CLINICAL STUDY

The associations between plasma adiponectin, ghrelin levels and cardiovascular risk factors

K M Choi, J Lee¹, K W Lee, J A Seo, J H Oh, S G Kim, N H Kim, D S Choi and S H Baik

Division of Endocrinology and Metabolism, Department of Internal Medicine and ¹ Department of Preventive Medicine, College of Medicine, Korea University, Seoul, Korea

(Correspondence should be addressed to S H Baik, Department of Internal Medicine, Korea University Guro Hospital, 80 Guro-Dong, Guro-Gu, Seoul 152-050, South Korea; Email: 103hyun@korea.ac.kr)

Abstract

Objective: Ghrelin is a recently discovered peptide, which is produced primarily in the stomach. This orexigenic peptide participates not only in the induction of mealtime hunger but also in long-term body weight regulation and energy homeostasis. Adiponectin is a protein secreted by adipocytes, and has been proposed to mediate obesity-related insulin resistance. Moreover, concentrations of adiponectin are reduced in individuals with obesity, insulin resistance and cardiovascular disease. However, human data are sparse about the direct relationship between adiponectin, ghrelin and cardiovascular risk factors including insulin resistance.

Design: Three hundred and thirty-eight elderly Korean women (mean age $\pm$ s.d., 72.3 ± 5.5 years) were included in the present study.

Methods: Plasma ghrelin and adiponectin levels were measured by RIA. Anthropometric measurements were taken and a 75 g oral glucose tolerance test performed. Fasting insulin and lipid profile were measured and insulin resistance was determined using the homeostasis model assessment insulin resistance index (HOMA-R) and the quantitative insulin sensitivity check index.

Results: Plasma adiponectin levels were negatively correlated with central obesity indices such as waist circumference ($r = -0.27$, $P < 0.001$) and waist-to-hip ratio (WHR) ($r = -0.32$, $P < 0.001$), and with insulin resistance indices such as fasting insulin ($r = -0.17$, $P = 0.004$) and HOMA-R ($r = -0.13$, $P = 0.035$). Plasma ghrelin levels were negatively correlated with WHR ($r = -0.12$, $P = 0.03$), but plasma adiponectin and ghrelin levels were not correlated ($r = 0.03$, $P = 0.66$). Multiple regression analysis showed that adiponectin was associated with WHR, fasting insulin and fasting glucose levels. When ghrelin was used as a dependent variable, only WHR remained in the final fitted model.

Conclusion: Fasting plasma adiponectin and ghrelin levels were found to be associated with central obesity or insulin resistance. However, plasma adiponectin and ghrelin concentrations were not associated with each other in elderly Korean women.

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Introduction

Ghrelin is a novel endogenous natural ligand of growth hormone (GH) secretagogue receptor (1), and is recognized an important regulator of GH secretion and energy homeostasis (2). Ghrelin is an orexigenic peptide that antagonizes leptin action and causes weight gain by increasing food intake and reducing fat utilization in rodents and humans (3–5). Human plasma ghrelin levels increase sharply before and decrease after every meal, which is consistent with a physiological role of ghrelin in mealtime hunger and meal initiation (6). In humans, circulating ghrelin levels are reduced in a chronic state of positive energy balance such as obesity, as well as in an acute state of positive energy balance such as food intake (6, 7).

On the other hand, ghrelin levels are increased by fasting (3) and in cachectic patients with anorexia nervosa (8).

Adiponectin has been recently identified as a protein secreted by adipocytes, which are important in metabolic and vascular diseases. Plasma adiponectin concentrations are reduced in obese individuals (9), and in those showing insulin resistance (10). Furthermore, adiponectin concentrations were found to be lower in diabetics than in non-diabetics (9), and were particularly low in subjects with coronary artery disease (11).

Previous studies have reported that ghrelin and adiponectin levels increase after gastric bypass surgery in obese humans (12). Moreover, chronic ghrelin administration to adipocytes strongly impaired adiponectin expression (13). Although both ghrelin and adiponectin

are associated with obesity and the status of long-term energy homeostasis, human data are scanty about the relationship between adiponectin, ghrelin and cardiovascular risk factors, including insulin resistance.

In this study, we evaluated a relationship between plasma adiponectin, produced by adipose tissue, and plasma ghrelin, produced by the stomach. Accordingly, we tried to find an interaction between stomach and adipose tissue in the control of energy homeostasis. In addition, we examined the effects of cardiovascular risk factors such as obesity and insulin resistance on plasma adiponectin and ghrelin levels in elderly Korean women.

