Predictors of Gastric Myoelectrical Activity in Type 2 Diabetes Mellitus

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Background: Previous studies have clearly demonstrated the delayed gastric emptying of solid meals in diabetics, whereas their gastric myoelectrical activity, which primarily determines gastric motility, has not yet been fully confirmed.

Goals: This study aimed to clarify the characteristics and potential predictors of gastric myoelectrical activity in type 2 diabetics.

Study: Twenty-eight diabetics and 18 healthy controls participated. Duodenal biopsy sample was used for reverse transcription-polymerase chain reaction to evaluate cholecystokinin and motilin mRNA contents. Electrogastrography was performed before and after the test meal, and was assessed in terms of dominant frequency; dominant frequency instability coefficient; and the percentage of bradygastria, normogastria, and tachygastria.

Results: Over the entire recording period, dominant frequency was significantly lower, and dominant frequency instability coefficient and the percentage of bradygastria were significantly higher in diabetics than in controls. In diabetics, the multiple regression analysis demonstrated that dominant frequency instability coefficient and the percentage of tachygastria in the fasting period were dependent on fasting plasma glucose level and HbA1c, respectively. Moreover, dominant frequency over the entire period and the postprandial percentage of bradygastria were significantly associated with body mass index; the fasting percentage of bradygastria and postprandial dominant frequency instability coefficient were associated with fasting serum leptin level; the postprandial percentage of bradygastria was also associated with cholecystokinin mRNA content.

Conclusions: Gastric myoelectrical activity in type 2 diabetics is impaired on dominant frequency, dominant frequency instability coefficient, and the percentage of bradygastria and predicted by body mass index, fasting serum leptin level, and cholecystokinin mRNA content besides the glycemic status.

Key Words: body mass index, cholecystokinin, electrogastrography, leptin, type 2 diabetes mellitus

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Gastroenteropathy occurs in as many as 76% of patients with diabetes and includes diabetic gastroparesis, which may cause upper gastrointestinal symptoms such as early satiety, nausea, vomiting, anorexia, fullness, abdominal discomfort, and epigastric pain.[1,2] Until now, in diabetics, the delayed gastric emptying has been clearly demonstrated by scintigraphic techniques[3,4] and lower contraction amplitudes, a smaller motility index, and pyloric spasm have been shown in manometric studies.[5–7] Moreover, it is revealed that the interstitial cells of Cajal that are fundamental in the generation of gastric slow waves are depleted in diabetics.[8] However, the characteristics of gastric myoelectrical activity resulting from the depletion of the interstitial cells of Cajal in diabetics have not yet been fully confirmed.

Traditionally, diabetic gastroparesis had been considered to be caused by irreversible autonomic nerve damage[9]; however, recent study showed that the correlation between cardiovascular autonomic neuropathy and gastric motility was poor.[10] Moreover, acute changes in plasma glucose levels rather than neuropathy have been demonstrated to have a major reversible effect on gastric and intestinal motility in diabetics.[11] On the other hand, since the relationship between body mass index (BMI) and gastrointestinal symptoms or gastric myoelectrical activity in normal subjects, and the delayed gastric emptying in obese patients have been suggested,[12–14] increased BMI that is commonly seen in type 2 diabetics might be involved in diabetic gastroparesis. Surprisingly, however, the relationship between BMI and gastric motility in diabetics has been little investigated.[15] Another factor that may play an important role in the regulation of gastric motility is the gut neuroendocrine system. Although plasma levels of gut hormones that are suspected to influence gastric motility have been assessed in diabetics, the results are still controversial. In this study, we measured the mRNA contents of gut hormones to estimate their local release amount,[16,17] because plasma hormone level do not necessarily reflect local interaction with specific receptors located on nearby target cells.[18] In addition, although it has been suggested that gastrointestinal symptoms in diabetics result from disturbed gastrointestinal motility,[19] the relationship between the two is actually weak.[20] Thus, determinants of gastric motility and gastrointestinal symptoms in diabetics are poorly defined.

Therefore, in this study, we aimed to clarify the characteristics of gastric myoelectrical activity in type 2 diabetics using electrogastrography (EGG) and to determine the potential predictors of gastric myoelectrical activity and gastrointestinal symptoms in these patients.

MATERIALS AND METHODS

Subjects

Diabetic patients were randomly selected from patients attending the Department of Internal and Geriatric

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Medicine, Kobe University Hospital, and volunteers were recruited as healthy controls on the basis of having no gastrointestinal symptoms. None of them had a medical history of digestive diseases, and none was taking medications known to alter gastrointestinal motility. A written informed consent was obtained in all cases, and the study was approved by the ethics committee of the Kobe University Graduate School of Medicine. Upper gastrointestinal endoscopy confirmed that none of the subjects had "substantial abnormalities," such as peptic ulcers, cancers, or deformities, in any location.

