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Relationships between fasting plasma ghrelin levels and metabolic parameters in children and adolescents☆

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Abstract

Recent findings suggest that ghrelin may have a beneficial effect on vasculature. In the present study, we examined the associations between plasma ghrelin concentration and metabolic parameters in children and adolescents. We measured fasting plasma ghrelin concentrations in 50 Korean children and adolescents (28 boys and 22 girls, mean ± SD age 12.6 ± 2.7 years, body mass index 22.7 ± 5.1 kg/m^2), and analyzed the associations between fasting plasma ghrelin level and anthropometric measurements, metabolic parameters, leptin concentration, and fasting insulin level. We found that fasting plasma ghrelin concentration was negatively correlated with height, weight, body mass index, percent body fat, waist circumference, and hip circumference in both boys and girls. Fasting plasma ghrelin levels were significantly negatively correlated with triglycerides and fasting insulin levels and positively correlated with high-density lipoprotein cholesterol in boys, but not in girls. Our results thus demonstrate that higher plasma ghrelin levels have beneficial effects on metabolic parameters in boys and that the relationships between fasting plasma ghrelin levels and metabolic parameters differed according to sex.

1. Introduction

Ghrelin is a potent stimulator of growth hormone release [1,2] that has been implicated in the control of food intake and energy homeostasis in human beings [3] and rodents [4,5]. Ghrelin causes weight gain by increasing food intake and reducing fat use [6,7]. Previous studies have found that plasma ghrelin levels are lower in obese subjects than in control subjects [4,8,9]. Plasma ghrelin concentration is negatively associated with percent body fat [4,10] and fasting plasma insulin [4,10-14] and leptin concentrations [4,15]. It has been hypothesized that plasma ghrelin concentrations are lowered in obese subjects as an adaptive response to decrease weight or adiposity [16].

More recently, it has been proposed that ghrelin has a beneficial effect on vasculature based on results showing that intravenous ghrelin injections decrease mean arterial blood pressure and cardiac afterload [17]. Ghrelin has also been shown to produce endothelium-independent vasodilation via direct action on smooth muscle [18]. Thus, the low plasma ghrelin concentration found in obese subjects may have a detrimental effect on vasculature and promote the development of cardiovascular disease. In addition, a recent study found that low plasma ghrelin is associated with hypertension and the prevalence of type 2 diabetes in adults [19].

Previous studies have not, however, fully investigated the relationships between plasma ghrelin concentration and metabolic parameters in children and adolescents. In addition, there is insufficient data to determine whether there are sex differences in those relationships. Therefore, the present study was undertaken to examine the association between fasting plasma ghrelin concentration and metabolic parameters according to sex in Korean children and adolescents.

2. Materials and methods

Fifty Korean children and adolescents (28 boys, 22 girls) were recruited via advertisements on the bulletin board of Asan Medical Center. Informed consent was obtained from

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Table 1
Basic characteristics of the study subjects

		Boys (n = 28)	Girls (n = 22)
		n (%)	n (%)
Age (y)	<10	4 (14.3)	3 (13.6)
	11-14	19 (67.9)	13 (59.1)
	≥15	5 (17.9)	6 (27.3)
BMI (kg/m^2)	<20	8 (28.6)	7 (31.8)
	20-24.9	10 (35.7)	10 (45.5)
	25-29.9	7 (25.0)	4 (18.2)
	<30	3 (10.7)	1 (4.6)
		Mean ± SD	Mean ± SD
Age (y)		12.4 ± 2.5	12.8 ± 3.0
BMI (kg/m^2)		23.0 ± 5.3	22.2 ± 4.9
Waist circumference (cm)		79.2 ± 13.5	73.5 ± 11.6
		Median (range)	Median (range)
Leptin (ng/mL)*		4.76 (0.83-18.48)	9.09 (2.82-19.31)
Insulin (μU/mL)		5.52 (1.2-15.2)	5.32 (1.2-16.96)
Ghrelin (fmol/mL)*		133.2 (69.4-394.5)	189.6 (113.7-389.4)

* $P < .05$ by Wilcoxon rank sum test.

the parents all subjects. Exclusion criteria included obesity secondary to endocrine disease, secondary hypertension, type 1 diabetes mellitus, familial lipid disorders, and genetic disorders. Ages ranged from 7 to 19 (mean ± SD age 12.6 ± 2.7) years. This study was approved by the institutional review board of Asan Medical Center.

