

Metabolic Syndrome is Associated with Lower Plasma Levels of Desacyl Ghrelin and Total Ghrelin in Asymptomatic Middle-aged Korean Men

Young Hye Cho¹, Sang Yeoup Lee^{1,2*}, Dong Wook Jeong¹, A Ra Cho¹, Jeong Suk Jeon¹, Yun Jin Kim³, Jeong Gyu Lee³, Yu Hyeon Yi³, Young Jin Tak³, Hye Rim Hwang³, Seung-Hun Lee³, Sun Min Lee⁴

¹Family Medicine Clinic, Obesity, Metabolism and Nutrition Center and Research Institute of Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan; ²Medical Education Unit, Pusan National University, School of Medicine, Yangsan; ³Department of Family Medicine, Pusan National University Hospital, Busan; ⁴Department of Laboratory Medicine and Molecular Genetics and Research Institute of Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, Korea

Background: Desacyl ghrelin is acylated by ghrelin O-acyltransferase (GOAT) and converted to acyl ghrelin. To date, little is known about the relationship among the levels of these two forms of ghrelin, GOAT level, and insulin resistance in Asian individuals. The purpose of this study was to determine the relationship between insulin resistance and the levels of plasma acyl ghrelin, desacyl ghrelin, and GOAT in asymptomatic middle-aged Korean men.

Methods: This cross-sectional study evaluated 78 asymptomatic middle-aged Korean men with metabolic syndrome (MS). We examined the correlation between the plasma levels of acyl ghrelin, desacyl ghrelin, and GOAT and sociodemographic, dietary, anthropometric, and metabolic parameters, as well as the association between insulin resistance and plasma levels of acyl ghrelin, desacyl ghrelin, and GOAT.

Results: The levels of desacyl ghrelin and total ghrelin were significantly lower in the MS group than in the non-MS group ($P < 0.017$, $P = 0.01$, respectively). HOMA-IR values showed a significant negative correlation with desacyl ghrelin ($r = -0.271$, $P = 0.017$) and total ghrelin ($r = -0.271$, $P = 0.016$) levels. Acyl ghrelin and GOAT were not significantly correlated with HOMA-IR, and no correlation was found between the plasma levels of the two ghrelin types and GOAT.

Conclusion: The plasma levels of desacyl ghrelin and total ghrelin in middle-aged Korean men with MS were lower than in those without MS. A significant negative correlation was observed between desacyl ghrelin level and HOMA-IR; however, no correlation was found between plasma levels of acyl ghrelin and GOAT and HOMA-IR.

Key words: Ghrelin, Metabolic syndrome, Insulin resistance, Obesity

Received September 23, 2016

Reviewed October 27, 2016

Accepted December 12, 2016

*Corresponding author
Sang Yeoup Lee



<http://orcid.org/0000-0002-3585-9910>

Family Medicine Clinic, Pusan National University Yangsan Hospital, 20 Geumoro, Mulgeum-eup, Yangsan 50612, Korea
Tel: +82-55-360-2140
Fax: +82-51-510-8125
E-mail: saylee@pnu.edu

INTRODUCTION

Ghrelin is a 28-amino acid peptide hormone that is primarily secreted by the gastric mucosa and is known to be involved in glucose metabolism, food intake, and energy homeostasis.^{1,2} Low concentrations of total ghrelin are associated with obesity^{3,4}, insulin resistance^{5,6}, and metabolic syndrome.⁷ However, ghrelin occurs in two major forms—acyl ghrelin and desacyl ghrelin¹—and these

forms have distinct metabolic effects.⁸⁻¹¹ Several studies have evaluated both ghrelin forms and indicated that high level of acyl ghrelin and low level of desacyl ghrelin are associated with increased prevalence of metabolic syndrome (MS).^{12,13}

The enzyme responsible for ghrelin acylation, ghrelin O-acyltransferase (GOAT), is recognized as the fourth member of the membrane-bound O-acyltransferase superfamily.^{8,9} Because GOAT knockout mice present improved glucose tolerance, GOAT is be-

lieved to be involved in control of the metabolic state and glucose homeostasis.¹⁴ Several studies have demonstrated that GOAT mRNA expression is regulated by energy balance and is dependent on the metabolic state.^{15,16} More recently, a cross-sectional study has found that human plasma GOAT protein level is positively correlated with body mass index (BMI) and negatively correlated with plasma level of total ghrelin.¹⁷

