を確立した。このモデルでは摂食低下により体重減少を生じ、悪液質を来していると考えられた。このモデルにグレリンを投与することで、摂餌量、体重、筋重量、筋横断面積の減少を抑制し、IL-6などの血中炎症性サイトカイン濃度やAtrogin-1などの筋特異的ユビキチンリガーゼの発現上昇を抑制した。これらの効果は、グレリンの持つ抗炎症作用や、筋組織中のIGF1濃度を上昇させることが関与している可能性が考えられた。

F. 健康危険情報

総括研究報告書にまとめて記入。

G. 研究発表

1. 論文発表

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2. 学会発表

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H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

［Ⅲ］　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

	発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
1	Tsubouchi H, Yanagi S, Miura A, Iizuka S, Mogami S, Yamada C, Hattori T, Nakazato M.	Rikkunshito ameliorates bleomycin-induced acute lung injury in a ghrelin-independent manner.	J Physiol Lung Cell Mol Physiol	306	L233-245	2014
2	Yano Y, Nakazato M, Toshinai K, Inokuchi T, Matsuda S, Hidaka T, Hayakawa M, Kangawa K, Shimada K, Kario K.	Circulating des-acyl ghrelin improves cardiovascular risk prediction in older hypertensive patients.	Am J Hypertens	27	727-733	2014
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	発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
8	Yamamoto K, Takiguchi S, Miyata H, Miyazaki Y, Hiura Y, Yamasaki M, Nakajima K, Fujiwara Y, Kangawa K, Doki Y.	Reduced plasma ghrelin levels on day 1 after esophagectomy: a new predictor of prolonged systemic inflammatory response syndrome.	Surg Today	43	48-54	2013
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10	Mitsunaga S, Ikeda M, Shimizu S, Ohno I, Furuse J, Inagaki M, Higashi S, Kato H, Terao K, Ochiai A.	Serum levels of IL-6 and IL-1β can predict the efficacy of gemcitabine in patients with advanced pancreatic cancer.	Br J Cancer	108	2063-2069	2013
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［Ⅳ］　研究成果の刊行物・別刷

Circulating Des-acyl Ghrelin Improves Cardiovascular Risk Prediction in Older Hypertensive Patients

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BACKGROUND

We aimed to assess the predictive value of circulating levels of des-acyl ghrelin, an abundant form of ghrelin in humans, for the risk of cardiovascular disease (CVD) in older hypertensive patients. We simultaneously evaluated other biomarkers, such as high-molecular-weight (HMW) adiponectin, high-sensitivity C-reactive protein (hs-CRP), and plasminogen activator inhibitor 1 (PAI-1), for their usefulness in risk prediction.

METHODS

We enrolled 590 older hypertensive patients (mean age = 72.9 years; 41.0% men). The incidences of CVD, including coronary artery disease, stroke, congestive heart failure, and sudden death, were prospectively ascertained.

RESULTS

During an average duration of 2.8 (SD = 0.7) years (1,653 person-years), there were 42 CVD events. Patients with CVD events had lower levels of des-acyl ghrelin at baseline than those without CVD events (median = 78.2 vs. 114.7 fmol/ml; $P < 0.001$). No difference was found among other biomarkers between the patients with CVD events and those without such events. The Cox proportional hazards model adjusted by covariables revealed that the hazard ratio for CVD events in patients with a 1-SD decrease of log des-acyl ghrelin was 1.8 (95% confidence interval = 1.3–2.4). Incorporation of des-acyl ghrelin in the risk model (including age, current smoking, 24-hour systolic blood pressure, preexisting CVD, and carotid intima-media thickness) improved the C statistics (from 0.683 to 0.721; $P = 0.22$) and resulted in a net reclassification improvement of 20.5% ($P = 0.02$). In contrast, HMW adiponectin, hs-CRP, and PAI-1 provided no improvement in risk prediction.

CONCLUSIONS

Des-acyl ghrelin improved the prediction of CVD events in older hypertensive patients.

Keywords: blood pressure; cardiovascular disease; des-acyl ghrelin; geriatric; hypertension.

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Ghrelin, an endogenous ligand for growth hormone secretagogue receptor type 1a, is a peptide that has been shown to stimulate food intake and energy balance.[1–4] In recent clinical studies, ghrelin and the major form (80%–90%) of ghrelin in circulation, des-acyl ghrelin, have attracted much attention because of their beneficial effects on the cardiovascular system, such as on vasodilation, amelioration of endothelial dysfunction, and suppression of sympathetic nerve activity.[5–9] From the clinical point of view, these findings raise the possibility that circulating levels of ghrelin or des-acyl ghrelin could serve as a useful biomarker to predict cardiovascular disease (CVD).

Two prospective studies recently demonstrated that low circulating ghrelin levels were associated with increased risk of cardiovascular morbidity and mortality in hemodialysis patients.[10,11] In particular, low ghrelin values in protein-energy–wasted hemodialysis patients were linked to a markedly increased cardiovascular mortality risk. Ghrelin can reflect an individual's energy balance,[1–4] and thus ghrelin may be useful as a biomarker for predicting outcomes at the pathological state in which protein-energy wasting or frailty contributes to individual prognosis. This hypothesis can apply to older patients with hypertension,[12,13] but the prognostic value of circulating ghrelin or des-acyl ghrelin levels in older hypertensive patients has never been examined.

In this study, we examined the predictive value of circulating levels of des-acyl ghrelin for the risk of CVD events in older hypertensive patients. We also compared these values with those of other cardiovascular biomarkers, such as high-molecular-weight (HMW) adiponectin, high-sensitivity C-reactive protein (hs-CRP), and plasminogen activator inhibitor 1 (PAI-1).

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METHODS

Patient characteristics

The methods of the study have been detailed elsewhere.[14,15] Briefly, from July 2007 to March 2009, we initially enrolled 596 consecutive patients with essential hypertension (mean age = 72.9 ± 8.3 years; 42% men) who had been treated at our outpatient clinic (Kitaura National Health Insurance Hospital or Nango National Health Insurance Hospital, both in Miyazaki, Japan). Hypertension was defined as office blood pressure (BP) ≥140/90 mm Hg on at least 2 occasions or current use of antihypertensive drugs. The enrolled patients were ambulatory, lived independently, and were not nursing home residents. The exclusion criteria for this study were as follows: a recent history (within 6 months) of coronary artery disease (defined by myocardial infarction or events requiring treatment with percutaneous coronary intervention), stroke, or congestive heart failure; the presence of inflammatory diseases (acute infection, autoimmune diseases); the presence of malignant diseases; atrial fibrillation; previous gastrectomy; and the absence of or incomplete data sampling. At baseline, all patients underwent measurement of 24-hour ambulatory BP monitoring (ABPM), blood sampling, and cardiac and carotid artery ultrasonography (see Supplementary Material).

This study was approved by the institutional review board at Jichi Medical University, and written informed consent was obtained from all participants.