Assessment of Autonomic Neuropathy

The assessment of autonomic nerve function was essentially based on the protocol of Ewing and Clarke.[21] Parasympathetic nerve function was evaluated by both the heart rate variation during deep breathing for a minute and the immediate heart rate response to standing up. When both of these 2 tests were abnormal, parasympathetic neuropathy was defined. Sympathetic nerve function was assessed by the fall in systolic blood pressure in response to standing, and sympathetic neuropathy was diagnosed when this test was found to be abnormal.

Assessment of *Helicobacter pylori* Infection Status

Because *Helicobacter pylori* (Hp) infection has been suggested to be involved in gastric motility disorder,[22] 2 biopsy specimens from the gastric antrum and upper body along the greater curvature were obtained during endoscopy for a rapid urease test (Helicocheck, Otsuka Pharmaceutical Co Ltd, Tokyo, Japan). Moreover, polymerase chain reaction (PCR) using gastric juice as a template was performed to detect the UreA gene of Hp as described by Yoshida et al.[23] The subjects were considered to be Hp positive if the results of one or both of these 2 methods were positive, and Hp negative if both methods showed negative results.

Symptom Score

Each subject completed a modified dyspepsia questionnaire that was similar to that described by Tack et al[24] before undergoing EGG. The subject was asked to grade the intensity of 9 symptoms that consisted of anorexia, nausea, early satiety, bloating/fullness, vomiting, abdominal pain, dysphagia, heartburn, and acid regurgitation on a scale from 0 to 3. The scale for intensity was 0, absent; 1, mild, present but not very bothersome; 2, relevant, bothersome but not interfering with daily activities; and 3, severe, interfering with daily activities. The total score for each symptom was adopted as the symptom score.

Gene Expression of Gastrointestinal Hormone mRNA

The other biopsy specimen was obtained from the opposite site of a duodenal papilla during endoscopy. The total RNA in this specimen was extracted with the RNeasy kit (QIAGEN K.K., Tokyo, Japan) and converted into cDNA with SuperScript II RNase H^- reverse transcriptase (Invitrogen Japan K.K., Tokyo, Japan). cDNA was coamplified by multiplex PCR in a single tube, as described by Jensen et al,[25] using the following thermal protocol: initial denaturing step of 95°C for 3 minutes, followed by 25 cycles of 95°C for 45 seconds, of 55°C for 45 seconds, and of 72°C for 1 minute, and a final extension step of 72°C for 10 minutes.

The primer pairs of human gut hormones were as follows: cholecystokinin (CCK) (forward, 5′-AAG AAC CTG CAG AAC CTG GA-3′, and reverse, 5′-TGT GTG GTT GCA CTG GAC AA-3′; expected size, 282 bp),[26] and motilin (forward, 5′-GAT GGT ATC CCG TAA GGC TG-3′, and reverse, 5′-TCT GGA GTT CAT CCT CAT TCC-3′; expected size, 277 bp).[27] Human glyceraldehyde-3-phosphate dehydrogenase (forward primer, 5′-CCA CCC ATG GCA AAT TCC ATG GCA-3′, and reverse primer, 5′-TCT AGA CGG CAG GTC AGG TCC ACC-3′; expected size 593 bp)[28] was employed as an internal control.

PCR products were separated by electrophoresis through 6% polyacrylamide gels stained with ethidium bromide and photographed by charge couple device camera. The intensity of each band was quantified by a Lane Analyzer version 3 (Atto Corp, Tokyo, Japan) on a personal computer, and the mRNA content of each gut hormone was evaluated in terms of the ratio of intensity of the target band to that of the coamplified glyceraldehyde-3-phosphate dehydrogenase. In the preliminary studies, we empirically determined the range of the number of PCR cycles and the amount of each primer pair to maintain parallel exponential amplification of target gene and internal control. The accuracy of quantification was additionally confirmed by changing the amount of cDNA.

EGG

EGG was performed within a week after upper gastrointestinal endoscopy. After overnight fasting and prohibition of any drugs, 3 disposable silver/silver chloride electrocardiogram electrodes (Nihon Koden, Tokyo, Japan) were placed on the abdominal skin. The electrodes were connected to an EGG recording device (Microdigitrapper EGG, Medtronic-Synectics, Shoreview, MN) with low and high cutoff frequencies set at 0.5 and 18 cpm, respectively. The EGG signal was digitized with a sampling frequency of 4 Hz and stored on a portable recording device.

