Table 4 Association between significant SNPs and histological traits in NAFLD subjects

SNP ID	Steatosis grade		Lobular inflammation		Hepatocyte ballooning		NAS		Fibrosis stage	
	β	*P*-value	β	*P*-value	β	*P*-value	β	*P*-value	β	*P*-value
rs780094	0.013	0.76	0.006	0.89	0.004	0.92	0.002	0.99	0.049	0.42
rs2954021	0.005	0.92	-0.140	**0.0016**	-0.072	0.09	-0.214	**0.029**	-0.152	**0.014**

Data were derived from linear regression analysis. Each phenotype was adjusted for age, sex, logarithmically transformed BMI, and the presence of DM. Bold entries indicate *P*-value < 0.05.

Table 5 Association between significant SNPs and anthropometric parameters in NAFLD subjects

SNP ID	BMI		VFA*		SFA*		V/S ratio*	
	β	*P*-value	β	*P*-value	β	*P*-value	β	*P*-value
rs780094	-0.004	0.37	0.023	0.051	-0.011	0.26	0.036	**0.0067**
rs2954021	-0.007	0.11	-0.024	**0.048**	-0.023	**0.021**	0.002	0.87

Data were derived from linear regression analysis. Values of BMI, VFA, SFA, and V/S ratio were logarithmically transformed. BMI and V/S ratio were adjusted for age and sex. VFA and SFA were adjusted for age, sex, and logarithmically transformed BMI. Bold entries indicate *P*-value < 0.05. *, n = 439.

Table 6 Logistic analysis of 3 SNPs on the association between NAFLD and control subjects

Explanatory valuables	*P*-value	OR (95% CI)
rs738409	4.1×10^{-13}	2.20 (1.78 - 2.72)
rs2954021	9.7×10^{-5}	1.52 (1.23 - 1.88)
rs780094	0.0011	1.42 (1.15 - 1.76)

The *P*-value and OR were derived from the following logistic regression model:
$Y=\beta_0+\beta_1\times rs738409+\beta_2\times rs2954021+\beta_3\times rs780094+\beta_4\times age+\beta_5\times sex+\beta_6\times \log_{10}(BMI)+\beta7\times DM$.

between rs780094 with lower plasma glucose (P = 0.014) and higher triglycerides ($P = 2.5\times10^{-5}$) was also observed in the control subjects. Originally, rs2954021 in *TRIB1* was reported to be associated with ALT [16]; however, we observed no association with ALT or AST in the NAFLD and control subjects. The A-allele of rs2954021 in *TRIB1*, which is a risk allele of NAFLD, was associated with lower lobular inflammation, NAS, and fibrosis grade, and rs780094 in *GCKR* was not associated with histological phenotype (Table 4). We also examined anthropometric parameters in the NAFLD patients and found that rs780094 in *GCKR* was associated with increased V/S ratio (P = 0.0067) (Table 5).

Next, we tested SNP×SNP epistasis, including rs738409 in *PNPLA3*, which was an SNP for NAFLD susceptibility. No SNP pairs showed significant epistatic effects on NAFLD (data not shown). We performed multiple logistic regression analysis of three genotypes (rs780094, rs2954021, and rs738409), age, sex, logarithmically transformed BMI, and the presence of DM as independent variables and found that the effects of these SNPs were independent (Table 6).

Discussion

After the first report of an association of *PNPLA3* rs738409 with NAFLD [9], many replication studies and meta-analysis were performed in various populations, verifying the importance of rs738409 in the development of NAFLD [11-14, 26, 27]. GWAS for NAFLD and ALT yielded SNPs in genes other than *PNPLA3* [15-17]; however, a few replication studies produced conflicting results. Among the 18 SNPs in this study, only six SNPs (rs2499604, rs780094, rs2645424, rs1227756, rs2862954, and rs6487679) were included in our previous GWAS [14]. The *P*-values for six SNPs exceeded the cut-off levels (5.0×10^{-5}) and proceeded to the second stage of analysis. The JSNP database (http://snp.ims.u-tokyo.ac.jp/) was used as a control; we made no adjustment for age, sex, BMI, or the presence of DM because clinical information was not available. The NAFLD sample size in our GWAS was relatively small (n = 392). Therefore, we investigated 18 SNPs associated with NAFLD and ALT susceptibility, including the six SNPs described above, in a larger

set of NAFLD patients and control subjects for whom clinical information was available.