Biomarker analysis

Venous blood was obtained in the morning (8:00 and 8:30 AM) after the patient had fasted overnight. The plasma des-acyl ghrelin levels were determined using a commercially available enzyme-linked immunosorbent assay kit (Mitsubishi Kagaku Iatron, Tokyo, Japan).[16] The HMW adiponectin concentrations were measured using a two-step sandwich enzyme-linked immunosorbent assay system (FujiRevio, Tokyo Japan), and the serum levels of nitric oxide (NO) were assessed by nitrite/nitrate ($NO2^-$ and $NO3^-$, NOx) using a high-performance liquid chromatography ultraviolet system. The concentrations of hs-CRP (N hs–CRP; Dade Behring, Liederbach, Germany) and PAI-1 antigen (LPIA-NV7; Mitsubishi Chemical Medience, Tokyo, Japan) were measured by SRL Inc (Tokyo, Japan). The intraassay and interassay coefficients of the laboratory tests were all <7%.

Diabetes was defined as the use of antihyperglycemic agents or a serum fasting glucose level of ≥126 mg/dl. To estimate renal function, the glomerular filtration rate was estimated by the Modification of Diet in Renal Disease study equation modified for Japanese, using the following equation: estimated glomerular filtration rate (ml/min/1.73 m^2) = 194 × age (years)$^{-0.287}$ × serum creatinine (mg/dl)$^{-1.094}$ (for women, × 0.739).

ABPM and cardiac and carotid artery ultrasonography

Twenty-four-hour ABPM (TM-2425; A&D, Tokyo, Japan) was performed with an automatic oscillometric device at 30-minute intervals. For full details, see Supplementary Material. A trained physician (Y.Y.) who was unaware of the patient's laboratory and ABPM data performed cardiac ultrasonography and carotid artery ultrasonography on all participants.[14,15] M-mode left ventricular end-diastolic diameter and interventricular septum and posterior left ventricular wall thicknesses at end diastole were measured according to the guidelines of the American Society of Echocardiography.[17] None of the patients had an impaired (<40 %) ejection fraction in this study. The carotid arteries were examined bilaterally at the level of the common carotid artery, bulb, and internal-carotid artery (ICA), as measured from both transverse and longitudinal orientations. The region with the thickest intima-media thickness (IMT) was measured, and the values calculated as the mean of the single thickest point in the far wall on both sides were included in our analysis. A repeatability analysis[14,15] demonstrated the coefficient of variation of mean difference for repeat measures of carotid-artery IMT was within 10%.

Follow-up and events

Components of the CVD endpoints included the following: (i) acute myocardial infarction or events requiring urgent treatment by percutaneous coronary intervention for unstable angina (criteria for myocardial infarction included definite electrocardiographic findings (i.e., ST elevation), typical or atypical symptoms together with electrocardiographic findings and abnormal enzymes, or typical symptoms and abnormal cardiac enzymes with or without electrocardiographic findings); (ii) stroke, defined as sudden onset of a neurological deficit persisting for ≥24 hours in the absence of any other disease that could account for the symptoms (stroke events were categorized as cerebral infarction, cerebral embolism, or hemorrhagic based on the findings of brain computed tomography or magnetic resonance imaging); (iii) hospital admission for congestive heart failure, which was defined by the clinical symptoms, including dyspnea, shortness of breath, peripheral edema, and pulmonary edema on chest X ray; and (iv) unexplained sudden death within 6 hours of the abrupt onset of symptoms. If CVD events occurred on ≥2 occasions, the first occurrence of CVD was included in the analysis. Transient ischemic brain attacks (i.e., those in which the neurological deficit was completely resolved within 24 hours of the onset of symptoms) were not calculated as clinical CVD events.

Evidence regarding the above CVD outcomes was ascertained by ongoing reports from a general physician at each institute.[15] The incidences of CVD were also ascertained by at least annual reviews of the patient's medical records. When patients failed to come to the hospital, we interviewed them and/or their families by telephone: among these patients, 2 were shown to have had CVD events. With the exception of these 2 cases, all CVD events were diagnosed by the physicians who were caring for the patient at the time of the events, and independent physicians reviewed the cases and confirmed the diagnosis of CVD events. A final follow-up survey to reconfirm the clinical outcomes was performed from May 2011 to September 2011; the mean follow-up

period was 2.8 (SD = 0.7) years (1,653 person-years), and complete follow-up has been achieved for 99.7% (n = 594) of the cohort.

Statistical analysis

All statistical analyses were performed with SPSS version 18.0J software (SPSS, Chicago, IL) and STATA version 12.1 (STATACorp, College Station, TX). Clinical parameters in patients with or without CVD events were compared using the unpaired *t* test for variables with normal distribution or Mann–Whitney *U* test for variables with skewed distribution, and categorical parameters were compared with the χ^2 test.

We used the Cox proportional hazards model to examine the association between each biomarker and CVD events. A penalized cubic spline (knots at every 5 fmol/ml in des-acyl ghrelin) was drawn to assess the shape of the association between des-acyl ghrelin and CVD events. Because all biomarkers and carotid artery IMT levels had skewed distributions, they were logarithmically transformed before analysis. After making adjustments for several risk factors such as age, current smoking, 24-hour systolic BP (SBP), preexisting CVD, and log ICA-IMT, we calculated the hazard ratio (HR) and 95% confidence interval (CI) for clinical CVD events for each biomarker. The likelihood ratio, χ^2, statistic was used to evaluate the goodness-of-fit of predictive models. Comparison of the predictive power of predictive models was conducted by *C* statistics (Harrell's *C*).[18] In addition, risk reclassification was assessed by categorizing the predicted risk for each model into categories of <10%, 10% to <20%, and ≥20%.[19] Because no established categories exist that guide clinical decisions for CVD risk in older hypertensive patients, the category-based net reclassification improvement (NRI) was based on the standard categories for 10-year CVD risk.[19,20] We calculated the proportion of participants who were reclassified by the comparison model compared with the reference model. We computed the NRI,[21] which compares the shifts in reclassified categories by observed outcome, and the integrated discrimination improvement (IDI), which compares the integrals of sensitivity and specificity under 2 models. A two-sided $P < 0.05$ was defined as statistically significant.

RESULTS

Clinical characteristics

Among the 594 hypertensive patients who were successfully followed in our study, 4 patients with unsatisfactory blood sampling of des-acyl ghrelin were excluded from this analysis. Thus, 590 patients were included in the final analysis (Table 1).

Associations of biomarkers with various clinical parameters

Associations of des-acyl ghrelin, HMW adiponectin, hs-CRP, and PAI-1 levels with various clinical parameters at baseline are shown in Supplementary Table S1. The des-acyl ghrelin levels were positively associated with the circulating NOx levels ($r = 0.16$) and inversely associated with ICA-IMT ($r = -0.12$; both $P < 0.01$); these associations remained significant even after adjustments for age, sex, and body mass index (both $P < 0.05$).

Associations of biomarkers with CVD events

The baseline clinical characteristics of patients by clinical CVD events are shown in Table 1. During an average duration of 2.8 ± 0.7 years (1,653 person-years), 42 CVD events occurred (25.4 events/1,000 person-years); these include 13 coronary artery disease events, 16 strokes (ischemic = 14; hemorrhagic = 2), 9 congestive heart failure events, and 4 sudden deaths. Patients with CVD events had lower des-acyl ghrelin levels than those without such events. Other biomarkers showed no significant differences between patients with and without CVD events. Although there was a baseline difference in high-density lipoprotein between groups, after adjustment for covariables, the difference was no longer significant ($P = 0.09$).