The subjects underwent a 1-hour fasting EGG recording before consuming the standardized test meal, which consisted of a piece of buttered toast, a hard-boiled egg, and 200 mL of milk (total 380 kcal). After the consumption of the test meal, an additional 1 hour of EGG recording was continued.

EGG Data Analysis

All EGG data in the recording device were uploaded to a personal computer, and the sections contaminated by motion artifacts were deleted visually. The remaining EGG data were then submitted to evaluation with software for EGG analysis (Multigram Version 6.31, Medtronic-Synectics). The following parameters were assessed for each period of interest:

1. Dominant frequency, which indicates the frequency at which the EGG signal reaches peak power and reflects the frequency of the gastric slow waves.
2. Dominant frequency instability coefficient, which specifies the stability of the gastric electrical peak and reflects fine changes in the gastric slow waves.
3. The percentage of bradygastria, normogastria, and tachygastria, which represent the percentage time of dominant in, respectively, the slow (0.5 to 2.0 cpm), normal (2.0 to 4.0 cpm), and fast (4.0 to 9.0 cpm) ranges during the observation period.

4. Power ratio, which is expressed as the ratio of fed to fasting EGG power at the dominant frequency in the power spectrum.

Plasma Glucose and Serum Insulin and Leptin Levels

During the recording of EGG, venous blood samples were taken 10 minutes before the ingestion of the test meal and then at 0, 15, 30, and 60 minutes thereafter. At each time, plasma glucose and serum insulin levels were determined with the glucose hexokinase method and an immunoradiometric assay (BML Inc, Tokyo, Japan), respectively. Postprandial glucose and insulin responses were evaluated with the area under the curve (AUC) for concentration versus time ($AUC_{Glucose}$, $AUC_{Insulin}$). Fasting serum leptin level was also determined by means of radioimmunoassay (BML Inc).

Data Analysis and Statistics

All data were expressed as mean ± SD, if not otherwise stated. Comparisons of sex and Hp infection status between diabetics and controls were assessed with the χ^2 test, and the Mann-Whitney U test was performed for other comparisons. The Pearson correlation was used to look at the relationship between parameters for EGG analysis or symptom score and other variables. A forward stepwise multiple regression analysis was carried out to determine the independent predictors of EGG analysis parameters; the variables that in univariate analysis correlated with each EGG analysis parameter with a P value < 0.1 were forced into the model. A value of $P < 0.05$ was accepted as statistically significant. All calculations were carried out using SPSS software version 12 (SPSS Inc, Chicago, IL).

RESULTS

Characteristics of Type 2 Diabetics and Normal Controls

Twenty-eight type 2 diabetic patients (9 males and 19 females, age 65.3 ± 13.4 y) and 18 healthy controls (9 males and 9 females, age 64.3 ± 15.0 y) participated in this study. Seventeen diabetic patients were receiving oral antidiabetics, 8 patients insulin therapy, and 3 patients dietary modification. There were no statistical differences in age, sex, BMI, $AUC_{Glucose}$, fasting serum insulin level, fasting serum leptin level, and Hp infection status between diabetics and controls. HbA1c and fasting plasma glucose level were significantly higher, and $AUC_{Insulin}$ was significantly lower in diabetics than in controls ($P < 0.05$, $P < 0.01$, $P < 0.01$, respectively). Among 28 diabetics, 17 patients accepted the assessment of autonomic nerve function, but parasympathetic function could not be evaluated in 1 patient because of atrial fibrillation. Parasympathetic dysfunction was found in 3 patients, and 5 had sympathetic dysfunction. Motilin mRNA content in diabetics was significantly higher than that in controls ($P < 0.05$), whereas there was no significant difference in CCK mRNA content between diabetics and controls. Symptom score in diabetics was 1.2 ± 2.0. All characteristics of the subjects are shown in Table 1.

