I (ng/ml)[a]	102 (97–107)	81 (76–86)	73 (68–78)	65 (60–70)	<0.001	<0.001
IGFBP-1 (ng/ml)[a]	4.0 (3.4–4.5)	4.1 (3.6–4.7)	4.1 (3.5–4.7)	4.9 (4.4–5.5)	0.345	0.732
Fasting insulin (mU/l)	15.7 (14.3–17.0)	14.2 (12.9–15.6)	13.2 (11.9–14.6)	11.7 (10.4–13.1)	<0.001	0.001
QUICKI	0.59 (0.57–0.60)	0.60 (0.59–0.61)	0.60 (0.59–0.61)	0.64 (0.62–0.65)	<0.001	0.001
Age (years)	52 (52–53)	50 (50–51)	51 (51–52)	52 (51–53)	0.001	
BMI (kg/m^2)	28.6 (28.1–29.2)	27.8 (27.3–28.4)	27.5 (27.0–28.1)	27.1 (26.5–27.6)	0.001	
Hypertensive/control cohort (n)	142/105	112/136	124/124	132/120	0.050§	
Males/Females (n)	131/116	116/132	114/134	123/129	0.397§	

Data are means and 95% CI. Means are adjusted for sex and study group
[a]Means are adjusted for sex, study group and age
*p-values for the differences between the ghrelin quartiles obtained by ANCOVA adjusted for sex and study group
**p-values for the differences between the ghrelin quartiles obtained by ANCOVA adjusted for sex, study group, BMI and age
§p-values for the differences between the ghrelin quartiles obtained by chi-square test

Table 2 Partial correlation coefficients (r) with p-values for ghrelin, IGF-I, IGFBP-1, BMI and insulin adjusted for age, sex and study group

	IGF-I		IGFBP-1 IGFBP-MI		BMI		Insulin	
	r	p	r	p	r	p	r	p
Ghrelin	−0.35	<0.001	0.07	0.028	−0.12	<0.001	−0.17	<0.001
IGF-I	–	–	−0.07	0.029	−0.14	<0.001	−0.03	0.365
IGFBP-1	–	–	–	–	−0.38	<0.001	−0.44	<0.001
BMI	–	–	–	–	–	–	0.53	<0.001

Results

The mean fasting plasma ghrelin concentration of the whole study cohort was 668 pg/ml (range 117–1,513 pg/ml). The mean values of ghrelin concentration were 657 and 678 pg/ml in males and females, respectively (p=0.169), and 661 and 674 pg/ml in the hypertensive and control cohorts, respectively (p=0.359). The main characteristics of the study subjects by ghrelin quartiles (Table 1) showed ghrelin concentration to be negatively associated with IGF-I and fasting insulin concentrations and BMI. There was a pos-