Materials and methods

This study was conducted in regional senior welfare centers of the Seoul Metropolitan Government by medical personnel of Korea University. A total of 338 elderly Korean women (between 61 and 89 years of age) who had no apparent disease history participated in this study. An informed consent has given by all subjects before participating in the study, which was approved by the ethical committee of our institutions. After history taking, a 75 g oral glucose tolerance test was been performed. Blood samples were drawn after an overnight fast and immediately centrifuged. Blood chemistry was measured at the laboratory of the Korea University Guro Hospital (Seoul, Korea). Serum total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol were determined by enzymatic methods with a chemistry analyzer (Hitachi 747, Tokyo, Japan). Low-density lipoprotein (LDL) cholesterol was calculated using the formula of Friedewald *et al.* (14). Plasma glucose was measured using the glucose oxidase method, while insulin was measured using a human insulin-specific RIA kit (Linco Research Inc., St Charles, MO, USA), which had reactivity of less than 0.2% with human proinsulin. Insulin resistance was assessed by determining the homeostasis model assessment insulin resistance index (HOMA-R) (15) and the quantitative insulin sensitivity check index (QUICKI) (16). HOMA-R was calculated as (fasting glucose (mmol/l) $\times$ fasting insulin (μ U/ml)/22.5), and QUICKI as $(1/(\log \text{fasting insulin } (\mu\text{U/ml}) + \log \text{fasting glucose (mg/dl)}))$. Plasma adiponectin was measured using a human adiponectin RIA kit (Linco Research) (human adiponectin sensitivity 1 ng/ml using a 100 μ l sample size; intra-assay coefficient of variation (CV) 8.7%). A human ghrelin (total) RIA kit (Linco Research) (human ghrelin sensitivity 100 pg/ml using a 100 μ l sample size; intra-assay CV 7.5%) was used for measuring the plasma ghrelin level.

Data were expressed as means $\pm$ S.D. (geometric mean and its S.D. for variables not normally distributed are presented). Correlation analysis between plasma adiponectin, ghrelin and risk factor variables

of cardiovascular disease was performed. Multiple linear regression models using adiponectin or ghrelin as dependent variables were conducted to obtain relative contributions of each variable to the outcome variable. Significant independent variables were chosen by using a stepwise variable selection method with $\alpha = 0.15$. A value of $P < 0.05$ was used to indicate statistical significance. Data were analyzed using SPSS for Windows (version 10.0; SPSS Inc., Chicago, IL, USA).

Results

The characteristics of study subjects were summarized in Table 1. Study subjects were elderly women aged over 60 years and relatively overweight (mean body mass index (BMI) $\pm$ S.D., 25.0 ± 3.1). By correlation analysis, plasma adiponectin levels were found to be negatively correlated with central obesity parameters such as waist circumference ($r = -0.27$, $P < 0.001$) and waist-to-hip ratio (WHR) ($r = -0.32$, $P < 0.001$), as well as with insulin resistance parameters such as fasting insulin ($r = -0.17$, $P = 0.004$) and HOMA-R ($r = -0.13$, $P = 0.035$). Plasma ghrelin levels correlated negatively with WHR ($r = 0.12$, $P = 0.03$). However, plasma adiponectin and ghrelin concentrations were not correlated ($r = 0.03$, $P = 0.66$). Stepwise multiple regression analyses were performed with adiponectin or ghrelin as dependent variables. Age, systolic and diastolic blood pressure, BMI, WHR, total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol, fasting and post-load 2 h glucose, fasting insulin, HOMA-R, QUICKI, adiponectin and ghrelin were employed as independent variables (Table 2). Multiple regression analysis showed that adiponectin was associated with WHR, fasting insulin and fasting glucose levels. On the other hand, WHR was the only

Table 1 Characteristics of study subjects.

Age (years)	72.3 $\pm$ 5.5
Systolic blood pressure (mmHg)	141.2 $\pm$ 20.3
Diastolic blood pressure (mmHg)	84.4 $\pm$ 10.1
BMI	25.0 $\pm$ 3.1
Waist circumference (cm)	86.4 $\pm$ 8.9
WHR	0.92 $\pm$ 0.08
Total cholesterol (mmol/l)	5.3 $\pm$ 0.9
HDL cholesterol (mmol/l)	1.5 $\pm$ 0.3
Triglycerides (mmol/l)*	1.4 $\pm$ 1.6
LDL cholesterol (mmol/l)	3.1 $\pm$ 0.8
Fasting glucose (mmol/l)	5.6 $\pm$ 1.1
Post-load 2 h glucose (mmol/l)	7.7 $\pm$ 2.5
Fasting insulin (μ U/ml)	8.9 $\pm$ 5.0
HOMA-R	2.2 $\pm$ 1.4
QUICKI	0.35 $\pm$ 0.06
Adiponectin (ng/ml)*	12.8 $\pm$ 2.3
Ghrelin (pg/ml)	1907 $\pm$ 547

* Geometric mean $\pm$ geometric S.D.

Table 2 Correlations among adiponectin, ghrelin and the risk factor variables of cardiovascular disease.