Standing height and weight were measured, and body mass index (BMI) was calculated. The BMI ranged from 14.7 to 37.4 (mean ± SD BMI 22.7 ± 5.1) kg/m^2. Body fat was measured using bioimpedance analysis (Inbody 3.0, Biospace, Korea) [20]. Waist and hip circumferences were measured with a tape measure. To minimize variations in anthropometric measurements, all measurements were obtained by the same experienced staff member. Waist circumference was measured at a point midway between the lower border of the rib cage and the iliac crest at the end of normal expiration; hip circumference was measured at the widest part of the hip, and waist/hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference.

Cardiovascular risk factors, including systolic blood pressure, diastolic blood pressure, fasting plasma glucose, total cholesterol, low-density lipoprotein cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol, as well as other metabolic variables, including plasma leptin, fasting insulin, and plasma ghrelin concentrations, were measured in all subjects. Blood pressure was measured with a mercury sphygmomanometer with the individual in a sitting position; participants were required to rest for 10 minutes before blood pressure measurement. Cuff size was selected according to the arm circumference of the participant. The first appearance of sound (phase 1 Korotkoff sound) was used to define systolic blood pressure, and the disappearance of sound (phase 5 Korotkoff sound) was used to define diastolic blood pressure. Two readings were recorded for systolic and diastolic blood pressure, and the average of each was used.

Blood samples were obtained from each subject after a 12-hour overnight fast. Fasting plasma glucose was measured using the glucose oxidase method, and total cholesterol and triglyceride levels were measured using enzymatic procedures with an autoanalyzer (Hitachi-747, Japan). The HDL cholesterol fraction was measured enzymatically after precipitation of apolipoprotein B–containing lipoproteins with $MnCl_2$. Low-density lipoprotein cholesterol was calculated using the Friedewald equation if the serum triglyceride levels were below 400 mg/dL [21]. Fasting plasma leptin concentrations were determined using a highly sensitive commercial human leptin-specific double-antibody radioimmunoassay kit (Linco Research, St Charles, Mo). Fasting insulin was measured by radioimmunoassay (Dianabott, Japan). Total plasma ghrelin concentration was measured by radioimmunoassay (Phoenix Pharmaceuticals, Belmont, Calif). The interassay and intraassay coefficients of variation, respectively, were 3.4% and 2.5% for leptin, 7.0% and 7.8% for insulin, and 13.6% and 5.3% for ghrelin.

All anthropometric measurement data for the study subjects are presented as mean ± SD. The values for

Table 2
Correlations between anthropometric measurements and plasma concentrations of leptin, insulin, and ghrelin after adjustment for age

	Leptin[a]		Insulin[a]		Ghrelin[a]	
	Boys	Girls	Boys	Girls	Boys	Girls
Age[b]	−0.11	0.30	0.26	0.05	−0.57**	−0.03
Height	0.21	0.15	0.16	0.05	−0.38*	−0.53*
Weight	0.81**	0.71**	0.50*	0.57*	−0.71**	−0.71**
BMI	0.89**	0.74**	0.58**	0.63**	−0.74**	−0.59**
Percent body fat	0.91**	0.89**	0.59**	0.48*	−0.74**	−0.48*
Waist circumference	0.90**	0.81**	0.54**	0.61**	−0.76**	−0.51*
Hip circumference	0.82**	0.72**	0.55**	0.50*	−0.69**	−0.73**
WHR	0.79**	0.65**	0.37*	0.50*	−0.66**	−0.15

[a] Log-transformed values were used.
[b] Correlation coefficient not adjusted.
* $P < .05$, correlation coefficient.
** $P < .005$, correlation coefficient.