The studies that evaluated the associations between the two ghrelin forms and metabolic syndrome had several limitations, including a relatively small sample size and the evaluation of Caucasian subjects only. However, there are many interethnic differences in underlying pathophysiological and lifestyle factors between Asian and Caucasian individuals. In addition, little is known about the relationships between the levels of the two ghrelin forms, GOAT, and insulin resistance. Therefore, the aim of the present study was to identify the relationships between insulin resistance and the levels of acyl ghrelin, desacyl ghrelin, and GOAT in asymptomatic middle-aged Korean men.

METHODS

Study subjects and ethics

This cross-sectional study was approved by the Institutional Review Board of Pusan National University Yangsan Hospital (L-2013-230), and informed written consent was provided by all subjects before participating in the study. Only men were recruited in order to avoid the effects of the menstrual cycle and oral contraceptives on insulin sensitivity. The study subjects included 80 asymptomatic men aged 40 to 60 years who visited the health promotion center of Pusan National University Yangsan Hospital. Subjects had not taken any anti-diabetic drugs, steroids, or hormonal products. Subjects with a history of malignancy or a cardiovascular event and subjects who received medication for acute diseases, including myocardial infarction and angina pectoris, were excluded. Ultimately, 78 middle-aged men were enrolled in the study.

Measurements

Height and weight were measured using standard protocols with the subjects wearing a light gown and no shoes. BMI was calculated as weight (kg) divided by height squared (m^2). According to the

guidelines of the World Health Organization, waist circumference (WC) was measured by placing a tape measure on the thinnest area between the lowest part of the ribs and iliac crest of the pelvis, during normal exhalation, and data were recorded to the nearest 0.1 cm. A mercury sphygmomanometer was used to measure blood pressure (BP) after a 10-minute resting period, with the subjects in the sitting position. Two readings of systolic BP (SBP) and diastolic BP (DBP) were recorded at 3 minutes intervals, and averages were included in the analysis.

Following an overnight fast, blood samples were obtained from an antecubital vein between 8 a.m. and 9 a.m. Plasma was immediately isolated by centrifugation at 4°C and stored at -80°C until assayed. The liver enzymes alanine aminotransferase and γ -glutamyl transferase (GGT) were measured in a Hitachi 7600 Analyzer (Hitachi Co., Ltd, Tokyo, Japan) using an enzymatic colorimetric method. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured in a biochemical autoanalyzer (Toshiba TBA200FR, Toshiba Co. Ltd., Tokyo, Japan) using a direct measurement method; triglycerides were measured using lipase, glycerol kinase (GK), glycerol 3-phosphate oxidase (GPO), peroxidase (POD), and a glycerol blank. Fasting glucose was measured using a glucose oxidase test method (LX-20, Beckman Coulter, Fullerton, CA, USA), and plasma insulin was measured in duplicate by radioimmunoassay using a solid-phase single antibody assay (Coat-a-Count Insulin, TKIN2, Diagnostic Products Corporation, Los Angeles, CA). C-reactive protein (CRP) was measured using a Behring BN II nephelometer (Dade Behring, Marburg, Germany). Acyl ghrelin and desacyl ghrelin were measured using a commercial ELISA kit (Cusabio Biotech, Wuhan, China). The inter- and intra-assay coefficients of variation (CV) were 7.0% and 5.2%, respectively, for acyl ghrelin and 8.6% and 6.5% for desacyl ghrelin. Plasma GOAT level was measured using the Human GOAT ELISA Kit (Cusabio Biotech, Wuhan, China), and the inter- and intra-assay CV were 7.6% and 5.8%, respectively.

Abdominal fat was assessed using computed tomography (CT) scans acquired at the L4-L5 level. Abdominal fat was defined as the area corresponding to the pixel range from -190 to -30 Hounsfield units.¹⁸ The areas of visceral and subcutaneous abdominal adipose tissue were measured. Fat inside the peritoneum was considered visceral adipose tissue, and fat between the dermis and muscle fas-

cia was considered subcutaneous adipose tissue. Whole body composition scans were obtained using a Hologic Discovery instrument (Hologic Inc., Bedford, MA, USA).