	Adiponectin		Ghrelin	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age	−0.030	0.619	−0.046	0.397
Systolic blood pressure	0.024	0.693	−0.001	0.981
Diastolic blood pressure	0.051	0.395	−0.016	0.772
BMI	0.011	0.857	0.023	0.682
Waist circumference	−0.272	< 0.001	−0.049	0.370
WHR	−0.323	< 0.001	−0.119	0.030
Total cholesterol	0.041	0.495	−0.019	0.730
HDL cholesterol	0.048	0.426	0.035	0.518
Triglycerides	−0.030	0.621	−0.062	0.260
LDL cholesterol	0.053	0.383	−0.013	0.816
Fasting glucose	0.126	0.035	0.091	0.096
Post-load 2 h glucose	0.078	0.230	0.053	0.374
Fasting insulin	−0.173	0.004	0.014	0.803
HOMA-R	−0.126	0.035	0.030	0.587
QUICKI	0.077	0.199	0.002	0.971
Adiponectin	1.000	—	0.026	0.661
Ghrelin	0.026	0.661	1.000	—

significant independent variable in multiple regression analysis using ghrelin as a dependent variable.

Discussion

The peripheral or central administration of ghrelin to rodents has been found to stimulate short-term food intake as potently as any known compound (17). This orexigenic action is mediated via the hypothalamic arcuate nucleus, which co-expresses neuropeptide Y and agouti-related protein (2). Several studies have suggested that ghrelin may participate not only in mealtime hunger and meal initiation, but also in long-term body weight regulation (18). Moreover, continuous or repeated ghrelin administration increases body weight (3, 19). These observations distinguish ghrelin from cholecystokinin, which can alter meal patterns, but not body weight when injected long-term (20). The blockade of endogenous ghrelin signaling in the brain can reduce spontaneous food intake and body weight (21, 22). In addition, it has been reported that ghrelin administration not only increases food intake but also decreases metabolic rate (17) and fat catabolism (3). These studies intimate that ghrelin has an important role in long-term energy homeostasis. Indeed, elevated ghrelin levels were found during weight loss resulting from caloric restriction (23), anorexia nervosa (8) and cancer anorexia (24). These results suggest a role for ghrelin in the adaptive response to body weight changes.

Previous studies reported that ghrelin reduces insulin secretion in the isolated rat pancreas and in humans (25, 26). Tschop *et al.* (27) found a negative correlation between plasma ghrelin and insulin concentrations. In contrast, no relationship was found

between plasma ghrelin and insulin concentrations in a recent study (28). In the present study, plasma ghrelin levels were only associated with central obesity parameters such as WHR.

We measured only fasting ghrelin levels in our study. Cummings *et al.* (6) reported that plasma ghrelin levels nearly doubled immediately before each meal, and fell within 1 h after eating, a pattern which is the reciprocal of that of insulin levels. They suggested that a single measurement of plasma ghrelin before breakfast might serve as a surrogate for the estimation of overall ghrelin levels, because this measure correlated strongly with 24 h integrated area under the curve values (6).

Recently, it was found that adipose tissue is not merely an inert energy storage depot, but rather an active organ which secretes various hormones and metabolites that are thought to regulate energy metabolism and insulin sensitivity (29). Both excess adipose tissue in obesity and a lack of adipose tissue in a lipodystrophic state were associated with insulin resistance and type 2 diabetes in human and animal studies (30, 31). Adiponectin has been recently identified as a protein secreted by adipocytes, which are important in metabolic and vascular diseases. Adiponectin is exclusively expressed in adipose tissue and released into the circulation (32). Paradoxically, obese individuals have reduced plasma adiponectin concentrations (10). Decreased adiponectin concentrations were also reported in individuals with type 2 diabetes (33), insulin resistance (9) or cardiovascular disease (11). Interestingly, chronic administration of adiponectin prevented a high-fat diet-induced weight gain, despite the fact that food consumption was unaffected (34).

In the present study, we evaluated an inter-relationship between ghrelin and adiponectin. Previous studies have reported that both ghrelin and adiponectin are associated with obesity and the status of long-term energy homeostasis. Holdstock *et al.* (12) reported that ghrelin and adiponectin levels increased after gastric bypass surgery in obese humans. Their study, in agreement with the present study, found no correlation between ghrelin and adiponectin. In addition, they reported correlations between insulin and ghrelin in univariate analysis. However, we could not find such a relationship in our study. Their study included younger subjects with higher BMIs than ours.

In the study of Ott *et al.* (13), chronic ghrelin administration to adipocytes strongly impaired adiponectin expression, although it did not significantly alter the gene expression of thermogenic uncoupling protein-1. Therefore, we hypothesized an interaction between ghrelin produced by the stomach and adiponectin produced by adipose tissue. However, although plasma adiponectin and ghrelin concentrations are individually related to obesity indices or insulin resistance indices, their levels were not found to be associated with each other in elderly Korean women.

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