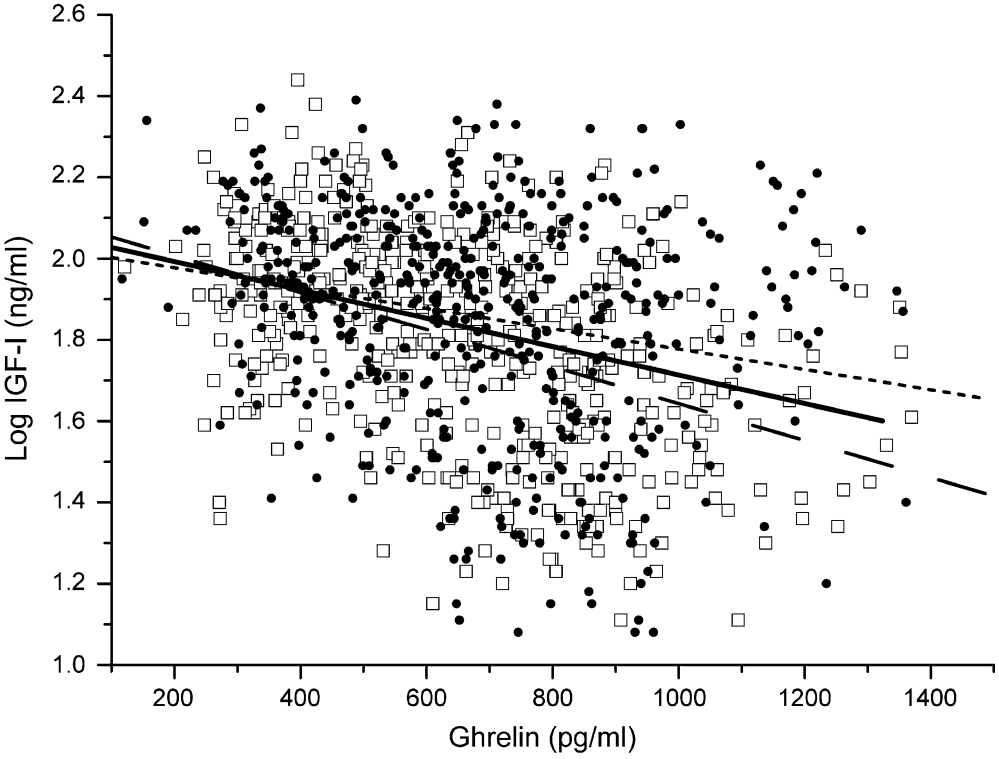

Fig. 1 Linear regression between ghrelin and IGF-I concentrations. *Open square and segment line*, males: n=484, r^2=19.1%, p<0.001; *black dot and dash line*, females: n=511, r^2=4.6%, p<0.001; *solid line*, all subjects: n=995, r^2=9.9%, p<0.001

itive association between ghrelin and insulin sensitivity, but no association between ghrelin and IGFBP-1 levels. Correlation analyses showed statistically significant inter-correlation between ghrelin, IGF-I, IGFBP-1, BMI and insulin (Table 2).

Linear regression analyses of all study subjects showed IGF-I concentration to be negatively associated with ghrelin concentration before ($p<0.001$) (Fig. 1, Table 3) and after adjustments for the potential confounding factors ($p<0.001$) (Table 3). Out of the individual factors, IGF-I was the most significant determinant of the plasma ghrelin concentration explaining 9.8% (adjusted r^2) of the variation in ghrelin concentrations. Since the interaction between IGF-I and sex was statistically significant in the multivariate analysis as a predictor of ghrelin concentration (ANCOVA, $p<0.05$), the analyses were further carried out in both sexes separately (Table 3). IGF-I explained a higher proportion of the variation in ghrelin concentration in males (adjusted $r^2=18.9\%$) compared to females (adjusted $r^2=4.4\%$).

In order to assess whether glycaemic status affects the association between ghrelin and IGF-I, the interaction between glycaemic status (no IR, IR or type 2 diabetes) and IGF-I concentration was tested in the multivariate analysis of all study subjects (ANCOVA), and it was statistically significant ($p<0.05$). The negative association between ghrelin and IGF-I concentrations is illustrated by simple linear regression (Fig. 2) and stepwise multivariate linear regression analyses (Table 4) separately for the subjects with no IR, with IR and with type 2 diabetes. IGF-I was negatively associated with ghrelin levels in all groups. However, the regression models explained the highest proportion of variation in subjects with type 2 diabetes ($r^2=33.7\%$) and they had the highest regression coefficient for IGF-I as well ($\beta=-0.56$, $p<0.001$). When the sexes were analysed separately, the trends were similar in both groups (data not shown). The subjects with IR had lower IGFBP-1 concentrations (3.2 ng/ml) compared to the subjects without IR (4.6 ng/ml) or with type 2 diabetes (5.9 ng/ml) (ANCOVA adjusted for BMI, age, sex and study group, $p<0.001$ in pairwise comparisons).

Figure 3 shows the variation of ghrelin concentration in relation to BMI and IGF-I levels in both sexes. The interactions between sex and IGF-I as well as between BMI and IGF-I as a predictor of ghrelin concentrations were significant ($p<0.05$). When IGF-I and fasting insulin levels

Table 3 Models explaining the variation of fasting plasma ghrelin concentration

Model	Variables entered	All subjects (n=995)			Males (n=484)			Females (n=511)		
		β	Significance	r^2 (%)	β	Significance	r^2 (%)	β	Significance	r^2 (%)
1	IGF-I	−0.32	***	9.8	−0.44	***	18.9	−0.22	***	4.4
2	Fasting insulin	−0.18	***	3.0	−0.14	**	1.6	−0.21	***	4.2
3	BMI	−0.13	***	1.5	−0.07	NS	0.3	−0.17	***	2.8
4	IGF-I	−0.40	***	16.1	−0.50	***	23.7	−0.28	***	10.0
	Fasting insulin	−0.15	***		−0.15	**		−0.13	*	
	BMI	−0.13	**		−0.10	NS		−0.14	*	
	IGFBP-1	−0.07	*		−0.10	*		−0.01	NS	
	Age	−0.10	**		−0.07	NS		−0.06	NS	
	Study group	0.04	NS		0.08	NS		0.04	NS	
	Sex	0.05	NS		–	–		–	–	