Both diet and physical activity were assessed because of their possible effects on insulin sensitivity. Diet was monitored by a semi-quantitative food frequency questionnaire¹⁹, and physical activity was assessed using the International Physical Activity Questionnaire.²⁰ Physical activity level was expressed in metabolic equivalent- (MET-) minutes. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using the formula: [fasting plasma insulin ($\mu\text{U}/\text{mL}$) \times fasting plasma glucose (mg/dL)]/(22.5 \times 18.182).²¹

Definition of metabolic syndrome

MS was defined according to the 2005 American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI).²² The waist circumference threshold for abdominal obesity in Korean men is 90 cm.²³

Statistical analysis

Descriptive data are expressed as means (standard deviation, SD) or numbers (%). The mean and SD of ghrelin level determined in a previous study⁷ were used to calculate the statistical power for this study. At least 19 subjects per group would be required to fulfill these assumptions (power = 90%, alpha error = 0.05). We assumed a prevalence of MS of 25% in middle-aged men and a dropout rate of 5%. Therefore, 80 subjects were selected for the present study. The study subjects were divided into two groups: those that met the AHA/NHLBI criteria for MS (the MS group) and those that did not meet these criteria (the non-MS group). Total ghrelin level was calculated by summing acyl ghrelin and desacyl ghrelin levels. The Kolmogorov-Smirnov test was used to test variable normality. Between-group comparisons were performed using the two-sample *t*-test or the Mann-Whitney U test for continuous variables or the chi-square test for categorical variables. Correlations between variables were tested using Spearman correlation coefficients. Receiver-operating characteristics (ROC) curve and area under the ROC curve (AUC) analyses were performed to identify correlations between desacyl ghrelin and total ghrelin and other biochemical factors. The analysis was conducted

using SPSS software version 18.0 for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc version 9.6.4.0 for Windows (MedCalc Software, Mariakerke, Belgium). Statistical significance was accepted for *P* smaller than 0.05.

RESULTS

The general characteristics of the study subjects are shown in Table 1. The overall average age was 51.2 years, and the mean BMI was 24.6 kg/m².

Sociodemographic, dietary, anthropometric, and metabolic parameters

No differences in age, alcohol consumption, physical activity, or dietary intake were observed between the MS and non-MS groups. However, the prevalence of current smoking was significantly higher in the MS group (*P* = 0.009, Table 2).

BMI and WC were significantly higher in the MS group than in the non-MS group (*P* < 0.001 and *P* < 0.001, respectively). Similarly, the CT-measured abdominal visceral fat area and subcutaneous fat area were significantly higher in the MS group than in the non-MS group (*P* < 0.001 and *P* = 0.006, respectively). However, no dif-

Table 1. Characteristics of the study participants

Characteristics	Value
No. of participants	78
Age (yr)	51.2 \pm 5.8
Height (cm)	171.5 \pm 5.2
Weight (kg)	72.3 \pm 9.4
BMI (kg/m ²)	24.6 \pm 2.7
Waist circumference (cm)	86.4 \pm 7.6
Smoking	34 (43.6)
Alcohol drinking	63 (80.8)
Metabolic syndrome components	
Abdominal obesity*	23 (29.5)
Elevated blood pressure [†]	42 (53.8)
Elevated fasting plasma glucose [‡]	31 (39.7)
Reduced HDL-C [§]	11 (14.1)
Elevated triglycerides	28 (35.9)

Data are expressed as means \pm SEs or numbers (%).

*Waist circumference \geq 90 cm; [†]Blood pressure \geq 130/85 mmHg or use of medication for hypertension; [‡]Fasting plasma glucose \geq 100 mg/dL or use of medication for diabetes; [§]HDL-C < 40 mg/dL or use of medication for dyslipidemia; ^{||}Triglycerides \geq 150 mg/dL or use of medication for dyslipidemia.

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol.

ferences in SBP and DBP were observed between the two groups. Fasting glucose, insulin, HOMA-IR, triglycerides, GGT, and CRP were higher in the MS group ($P < 0.001$ for all variables, except

CRP, with $P = 0.005$). On the other hand, the mean HDL-C level was lower in the MS group ($P < 0.001$).