Table 2 shows the adjusted HR for CVD events for a 1-SD change in log des-acyl ghrelin and other biomarkers after adjusting for age, current smoking, 24-hour SBP, preexisting CVD, and log ICA-IMT. Improvement in *C* statistics in the model with and without biomarkers is also shown. The 1-SD change in log des-acyl ghrelin but not in other biomarkers was associated with increased risk for CVD events (HR = 1.8; 95% CI = 1.3–2.4; $P < 0.001$). When we analyzed the CVD risk of low des-acyl ghrelin among patients without preexisting CVD at baseline (n = 531), the conclusions remained unchanged (HR = 1.7; 95% CI = 1.2–2.5; $P = 0.002$). Moreover, CVD risk associated with low des-acyl ghrelin remained significant even after further adjustments for any 1 of the following variables: sex, body mass index, physical activity, use of statin or all classes of antihypertensive medication, 24-hour pulse pressure, glucose and lipid variables, and serum NOx levels (data not shown). Penalized cubic spline demonstrated a graded increase in the risk of CVD events with decreasing des-acyl ghrelin levels (Figure 1).

The *C* statistics for the reference model (including age, current smoking, 24-hour SBP, preexisting CVD, and log ICA-IMT) was 0.683, which showed a tendency toward improvement with addition of log des-acyl ghrelin levels (*C* statistics = 0.721; *P* for improvement = 0.22). Table 3 shows the cross-tabulation between predicted risk obtained using a reference model with and without log des-acyl ghrelin in patients who experienced CVD events and those who did not. The overall NRI was 20.5% for des-acyl ghrelin ($P = 0.03$). The estimated IDI was 0.04 and was significant ($P < 0.001$). For HMW adiponectin, hs-CRP, and PAI-1, the overall NRI was small (0%–3%) and nonsignificant ($P = 0.52$–1.00), and the IDI was also nonsignificant ($P = 0.20$–0.84).

DISCUSSION

In this prospective study with older hypertensive patients, we have demonstrated for the first time that the circulatory levels of des-acyl ghrelin, rather than those of HMW adiponectin, PAI-1, or hs-CRP, improved CVD risk prediction

Table 1. Baseline clinical characteristics of the study population according to the occurrence of cardiovascular disease events

	All subjects (n = 590)	CVD events (−) (n = 548)	CVD events (+) (n = 42)	*P* value
Patient characteristics				
Age, years	72.9±8.2	72.6±8.3	76.5±7.3	0.003
Male, %	41.0	40.9	42.9	0.87
Body mass index, kg/m^2	24.5±3.5	24.5±3.5	25.5±3.5	0.08
Current smoker, %	8.3	7.7	16.7	0.049
Daily drinker, %	27.6	27.4	31.0	0.72
eGFR, ml/min/1.73 m^2	68.8±18.7	69.5±18.6	60.3±19.0	0.002
Comorbidity				
Type 2 diabetes, %	16.8	16.8	16.7	1.00
Use of statin, %	18.8	19.2	14.3	0.54
Preexisting CVD, %	10.0	8.6	28.6	<0.001
Antihypertensive medication, %		78.8	85.7	0.33
Duration of antihypertensive medication, years	7.0 (1.0–15.0)	7.0 (1.0–15.0)	9.5 (3.8–20.0)	0.12
Number of antihypertensive drugs, no.	1.0 (1.0–2.0)	1.0 (1.0–2.0)	2.0 (1.0–3.0)	0.03
Calcium-channel blockers, %	58.1	57.3	69.0	0.15
ACE inhibitors, %	12.0	12.4	7.1	0.35
Angiotensin receptor blockers, %	44.9	44.0	57.1	0.11
Diuretics, %	19.3	18.6	28.6	0.15
Alpha-blockers, %	2.7	2.4	7.1	0.10
Beta-blockers, %	6.3	6.2	7.1	1.00
24-hour BP measurements				
24-hour SBP/ DBP, mm Hg	134/77±15/8	133/77±14/8	137/77±18/8	0.11/ 0.97
24-hour PP, mm Hg	57±11	57±10	61±13	0.03
24-hour PR, bpm	67±7	67±7	66±7	0.35
Laboratory data				
Fasting glucose, mg/dl	98.0 (92.0–110.0)	98.0 (92.0–110.0)	98.0 (92.8–112.3)	0.71
Triglycerides, mg/dl	94.0 (71.0–128.3)	94.0 (71.0–130.0)	95.0 (71.8–122.3)	0.64
High-density lipoprotein, mg/dl	55.0 (45.8–65.0)	55.0 (46.0–66.0)	49.0 (43.0–55.5)	0.002
Low-density lipoprotein, mg/dl	111.0 (93.0–130.0)	111.0 (93.0–129.8)	116.0 (91.0–140.3)	0.38
High-sensitivity C-reactive protein, mg/L	0.52 (0.26–1.07)	0.51 (0.26–1.07)	0.59 (0.36–1.12)	0.22
Plasminogen activator inhibitor-1, ng/ml	27.0 (19.0–39.0)	27.0 (19.0–39.0)	30.0 (22.0–43.0)	0.22
High-molecular-weight adiponectin, μg/ml	6.8 (4.2–10.3)	6.6 (4.1–10.3)	7.9 (5.7–11.0)	0.06
Des-acyl ghrelin, fmol/ml	112.4 (68.3–169.3)	114.7 (70.8–171.7)	78.2 (43.7–116.9)	<0.001
NOx, μmol/L	35.0 (23.0–57.3)	35.0 (23.0–58.0)	32.0 (22.5–51.5)	0.37
Target-organ damage				
CCA-IMT, mm	0.9 (0.8–1.0)	0.9 (0.8–1.0)	1.0 (0.8–1.1)	0.02
ICA-IMT, mm	1.1 (0.9–1.4)	1.1 (0.9–1.4)	1.4 (1.1–1.7)	<0.001

Variables with skewed distributions are expressed as median (interquartile range). All other data are expressed as means ± SD unless otherwise noted. *P* values were calculated by unpaired *t* test, χ^2 test, or Mann–Whitney *U* test (variables with skewed distributions). Statistical significance was defined as $P < 0.05$.

Abbreviations: ACE, angiotensin-converting enzyme; BP, blood pressure; CCA, common-carotid artery; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ICA, internal-carotid artery; IMT, intima-media thickness; NO, nitric oxide; PP, pulse pressure; PR, pulse rate; SBP, systolic blood pressure.