TABLE 1. Characteristics of Type 2 Diabetics and Normal Controls

	Diabetics (n = 28)	Controls (n = 18)
Age (y)	65.3 ± 13.4	64.3 ± 15.0
Sex (male/female)	9/19	9/9
Therapeutic regimen (diet/oral antidiabetics/insulin)	3/17/8	
Body mass index (kg/m^2)	24.6 ± 4.5	23.3 ± 3.2
HbA1c (%)	8.5 ± 2.0*	5.2 ± 0.5
Fasting plasma glucose (mg/dL)	156.5 ± 44.3**	92.1 ± 10.8
$AUC_{Glucose}$ (mg·h/dL)	195.1 ± 54.1	219.7 ± 47.5
Fasting serum insulin (μU/mL)	8.8 ± 10.0	6.2 ± 2.5
$AUC_{Insulin}$ (μU·h/mL)	20.9 ± 16.5**	56.4 ± 16.6
Fasting serum leptin (ng/mL)	7.5 ± 6.9	8.3 ± 4.7
Parasympathetic neuropathy (+/−)	3/13	—
Sympathetic neuropathy (+/−)	5/12	—
Helicobacter pylori infection (+/−)	20/8	10/8
CCK (/GAPDH mRNA)	1.18 ± 0.46	1.05 ± 0.48
Motilin (/GAPDH mRNA)	1.31 ± 0.59*	1.03 ± 0.64
Symptom score	1.2 ± 2.0	—

Results are expressed as mean ± SD or number of subjects.
*$P < 0.05$, **$P < 0.01$ versus controls.
AUC indicates area under the curve; CCK, cholecystokinin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

EGG

Twenty patients among 28 diabetics and 10 subjects among 18 controls tolerated EGG recording. EGG data obtained during the fasting and postprandial period are shown in Table 2.

Fasting Period

The dominant frequency was significantly lower in diabetics than in controls ($P < 0.01$), though dominant frequency instability coefficient in diabetics was significantly higher than that in controls ($P < 0.05$). The percentage of bradygastria in diabetics was significantly higher than that in controls ($P < 0.05$), and the significantly lower percentage of normogastria was observed in diabetics as compared with controls ($P < 0.05$).

TABLE 2. Parameters for EGG Analysis in Type 2 Diabetics and Normal Controls

	Diabetics (n = 20)	Controls (n = 10)
Fasting period		
DF (cpm)	1.4 ± 1.3**	2.9 ± 1.0
DFIC (%)	76.9 ± 27.1*	49.4 ± 21.5
Bradygastria (%)	45.8 ± 29.3*	23.1 ± 20.7
Normogastria (%)	49.7 ± 28.0*	72.2 ± 23.3
Tachygastria (%)	4.5 ± 6.3	4.7 ± 6.9
Postprandial period		
DF (cpm)	1.9 ± 1.4*,†	3.3 ± 0.3
DFIC (%)	63.8 ± 28.0**	33.1 ± 13.4
Bradygastria (%)	32.8 ± 21.5*,†	13.6 ± 10.5
Normogastria (%)	61.0 ± 25.6*,†	84.0 ± 10.0
Tachygastria (%)	6.0 ± 10.2	2.3 ± 3.4
PR	2.3 ± 2.9	3.1 ± 2.6

Results are expressed as mean ± SD.
*$P < 0.05$, **$P < 0.01$ versus controls.
†$P < 0.05$ versus each in fasting period.
DF indicates dominant frequency; DFIC, dominant frequency instability coefficient; EGG, electrogastrography; PR, power ratio.

Postprandial Period

Although a significant increase of the dominant frequency after the test meal was observed only in diabetics, it was still significantly lower than in controls ($P < 0.05$). Furthermore, dominant frequency instability coefficient in diabetics was significantly higher ($P < 0.01$), as seen in the fasting period, because the postprandial decrease of dominant frequency instability coefficient in both groups was comparable. Significant decreased percentage of bradygastria and increased percentage of normogastria were caused in only diabetics; however, the percentage of bradygastria in diabetics was still significantly higher than in controls ($P < 0.05$), and the percentage of normogastria in diabetics was significantly lower ($P < 0.05$). On the other hand, power ratio tended to be lower in diabetics than in controls; however, the difference did not reach statistical significance.

Univariate Analysis of Relationship Between Parameters for EGG Analysis and Other Variables in Diabetics