Table 2. Differences in the characteristics of the study patients

Variables	Non-MS (n=58)	MS (n=20)	P
Socio-demographic parameters			
Age (yr)	50.6±5.8	52.6±5.9	0.184
Smoking status			0.009
Never	38 (65.5)	6 (30.0)	
Current	20 (34.5)	14 (70.0)	
Alcohol consumer	45 (77.6)	18 (90.0)	0.191
Activity (METS/week)*	1,315.1±1,738.9	1,111.3±1,091.2	0.870
Dietary parameters			
Energy intake (kcal/day)	1,986.9±294.8	2,127.0±387.4	0.096
Protein intake (g/day)	82.5±19.7	91.6±23.1	0.093
Fat intake (g/day)	45.6±13.0	51.5±17.6	0.119
Carbohydrate intake (g/day)	311.5±44.5	324.4±52.8	0.293
Anthropometric parameters			
Body mass index (kg/m ²)	23.9±2.5	26.4±2.6	<0.001
Waist circumference (cm)	84.6±6.7	91.7±7.6	<0.001
DXA-measured fat			
Upper arm fat (%)	24.8±4.7	27.8±5.0	0.020
Lower leg fat (%)	23.9±4.4	26.1±5.1	0.069
Trunk fat (%)	28.2±5.0	31.4±4.6	0.013
Total fat (%)	26.1±4.1	28.8±4.3	0.014
CT-measured abdominal fat area			
VFA (cm ²)	91.0±30.0	141.9±50.0	<0.001
SFA (cm ²)	119.4±59.7	160.6±44.6	0.006
Metabolic parameters			
SBP (mmHg)	120.5±13.4	124.5±14.6	0.259
DBP (mmHg)	81.5±10.1	84.1±11.5	0.350
Fasting plasma glucose (mg/dL)	98.1±30.5	121.3±35.8	<0.001
Fasting insulin (μIU/mL)*	3.8±1.7	7.4±5.5	<0.001
HOMA-IR*	0.92±0.46	2.07±1.45	<0.001
HDL-cholesterol (mg/dL)*	53.9±11.1	43.9±7.9	<0.001
Triglycerides (mg/dL)*	131.8±75.3	210.6±98.3	<0.001
ALT (IU/L)	30.8±15.9	40.7±16.9	0.014
GGT (IU/L)*	57.8±61.3	109.7±94.5	<0.001
CRP (mg/dL)*	0.11±0.12	0.26±0.29	0.005
Ghrelin profiles			
Desacyl ghrelin (pg/mL)*	315.5±188.2	205.2±144.4	0.017
Acyl ghrelin (pg/mL)*	45.1±34.7	31.7±28.1	0.114
Total ghrelin (pg/mL)*	360.6±188.8	236.9±148.5	0.010
GOAT (pg/mL)	31.4±52.1	26.1±14.8	0.655

Data are expressed as means ± SEs or numbers (%).

P values were determined using the 2-sample t-test or the chi-square test.

*P values were determined using the Mann-Whitney U test.

MS, metabolic syndrome; VFA, visceral fat area; SFA, subcutaneous fat area; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment insulin resistance index; HDL, high-density lipoprotein; ALT, alanine aminotransferase; GGT, γ -glutamyl transferase; CRP, C-reactive protein; GOAT, ghrelin O-acyltransferase; DXA, dual-energy X-ray absorptiometry; CT, computed tomography. One MET is roughly equivalent to 1 kcal/min for a 60 kg person.

Ghrelin levels

Desacyl ghrelin and total ghrelin levels were significantly lower in the MS group ($P < 0.017$, $P = 0.01$, respectively). However, no significant differences in acyl ghrelin and GOAT levels were observed between the two groups (Table 2).

The ROC curves of desacyl ghrelin, total ghrelin, GGT, and HOMA-IR for the diagnosis of MS are shown in Fig. 1. The AUC of HOMA-IR had the greatest diagnostic value for MS (0.890; 95% CI, 0.798-0.949). The AUC of desacyl ghrelin was 0.680 (95% CI, 0.564-0.781), and that of total ghrelin was 0.694 (95% CI, 0.579-0.793) for MS. The AUC of HOMA-IR differed significantly from the AUCs of desacyl ghrelin and total ghrelin ($P = 0.002$, $P = 0.003$).