Table 2. Model fit, hazard ratio, and *C* statistics for cardiovascular disease events (n = 590)

Variable	−2 log likelihood	*P* value	HR (95% CI)	*P* value	*C* statistics (95% CI)	*P* value
Reference model	485.0	—	—	—	0.683 (0.557–0.809)	—
Reference model +des-acyl ghrelin	471.1	<0.001	1.8 (1.3–2.4)	<0.001	0.721 (0.586–0.855)	0.22
Reference model + HMW adiponectin	483.7	0.26	1.2 (0.9–1.7)	0.27	0.684 (0.557–0.811)	0.55
Reference model + hs-CRP	485.0	1.00	1.0 (0.7–1.4)	1.00	0.660 (0.531–0.789)	0.01
Reference model + PAI-1	484.8	0.63	1.1(0.8–1.5)	0.63	0.679 (0.553–0.806)	0.74

The likelihood ratio test was used to evaluate the goodness-of-fit of predictive models. Adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for cardiovascular disease (CVD) events for a 1-SD change in log des-acyl ghrelin (−1 SD) and other biomarkers (+ 1 SD) are shown. All models were adjusted by age, current smoking, estimated glomerular filtration rate, preexisting CVD, 24-hour systolic blood pressure, log internal-carotid artery–intima-media thickness. One-SD change in log des-acyl ghrelin (−1 SD) and other biomarkers (+ 1 SD) were as follows: log des-acyl ghrelin = −0.31; log high-molecular-weight (HMW) adiponectin = 0.28; log high-sensitivity C-reactive protein (hs-CRP) = 0.51; log plasminogen activator inhibitor 1 (PAI-1) = 0.24. Significance was defined as $P < 0.05$.

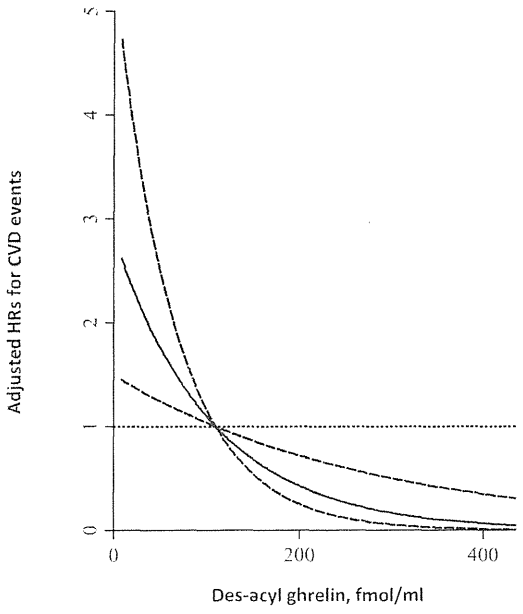

Figure 1. Association of des-acyl ghrelin and cardiovascular (CVD) events. The regression spline curve based on the Cox regression model (adjusted by age, current smoking, 24-hour systolic blood pressure, preexisting CVD, and log internal-carotid artery–intima-media thickness) is used to examine the association of des-acyl ghrelin with CVD events. The adjusted hazard ratios (HRs) for CVD events in relation to des-acyl ghrelin as function of the penalized regression spline are shown. Reference is defined as des-acyl ghrelin of 112 fmol/mL (median). Solid line indicates HRs, and dashed line indicates 95% confidence intervals.

beyond several risk factors. The addition of des-acyl ghrelin to a risk model (including age, current smoking, 24-hour SBP, preexisting CVD, and ICA-IMT) improved the risk classification, with an NRI value of 20.5%, suggesting that the results were not only statistically significant but clinically meaningful.[22] An improvement in the *C* statistics with the addition of des-acyl ghrelin did not reach statistical significance; this can be explained by the property of *C* statistics: they hardly move when a few good risk factors are included in the model and consequently require a large HR to achieve statistical significance.[21,22] To account for the short follow-up period (mean = 2.8 years), the assessment of des-acyl ghrelin in older hypertensive patients may be useful for identifying patients who are likely to develop CVD events in the relatively near future. Despite our in-depth analysis and suggestion for a predictive ability of des-acyl ghrelin beyond clinically appreciable markers, further detailed studies, including an examination of cost effectiveness, are warranted.

Several experimental and clinical studies have demonstrated the respective abilities of ghrelin and/or des-acyl ghrelin to exert beneficial effects on the cardiovascular system.[5–9] The previous experimental data suggested that ghrelin administration *in vivo* and *in vitro* inhibits proinflammatory responses and nuclear factor-κB activation in human endothelial cells, monocytes, and T cells.[23] Of particular interest are the results of studies demonstrating the widespread expression of receptors shared by both ghrelin and des-acyl ghrelin in human endothelial cells and vascular smooth cells, and the administration of ghrelin as well as des-acyl ghrelin in humans *in vivo* can lead to an improvement of endothelial dysfunction through increases in NO bioavailability within the vasculature.[5–7] NO possesses some antiatherogenic and plaque-stabilizing properties, such as regulation of vascular tone and arterial wall stress, protection of oxidative stress, and inhibition of leukocyte and platelet adhesion.[24] In this study, a positive association between circulating des-acyl ghrelin and NOx levels was observed. However, the relationship was weak ($r = 0.16$), and the NOx level in the blood depends not only on NO production/endothelial function but also other factors such as nitrate/nitrite intake and renal function. Thus, the interpretation of this relationship needs further attention.

We found that an association of low des-acyl ghrelin with CVD events was independent of serum NOx levels. Other mechanisms that were left unmeasured in this analysis, such as psychological stress and sympathetic nervous system status, both of which could be affected by ghrelin,[8,25] may also partly account for this association. Because this study was a hypothesis-generating survey, our results remain suggestive

Table 3. Change in risk stratification by des-acyl ghrelin for cardiovascular disease events (n = 590)

Reference model, predicted risk	Reference model + des-acyl ghrelin			Total
	<10 %	≥10 % to <20%	≥20%	
Participants who experienced CVD events				
<10 %	16	6	1	23
≥10 % to < 20%	2	3	4	9
≥20 %	0	0	10	10
Total	18	9	15	42
Participants who did not experience a CVD event				
<10 %	421	29	4	454
≥10 % to < 20%	29	25	14	68
≥20 %	2	11	13	26
Total	452	65	31	548

Reclassification tables are separated for cases (top) and non-cases (bottom), with rows indicating the risk categories based on the reference model (age, current smoking, estimated glomerular filtration rate, preexisting cardiovascular disease (CVD), 24-hour systolic blood pressure, log internal-carotid artery–intima-media thickness) without des-acyl ghrelin and columns indicating the new risk stratification after the addition of des-acyl ghrelin to the basic model. The cells give the number of subjects reclassified by predicted risk.

and speculative. Further studies to elucidate the pathogenic mechanism underlying the association between des-acyl ghrelin with CVD events might help to clarify the clinical values of circulating des-acyl ghrelin levels.

The HMW adiponectin, PAI-1, and hs-CRP levels were not associated with an increased risk of CVD events in this study. The role of some biomarkers diminish with advancing age.[12,26] Recent studies conducted in older populations demonstrated that, as opposed to the findings from younger cohorts, high adiponectin levels were associated with CVD morbidity and mortality.[27,28] The predictive value of hs-CRP has also been shown to decrease in older populations.[29,30] Because circulating levels of adiponectin, hs-CRP, and PAI-1 are easily influenced by the amount of adiposity,[31] their interpretation in older persons may be confounded by the existence of the obesity paradox.[13,26] Specifically, high adiponectin levels in older persons are a consequence of wasting or frailty with aging.[32] A statistically significant reduction of the *C* statistics with the addition of hs-CRP to the reference model was observed. This reduction might be due to the existence of confounders, but the exact mechanism remains uncertain and is worth of further investigations.