In this study, we confirmed the association of rs780094 in *GCKR* and rs2954021 in *TRIB1*. The A-allele of rs780094 in *GCKR* was associated with NAFLD in subjects of European descent [11, 28, 29]. No association between the A-allele of rs780094 and NAFLD was observed in African American and Hispanic Americans [28, 29]. A study in Asian populations (Indian, Malay, and Chinese) was not conclusive, due to small sample size [30]. GWAS in the Japanese population reported by Kawaguchi *et al.* showed a weak association ($P = 0.011$) [12]. Therefore, rs780094 in *GCKR* is associated with NAFLD in the Japanese population. Other NAFLD susceptibility SNPs in *LYPLAL1*, *PPP1R3B,* and *NCAN* were not associated with NAFLD in our study. *LYPLAL1* rs12137855 and *NCAN* rs2228603 were not associated with NAFLD in another Japanese study [12]. This may be due to the relatively lower power of this study, since the MAFs were no more than 0.05. These results may be also due to ethnic differences in linkage disequilibrium (LD) patterns, ethnic-specific association, and gene/environment interactions.

The A-allele of rs780094 in *GCKR* was associated with decreased fasting plasma glucose and increased triglycerides, as reported by Speliotes *et al.* [11]. An association between increased triglycerides and rs780094 has been reported in previous GWAS [31]. The A-allele of rs780094 in *GCKR* was associated with an increased V/S ratio in our study. These data suggest the A-allele of rs780094 in *GCKR* is related to the development of NAFLD through the increased serum triglycerides caused by visceral fat accumulation.

TRIB1 rs2954021 is associated with increased ALT [16]. *TRIB1* rs17321515, which is in LD ($r^2 = 1.00$), is associated with increased triglycerides [32]. Although rs2954021 was associated with NAFLD, this SNP was not associated with ALT or triglycerides in our study. Although further study is necessary, these and previous results suggest *TRIB1* rs2954021 is related to the development of NAFLD through increased triglycerides.

We previously showed that *PNPLA3* rs738409 is associated with NAFLD severity [14]. *GCKR* rs780094 and *TRIB1* rs2954021 were not associated with histological traits and ALT levels, suggesting these SNPs are not related to NAFLD severity. *PNPLA3* rs738409 was not associated with metabolic syndrome traits [14]. These results were confirmed in this study (490 subjects overlapped) and *PNPLA3* rs738409 was not associated with VFA ($P = 0.32$) or V/S ratio ($P = 0.14$). The effects of *GCKR*, *TRIB1*, and *PNPLA3* on NAFLD were different. Indeed, *GCKR* rs780094, *TRIB1* rs2954021, and *PNPLA3* rs738409 were independently associated with NAFLD.

In conclusion, *GCKR* rs780094 may be involved in the development of NAFLD but does not affect disease severity. Our study suggests *GCKR* rs780094, *TRIB1* rs2954021, and *PNPLA3* rs738409 affect NAFLD through different mechanisms.

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Disclosure Statement

The authors have nothing to disclose.

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Original Article

Early Effect of Single-dose Sitagliptin Administration on Gastric Emptying: Crossover Study Using the ^{13}C Breath Test

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Background/Aims
The gastrointestinal motility effects of endogenous incretin hormones enhanced by dipeptidyl peptidase-IV (DPP-IV) inhibitors have not yet been sufficiently investigated. The aim of this study was to determine whether single pre-prandial sitagliptin, the DPP-IV inhibitor, administration might have an effect on the rate of liquid gastric emptying using the ^{13}C-acetic acid breath test.