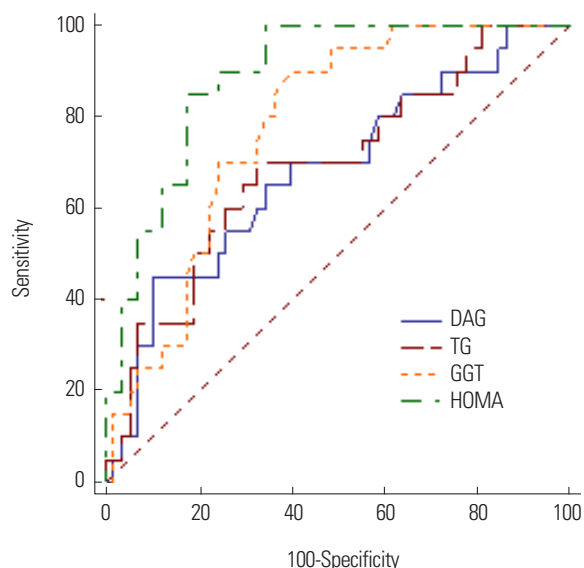


Figure 1. Receiver-operating characteristic (ROC) curves of desacyl ghrelin (DAG), total ghrelin (TG), γ -glutamyl transferase (GGT), and homeostatic model assessment insulin resistance index (HOMA-IR) for metabolic syndrome according to the AHA/NHLBI criteria. The area under the ROC curves \pm standard errors (95% CI) were: DAG, 0.680 ± 0.065 (0.564-0.781); TG, 0.694 ± 0.064 (0.579-0.793); GGT, 0.779 ± 0.066 (0.671-0.865); and HOMA-IR, 0.890 ± 0.050 (0.798-0.949).

Correlations between HOMA-IR, desacyl ghrelin, acyl ghrelin, and GOAT

HOMA-IR values were significantly negatively correlated with desacyl ghrelin ($r = -0.271$, $P = 0.017$) and total ghrelin ($r = -0.271$, $P = 0.016$) (Table 3), but not significantly correlated with acyl ghrelin or GOAT level. No correlations were found between acyl ghrelin, desacyl ghrelin, and GOAT levels.

DISCUSSION

In the present study, we investigated the associations between two types of ghrelin and MS in middle-aged Korean men. Desacyl ghrelin and total ghrelin levels in the MS group were lower than in the non-MS group; however, no intergroup difference in the levels of acyl ghrelin and GOAT were observed. Furthermore, a significant negative correlation was found between desacyl ghrelin and HOMA-IR, but no correlation was found between acyl ghrelin or GOAT and HOMA-IR.

Desacyl ghrelin is acylated by GOAT to form acyl ghrelin. Acyl ghrelin and desacyl ghrelin are detectable in the circulation at ratios of 1:4 to 1:9 depending on nutritional status and the sample collection method used.^{1,8} Acyl ghrelin exerts central and peripheral effects via growth hormone secretagogue receptor (GHSR) type 1a.¹ Desacyl ghrelin was initially believed to be an inactive form because it does not bind or activate GHSR. However, several studies have demonstrated that desacyl ghrelin antagonizes the inhibitory action of acyl ghrelin on insulin secretion independently of GHSR^{10,11}, although the desacyl ghrelin receptor has not been identified. Further investigation is needed to understand the actions of acyl ghrelin and desacyl ghrelin on pancreatic islet cell function.

Previous studies have shown that low total ghrelin level is associ-

Table 3. Correlations between HOMA-IR and desacyl ghrelin, acyl ghrelin, and GOAT levels

	HOMA-IR	Desacyl ghrelin	Acyl ghrelin	Total ghrelin	GOAT
HOMA-IR	1	-0.271*	-0.017	-0.271*	0.041
Desacyl ghrelin			0.050	0.981 [†]	0.023
Acyl ghrelin				0.213	-0.083
Total ghrelin					-0.011
GOAT					1

P values were determined using the using Spearman correlation coefficients.