Pearson correlation coefficients for the relationships between clinical characteristics and EGG analysis parameters in diabetics were calculated. In the fasting period, BMI negatively correlated with dominant frequency ($P < 0.01$) and the percentage of normogastria ($P < 0.05$), and positively with the percentage of bradygastria ($P < 0.05$). In the postprandial period, BMI also negatively correlated with dominant frequency ($P < 0.01$) and positively with dominant frequency instability coefficient ($P < 0.05$) and the percentage of bradygastria ($P < 0.05$), but negative correlation between BMI and the percentage of normogastria was relatively weak ($P < 0.1$). HbA1c positively correlated with the percentage of tachygastria ($P < 0.05$) and negatively with dominant frequency instability coefficient weakly ($P < 0.1$) in the fasting period. The fasting plasma glucose level negatively correlated with dominant frequency instability coefficient ($P < 0.01$) and the percentage of bradygastria ($P < 0.05$) and positively with the percentage of normogastria ($P < 0.05$) in the fasting period, but $AUC_{Glucose}$ weakly correlated positively with dominant frequency and negatively with dominant frequency instability coefficient in the postprandial period (each $P < 0.1$). The fasting serum leptin level negatively correlated with dominant frequency ($P < 0.05$) and positively with dominant frequency instability coefficient ($P < 0.01$) in the postprandial period, but it weakly correlated positively with the percentage of bradygastria and negatively with the percentage of normogastria in the fasting period (each $P < 0.1$). The presence of parasympathetic neuropathy negatively correlated with dominant frequency instability coefficient in the fasting period ($P < 0.05$). On the other hand, CCK mRNA content positively correlated with the percentage of bradygastria in the postprandial period ($P < 0.05$); however, it weakly correlated positively with the percentage of bradygastria and negatively with the percentage of normogastria in the fasting period and negatively with the percentage of normogastria and power ratio in the postprandial period (each $P < 1.0$). Concerning the therapeutic regimen, daily practice of insulin injection positively correlated with the percentage of tachygastria ($P < 0.05$) in the fasting period. Other features did not correlate with any EGG analysis parameters.

Predictors of EGG in Diabetic Patients

Stepwise multiple linear regression analyses were performed to examine the independent predictors of EGG in diabetics. On the basis of univariate analysis, BMI, HbA1c, the fasting plasma glucose level, the fasting serum leptin level, the presence of parasympathetic neuropathy, CCK mRNA content, and the therapeutic regimen were included in the model in the fasting period, and BMI, $AUC_{Glucose}$, the fasting serum leptin level, and CCK mRNA content were selected for the model in the postprandial period. The results of stepwise regression analysis are shown in Table 3. The variations in dominant frequency in the fasting and postprandial period were explained by BMI (68.6%, $P = 0.006$, and 37.3%, $P = 0.009$, respectively). Dominant frequency instability coefficient variation was significantly dependent on the fasting plasma glucose level in the fasting period (56.2%, $P = 0.020$) and the fasting serum leptin level in the postprandial period (35.9%, $P = 0.011$). The fasting serum leptin level and the presence of parasympathetic neuro-

TABLE 3. Forward Stepwise Multiple Linear Regression Analysis for Each EGG Parameter in Type 2 Diabetic Patients

Dependent Variable	Independent Variable	Coefficient	SE	β	*P*	R^2 Change (%)
Fasting period						
DF	BMI	−0.211	0.054	−0.828	0.006	68.6
DFIC	FPG	−0.374	0.125	−0.750	0.020	56.2
Bradygastria	Leptin	4.551	0.847	0.834	0.002	66.0
$R^2 = 0.856$, $P = 0.003$	PN	−26.777	9.389	−0.443	0.029	19.6
Normogastria	Leptin	−4.056	1.197	−0.788	0.012	62.1
Tachygastria	HbA1c	2.168	0.491	0.858	0.003	73.6
Postprandial period						
DF	BMI	−0.208	0.070	−0.611	0.009	37.3
DFIC	Leptin	2.201	0.759	0.599	0.011	35.9
Bradygastria	BMI	2.440	1.032	0.462	0.033	30.1
$R^2 = 0.486$, $P = 0.009$	CCK mRNA	28.495	12.710	0.438	0.042	18.5
Normogastria		No significant association				
Tachygastria		No significant association				
PR		No significant association				

$P < 0.05$ is accepted as statistically significant.

BMI indicates body mass index; CCK, cholecystokinin; EGG, electrogastrography; FPG, fasting plasma glucose level; PN, parasympathetic neuropathy; PR, power ratio.

pathy were independently associated with the percentage of bradygastria in the fasting period (total $R^2 = 0.856$, $P = 0.003$); each accounted for 66.0% ($P = 0.002$) and 19.6% ($P = 0.029$), respectively, of the total variance. In the postprandial period, the percentage of bradygastria variation was explained by BMI (30.1%, $P = 0.033$) and CCK mRNA content (18.5%, $P = 0.042$); total $R^2 = 0.486$, $P = 0.009$. The percentage of normogastria variation in the fasting period was dependent on the fasting serum leptin level (62.1%, $P = 0.012$). HbA1c was a significant determinant of the percentage of tachygastria in the fasting period, accounting for 73.6% ($P = 0.003$) of its variability.

Univariate Analysis of Relationship Between Symptom Score and Other Variables in Diabetics

Pearson correlation coefficients for the relationships between symptom score and clinical characteristics or EGG analysis parameters in diabetics are shown in Table 4. There was no significant correlation between symptom score and other variables, though BMI showed a tendency to negatively correlate with symptom score.