Methods
Ten healthy male volunteers participated in this randomized, two-way crossover study. The subjects fasted for overnight and were randomly assigned to receive 50 mg sitagliptin 2 hours before ingestion of the liquid test meal (200 kcal per 200 mL, containing 100 mg ^{13}C-acetate) or the test meal alone. Under both conditions, breath samples were collected for 150 minutes following the meal. Liquid gastric emptying was estimated by the values of the following parameters: the time required for 50% emptying of the labeled meal ($T_{1/2}$), the analog to the scintigraphy lag time for 10% emptying of the labeled meal (T_{lag}), the gastric emptying coefficient and the regression-estimated constants (β and κ), calculated by using the $^{13}CO_2$ breath excretion curve using the conventional formulae. The parameters between the 2 test conditions were compared statistically.

Results
No significant differences in the calculated parameters, including $T_{1/2}$, T_{lag}, gastric emptying coefficient or β and κ, were observed between the 2 test conditions.

Conclusions
The present study revealed that single-dose sitagliptin intake had no significant influence on the rate of liquid gastric emptying in asymptomatic volunteers.
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Key Words
Breath tests; Gastric emptying; Sitagliptin

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Introduction

The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are peptides secreted from the intestine into the circulation in response to food ingestion, and they help manage glycemic control by regulating insulin and glucagon release, slowing gastric emptying, and reducing caloric intake.[1-4] Physiologically, the clinical utility of native GLP-1 and GIP is limited because they are rapidly degraded and inactivated by the enzyme dipeptidyl peptidase-IV (DPP-IV).[5,6]

Inhibition of this enzyme leads to an increase in circulating endogenous GLP-1 and GIP levels. Therefore, DPP-IV inhibitors are a novel therapeutic strategy for type 2 diabetes. Since the release of sitagliptin in 2006, numerous studies have documented the advantages of DPP-IV inhibitors in the management of type 2 diabetes mellitus.[7-10] However, the effect of DPP-IV inhibitor-induced enhancement of endogenous incretin hormones on gastrointestinal motility has not yet been sufficiently investigated.[11,12] In the present study, the pharmacological effects of pre-prandial single-dose sitagliptin administration on the rate of liquid gastric emptying were examined in healthy volunteers using a ^{13}C-acetic acid breath test.

Materials and Methods

Subjects

The subjects were 10 asymptomatic male volunteers (median age 34 years, range 27-50 years). The height and weight of the subjects were as follows: median height, 169 cm; height range, 162-181 cm; median weight, 64.5 kg; and weight range, 60-92 kg. None of the subjects were habitual drinkers. All were non-smokers and none had a history of gastrointestinal disease or abdominal surgery. None of the subjects was on any routine medication at the time of the study.

The study (Clinical trial registry number: UMIN 000006213) was conducted in accordance with the Declaration of Helsinki. Prior to study initiation, written informed consent was obtained from all participants. The study protocol using the ^{13}C-acetic acid breath test was approved by the Ethics Committee of Yokohama City University School of Medicine.

^{13}C-acetic Acid Breath Test

Ten subjects participated in this randomized, two-way crossover study (Fig. 1). After overnight fasting (at least 8 hours), the subjects received 50 mg sitagliptin orally 2 hours before ingestion of the test meal (sitagliptin condition) or the test meal alone (control condition) in a random sequence. The 2 test conditions were separated by a washout period of at least 7 days.

The test meal was a 200 kcal per 200 mL liquid meal (Racol with milk flavor, Otsuka Pharmaceutical, Co., Ltd., Tokyo, Japan) containing 100 mg of ^{13}C-acetic acid (Cambridge Isotope Laboratories, Inc., USA), and the subjects were requested to consume the meal within 5 minutes.