Linear regression analysis with standardised regression coefficients and adjusted R^2
NS Not significant
*$p<0.05$
**$p<0.01$
***$p<0.001$

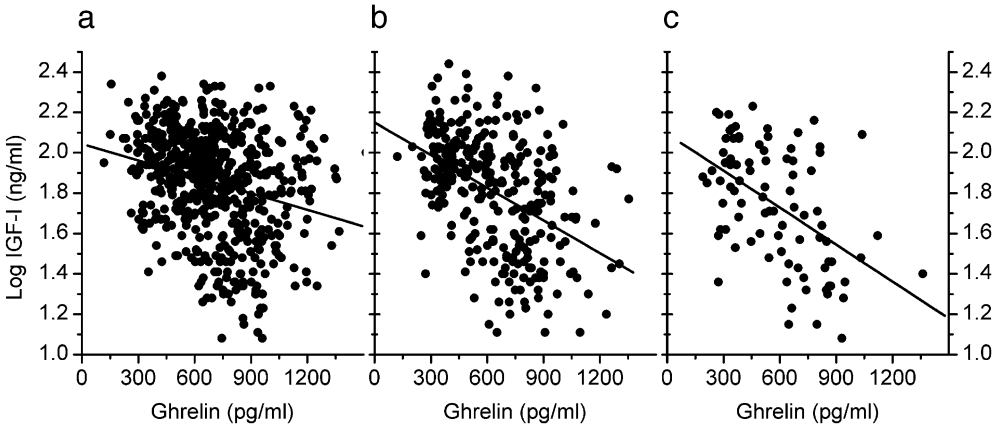

Fig. 2 Linear regression between ghrelin and IGF-I concentrations in relation to glycaemic status. **a** Subjects with no insulin resistance: n=602, $r^2=6.5\%$, $p<0.001$. **b** Subjects with insulin resistance: n=304, $r^2=21.0\%$, $p<0.001$. **c** Subjects with type 2 diabetes: n=89, $r^2=25.4\%$, $p<0.001$

Table 4 Models for fasting plasma ghrelin concentration in subjects with no insulin resistance (IR), with IR and with type 2 diabetes

Variables entered	No insulin resistance (n=602)			Insulin resistance (n=304)			Type 2 diabetes (n=89)		
	β	p	r^2 (%)	β	p	r^2 (%)	β	p	r^2 (%)
IGF-I	−0.28	<0.001	7.6	−0.49	<0.001	23.9	−0.56	<0.001	33.7
IGFBP-1	–	–		−0.17	0.002		–	–	
BMI	−0.12	0.002		−0.15	0.005		–	–	
Insulin	–	–		–	–		−0.25	0.005	
Age	–	–		–	–		−0.24	0.009	
Sex	–	–		–	–		–	–	
Study group	–	–		–	–		–	–	

Stepwise linear regression analysis with standardised regression coefficient and adjusted r^2

