Alteration of Plasma Ghrelin Levels Associated With the Blood Pressure in Pregnancy

Yasuo Makino, Hiroshi Hosoda, Kazuhiko Shibata, Ikuko Makino, Masayasu Kojima, Kenji Kangawa, Tatsuhiko Kawarabayashi

Abstract—Ghrelin, an endogenous ligand for the growth hormone secretagogues, was originally isolated from rat stomach. It stimulates the release of growth hormone from primary pituitary cell cultures. We investigated the plasma concentration of ghrelin peptide in 16 nonpregnant women, 18 normal pregnant women, 20 patients with pregnancy-induced hypertension, and 10 postpartum women. The plasma concentration of ghrelin in nonpregnant women was 239.5±16.9 fmol/mL. The plasma concentration of ghrelin in normal pregnant women at the third trimester was 127.1±6.6 fmol/mL. There was negative correlation between plasma ghrelin concentration and systemic blood pressure in normal pregnant women (systolic: \( r = -0.564, P < 0.05 \); diastolic: \( r = -0.610, P < 0.01 \)). Pregnant women with pregnancy-induced hypertension (177.9±14.6 fmol/mL, \( P < 0.05 \)) also had significantly higher levels of ghrelin compared with those of normal pregnant women. In addition, there was a significant correlation between plasma ghrelin levels and systemic blood pressure (systolic: \( r = -0.482, P < 0.05 \); diastolic: \( r = -0.466, P < 0.05 \)). These results suggest for the first time that ghrelin might have some role in cardiovascular control during normal pregnancy and in pathophysiological conditions in pregnancy, such as pregnancy-induced hypertension. (Hypertension. 2002;39:781-784.)

Key Words: blood pressure ■ growth substances ■ preeclampsia ■ pregnancy

G

hrelin, an endogenous ligand for the growth hormone (GH) secretagogues, was identified from rat stomach and subsequently cloned in rats and humans. \(^1\) Ghrelin stimulates GH release in rat pituitary cell cultures, and intravenous ghrelin administration resulted in an increase in serum GH in rat and human. \(^1\)\(^2\) Rat and human ghrelin differ by only 2 amino acids, suggesting an important physiological role. In addition, ghrelin immunoreactivity has been located in the hypothalamic arcuate nucleus. \(^1\) On the other hand, ghrelin receptors are expressed in various tissues such as the brain \(^3\)\(^-\)5 and blood vessels. \(^6\)

It has been recently demonstrated that intracerebroventricular injection of ghrelin strongly stimulates feeding in rats and increased body weight gain. \(^7\)\(^-\)\(^8\) These findings suggest that ghrelin may function not only as GH secretagogues but also as a regulator of energy balance.

It has been recently reported that ghrelin is present in other tissue, such as human placenta, both at the first trimester and after delivery, \(^9\) which suggests that this peptide may play an important role in the reproductive physiology. However, there have been no reports on the amount of ghrelin peptide in human fetal-maternal circulation in pregnancy.

Pregnancy-induced hypertension (PIH) is a common cause of maternal or fetal mortality. Shallow endovascular cytotrophoblast invasion in the spiral arteries and endothelial cell dysfunction are 2 key features in the pathogenesis of PIH; however, the etiology of preeclampsia is still unknown. \(^10\)

Therefore, in the present study, to elucidate whether ghrelin has any implications as a physiological or pathophysiological substance in pregnancy, we examined the plasma concentration of ghrelin in maternal venous blood and umbilical artery blood in pregnant women.

Methods

Subjects

The study population consisted of 16 nonpregnant women, 18 normal pregnant women, 20 patients with PIH, and 10 postpartum women, who were admitted to the Obstetrical Clinic of the School of Medicine of Fukuoka University between March 1, 1998, and April 30, 2001. The normotensive group had no abnormal alterations in blood pressure, proteinuria, or edema and did not undergo any drug administration during pregnancy. The diagnosis of PIH was made on the basis of systolic blood pressure of \( \geq 140 \) mm Hg or diastolic pressure of \( \geq 90 \) mm Hg, with or without proteinuria and edema. Only singleton pregnancies delivered from 28 to 41 weeks of gestation with no evidence of fetal anomalies were included in this study. There were no remarkable past histories except for previous cesarean section. This protocol was approved by the Human Investigational Review Board for the School of Medicine of Fukuoka University. From the Department of Obstetrics and Gynecology (Y.M., I.M., T.K.) and Research Laboratory of Biodynamics (K.S.), School of Medicine, Fukuoka University, Fukuoka, Japan; and National Cardiovascular Center Research Institute (H.H., M.K., K.K.), Osaka, Japan.