Correlations of BMI With Fasting Serum Leptin Level and mRNA Contents of CCK and Motilin

In the predictors of EGG analysis parameters in diabetics, each relationship among BMI, fasting serum leptin level, and CCK mRNA content was further investigated with univariate analysis (Fig. 1). BMI showed a significant positive correlation with fasting serum leptin level and CCK mRNA content ($P = 0.0229$, $P = 0.0259$, respectively), but there was no significant correlation between fasting serum leptin level and CCK mRNA content. Besides, motilin mRNA content, elevated in

TABLE 4. Pearson Correlation Coefficients for the Relationships Between Symptom Score and Clinical Characteristics, or EGG Parameters in Type 2 Diabetic Patients

Variables	
Age	−0.080
Sex	−0.160
BMI	−0.360†
HbA1c	−0.009
FPG	0.103
$AUC_{Glucose}$	0.333
FSI	−0.112
$AUC_{Insulin}$	−0.088
Leptin	−0.006
PN	−0.156
SN	−0.054
HP infection	0.207
CCK mRNA	−0.104
Motilin mRNA	−0.129
Fasting period	
DF	−0.060
DFIC	0.121
Bradygastria	−0.147
Normogastria	0.165
Tachygastria	−0.025
Postprandial period	
DF	−0.075
DFIC	−0.094
Bradygastria	−0.211
Normogastria	0.103
Tachygastria	0.165
PR	0.163
Therapeutic regimen	−0.023

†$P < 0.1$.

AUC indicates area under the curve; BMI, body mass index; CCK, cholecystokinin; DF, dominant frequency; DFIC, dominant frequency instability coefficient; EGG, electrogastrography; FPG, fasting plasma glucose level; FSI, fasting serum insulin level; HP, *Helicobacter pylori*; PN, parasympathetic neuropathy; PR, power ratio; SN, sympathetic neuropathy.

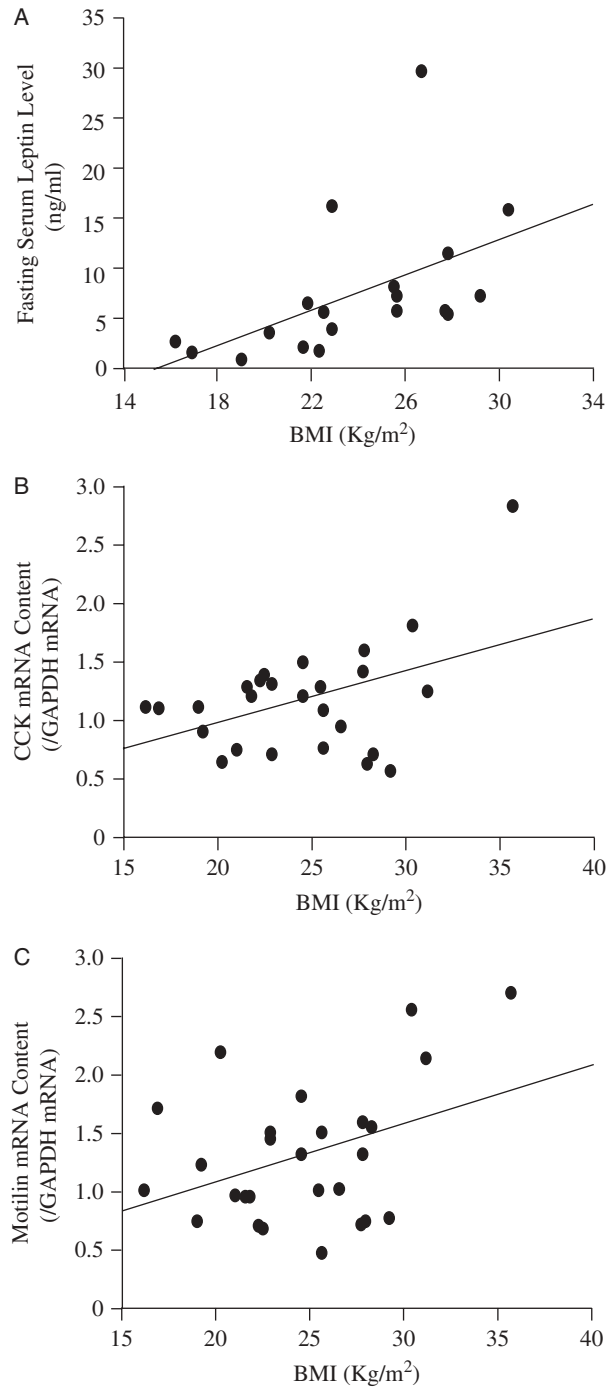

FIGURE 1. The relationship between BMI and fasting serum leptin level (A: $r = 0.514$, $P = 0.0229$), CCK mRNA content (B: $r = 0.434$, $P = 0.0259$), and motilin mRNA content (C: $r = 0.386$, $P = 0.0463$). BMI indicates body mass index; CCK, cholecystokinin.

diabetics, was also significantly correlated with BMI ($P = 0.0463$).