Circulating ghrelin levels can reflect individual short- and long-term energy homeostasis,[1–4] which may be one of the reasons why des-acyl ghrelin rather than HMW adiponectin, PAI-1, and hs-CRP was associated with poor outcomes in this older population. The small number of participants, short-term follow-up period, and ongoing medical therapy—in particular the administration of antihypertensive drugs that can influence the circulating levels of HMW adiponectin, hs-CRP, and PAI-1[33,34]—may have resulted in an underestimation of the strength of the associations between these biomarkers and CVD events. It will be important to duplicate these results in other longitudinal studies.

This study, although presenting novel data, has limitations. Because of a low event rate, we could not evaluate groups of CVD events separately and could not determine the appropriate cutoff values of des-acyl ghrelin for predicting CVD events. This limitation should be addressed using a longer follow-up period of this study or larger database in future studies. Second, our results cannot be applied to a younger cohort or other ethnicities. Lastly, we issue a cautionary note regarding the interpretation of NRI findings in the absence of an accepted risk grouping.

We conclude that this study provides novel data to suggest the clinical value of circulating des-acyl ghrelin because it might be a useful independent predictor of CVD events in older hypertensive patients. Further research is required to determine whether assessing circulating des-acyl ghrelin in older hypertensive patients improves clinical outcomes.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

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DISCLOSURE

The authors declared no conflict of interest.

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グレリンとオベスタチン*

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Key Words：ghrelin, des-acyl ghrelin, obestatin, ghrelin *O*-acyltransferase (GOAT)

はじめに

グレリンは，成長ホルモン(GH)分泌促進因子受容体(GHS-R)の内因性リガンドとしてラット胃から発見された摂食亢進ペプチドである．グレリンの末梢投与によるGH分泌促進作用や摂食亢進作用の発現に，グレリンの3番目のセリン残基への脂肪酸修飾が必須である．最近，グレリンの脂肪酸修飾に関与する酵素ghrelin *O*-acyltransferase(GOAT)が同定され，食事中の脂質が直接グレリンのアシル化に関与しグレリンが脂質センサーとして機能する可能性が示された．脂肪酸修飾を受けていないグレリン(デスアシルグレリン)は当初非活性型と考えられていたが，GHS-R非依存的な作用機序で多彩な生理作用を有することが報告されている．オベスタチンは，バイオインフォマティクス的手法を用いてグレリン前駆体蛋白より推定・同定されたペプチド断片であるが，その生理作用は一定せず疑問視されている．グレリンの持つ多彩な生理作用を応用して，心不全，慢性閉塞性肺疾患などに対するグレリンの臨床試験が開始されている．今回，グレリンおよびオベスタチンに関して，最近の知見を含めて紹介する．なお，誌面の関係で参考文献を割愛したので，最新の総説などを参考にしていただきたい[1]．

グレリンとグレリン受容体

1970年代からオピオイドペプチド誘導体の中に弱いGH分泌活性を示すものが発見されていた．その後，強力なGH分泌促進作用を有する化合物が合成され，それらは成長ホルモン分泌促進因子(GH secretagogues；GHSs)と呼ばれた．1996年にGHSの細胞内カルシウム上昇活性を指標とした発現クローニング法で，7回膜貫通型のG蛋白共役型受容体(G-protein-coupled receptor；GPCR)であるGHS-Rが同定された[2]．

グレリンは，1999年に国立循環器病センター研究所生化学部の児島将康博士(現久留米大学分子生命科学研究所遺伝情報研究部門)，寒川賢治博士によりGHS-Rの内因性リガンドとして単離・同定された28アミノ酸残基からなる生理活性ペプチドである[3]．グレリンの最も多く発現する組織が，それまでGH分泌調節とは無関係と考えられていた胃であった．グレリンをコードするヒトグレリン遺伝子は第3番染色体短腕(3p25-26)に存在し，4つのエキソンを有する．グレリンのmRNAは胃に最も多く発現し，小腸，大腸，膵臓，視床下部，胎盤，腎臓，副腎，脂肪細胞などに

* Ghrelin and obestatin.

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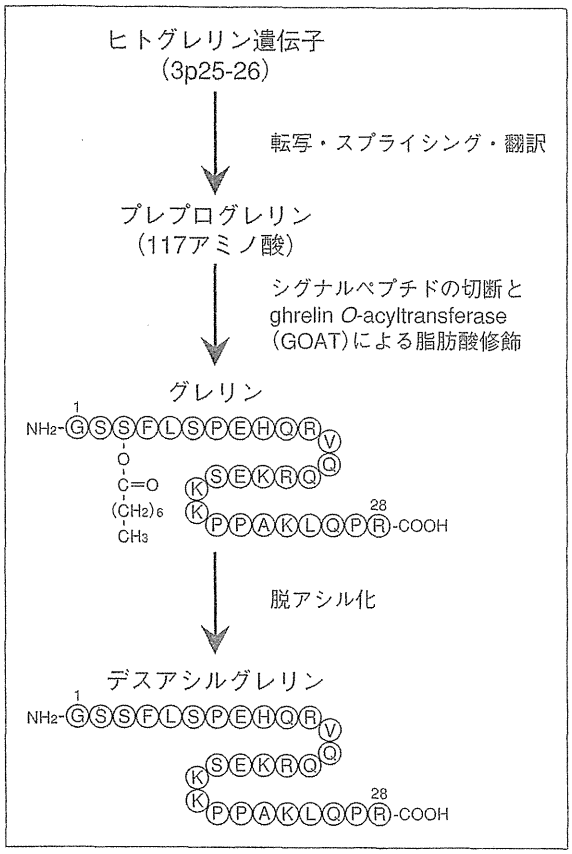

図1　グレリンのGOATによる脂肪酸修飾
ヒトグレリンのペプチド鎖は28アミノ酸残基からなり，3番目のセリン残基(S)の水酸基と炭素原子数8個からなる脂肪酸の*n*-オクタン酸がghrelin *O*-acyltransferase(GOAT)によりエステル結合してグレリンが形成される．流血中では速やかに脂肪酸が解離(脱アシル化)してデスアシルグレリンとなる．アミノ酸は一文字表記してある．

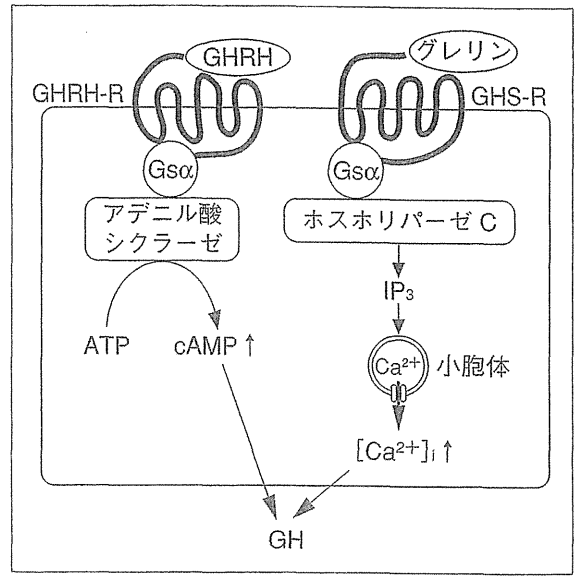

図2　GHRHとグレリンによるGH分泌の細胞内シグナル伝達機構
成長ホルモン(GH)は2種類の異なる受容体を介したシグナルにより分泌される．一つは成長ホルモン放出ホルモン(GHRH)がGHRH受容体(GHRH-R)に結合し，細胞内サイクリックAMP(cAMP)の上昇を介する系．もう一つはグレリンがGHS受容体(GHS-R)に結合し，細胞内カルシウム濃度($[Ca^{2+}]_i$)の上昇を介する系である．IP_3：イノシトール3リン酸．