Correspondence to Yasuo Makino, MD, Department of Obstetrics and Gynecology, School of Medicine, Fukuoka University, 45-1, 7-chome Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. E-mail makino-y@fukuoka-u.ac.jp

© 2002 American Heart Association, Inc.

Hypertension is available at http://www.hypertensionaha.org
### Qualification of Immunoreactive Ghrelin in Plasma

Blood samples were taken from the antecubital vein in the morning between 7:00 AM and 8:00 AM after an overnight fast, because plasma ghrelin level has been shown to be altered by food intake. Also, the RIA incubation mixture consisted of 100 μL of standard ghrelin or unknown sample, normal rabbit serum, and 200 μL of antiserum at a dilution of 1:10,000. After 12-hour incubation at 4°C, the absorbed materials were eluted with 3 mL saline and then loaded into a Sep-Pak C18 cartridge. After the column was washed with 5 mL each of saline and 5% acetonitrile containing 0.1% trifluoroacetic acid (TFA), and saline, Plasma (1000 μL) was diluted with 1000 μL saline and then loaded into a Sep-Pak C18 cartridge. The plasma concentration of ghrelin was 239.5 ± 16.9 and 221.5 ± 16.0 fmol/mL in the nonpregnant and postpartum women, respectively, and there was no significant difference between the 2 groups (Figure 1). On the other hand, the

### Statistical Analysis

Data for ghrelin peptide levels are expressed as the mean ± SEM. Data of clinical characteristics are expressed as the mean ± SD. The data were compared using Newman-Keul’s multiple comparison test (maternal blood) or Student’s t test (clinical data and umbilical cord blood). Correlations between peptide level and blood pressure was examined by linear regression analysis. A value of P < 0.05 was considered significant.

### Results

#### Subjects

There were no significant differences in maternal age and gestational age at sampling of the umbilical artery blood (Table). The systolic and diastolic blood pressures were significantly higher in the patients with PIH than in nonpregnant and normal pregnant women (P < 0.01) (Table).

#### Immunoreactive Ghrelin

The plasma concentration of ghrelin was 239.5 ± 16.9 and 221.5 ± 16.0 fmol/mL in the nonpregnant and postpartum women, respectively, and there was no significant difference between the 2 groups (Figure 1). On the other hand, the

---

**Figure 1.** Concentration of plasma ghrelin in maternal (A) and umbilical cord (B) blood. Values are mean ± SEM. *P < 0.05 vs normal pregnant women. Non-prev indicates non-pregnant women; pregnant, normal pregnant women.
plasma concentration of ghrelin in normal pregnant women at
the third trimester was 127.1 ± 5.6 fmol/mL. Moreover, the
plasma concentration of ghrelin was 177.9 ± 14.6 fmol/mL in
the pregnant women with PIH, and the levels were signifi-
cantly higher than those in the normal pregnant women
\( (P < 0.05) \). There was no significant difference between 8
normal pregnant women and 12 patients with PIH in regard to
concentration in the umbilical artery blood (183.9 ± 22.0 and
213.2 ± 19.1 fmol/mL, respectively). However, in the normal
pregnant women, the plasma ghrelin concentrations in um-
bilical artery blood were significantly higher than those in
maternal blood \( (P < 0.05) \).

The Relationship Between Plasma Ghrelin Level
and Systemic Blood Pressure

Figure 2 shows the relationship between plasma ghrelin
concentration and systemic blood pressure in 3 groups. In
nonpregnant women, there was no correlation between
plasma ghrelin concentration and systemic blood pressure.
However, in normal pregnant women (Figure 2B), plasma
ghrelin concentrations were negatively correlated with the
systemic blood pressure women (systolic: \( r = 0.564, \ P < 0.05 \);
diastolic: \( r = -0.610, \ P < 0.01 \)). Furthermore, plasma
ghrelin concentrations were also negatively correlated
with the systolic and diastolic blood pressure in the patients
with PIH pressure (systolic: \( r = -0.482, \ P < 0.05 \); diastolic:
\( r = -0.466, \ P < 0.05 \)).

Discussion

Ghrelin, an endogenous ligand for the GH secretagogues
receptor, was originally isolated from rat stomach. Ghrelin
stimulates GH release in rat pituitary cell cultures, and
intravenous ghrelin administration resulted in an increase in
serum GH in rat and human.\(^1\)\(^2\) It is well documented that GH
plays an important role in metabolic changes occurring in late
pregnancy in the rat.\(^14\) Also, it has been demonstrated that
plasma GH levels increased during pregnancy in human.\(^15\)
However, in the present study, the maternal plasma concen-
tration of ghrelin was markedly decreased in normal pregnant
women in the third trimester. The present results thus suggest
that plasma ghrelin levels do not play an important role in
maintaining circulating GH levels during pregnancy.