DISCUSSION

This study clearly demonstrated that gastric myoelectrical activity in type 2 diabetics was impaired on dominant frequency, dominant frequency instability coefficient, and the percentage of bradygastria throughout the recording period. In the previous studies, the high percentage of tachygastria and low dominant frequency in type 1 diabetics, high dominant frequency instability coefficient in types 1 and 2 diabetics and the low percentage of normogastria and power ratio especially in diabetics with autonomic neuropathy, and the increased percentage of bradygastria in type 1 diabetes have been observed.[29–31] These discrepancies in EGG findings could be the result of differences in types of diabetes, plasma glucose levels, status of complications, and symptoms, as well as a lack of standardization of EGG recording procedures. Our EGG data in type 2 diabetics were almost compatible with these previous results. Until now, the relationship between parameters for EGG analysis and gastric motor function has not yet been well determined. However, only one research which assessed EGG simultaneously with manometry in diabetics indicated that lower postprandial increase in myoelectrical activity and the dominant frequency instability coefficient were accompanied by that in antral motility.[32] Moreover, diabetic patients with delayed gastric emptying had a higher percentage of gastric dysrhythmias or a lower postprandial increase in the dominant power.[33,34] Therefore, our EGG findings must be reflecting the impaired gastric motor function in diabetics.

Furthermore, this study evaluated the potential predictors of gastric myoelectrical activity in diabetics. It is widely believed that glycemic status has major value in predicting gastric motility in diabetics. In our results, the fasting plasma glucose level was negatively associated with dominant frequency instability coefficient, and HbA1c was positively associated with the percentage of tachygastria in the fasting period. The former association seems to conflict with the previous work in which the HbA1c level was positively associated with dominant frequency instability coefficient in type 1 and type 2 diabetics,[30] though the latter association is quite likely to be supported by the study that hyperglycemia induced tachygastria in type 1 diabetics, using the glucose clamp technique.[35] The precise mechanism by which hyperglycemia disturbs gastrointestinal motility is not clear; however, decreased vagal-cholinergic activity by hyperglycemia might be involved.[36] Anyway, little information is available about the relationship between glycemic status and EGG especially in the fasting period, so our data need to be further confirmed.

Also, a negative association between the presence of parasympathetic neuropathy and the fasting percentage of bradygastria was found. As for the effect of autonomic neuropathy on gastric myoelectrical activity in diabetics, even though Mayaudon et al[29] found no correlation between the two, Kawagishi et al[30] revealed that power ratio in diabetics was negatively associated with autonomic neuropathy. In healthy children, furthermore, recent study using the spectral analysis of heart rate variability demonstrated that the indicator of sympathovagal balance was positively correlated with the postprandial increase in EGG-dominant power.[37] However, a negative association between the presence of autonomic neuropathy and the percentage of bradygastria has not been reported yet. Moreover, in this study, as actually only 3 diabetics were accompanied by parasympathetic neuropathy and the Pearson correlation indicated that there was no relationship between the presence of parasympathetic neuropathy and the fasting percentage of bradygastria, we could not determine the relationship between the two.

Besides glycemic status and the presence of autonomic neuropathy, the present study clearly demonstrated that BMI was negatively associated with dominant frequency throughout the recording period and positively associated with the postprandial percentage of bradygastria in diabetics. As there is no study about the relationship between BMI and gastric motility in diabetics but one which showed the negative correlation between the gastric emptying rate and BMI,[15] this is the first report demonstrating the impact of BMI on gastric myoelectrical activity in diabetics. As approach to the mechanisms by which increased BMI disturbs gastric myoelectrical activity, we subsequently investigated the role of serum leptin level. Leptin, a hormone secreted not only from adipose tissue, but also from gastric mucosa and that regulates food intake and energy balance,[38,39] has been well known to correlate with BMI. Moreover, leptin has been confirmed to reduce gastric motility via its central or peripheral effect.[40] In this study, the fasting percentage of bradygastria and the postprandial dominant frequency instability coefficient were positively associated with fasting serum leptin level in the stepwise regression analysis; furthermore, the fasting serum leptin level significantly correlated with BMI. Therefore, it is suggested that increased BMI impairs gastric motility via augmented leptin secretion.