* $P < 0.05$; [†] $P < 0.01$.

HOMA-IR, homeostatic model assessment-insulin resistance index; GOAT, ghrelin O-acyltransferase.

ated with a high risk of MS⁷, and that circulating acyl ghrelin level is elevated in obesity and MS, whereas desacyl ghrelin level is diminished in these conditions.¹² However, we found no significant difference in acyl ghrelin levels between the MS and non-MS groups. We believe that the failure to identify this difference was due the small intergroup difference in mean BMI (MS group, 26.4 kg/m²; non-MS group, 23.9 kg/m²). However, the present study focused on insulin resistance rather than obesity, and previous studies in mice and humans have shown that insulin-resistant subjects have lower levels of total ghrelin and desacyl ghrelin, which is consistent with our findings.^{24,25} These results indicate that insulin resistance reflects a relative deficiency in desacyl ghrelin, and that desacyl ghrelin plays an active role in the regulation of glucose and insulin metabolism.

GOAT, the enzyme responsible for ghrelin acylation, was discovered recently; therefore, few studies have evaluated the correlation between GOAT and ghrelin hormones. In a prospective intervention study in rats, GOAT and ghrelin mRNA levels in the stomach mucosa were found to be relatively stable during periods of decreased food intake.¹⁵ However, in situations of chronic food restriction, GOAT and ghrelin mRNA levels were increased.¹⁵ Moreover, a recent study evaluated the regulation of GOAT protein level under different metabolic conditions in the plasma of rats and mice by semi-quantitative Western blotting and found that an increase in circulating GOAT level increased ghrelin acylation in response to fasting.¹⁶ More recently, a cross-sectional study conducted in 45 human subjects found that plasma GOAT protein level was positively correlated with BMI and negatively correlated with plasma total ghrelin.¹⁷ Moreover, a semi-quantitative study indicated that plasma GOAT protein level was lower in anorexic subjects than in normal-weight controls (BMI, 18.5-25.0 kg/m²) and higher in subjects with obesity (BMI > 50 kg/m²).¹⁷

Because GOAT knockout mice that fasted for 16 hours had increased insulin secretion and improved glucose tolerance, GOAT is believed to play a major role in the regulation of insulin secretion and glucose metabolism. Accordingly, we expected that MS patients would have higher circulating GOAT level, and that GOAT level would correlate with HOMA-IR. However, our results showed no significant intergroup difference and no such correlation. We believe that this apparent discrepancy was caused by the

inclusion of extreme metabolic groups in the previous study, including a group with anorexia nervosa and a group with morbid obesity (BMI > 50 kg/m²). In addition, plasma GOAT level might not reflect GOAT mRNA level in the gastric mucosa. Herein, we did not assess GOAT mRNA level in the gastric mucosa and therefore could not assess the changes in GOAT mRNA expression.

This study has several limitations, including a relatively small-size cohort and a study population limited to relatively healthy middle-aged men, the latter of which was included to circumvent various unmeasured confounding factors. In addition, the BMI range of our subjects was restricted, and patients with anorexia and morbid obesity were not included. Further studies involving subjects with a wider range of BMIs and severe metabolic disturbances are required. Furthermore, the study had a cross-sectional design; therefore, temporal relations between ghrelin profiles and insulin resistance and MS could not be determined. Nonetheless, we believe that our findings are meaningful because this study is the first to evaluate the relations between circulating protein levels of desacyl ghrelin, acyl ghrelin, and GOAT.

In conclusion, desacyl ghrelin and total ghrelin levels in middle-aged Korean men with MS were lower than those in subjects without MS, and HOMA-IR was significantly and negatively correlated with desacyl ghrelin and total ghrelin, but not with acyl ghrelin or GOAT.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by the Research Institute for Convergence of Biomedical Science and Technology (30-2013-001), Pusan National University Yangsan Hospital.

REFERENCES

1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999;402:656-60.

2. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000;141:4255-61.
3. Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001;50:707-9.
4. Suh YS, Kim DH, Lee IK. Circulating ghrelin levels and fat distribution in obese patients. *Korean J Obes* 2002;11:356-61.
5. Kim JY, Shin HW, Jeong IK, Cho SW, Min SJ, Lee SJ, et al. The relationship of adiponectin, leptin and ghrelin to insulin resistance and cardiovascular risk factors in human obesity. *Korean J Med* 2005;69:631-41.
6. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, et al. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 2002;87:240-4.
7. Ukkola O, Pöykkö SM, Antero Kesäniemi Y. Low plasma ghrelin concentration is an indicator of the metabolic syndrome. *Ann Med* 2006;38:274-9.
8. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 2008;132:387-96.
9. Gutierrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, et al. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci U S A* 2008;105:6320-5.
10. Delhanty PJ, van der Lely AJ. Ghrelin and glucose homeostasis. *Peptides* 2011;32:2309-18.
11. Delhanty PJ, Neggers SJ, van der Lely AJ. Ghrelin: the differences between acyl- and des-acyl ghrelin. *Eur J Endocrinol* 2012;167:601-8.
12. Rodríguez A, Gómez-Ambrosi J, Catalán V, Becerril S, Sáinz N, Gil MJ, et al. Association of plasma acylated ghrelin with blood pressure and left ventricular mass in patients with metabolic syndrome. *J Hypertens* 2010;28:560-7.
13. Barazzoni R, Zanetti M, Ferreira C, Vinci P, Pirulli A, Mucci M, et al. Relationships between desacylated and acylated ghrelin and insulin sensitivity in the metabolic syndrome. *J Clin Endocrinol Metab* 2007;92:3935-40.
14. Zhao TJ, Liang G, Li RL, Xie X, Sleeman MW, Murphy AJ, et al. Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proc Natl Acad Sci U S A* 2010;107:7467-72.
15. González CR, Vázquez MJ, López M, Diéguez C. Influence of chronic undernutrition and leptin on GOAT mRNA levels in rat stomach mucosa. *J Mol Endocrinol* 2008;41:415-21.
16. Stengel A, Goebel M, Wang L, Taché Y, Sachs G, Lambrecht NW. Differential distribution of ghrelin-O-acyltransferase (GOAT) immunoreactive cells in the mouse and rat gastric oxyntic mucosa. *Biochem Biophys Res Commun* 2010;392:67-71.
17. Goebel-Stengel M, Hofmann T, Elbelt U, Teuffel P, Ahnis A, Kobelt P, et al. The ghrelin activating enzyme ghrelin-O-acyltransferase (GOAT) is present in human plasma and expressed dependent on body mass index. *Peptides* 2013;43:13-9.
18. Lee BK, Kim Y. Iron deficiency is associated with increased levels of blood cadmium in the Korean general population: analysis of 2008-2009 Korean National Health and Nutrition Examination Survey data. *Environ Res* 2012;112:155-63.
19. Lee S, Park HK, Son SP, Lee CW, Kim IJ, Kim HJ. Effects of oral magnesium supplementation on insulin sensitivity and blood pressure in normo-magnesium nondiabetic overweight Korean adults. *Nutr Metab Cardiovasc Dis* 2009;19:781-8.
20. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003;35:1381-95.
21. Lee S, Choi S, Kim HJ, Chung YS, Lee KW, Lee HC, et al. Cutoff values of surrogate measures of insulin resistance for metabolic syndrome in Korean non-diabetic adults. *J Korean Med Sci* 2006;21:695-700.
22. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735-52.
23. Lee SY, Park HS, Kim DJ, Han JH, Kim SM, Cho GJ, et al. Appropriate waist circumference cutoff points for central obe-

- sity in Korean adults. *Diabetes Res Clin Pract* 2007;75:72-80.
24. Pacifico L, Poggiogalle E, Costantino F, Anania C, Ferraro F, Chiarelli F, et al. Acylated and nonacylated ghrelin levels and their associations with insulin resistance in obese and normal weight children with metabolic syndrome. *Eur J Endocrinol* 2009;161:861-70.
25. Nonogaki K, Nozue K, Oka Y. Hyperphagia alters expression of hypothalamic 5-HT_{2C} and 5-HT_{1B} receptor genes and plasma des-acyl ghrelin levels in Ay mice. *Endocrinology* 2006; 147:5893-900.