We also demonstrated that plasma ghrelin concentrations
were negatively correlated with systemic blood pressure in
normal pregnant women but not in nonpregnant women.

During pregnancy, a number of physiological changes
occur in the maternal circulation to accommodate the grow-
ing fetus. These changes usually include an increase in
cardiac output and a decrease in arterial blood pressure and
total peripheral resistance. However, the mechanisms of this
physiological adaptation remain to be elucidated. On the
other hand, the cardiovascular effects of ghrelin are still
unknown at present. However, recent reports have demon-
strated that ghrelin peptide and its specific binding sites are
detected in cardiovascular systems, including the heart, kid-
ney, and blood vessels.\(^6\)\(^13\) Therefore, it is interesting to
speculate that ghrelin may have some role in the cardiovas-
cular system. In fact, very recently, Nagaya et al reported that
ghrelin decreased mean arterial pressure but did not increase
heart rate in healthy volunteers.\(^13\) They have also mentioned
that ghrelin induced hypotension in hypophysectomized rats
and speculated that ghrelin has a direct vasorelaxant effect.
From these findings, it is thus possible that ghrelin may be an
important mediator in cardiovascular control during
pregnancy.

Furthermore, plasma ghrelin concentration was signifi-
cantly higher in patients with PIH than in normal pregnant
women, and there was significant correlation between plasma
ghrelin concentration and systemic blood pressure in patients
with PIH. Therefore, we speculate that a compensatory
increase of ghrelin occurred in patients with PIH.

Previously, we developed 2 RIAs specific for the C- and
N-terminal regions of ghrelin.\(^16\) Using these 2 RIAs com-
bined with high-performance liquid chromatography, we
found 2 major molecular forms of ghrelin, an n-octanoylated
and des-acyle ghrelin, in rat stomach and intestine.\(^16\) RIA for
C-terminal region of ghrelin detected both n-octanoylated and
des-acyle ghrelin. RIA for N-terminal region of ghrelin

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Scatterplots for correlations between plasma ghrelin concentrations
and systemic blood pressure in nonpregnant women (A), normal pregnant
women (B), and patients with pregnancy-induced hypertension (C). Solid line
represents the regression line.}
\end{figure}
detected only n-octanoylated ghrelin. Des-acyl ghrelin has no biological activity to increase the intracellular calcium concentration. In the present study, we also measured the plasma ghrelin level by the RIA for N-terminal region of ghrelin in nonpregnant women. The plasma n-octanoylated ghrelin (active form) concentration in nonpregnant women was 26.7 ± 8.3 fmol/mL. Therefore, we think that the percentage of active form in plasma ghrelin is ≈10%. However, further studies are needed to determine whether des-acyl ghrelin is a premodified form or a n-octanoyl–deleted form.

Recently, it has been demonstrated that administration of ghrelin, in addition to its role in regulating GH secretion, caused weight gain by reducing fat utilization in mice and rats. It is well documented that energy requirements are increased during pregnancy. These increased demands can be met through metabolic adaptations and increased dietary intakes. The significant decrease in circulating maternal ghrelin during the third trimester of normal pregnancy is hypothesized to be a response to the marked changes in maternal weight and energy expenditure. Therefore, it is possible that the decreased plasma ghrelin concentrations during normal pregnancy may represent a physiological adaptation to the positive energy balance during pregnancy.

It has been recently demonstrated that plasma ghrelin concentration was decreased in obesity and that plasma ghrelin during the third trimester of normal pregnancy is significantly lower than those in maternal blood normal pregnant women, suggesting that the fetal-placental unit may contribute to its production. Furthermore, Gualillo et al. demonstrated that ghrelin mRNA and ghrelin peptides are present in the human and in rat placenta. These findings thus also suggest that ghrelin may have some role not only in maternal circulation but also in fetal-placental circulation.

In conclusion, although it still remains to be elucidated whether ghrelin has biological actions other than growth hormone secretion and food intake, our results suggest for the first time that ghrelin may have some important role in cardiovascular control during normal pregnancy and in pathophysiological conditions in pregnancy, such as PIH.

Acknowledgments

This work was supported in part by a grant from Japan Association of Obstetricians and Gynecologists. This work was also supported in part by the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research (OPSR) of Japan. We also thank Jane Huang (Fukuoka University) for her careful reading of the manuscript.

References

Alteration of Plasma Ghrelin Levels Associated With the Blood Pressure in Pregnancy
Yasuo Makino, Hiroshi Hosoda, Kazuhiko Shibata, Ikuko Makino, Masayasu Kojima, Kenji Kangawa and Tatsuhiko Kawarabayashi

Hypertension. 2002;39:781-784
doi: 10.1161/hy0302.105221

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/39/3/781

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/