Int J Clin Exp Med 2021;14(11):2539-2548
www.ijcem.com /ISSN:1940-5901/IJCEM0119531

Original Article

Changes in gastrointestinal hormones during a pancreatic exocrine function breath test using N-Benzoyl-L-Tyrosyl-[1-^{13}C] alanine sodium

Michiko Ishii[1,2], Yukimoto Ishii[1], Satoshi Asai[2]

[1]Division of Research Planning and Development, Medical Research Support Center, Nihon University School of Medicine, Tokyo 173-8610, Japan; [2]Division of Pharmacology, Department of Biomedical Sciences, Nihon University School of Medicine, Tokyo 173-8610, Japan

Received August 4, 2020; Accepted October 11, 2021; Epub November 15, 2021; Published November 30, 2021

Abstract: Objective: This study examined changes in gastrointestinal hormones associated with pancreatic exocrine secretion during a pancreatic exocrine function breath test using N-benzoyl-L-tyrosyl-[1-^{13}C] alanine (Bz-Tyr-Ala) to clarify the hormonal environment during this test. Materials and methods: After dissolving 5 mg/kg Bz-Tyr-Ala in distilled water, a 7-mM solution was orally administered to Sprague Dawley rats. Expired air was then collected at 10-min intervals for 60 min. Changes in expiratory $^{13}CO_2$ levels were measured using an infrared spectrometer ($\Delta^{13}CO_2$ ‰). After collecting expired air, blood was collected to measure the plasma levels of Bz-Tyr-Ala, N-benzoyl-L-tyrosine (Bz-Tyr), alanine, and tyrosine using liquid chromatography with tandem mass spectrometry as well as plasma levels of gastrin, cholecystokinin, secretin, and somatostatin using enzyme-linked immunosorbent assay. Results: Expiratory $^{13}CO_2$ levels (Δ‰) reached the maximum at 20 min after the start of testing, whereas plasma Bz-Tyr-Ala and Bz-Tyr levels reached the maximum after 30 min. Only plasma alanine levels increased after 10 min, and no changes in plasma tyrosine levels were observed during measurement. Our findings showed a significant increase in plasma gastrin levels but a decrease in plasma cholecystokinin levels with Bz-Tyr-Ala. A significant decrease in secretin levels and increase in somatostatin levels was observed at 60 min. Conclusion: The following hormonal conditions were observed during the Bz-Tyr-Ala breath test: elevated gastrin secretion, decreased or basal cholecystokinin and secretin secretion, and basal or elevated somatostatin secretion.

Keywords: ^{13}C-breath tests, pancreatic exocrine function, gastrin, cholecystokinin (CCK), secretin, somatostatin

Introduction

To diagnose pancreatic exocrine dysfunction, a direct function test, namely the secretin-cholecystokinin test, has proven useful. However, this test is not routinely performed given its complexity and the stress it places on the subjects. Thus, the following indirect pancreatic exocrine function breath tests using a stable isotope, ^{13}C, have been reported: ^{13}C-mixed triglycerides [1-12], ^{13}C-tripalmitin [13], ^{13}C-triolein, hiolein [14, 15], and ^{13}C-trioctanain [16] tests for investigating pancreatic fluid lipase; ^{13}C-corn starch test [7, 17] for investigating amylase; and ^{13}C-cholesteryl octanoate test [18] for investigating esterase. In addition, studies have reported a pancreatic exocrine function breath test using N-benzoyl-L-tyrosyl-[1-^{13}C] alanine sodium salt (Bz-Tyr-Ala), in which L-[1-^{13}C] alanine was introduced at the C-terminal, to test for pancreatic fluid carboxypeptidase [19-23].

To assess pancreatic exocrine function using the previously reported ^{13}C breath tests, several methods have been reported. These include providing a specific loading diet before testing, reagent stimulation of pancreatic fluid secretion, and testing after fasting without stimulating pancreatic fluid secretion. However, only few studies have examined changes in hormone levels associated with pancreatic exocrine function during a breath test. The Bz-Tyr-Ala breath test, which has been used to measure basic pancreatic exocrine function, is performed after fasting without stimulating pan-

creatic fluid secretion and then measured. However, no study has investigated changes in the levels of gastrointestinal hormones [cholecystokinin (CCK), secretin, and somatostatin] that play an important role in regulating pancreatic fluid secretion [19-23]. The present study, therefore, aimed to examine hormone levels during the Bz-Tyr-Ala breath test and clarify changes in gastrointestinal hormones associated with pancreatic exocrine function during this test.

Materials and methods

Animals

Male Sprague Dawley (SD) rats (body weight: approximately 200-210 g) were purchased from CLEA Japan, Inc. (Shizuoka City, Shizuoka Prefecture, Japan) and acclimated for 1 week before the start of experiments under the following conditions: room temperature, 22°C±2°C; humidity, 55%±15%; and lighting cycle, 12 h. To prevent cannibalism, each rat was individually housed in a mesh cage and provided no food from 12 h before each experiment; however, water was given ad libitum during this period.

This animal study was conducted in accordance with the Nihon University Regulations for the Acclimation and Use of Laboratory Animals and Animal Research: Reporting of In Vivo Experiment Guideline (June 2010). The study protocol was approved by the Nihon University Animal Experiment Committee (AP17M009-1).