Table 3
Correlations between metabolic parameters and plasma concentrations of leptin, insulin, and ghrelin after adjustment for age

	Leptin[a]		Insulin[a]		Ghrelin[a]	
	Boys	Girls	Boys	Girls	Boys	Girls
Systolic blood pressure	0.27	0.30	0.36	0.28	−0.34	−0.20
Diastolic blood pressure	0.30	−0.06	0.37	−0.09	−0.22	−0.09
Fasting plasma glucose	−0.22	−0.37	−0.11	−0.27	0.13	0.27
Total cholesterol	0.24	0.40	0.23	0.05	−0.20	0.02
Triglycerides[a]	0.53**	0.29	0.43*	0.74**	−0.60**	−0.38
HDL cholesterol	−0.37	−0.15	−0.27	−0.39	0.66**	0.24
Ghrelin[a]	−0.71**	−0.46*	−0.59**	−0.24		
Insulin[a]	0.71**	0.44*				

[a] Log-transformed values were used.
* $P < .05$, correlation coefficient.
** $P < .005$, correlation coefficient.

triglycerides, leptin, insulin, and ghrelin were not normally distributed independently for age or BMI. They were log-transformed to generate normally distributed data before statistical analysis, and their concentrations are presented as medians. Anthropometric measurements of boys and girls were compared using Student t test, and sex differences in leptin, insulin, and ghrelin concentrations were compared using Wilcoxon rank sum test. Partial correlation coefficients were used to estimate the correlations between the concentrations of leptin, insulin, or ghrelin and anthropometric measurements and metabolic variables after adjustment for age in boys and girls. All analyses were 2-tailed, and a P value $<.05$ was considered statistically significant. All statistical analyses were performed using SAS 6.12 for Windows (SAS institute Inc, Cary, NC).

3. Results

The basic characteristics of the study subjects are listed in Table 1. The distribution of age and BMI was not different between boys and girls. Plasma leptin levels and fasting plasma ghrelin levels were significantly higher in girls than in boys, whereas fasting insulin levels were similar in boys and girls.

The correlations between age, anthropometric measurements, and fasting plasma concentrations of leptin, insulin, and ghrelin are shown in Table 2. In both sexes, circulating leptin and insulin levels did not correlate with age or height. Fasting leptin and insulin levels were positively correlated with weight, BMI, percent body fat, waist circumference, hip circumference, and WHR. In contrast, fasting plasma ghrelin levels were negatively correlated with age in boys and with height in both sexes. Fasting plasma ghrelin levels were negatively correlated with weight, BMI, percent body fat, waist circumference, and hip circumference in both sexes.

Table 3 lists the correlations between fasting concentrations of leptin, insulin, ghrelin, and metabolic parameters after adjustment for age. Leptin concentration was positively correlated with triglycerides concentration only in boys. Fasting insulin levels were positively correlated with triglycerides in both boys and girls. Fasting plasma ghrelin levels were negatively correlated with fasting insulin and triglycerides and positively correlated with HDL cholesterol only in boys. No significant correlations were observed between fasting plasma ghrelin concentration and metabolic parameters in girls.