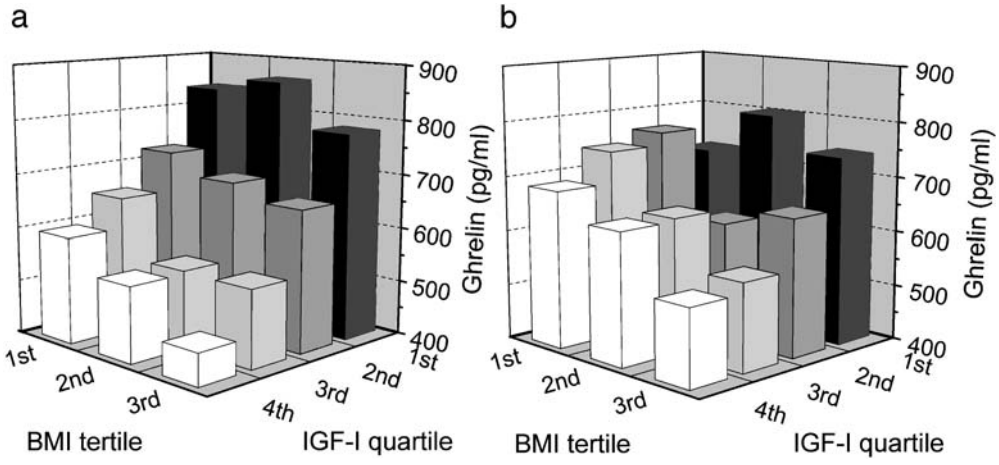

Fig. 3 Variation of fasting plasma ghrelin concentrations in relation to sex-specific BMI tertiles and IGF-I quartiles. The first BMI tertile and the first IGF-I quartile refer to the lowest BMI and IGF-I values, respectively. **a** Males (n=484). **b** Females (n=511)

Table 5 IGF-I and insulin as predictors of fasting plasma ghrelin concentration in sex-specific BMI tertiles

Linear regression analysis with standardised regression coefficient and adjusted r^2

		First BMI tertile			Second BMI tertile			Third BMI tertile		
		β	p	r^2 (%)	β	p	r^2 (%)	β	p	r^2 (%)
Males	IGF-I	−0.37	<0.001	15.9	−0.51	<0.001	24.6	−0.49	<0.001	23.5
	Insulin	−0.15	0.038		−0.11	0.101		−0.10	0.152	
Females	IGF-I	−0.04	0.652	1.6	−0.23	0.002	6.2	−0.44	<0.001	21.4
	Insulin	−0.16	0.041		−0.10	0.173		−0.21	0.003	

were entered into the linear regression analysis stratified according to sex and sex-specific BMI tertiles, IGF-I was the only statistically significant predictor of ghrelin in males in all BMI tertiles (p<0.001), whereas in females, IGF-I was statistically significant only in the second (p=0.002) and third BMI tertiles (p<0.001) (Table 5).

Discussion

Our findings in the large, population-based cohort of middle-aged subjects pointed out that IGF-I concentration is a significant determinant of ghrelin concentration, being negatively associated with it. This association was stronger in males and in subjects with high BMI, IR or type 2 diabetes. BMI explained a larger proportion of the variation in ghrelin concentrations in women than in men. IGFBP-1 concentrations were associated with ghrelin concentrations only in subjects with IR.

Since ghrelin has a powerful GH-releasing effect in experimental settings [1, 8, 17, 18], it has been proposed to serve as a regulator of somatotroph function together with GHRH and somatostatin [39, 40]. However, the physiological role of ghrelin in the regulation of GH/IGF-I axis has not yet been established. Several lines of evidence argue against the role of ghrelin in this regulation. Previous studies have shown no change in ghrelin levels in GH-deficient subjects after GH treatment [41, 42]. Pubertal GH surge is not associated with ghrelin levels [27]. Furthermore, GH pulsatility is not explained by changes in ghrelin concentration [43] and acute GH response in exercise is not associated with the changes in ghrelin levels [25, 31]. Interestingly, ghrelin-deficient mice are viable and exhibit a normal growth rate, demonstrating that ghrelin is not critical for normal growth [23]. On the other hand,

contrasting findings exist reporting a decrease in ghrelin levels after GH treatment [19], hyperghrelinemia in GH deficiency [20] and relatedness between ghrelin and GH pulsatility [21].

IGF-I has been considered a surrogate measure of GH secretion because of its long circulating half-life and its regulation by GH. Even though IGF-I is not universally regarded as an ideal discriminator of GH status [44], IGF-I measurement is currently the best indirect method to assess GH secretion [45]. The recent studies have reported negative association between ghrelin and IGF-I concentrations in children and adolescents [27–30] whereas nihilistic results has been reported in adult subjects [31–33].

To our best knowledge the results of the present study for the first time show clear and independent negative association between ghrelin and IGF-I concentrations in adult subjects. Our large sample size compared to the sample sizes of the previous studies might explain why association was detected in this study. However, the clear association between IGF-I and ghrelin was somewhat unexpected finding in the light of the several previous studies showing no association between ghrelin and GH/IGF-I axis. Nonetheless, our finding implies that there might be a negative feedback effect of either GH or IGF-I or both on ghrelin concentrations. The elevated ghrelin concentrations and decreased IGF-I concentrations, despite the high GH concentrations, in subjects with chronic liver disease [46] suggest that the regulation may preferably take place between ghrelin and IGF-I and not between ghrelin and GH.