も発現が広く認められる．胃において，グレリンは117アミノ酸残基のグレリン前駆体蛋白からシグナルペプチドが切断され，3番目のセリン残基が炭素数8個の脂肪酸である*n*-オクタン酸により修飾を受け，prohormone convertase 1/3(PC1/3)による分解を経て28アミノ酸残基のグレリンが生成される[4](図1)．グレリンが発見されるまで，同様の脂肪酸修飾を受けた生理活性ペプチドはいままでまったく知られていなかった．脂肪酸による修飾を受けていないグレリン(デスアシルグレリン)は，末梢投与でGH分泌促進作用や摂食亢進作用を認めず，脂肪酸修飾がグレリンの末梢での生物活性に必須であった．

成長ホルモン放出ホルモン(GHRH)受容体(GHRH-R)とGHS-Rはともに7回膜貫通型のGPCRであるが，GHRH-Rがセカンドメッセンジャーとしてアデニル酸シクラーゼを活性化して細胞内cAMPを上昇させるのに対し，GHS-RはホスホリパーゼCの活性化とイノシトール3リン酸の産生を介して細胞内カルシウムを上昇させてGH分泌を促進する(図2)．

グレリンの脂肪酸修飾酵素GOAT

2008年に2つの研究グループにより，*n*-オクタン酸によるグレリンの脂肪酸修飾は細胞膜に存在する脂肪酸転移酵素GOATまたはmembrane-bound-*O*-acyltransferase domain containing 4(MBOAT4)が関与することが報告された[5][6]．GOATは胃や膵臓などグレリンが発現する部位に多く存在し，マウス，ラット，ゼブラフィッシュを含む脊椎動物間で保存されていた．In vivoでマウス，ラット，ゼブラフィッシュのGOATがヒトグレリンの脂肪酸修飾を行うことが可能であった．GOAT(またはMBOAT4)遺伝子欠損マウスでは，血中にグレリンはまったく認められずデスアシルグレリンのみであったことから，GOATがグレリンの脂肪酸修飾酵素であることが証明

された．GOATはグレリンの3番目のセリン残基にのみ脂肪酸を修飾すること，3番目がスレオニンに置換されても脂肪酸修飾すること，1番目のグリシンと4番目のフェニルアラニンを他のアミノ酸に置換するとGOATによる脂肪酸修飾がみられないことから，GOATによるグレリンの脂肪酸修飾にグレリンの3番目のセリン残基前後のアミノ酸配列が重要とされている[5]．GOATの至適pHは7～8[7]であり，プレプログレリンのプロセッシングに関与するPC1/3の至適pHや分泌顆粒内pHは5～6であるため，GOATによるグレリンのアシル化はプログレリンの段階で行われ，その後PC1/3により分解されアシル化グレリンが生成されることが推定されている．

最近，体内の脂質でなく食事中の脂質が直接グレリンのアシル化に関与することや，GOAT欠損マウスでは高脂肪食負荷で脂肪蓄積が少なくGOATおよびグレリン過剰発現マウスでは体脂肪蓄積が多い知見が報告され，グレリンは空腹シグナルとしてよりも，脂肪摂取を脳に知らせ体内に脂肪蓄積を指令する脂質センサーとして機能するという概念が提唱されている[8]．

グレリンの摂食亢進およびGH分泌促進作用

グレリンはin vivoとin vitroで強力なGH分泌促進作用を有し，ヒトにグレリンとGHRHを同時に静注すると相乗的にGH分泌が促進される．グレリンをラットやマウスの中枢または末梢に投与すると，摂食亢進作用，脂肪蓄積作用を示すが，グレリンの摂食亢進作用は弓状核のニューロペプチドY(NPY)やアグーチ関連ペプチド(AgRP)を介して機能することが示されている[9]．健常者にグレリンを5 pmol/kg/分で持続静注しながら昼食を自由摂食させると，平均摂取カロリーは生食投与群の1,130kcalに対してグレリン投与群では1,440kcalと増加し，グレリン末梢投与での摂食亢進作用がヒトにおいても認められている．グレリンによる摂餌量増加はレプチンの脳室内同時投与により有意に抑制され，グレリンを最初に投与しその1時間後にレプチンを投与するとレプチンによる摂餌量減少は抑制されたことより，摂食に関してグレリンとレプチンは少なくともNPYを介して拮抗的に作用していると考えられている．

AMP依存性プロテインキナーゼ(AMPK)は視床下部のエネルギーセンサーとして機能する分子であるが，視床下部のAMPK活性はグレリンで増加しレプチンで低下することから，AMPK活性化においてもグレリンとレプチンは拮抗的に作用していることが示されている．

その他，大麻の主成分の受容体として知られるカンナビノイド受容体は快楽・報酬系への作用および摂食調節に関与するが，グレリンによる摂食亢進作用はカンナビノイド受容体拮抗剤であるリモナバンの併用で抑制され，グレリンの摂食亢進作用の一部はカンナビノイド系を介していることが報告されている．

胃で産生されたグレリンの中枢へのシグナル伝達

グレリンは末梢投与で摂食亢進作用やGH分泌亢進作用を示すが，胃から分泌されたグレリンの中枢への情報伝達に迷走神経が重要であると考えられている．迷走神経を物理的または化学的に遮断したラットでは，グレリン末梢投与による摂食亢進作用やGH分泌促進作用が減弱していた．ヒトでも胃全摘術などに伴い迷走神経を切断されている症例では，グレリンを持続静注しても摂食量は増加しなかったと報告されている．以上の知見などから，胃から分泌されたグレリンの情報は，迷走神経求心路・延髄孤束核を経由する神経路を介して視床下部に伝達されることが示されている[10](図3)．

グレリンの血中動態と分泌調節

ヒト血漿グレリン濃度は100～120 fmol/mlでその約90%がデスアシルグレリンとして存在している．胃切除後や胃バイパス術後の症例では血中グレリン濃度が低下することから，血中グレリンの主な供給源は胃である[11]．健常者での血中グレリン濃度は，空腹で増加し食後に低下するが，胃の機械的伸展では変化はない．血中グレリン濃度は，GH, ソマトスタチンアナログやurocortin-1投与で低下するが，レプチン投与では変動しない．豚を用いた研究で，トリプトファ