Figs. 1 and 2 show scatter plots with regression lines of the relationship between the fasting plasma ghrelin concentration and several metabolic parameters with untransformed data. In both sexes, a negative correlation is observed between the fasting plasma ghrelin and leptin concentrations. However, fasting plasma ghrelin showed significant negative correlations with fasting insulin and triglycerides

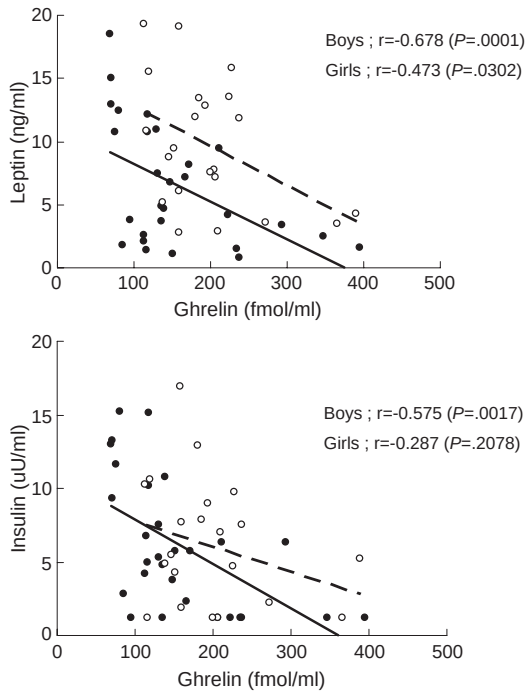

Fig. 1. Relationships of fasting plasma ghrelin concentrations with plasma leptin and fasting insulin levels in boys and girls. Closed circles and solid line, boys; open circles and dashed line, girls.

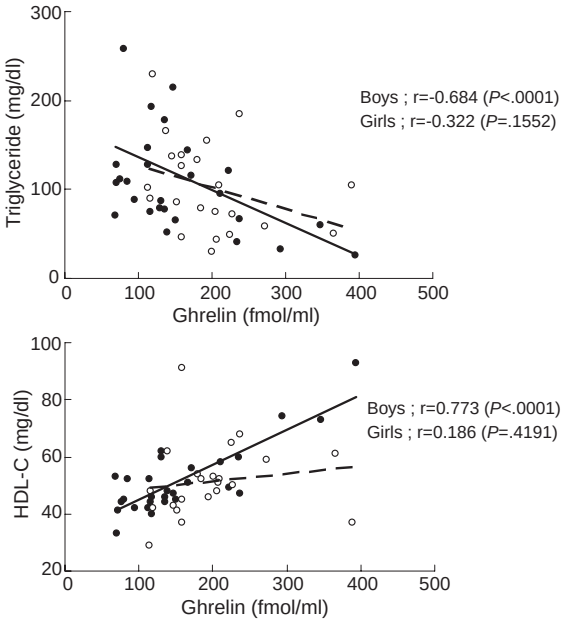

Fig. 2. Relationships of fasting plasma ghrelin concentrations with triglycerides and HDL cholesterol in boys and girls. Closed circles and solid line, boys; open circles and dashed line, girls.

and a positive correlation with HDL cholesterol in boys but not in girls.

4. Discussion

In this study, fasting plasma ghrelin levels were significantly negatively correlated with triglycerides and positively correlated with HDL cholesterol in boys but not in girls. The mechanism underlying the observed associations between fasting plasma ghrelin concentration and metabolic parameters is currently unknown. However, these findings are in accord with the results of a recent study conducted in middle-aged adults, which found that triglycerides decreased and HDL cholesterol increased significantly with increasing plasma ghrelin concentration [19].

We observed a negative association between plasma ghrelin concentration and plasma leptin in both sexes. By contrast, a significant negative correlation between plasma ghrelin concentration and fasting insulin was observed in boys but not in girls. A previous study of obese children and adolescents, which included boys and girls in a single subject group, disclosed a negative correlation between fasting plasma ghrelin concentration and insulin resistance but found no significant correlation between fasting plasma ghrelin concentration and leptin level [14]. Thus, the relationships between plasma ghrelin concentration and plasma leptin, fasting insulin, and insulin resistance in children and adolescents require further investigation and may depend on sex, age, pubertal stage, and severity of obesity.