On the other hand, it is well known that CCK delays gastric emptying physiologically,[41] thus we analyzed CCK mRNA content in duodenal mucosa to estimate the local CCK release, which may be modulating the vagal tone directly. In the current study, CCK mRNA content was positively associated with the postprandial percentage of bradygastria in diabetics, which seems to conflict with the results in previous research that CCK decreased the postprandial EGG amplitude without the effect to the frequency and regularity of the gastric slow wave.[42] However, power ratio which showed the tendency to be negatively associated with CCK mRNA content in univariate analysis was partially supported by the above reports. Anyway, there is no research to estimate the relationship between CCK mRNA content and gastric myoelectrical activity, therefore, our result needs to be further confirmed. Furthermore, our research showed that CCK mRNA content positively correlated with BMI in diabetics. As for the relationship between CCK and BMI, the results of a rat study that leptin secreted into the gastric juice stimulates CCK release and that CCK further stimulates leptin secretion into gastric juice through a positive feedback loop may be relevant.[43] However, as we could not determine the leptin level in gastric juice, the precise mechanisms through which increased BMI induces the augmentation of CCK mRNA content could not be clarified in this study.

Concerning motilin, which is well known to induce phase III contractions of the interdigestive migrating complex,[44] its mRNA content was significantly higher in

diabetics than in controls, but it did not correlate with any EGG analysis parameters. This high motilin mRNA content might be consistent with the high basal plasma motilin level noted in diabetics, especially in patients with neuropathy.[45] Persistent gastric dilation or retained food in the gastrointestinal tract in diabetic gastroparesis may contribute to elevated basal motilin levels.[46] Moreover, BMI also significantly correlated with motilin mRNA content in diabetics, which may be supported by the recent finding that leptin stimulated motilin mRNA expression in T84 cells,[47] though no significant correlation was found between motilin mRNA content and fasting serum leptin level.

Meanwhile, our data in univariate analysis demonstrated that there was no correlation between Hp infection status and any EGG analysis parameters in diabetics. In functional dyspepsia, Shiotani et al[48] showed that the fasting percentage of tachygastria was significantly higher in Hp-positive than Hp-negative patients and Lu et al[49] indicated the increased percentage of normal slow waves after Hp eradication. Moreover, in mice, Bercík et al[50] showed that chronic Hp infection induced impaired antral relaxation and acetylcholine release as well as altered content of sensory neurotransmitters in the stomach and spinal cord. However, in diabetics, a number of studies revealed that Hp infection was not associated with delayed gastric emptying,[51,52] which might support our result.

In this study, although no antidiabetic agents were administered to all diabetics before EGG recording, diabetics who had been treated with daily insulin injection showed the high percentage of tachygastria in fasting period in univariate analysis. Concerning the effect of insulin infusion on gastric motility, Kong et al[53] showed euglycemic hyperinsulinemia delayed gastric emptying of the meal in normal subjects, but it does not affect gastric emptying in type I and type II diabetes mellitus,[54] and Hasler et al[55] reported that acute hyperglycemia, but not hyperinsulinemia, inhibited fed antral motility and induced gastric dysrhythmias in healthy volunteers. However, these studies did not investigate the effect of long-term insulin injection on gastric motility, but the effect of short-term one. Coulson et al[56] clarified the role of long-term insulin injection in gastrointestinal dysmotility in diabetic rat study, in which lack of insulin, or excess insulin, increases M2 muscarinic receptor function of the ileum in vitro, leading to decreased acetylcholine release, and resulting in gastrointestinal dysmotility. These results may partially support our results. Anyway, as the percentage of tachygastria variation in fasting period was not explained by therapeutic regimen in stepwise regression analysis, the impact of it was considered to be relatively week.

We also attempted to determine the predictors of gastrointestinal symptoms in diabetics. Although weak correlation was detected between BMI and symptom score, any clinical characteristics and parameters for EGG analysis did not statistically contribute to symptom score, which appeared to be consistent with previous reports.[57]

In conclusion, type 2 diabetics are characterized by low dominant frequency, high bradygastria, and high dominant frequency instability coefficient in their gastric myoelectrical activity throughout the fasting and postprandial period. Besides glycemic status, BMI, fasting serum leptin level, and duodenal CCK mRNA content independently predict gastric myoelectrical activity in type 2 diabetics. It is suspected that increased BMI impairs gastric myoelectrical activity in type 2 diabetics through augmented CCK release secondary to the hypersecretion of leptin. Therefore, weight reduction may contribute to the improvement of gastric motility in type 2 diabetics, although it needs to be further investigated.

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