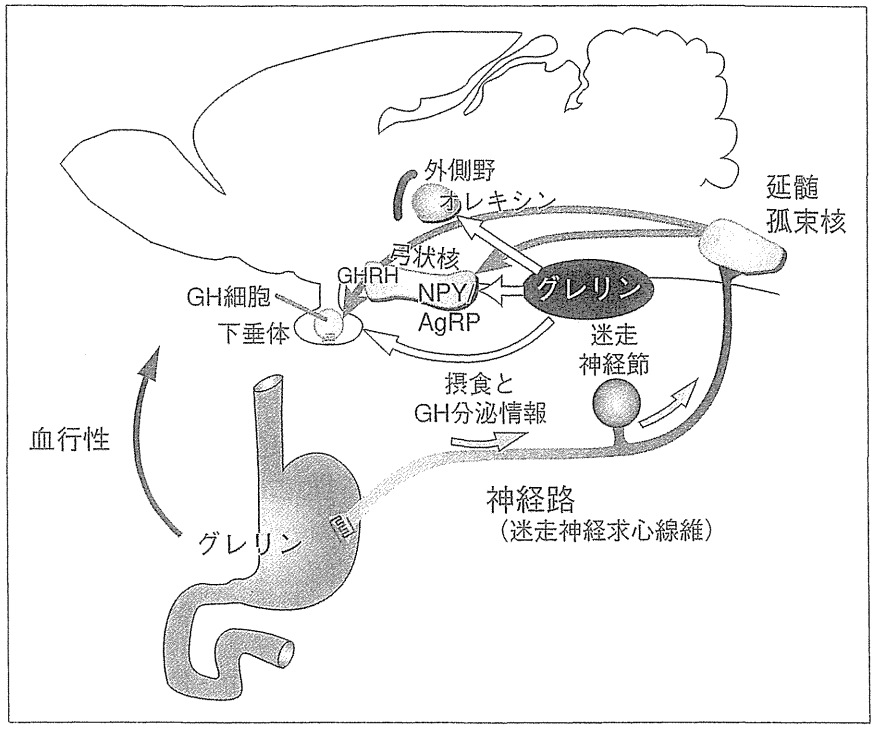

図3　胃グレリンの生体内情報伝達経路
グレリンの摂食亢進とGH分泌促進の情報は延髄孤束核を経由して視床下部のNPY/AgRPニューロンとGHRHニューロンに伝達される．血中を介して直接下垂体に末梢のグレリン情報が伝達される血行性経路もある．

ン(tryptophan)とzinc oxide(ZnO)がグレリン分泌を促進することが報告されている．

健常者では，早朝空腹時の血漿グレリン値はbody mass index(BMI)と負の相関を示す．著明なやせを呈する神経性食思不振症患者では早朝空腹時の血漿グレリン値が正常体重の健常者よりも高値で，治療により体重が増加してくると血漿グレリン値は低下する．また，健常者では摂食やグルコースの経口または経静脈的投与により血漿グレリン濃度は速やかに低下しその後徐々に回復するが，神経性食思不振症患者，神経性大食症患者および高度肥満者では摂食後にみられる血漿グレリン値の速やかな低下はみられない．

食欲亢進，肥満などを呈するPrader-Willi症候群では，早朝空腹時の血漿グレリン値は体脂肪量で補正した正常対照よりも高値である[12]．

グレリンの多彩な生理作用

グレリンの主な作用はGH分泌促進と摂食亢進であるが，そのほかに心機能改善作用，胃蠕動運動亢進や胃酸分泌促進作用，交感神経系抑制作用，糖代謝改善作用などが報告されている．

慢性閉塞性肺疾患や心不全でカヘキシアを呈する症例に対してグレリンを投与すると，摂食量増加，呼吸機能改善，運動耐容能改善などquality of lifeの改善につながる効果が報告されている[13]．やせを伴う慢性下気道感染症患者にグレリンを投与すると，摂食量や体重の増加に加え，喀痰量減少，喀痰中の好中球，IL-8, TNF-αの減少，6分間歩行距離の延長など，抗炎症作用を含めたグレリンの広範囲な臨床効果が確認されている．ラットにおいてグレリンがストレプトゾトシン誘発の糖尿病性神経障害を抑制および改善する作用があることが報告され[14]，グレリンの糖尿病性神経障害への臨床応用が期待されている．

デスアシルグレリンの生理作用

脂肪酸修飾されていないデスアシルグレリンは血中にグレリンよりも多く存在するが，グレリンの末梢投与で認められる摂食亢進作用やGH分泌促進作用がなく非活性型と考えられていた．しかし，デスアシルグレリンをラット脳室内に投与すると，グレリン投与に比べると弱いなが

らも摂食亢進作用を示し，デスアシルグレリンは中枢においてGHS-R以外の未知の受容体または結合蛋白に作用し，オレキシン系を介して摂食亢進作用を発揮することが報告されている[15]．また，デスアシルグレリンを過剰発現させたトランスジェニックマウスでは白色脂肪の低形成と糖代謝やインスリン感受性の亢進を認め，デスアシルグレリンが末梢で生理作用を有することが示されている[16]．また最近，デスアシルグレリンが2型糖尿病患者の血管内皮前駆細胞(EPC)機能を改善することが報告[17]され，動脈硬化を有する糖尿病患者への新たな治療法開発に向けた臨床研究が期待される．

デスアシルグレリンが，心筋細胞株のH9c2細胞，GHS-Rを発現しないラット脂肪細胞，前立腺癌細胞株に作用することが報告され，非活性型と考えられていたデスアシルグレリンが未知の受容体を介して作用している可能性が考えられている．しかし，ヒトゲノムのBLAST検索ではヒトグレリン受容体のホモログはいまだ同定されていない．

オベスタチンの同定とその生物活性に関する疑問

オベスタチンは，2005年にバイオインフォマティクス的手法を用いてグレリンの前駆体蛋白より推定され，ラット胃から同定された23アミノ酸残基からなるペプチドである[18]．グレリンの摂食亢進や脂肪蓄積作用と異なり，オベスタチンに摂食抑制作用，空腸収縮阻害作用，体重増加抑制作用を認め，同一の前駆体蛋白に由来するグレリンとオベスタチンは拮抗する機能を有すると報告され注目を集めた．

しかし，オベスタチンの報告後に各研究室で追試がなされ，その生物活性やGPR39の内在性リガンドに関する知見が再現されず，その生物活性に疑問が投げかけられている．UCLAのTachéらのグループは，オベスタチン末梢投与はラットおよびマウスの摂餌量やラット胃排出能を変化させず，また，オベスタチンを大量投与してもコレシストキニン(CCK)末梢投与による胃運動や迷走神経活動に影響しないこと，GPR39発現細胞の細胞内cAMP上昇活性を有さない知見から，オベスタチンの名称をghrelin-associated peptide(GAP)に変更することを提案している[19]．われわれも，ラット中枢および末梢へのオベスタチン投与後の摂食調節作用やグレリンとの相互作用を検討したが，オベスタチンは摂食に影響を与えなかった．オベスタチンを最初に報告したグループによる追試で，オベスタチンの摂食抑制作用は中枢投与15分後でのみ認められたと報告された[20]．ラット中枢へのオベスタチン投与で飲水が抑制されたとの報告も散見されるが，オベスタチンの種族間で異なるアミノ酸配列やグレリンと比較しその血中濃度や組織含量の著しい低値から，プロテアーゼによるペプチド断端との意見もある[1]．