Gastric emptying was measured using the ^{13}C-acetic acid breath test while the subjects were seated. Breath samples were collected in air bags at baseline (before test meal) and at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135 and 150 minutes after completion of the test meal ingestion. The $^{13}CO_2/^{12}CO_2$ ratio in collected breath samples was determined as the difference above baseline using non-dispersive infrared spectrophotometry (POCone, Otsuka Electronics Co., Ltd., Osaka, Japan).

Data Analysis

In accordance with the method reported by Ghoos et al,[13] the percentage of $^{13}CO_2$ recovery in expired breaths per hour (percent dose per hour) against time was fitted to the formula $y(t) = at^{b}e^{-ct}$ by non-linear regression analysis, where y is the percentage of ^{13}C excretion in breath per hour, t is time in hours, and a, b, and c are constants. The time-course of cumulative $^{13}CO_2$ recovery in expired breaths can be fitted to another formula, $z(t) =$

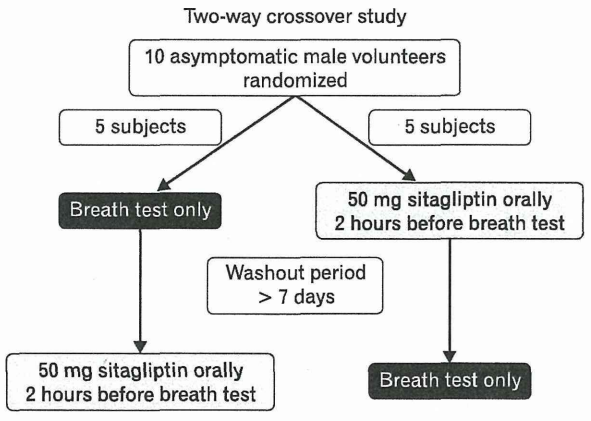

Figure 1. The flow of volunteers throughout the trial: two-way crossover study.

$m(1-e^{-kt})^{\beta}$, where z is the percentage of the cumulative ^{13}C excretion in expired breaths and also an integral of y(t), m is the cumulative $^{13}CO_2$ recovery at an infinite time, and β and κ are regression-estimated constants. Using the mathematical curve-fitting technique, β and κ were determined. A larger β indicates slower emptying in the early phase, and a larger κ indicates faster emptying in the later phase. The opposites are also true. The time required for 50% emptying of the labeled meal ($T_{1/2}$), the analog to the scintigraphy lag time for 10% emptying of the labeled meal (T_{lag}) and the gastric emptying coefficient (GEC) were calculated as overall measures of gastric emptying: $T_{1/2} = -[\ln(1-2^{-1/\beta})]/\kappa$, $T_{lag} = (\ln\beta)/\kappa$ and GEC = ln(a).[13-15] These parameters were calculated using the Solver procedure in Excel 2010 (Microsoft Corp., Redmond, WA, USA).

Statistical Methods

Statistical evaluation was carried out using the Wilcoxon's signed-rank test. The level of significance was set at *P*-value < 0.05. We previously estimated that 90% of the subject delayed liquid gastric emptying in sitagliptin condition compare to control condition. The required sample size was therefore estimated to be 10 per group to have 80% power to detect differences at *P* < 0.05 level. All the statistical analyses were performed using Stat View software (SAS Institute, Cary, NC, USA).

Results

All 10 subjects completed this study, and no adverse events occurred during the study. No significant differences were observed in the $T_{1/2}$ ([91.8: 72.2-98.4] vs. [94.2: 81.2-106.6]), T_{lag} ([52.8: 41.7-70.1] vs. [56.0: 44.8-65.5]), GEC ([4.19: 3.76-4.48] vs. [4.17: 3.30-4.52]), β ([2.05: 1.71-3.23] vs. [2.09: 1.86-2.65]) and κ ([0.88: 0.76-1.04] vs. [0.86: 0.66-0.94]) (median: range, control vs. sitagliptin) between the control and experimental conditions (Fig. 2). These results indicated that sitagliptin had no significant effect on the rate of liquid gastric emptying.