Among boys, the relationships between fasting plasma ghrelin levels and triglycerides and HDL cholesterol levels were more prominent than the relationship between plasma leptin concentration and those metabolic parameters. A previous report has suggested that exogenous administration of ghrelin decreases mean arterial blood pressure and improves forearm blood flow in healthy individuals [17]. Ghrelin has also been shown to possess direct vasorelaxant properties in endothelium-denuded human internal mammary arteries and to potently reverse endothelin 1–induced contractions [22]. Moreover, a recent study suggested that the native receptor for [^{125}I-His(9)]-ghrelin is widely distributed in the human cardiovascular system [23]. Furthermore, a link has been established between the density of ghrelin receptors in an individual and the likelihood that individual will develop atherosclerosis, and thus these receptors may represent a novel therapeutic target in the treatment of cardiovascular disease [23]. When it is considered that the atherosclerotic process begins at a young age [24,25], the relationships between plasma ghrelin concentration and metabolic parameters may influence the development of atherosclerosis even in adolescents.

One previous study reported a decrease in plasma ghrelin levels with age [13], whereas others have reported similar ghrelin levels in healthy lean children and adults, with no dependence on sex or pubertal status [11]. In our study, there was a significant correlation between plasma ghrelin levels and age only in boys, not in girls. These results are in accordance with a previous report that the decrease in ghrelin with advancing age or pubertal stage was more marked in boys than in girls [26]. Further studies are needed to fully elucidate the sex difference observed in the effects of plasma ghrelin.

In contrast to our findings in boys, we observed no correlations between plasma ghrelin concentration and metabolic parameters including fasting insulin levels in girls. This sex difference is difficult to explain. One possible explanation would be the matter of sex differences in levels of sex steroids. Further differences in sex steroids in female children and postmenarchal adolescents may have altered the findings.

The present study has several limitations. The cross-sectional design of the study prevented us from establishing casual relationships; hence, the results presented here reflect only correlations between fasting plasma ghrelin and metabolic parameters. In addition, we could not measure acylated ghrelin and des-acylated ghrelin separately. Current evidence suggests that the biologic activity of ghrelin is dependent on its acylation in serine [27,28]. Several studies have reported that des-acylated ghrelin is metabolically active, as it shares with acylated ghrelin some nonendocrine actions such as cardiovascular effects, modulation of cell proliferation, and even some influence on adipogenesis [27,29,30]. Further studies will be needed to unravel the relationship between the 2 major molecular forms of ghrelin (des-acylated and acylated forms) and metabolic parameters. An additional limitation of the present work is that single morning fasting measurements of metabolic parameters including plasma ghrelin levels as

well as other parameters might not represent the full nature of the reciprocal relationships because of the multifactorial dynamic nature of those parameters.

In conclusion, we found that fasting plasma ghrelin levels were significantly negatively correlated with triglycerides and fasting insulin levels and positively correlated with HDL cholesterol in boys. These findings suggest that high plasma ghrelin levels may have beneficial effects on the metabolic parameters. In addition, our results indicated that the relationships between fasting plasma ghrelin concentration and metabolic parameters were sex-dependent in children and adolescents. The mechanism underlying this sex difference should be examined in a larger cohort.

References

[1] Arvat E, Di Vito L, Broglio F, et al. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)–receptor ligand, strongly stimulates GH secretion in humans. J Endocrinol Invest 2000;23:493-5.

[2] Peino R, Baldelli R, Rodriguez-Garcia J, et al. Ghrelin-induced growth hormone secretion in humans. Eur J Endocrinol 2000;143:R11-4.

[3] Wren AM, Small CJ, Abbott CR, et al. Ghrelin causes hyperphagia and obesity in rats. Diabetes 2001;50:2540-7.

[4] Tschop M, Weyer C, Tataranni PA, et al. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001;50:707-9.

[5] Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407:908-13.

[6] Nakazato M, Murakami N, Date Y, et al. A role for ghrelin in the central regulation of feeding. Nature 2001;409:194-8.