おわりに

グレリンは末梢および中枢投与で機能する唯一の摂食亢進物質で，その発見以降レプチンと並んで摂食・エネルギー代謝調節メカニズムの解明に寄与している．今後さらにグレリンおよびデスアシルグレリンの多彩な生理作用やghrelin *O*-acyltransferase(GOAT)の調節機構が明らかにされ，グレリンやGOATの作動薬・拮抗薬の開発研究の進歩が期待される．

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グレリンによる摂食調節機構

A Mechanism of Feeding Regulation by Ghrelin

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Key Words
- グレリン
- アシル化修飾
- 迷走神経
- 視床下部
- 弓状核

Summary

Ghrelin is a 28-amino-acid peptide that was isolated from the stomach in 1999, and discovered as the endogenous ligand of growth hormone secretagogue receptor. Ghrelin induces a positive energy balance and weight gain by stimulating food intake through GH-independent mechanisms. Peripherally and centrally administered ghrelin induces appetite and increases food intake. Ghrelin stimulates appetite by peripheral pathway via the vagus nerve and it is the only known peptide that transmits a starvation signal from a peripheral organ to the central nervous system. In hypothalamus, ghrelin activates the arcuate nucleus and orexigenic peptides (neuropeptides Y and agouti-related peptide) are produced in the nucleus. Clinical applications of ghrelin have been investigated in obesity, anorexia nervosa, cachexia, and sarcopenia.

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呼吸器内科を専門としており，慢性呼吸不全や癌カヘキシアへのグレリン臨床応用に奮闘しています。グレリンはさまざまな治療への可能性を秘めたホルモンです。

はじめに

グレリンは，1999年にKojima, Kangawaらによってヒトの胃から発見されたペプチドで，当初は成長ホルモン（GH）分泌を強力に刺激するgrowth hormone secretagogue receptor（GHS-R）の内因性リガンドとして知られていた[1]。グレリンは胃底腺のA/X様細胞から産生され，ヒトにおいて遺伝子は3番染色体短腕に局在している。94個のプログレリンよりシグナルペプチドが切断され，C末端側はオベスタチンとして分泌されている。グレリンは28アミノ酸からなるペプチドで，3番目のセリン残基がオクタン酸によりアシル化されており，このアシル化修飾がグレリンの生物活性にとって必須のものと考えられている[1]。グレリンのアシル化はグレリン-O-アシル-トランスフェラーゼにより修飾される。血中グレリンの半減期は約10分と短く，容易に不活化されてデスアシル体となる。

グレリンを実験動物に中枢あるいは末梢に投与すると，摂食が亢進し，体重が増加する。一方，抗グレリンIgGを脳室内または静脈内へ投与すると，

摂食は抑制されることから，グレリンは内在性の摂食亢進物質であると考えられる[2]。マウス脳室内へのグレリン投与により，摂食が亢進し，体重と脂肪量が増加する。この現象はGH欠損ラットへのグレリン投与でも同じように確認されており，グレリン投与によるこれらの作用はGH非依存性と考えられる[3]。また，ヒトにおいてもグレリンの摂食亢進作用は確認されており，健常人にグレリンを5 pmol/kg/分で静脈内持続投与しながら食事をすると，摂取エネルギー量は約28%増加すると報告されている[4]。その後，グレリンは摂食亢進だけでなく，エネルギー同化，抗炎症，心機能改善，自律神経調整，消化管運動刺激などさまざまな生理作用を有することが明らかとなってきた。現在，これらの多彩な生理作用を利用して，人工関節置換術後，消化器癌術後，摂食障害，機能性ディスペプシア，癌カヘキシア，慢性心不全カヘキシア，慢性腎不全カヘキシアにグレリンを投与する臨床試験が行われている。本稿では，グレリンの摂食調節機構について概説する。

グレリン分泌とその制御

グレリンは，主に胃のA/X様細胞といわれていた貯蔵顆粒含有内分泌細胞から分泌される。一方，グレリン受容体は迷走神経節の細胞体で産生され，求心線維末端胃側まで軸索輸送される。ここでグレリンと結合し，迷走神経求心路の電気活動を抑制する。このシグナルが延髄孤束核でシナプスを変え，ノルアドレナリン含有ニューロンに伝達され，グレリンのシグナルは視床下部に伝達される[5]。

末梢血中のグレリン濃度は空腹感とよく相関し，食前に高値で食後には血中濃度が低下する[6)-8)]。高カロリー摂取であるほど食後の血中グレリン濃度抑制が強く[9]，脂質やたんぱく質よりも特に炭水化物による抑制効果が強い[10]。摂食に伴うグレリン分泌制御メカニズムの詳細ははっきりとは解明されていない。しかしながら，摂食後に分泌されるインスリン[11]やペプチドYY，コレシストキニン[12)13)]などの消化管ホルモンがグレリン分泌抑制に作用していると考えられる。また，グレリンはBMI（body mass index）と負に相関し，体重減少とは非常によく相関することが知られている[14)15)]。

グレリンによる食欲亢進作用

グレリンを末梢に投与すると，ヒト，実験動物のどちらにおいても食欲が刺激され，摂食が亢進する[4)16)]。グレリンは，末梢投与により食欲を刺激することが知られている唯一のホルモンである。グレリン末梢投与による摂食亢進作用の主な作用経路は迷走神経求心路と考えられている[5]。迷走神経は，消化管からの種々の情報を，脳幹を経て間脳や新皮質に伝達する役割の脳神経である。迷走神経は運動と感覚の両線維を有しており，感覚神経の細胞体は迷走神経節に存在する。腹部迷走神経線維の約90%は感覚線維（求心性線維）であるといわれている。迷走神経終末は胃の粘膜層にも存在し，同部にはグレリンを産生するA/X様細胞も存在する。A/X様細胞はグレリン-O-アシル-トランスフェラーゼを発現しているため，局所で活性型グレリンを産生することができる。グレリン受容体であるGHS-R1aは迷走神経求心性ニューロンで産生され，求心線維末端へ輸送されるため，グレリンは胃粘膜局所で活性型として産生されると，胃粘膜に存在する迷走神経求心線維末端のGHSR-1aと結合して，そのシグナルが求心性に延髄孤束核へ伝達される。ここでいったんニューロンを変えてこの情報は視床下部のニューロペプチドY（NPY）/agouti関連蛋白（aouti-related protein：AgRP）ニューロンとgrowth hormone releasing hormone（GHRH）ニューロンへ伝えられる。GHRHニューロンへ伝達された情報は下垂体からのGH分泌を刺激し，NPY/AgRPニューロンへ伝えられた情報は食欲を刺激する（図1）。視床下部に存在する弓状核は摂食調節を中心的に担っており，食欲を亢進させ摂食を促進するNPY/AgRPニューロンと摂食行動を抑制するプロオピオメラノコルチン（POMC）ニューロンが存在する。末梢投与されたグレリンの刺激は，迷走神経，延髄孤束核を介して視床下部弓状核においてNPYやAgRP産生を促進する[17]。これらの摂食促進蛋白は脳室内投与により摂食促進作用を有することが知られている[18]。視床下部弓状核に存在するNPY/AgRPニューロンにはグ