Discussion

The present study was conducted to examine the changes in the rate of liquid gastric emptying after single pre-prandial administration of sitagliptin 50 mg during the first 2.5 hours after ingestion of a liquid meal in healthy volunteers. There were no significant differences in any of the liquid gastric emptying parameters measured using the ^{13}C-acetic acid breath test between the 2 test conditions, either ingestion of sitagliptin before the meal or the test meal alone. These results indicate that sitagliptin does not influence the rate of liquid gastric emptying.

After the introduction of DPP-IV inhibitors, numerous studies documenting their advantages in the management for type 2 diabetes mellitus patients have been published.[8-10] However, to date, there have been a few studies reporting the pharmacological effects of DPP-IV inhibitors on the gastric emptying rate. In a previous study, DeFronzo et al[12] reported that 100 mg sitagliptin once a day for 2 weeks had no effect on the rate of gastric emptying in type 2 diabetes patients by an acetaminophen absorption method. Vella et al[11] described that gastric emptying assessed by scintigraphy did not differ between type 2 diabetes patients treated with 50 mg vildagliptin twice a day and placebo for 10 days. Our study was novel in that it examined the effect of single-dose pre-prandial sitagliptin 50 mg on the rate of gastric emptying measured by a ^{13}C-acetic acid breath test using a liquid meal in healthy volunteers.

One of the limitations in this study was the lack of information about actual serum GLP-1 concentrations enhanced by sitagliptin. Steady-state trough concentrations of sitagliptin have been reported to be achieved within 2 to 3 days of administration.[14] On the other hand, it has also been reported that single administration of sitagliptin shows an equivalent pharmacokinetic profile compared with once-daily dosing in healthy subjects.[14-16] Furthermore, single administration of sitagliptin 50 mg produced by 80% or greater inhibition of DPP-IV activity at 2 hours after administration and over the following 12-hour period, and approximately 2-fold augmentation of postprandial active GLP-1 concentrations compared with placebo in healthy subjects was also observed.[15] Herman et al[17] reported that single administration of sitagliptin 25 and 200 mg inhibited the enzymatic activity of DPP-IV by 80 to 96% at 2 hours after administration, respectively, and active GLP-1 levels increased greater than 2-fold after both doses in response to an oral glucose tolerance test (OGTT) at 2 hours after administration in patients with type 2 diabetes. They also showed that the near maximal glucose-lowering efficacy of single oral dose of sitagliptin was associated with 80% or greater plasma inhibition of DPP-4 activity. This level of DPP-IV inhibition corresponds to a plasma sitagliptin concentration of 100 nM or greater and an augmentation of active GLP-1 and GIP levels of 2-fold or higher after an OGTT.[17]

Hence, active GLP-1 concentrations reached potent levels in this present study after single administration of sitagliptin 50 mg. However, these levels of serum GLP-1 concentration en-