[7] Wren AM, Small CJ, Ward HL, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 2000;141:4325-8.

[8] Rosicka M, Krsek M, Matoulek M, et al. Serum ghrelin levels in obese patients: the relationship to serum leptin levels and soluble leptin receptors levels. Physiol Res 2003;52:61-6.

[9] Shiiya T, Nakazato M, Mizuta 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.

[10] Bunt JC, Salbe AD, Tschop MH, et al. Cross-sectional and prospective relationships of fasting plasma ghrelin concentrations with anthropometric measures in Pima Indian children. J Clin Endocrinol Metab 2003;88:3756-61.

[11] Bellone S, Rapa A, Vivenza D, et al. Circulating ghrelin levels as function of gender, pubertal status and adiposity in childhood. J Endocrinol Invest 2002;25:RC13-5.

[12] Broglio F, Arvat E, Benso A, et al. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. J Clin Endocrinol Metab 2001;86:5083-6.

[13] Haqq AM, Farooqi IS, O'Rahilly S, et al. Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. J Clin Endocrinol Metab 2003;88:174-8.

[14] Ikezaki A, Hosoda H, Ito K, et al. Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. Diabetes 2002;51:3408-11.

[15] Shintani M, Ogawa Y, Ebihara K, et al. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 2001;50:227-32.

[16] Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M. Minireview: ghrelin and the regulation of energy balance—a hypothalamic perspective. Endocrinology 2001;142:4163-9.

[17] Nagaya N, Kojima M, Uematsu M, et al. Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. Am J Physiol Regul Integr Comp Physiol 2001;280:R1483-7.

[18] Katugampola SD, Maguire JJ, Kuc RE, Wiley KE, Davenport AP. Discovery of recently adopted orphan receptors for apelin, urotensin II, and ghrelin identified using novel radioligands and functional role in the human cardiovascular system. Can J Physiol Pharmacol 2002;80:369-74.

[19] Poykko SM, Kellokoski E, Horkko S, et al. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes 2003;52:2546-53.

[20] Cha K, Chertow GM, Gonzalez J, Lazarus JM, Wilmore DW. Multifrequency bioelectrical impedance estimates the distribution of body water. J Appl Physiol 1995;79:1316-9.

[21] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

[22] Wiley KE, Davenport AP. Comparison of vasodilators in human internal mammary artery: ghrelin is a potent physiological antagonist of endothelin-1. Br J Pharmacol 2002;136:1146-52.

[23] Katugampola SD, Pallikaros Z, Davenport AP. [^{125}I-His(9)]-ghrelin, a novel radioligand for localizing GHS orphan receptors in human and rat tissue: up-regulation of receptors with atherosclerosis. Br J Pharmacol 2001;134:143-9.

[24] Berenson GS, Srinivasan SR, Bao W, et al. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998;338:1650-6.

[25] Mahoney LT, Burns TL, Stanford W, et al. Coronary risk factors measured in childhood and young adult life are associated with coronary artery calcification in young adults: the Muscatine Study. J Am Coll Cardiol 1996;27:277-84.

[26] Whatmore AJ, Hall CM, Jones J, Westwood M, Clayton PE. Ghrelin concentrations in healthy children and adolescents. Clin Endocrinol (Oxf) 2003;59:649-54.

[27] Muccioli G, Tschop M, Papotti M, et al. Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. Eur J Pharmacol 2002;440:235-54.

[28] Kojima M, Hosoda H, Matsuo H, Kangawa K. Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol Metab 2001;12:118-22.

[29] Cassoni P, Papotti M, Ghe C, et al. Identification, characterization, and biological activity of specific receptors for natural (ghrelin) and synthetic growth hormone secretagogues and analogs in human breast carcinomas and cell lines. J Clin Endocrinol Metab 2001;86:1738-45.

[30] Baldanzi G, Filigheddu N, Cutrupi S, et al. Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. J Cell Biol 2002;159:1029-37.