The interpretation of our cross-sectional results is complicated by the complex regulation of IGF-I levels by a series of IGFBPs that control the bio-availability of IGFs [47], and by several other factors like age, sex, nutritional status and peripheral hormones, such as insulin and glucocorticoids that, in addition to GH status, affect IGF-I and/or ghrelin concentrations. In order to control some of these potentially confounding factors, we added them into the multivariate analyses as covariates. In addition, the analyses were carried out as stratified by sex, glycaemic status and BMI tertile. The multivariate analysis of all subjects showed that the association between ghrelin and IGF-I was statistically significant even after adjustments for sex, age, insulin concentration and BMI. In further analyses both sex, BMI and glycaemic status were found to modify the association between ghrelin and IGF-I levels. Nevertheless, the effect of IGF-I in the models remained even after stratifications. However, based on our cross-sectional results we can not definitively tell whether this association reflects mutual regulation between ghrelin and IGF-I or similar regulatory pathways behind ghrelin and IGF-I.

Interestingly, the negative association between ghrelin and IGF-I concentrations was clearly modified by IR and type 2 diabetes. This association was more pronounced among subjects with IR and particularly in a state of extreme insulin resistance, type 2 diabetes. Therefore, serum insulin concentrations seem to be an important determinant of the relationship between ghrelin and IGF-I. Subjects with type 2 diabetes also have decreased ghrelin [15] and IGF-I concentrations [48], which might also modify the association. The roles of IGF-I [49] and ghrelin [50] in the pathophysiology of insulin metabolism are complicated and not yet fully clarified. Whether ghrelin deficiency has a functional role in the development of IR and type 2 diabetes (e.g. by decreasing insulin sensitivity), remains to be explored in further prospective and experimental studies. Based on our findings we can only speculate that ghrelin/IGF-I interactions might also be involved in the development of IR and type 2 diabetes.

We found the association between ghrelin and IGF-I to be modulated by sex, suggesting that there might be a sex-dependent difference in the regulation of ghrelin despite the sex-independent GH-releasing effect of ghrelin [51] and the sex-independent ghrelin levels [15, 29]. Ghrelin concentrations have been reported to depend on sex only in young subjects [52]. The sex difference in the role of ghrelin in the regulation of GH has been previously shown in animal models [53, 54].

To obtain a measure of overall ghrelin concentrations, we used fasting plasma ghrelin concentrations, which have been shown to correlate strongly with the 24-h integrated AUC values [37]. Therefore, the single measurement of fasting plasma ghrelin concentration is well reasoned. Fasting plasma ghrelin concentrations were analysed using antibody that recognises both acylated and des-acylated ghrelin. Although only acylated ghrelin is thought to have endocrine activity [1], non-endocrine functions have been reported for the non-acylated form of ghrelin [55] and, therefore, the measurement of total ghrelin is reasoned. Another reason for the measurement of total ghrelin concentration is that total ghrelin concentration remains significantly better in all conditions compared to acylated ghrelin concentration [56]. Furthermore, total ghrelin is a good surrogate of acylated ghrelin since they are well correlated [57], and the ratio of these two remains constant under a wide variety of conditions [58].

In conclusion, we showed in a large, population-based study that there is an independent negative association between ghrelin and IGF-I concentrations. This association is modified by BMI, gender, IR and type 2 diabetes. Further studies are warranted to elucidate the role of ghrelin in the development of obesity and type 2 diabetes.

Acknowledgements The Research Council for Health of the Academy of Finland, the Finnish Foundation for Cardiovascular Research, the Finnish Medical Foundation, the Aarne Koskelo Foundation, and the Ida Montin Foundation supported this study. We acknowledge the excellent technical assistance of Ms. Helena Kalliokoski, Ms.Saija Kortetjärvi, Ms. Liisa Mannermaa, and Ms. Sirpa Rannikko.

Duality of Interest

There is no duality of interest concerning any financial or other interests directly or indirectly related to the subject of this manuscript.

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