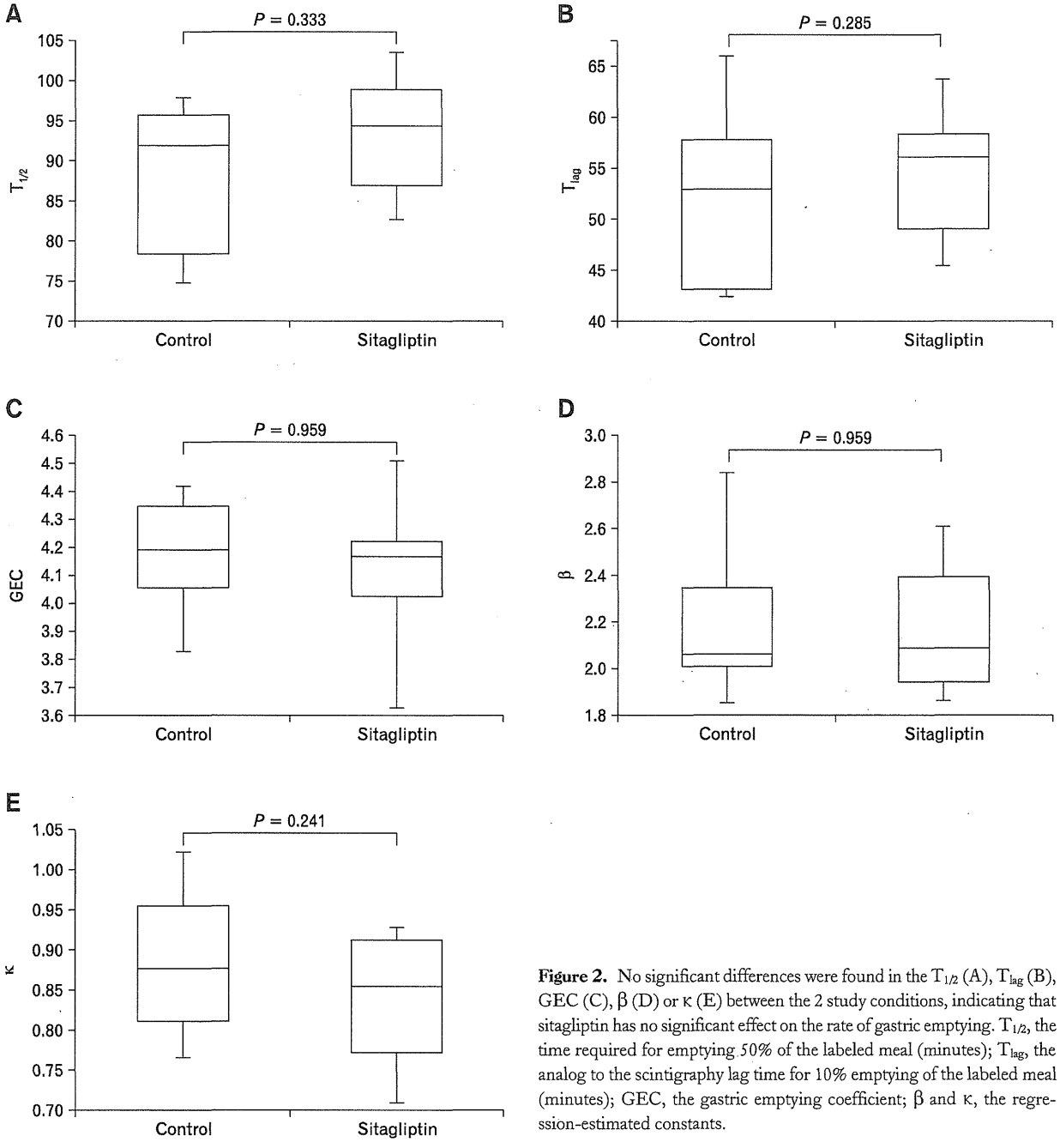

Figure 2. No significant differences were found in the $T_{1/2}$ (A), T_{lag} (B), GEC (C), β (D) or κ (E) between the 2 study conditions, indicating that sitagliptin has no significant effect on the rate of gastric emptying. $T_{1/2}$, the time required for emptying 50% of the labeled meal (minutes); T_{lag}, the analog to the scintigraphy lag time for 10% emptying of the labeled meal (minutes); GEC, the gastric emptying coefficient; β and κ, the regression-estimated constants.

hanced by sitagliptin could be within the physiologic range. Thus, the explanation for the lack of effect on gastric emptying may be due to insufficient concentrations of active GLP-1 to delay gastric emptying, though active GLP-1 concentrations were sufficiently enhanced by sitagliptin to improve glycemic control.

This study was conducted in healthy, normoglycemic male subjects, which limited the extent to which the data can be extrapolated to patients with type 2 diabetes. As mentioned above, on the point of view of drug efficacy, the pharmacokinetic and pharmacodynamics profiles of sitagliptin are reported to be sim-

ilar in healthy individuals and in those with type 2 diabetes.[14-18] However, gastric emptying rates in the type 2 diabetes population have been reported to be delayed, unchanged, or accelerated.[19-23] The investigation of gastric emptying rate in healthy subjects might be advantageous for understanding of the natural characteristics of pharmaceutical preparations in contrast to diabetic patients with high heterogeneity in their rates of gastric emptying.

It is also known that there are fundamental differences in the regulatory mechanisms underlying gastric emptying of solids and liquids.[24,25] Additionally, the GLP-1 secretory patterns can be modulated by various ingested nutrients.[26-28] Solid test meals, as mentioned in previous reports,[11,12] may be more useful in clinical application.

Although scintigraphy is the current standard method for assessing gastric emptying,[29,30] it is expensive, involves radiation exposure and requires the facilities of a department of nuclear medicine. The evaluation of gastric emptying using the ^{13}C-acetic acid breath test has been developed as a non-radioactive alternative. The subject ingests ^{13}C-labeled acetic acid, which passes through the stomach and is absorbed in the duodenum and superior small bowel. The ^{13}C-labeled acetic acid is then metabolized in the liver and excreted from the lungs as $^{13}CO_2$. This pathway enables gastric emptying to be measured in a noninvasive manner.[31-41] The accuracy of the breath test for measuring gastric emptying has been well supported by several validation studies demonstrating a strong correlation between the breath test and the scintigraphy.[13,42-46]

Ultimately, as demonstrated in previous studies, sitagliptin had no effect on the rate of liquid gastric emptying in asymptomatic volunteers.

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CASE REPORT Open Access

Postpyloric decompression tube placement through a gastrostomy for malignant bowel obstruction

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Abstract

Background: Malignant bowel obstruction affect a patient's quality of life, but, management of MBO is controversial.

Case presentation: A 51-year-old woman who had been diagnosed as uterine cervix cancer 2 years ago and had undergone surgery, chemotherapy and radiotherapy, was admitted to our hospital. She was diagnosed as having a recurrence of peritoneal metastasis and bowel obstruction. For her nasal pain, we considered insertion of a postpyloric decompression tube through the gastrostomy instead of via the nasal cavity. After insertion of a percutaneous gastrostomy tube was performed endoscopically, we introduced a postpyloric decompression tube through her gastrostomy. She could be discharged home, and 91 days later, she died in her home under hospice care, as she had wished.

Conclusions: Insertion of a postpyloric decompression tube through a gastrostomy might be useful in the management of advanced cancer patients with bowel obstruction.

Keywords: Malignant bowel obstruction, Gastrostomy, Palliative care, Quality of life

Background

Malignant bowel obstruction (MBO), a common complication in patients with advanced cancer, can significantly affect a patient's quality of life [1-3]. However, management of MBO is controversial [4-6].

Case presentation

A 51-year-old woman who had been diagnosed as having stage 2b uterine cervix cancer 2 years ago and had undergone surgery, chemotherapy and radiotherapy, was admitted to our hospital with nausea and abdominal pain. She was diagnosed as having a recurrence of peritoneal metastasis with complicating ascites and bowel obstruction. We first treated her conservatively however, a month later, her symptoms recurred and a postpyloric decompression tube was introduced via the nasal cavity. After the procedure, she complained of severe nasal pain and expressed her wish for treatment by a different method.

We therefore considered insertion of a postpyloric decompression tube through the gastrostomy instead of via the nasal cavity. Following obtainment of informed consent, insertion of a percutaneous gastrostomy tube was performed endoscopically (Figure 1). Two weeks later, we introduced a postpyloric decompression tube through her gastrostomy instead of via the nasal cavity. The postpyloric decompression was effective (Figure 2), she was discharged home, and 91 days later, she died in her home under hospice care, as she had wished.

Conclusions

Palliation of symptoms is the treatment goal terminal disease patients with MBO. Hospitalization and conservative management by nasogastric tube decompression and bowel rest is the first step in the management of MBO. However, when continuous postpyloric decompression is required, insertion of the postpyloric decompression tube through the gastrostomy instead of via the nasal cavity

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