^{13}C breath test

Bz-Tyr-Ala breath test: The Bz-Tyr-Ala sodium salt was synthesized from 1-^{13}C-L-alanine (Cambridge Isotope Laboratories Massachusetts, USA) as the starting material at Peptide Institute Inc. (Osaka, Japan). Its chemical purity was confirmed to be 99.6% via thin-layer chromatography and high-performance liquid chromatography. Bz-Tyr-Ala (5 mg/kg) was dissolved in distilled water (7 mM) and administered orally to the rats.

L-[1-^{13}C] alanine breath test (ABT): For the ^{13}C-ABT, 7 mM [1-^{13}C] alanine, an amount equimolar to that contained in Bz-Tyr-Ala, was administered orally, after which breath samples were collected as in the Bz-Tyr-Ala breath test.

Breath test system

The breath test was performed as per the method reported by Uchida et al. [20, 24-26]. The desiccator (animal chamber) and aspiration pump (Masterflex L/S, Cole-Palmer Inst. Co., USA) were selected. POCone and UBiT apparatus (Fukuda Electronics, Co., Ltd., Japan) were selected as they enable the simple and effective measurement of $^{13}CO_2$. A 2,000-mL desiccator was used to allow the free movement of rats within the chamber and for the effective collection of expired air via the breath-sampling bag (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Aspirating expired air led to fresh air being automatically drawn into the desiccator via a hole that passed through the aspiration tube in the chamber's side. Air in the chamber was continuously aspirated during the experimental period. CO_2 concentration in the chamber was maintained at 3.5%±0.5% during the experiment. Aspirated air was discharged outside the breath test system, except during the collection of expired air into the breath-sampling bag.

Rats were placed in the chamber immediately after the oral administration of the reagent containing Bz-Tyr-Ala or 1-^{13}C-L-alanine. Expired $^{13}CO_2$ air was collected and measured at 10-min intervals for 60 min after reagent administration. The results of the breath test were expressed as Δ‰. $\Delta^{13}CO_2$ (‰) = {($^{13}CO_2/^{12}CO_2$ t min - $^{13}CO_2/^{12}CO_2$ 0 min)/$^{13}CO_2$ std} × 10^3.

Blood samples

Controls were established for all breath tests (Bz-Tyr-Ala: 10, 30, and 60 min; ABT: 10, 30, and 60 min); the controls received distilled water at a volume similar to that of the respective reagents. They were housed in a desiccator and their blood was collected under the same conditions as the reagent-treated group. For blood collection, they were euthanized using CO_2 after the collection of expired air. Each group comprised five to eight rats.

Measurement of gastrin, CCK, secretin, and somatostatin

Plasma gastrin and CCK levels were measured using the RayBio gastrin enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech, Norcross, GA, USA), plasma secretin levels were

Table 1. Measurement conditions of N-benzoyl-L-tyrosyl-[1-^{13}C] alanine and benzoyl-L-tyrosyl

Chromatographic conditions	Manufacturer	Waters
	Model	Acquity UPLC
	Column	ACQUITY UPLC CSH C18 1.7 μm, 2.1 × 50 mm
	Column temperature	40 °C
	Mobile Phase A	Water and 0.1% formic acid
	Mobile phase B	Acetonitrile 0.1% formic acid
	Gradient elution	B: 5% (0.2 min), 50% (6.2 min), 98% (6.3 min), 5% (7.2 min)
	Flow rate	0.3 mL/min
	Injection volume	5 μL
Mass spectrometric conditions	Manufacturer	Waters
	Model	Xevo TQ-S micro
	Electrospray ionization	Positive
	Monitor ion	
	N-benzoyl-L-tyrosyL-[1-^{13}C] alanine	358>240, 358>268. Precursor ion > Product ion
	N-benzoyl-L-tyrosine	286>104, 286>135. Precursor ion > Product ion
	N-benzoyl-L-phenylalanine	270>104, 270>119. Precursor ion > Product ion

measured using the Cusabio secretin ELISA kit (Wuhan Huamei Biotech Co., Wuhan, China), and plasma somatostatin levels were measured using the Cloud Clone somatostatin ELISA kit (Cloud Clone Corp, Huston, TX, USA) as per the manufacturers' protocols.

Measurement of Bz-Tyr-Ala, Bz-Tyr, alanine, and tyrosine

The plasma levels of Bz-Tyr-Ala, N-benzoyl-L-tyrosine (Bz-Tyr), alanine, and tyrosine were measured using liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Acquity UPLC, MS/MS Waters Xevo TQ-S micro, Waters Corporation, Milford, Massachusetts, USA).

Sample preparation

Measurement of Bz-Tyr-Ala and Bz-Tyr: Internal standard solution (L-benzoyl phenylalanine) (5 μL; 500 ng/mL) was added to 20 μL sample plasma, mixed with 90 μL 50% methanol, and centrifuged at 13,000 rpm and 4°C for 3 min. The supernatant (10 μL) was mixed with 70 μL formic acid solution (0.1%) and used as the sample solution. The LC-MS/MS measurement conditions are presented in **Table 1**.

Measurement of alanine and tyrosine: Internal standard solution (20 μL; 0.1 N HCl tyrosine: 10 μg/mL, alanine: 10 μg/mL) and methanol (360 μL) were added to 20 μL sample plasma and centrifuged at 13,000 rpm and 4°C for 3 min. The supernatant (120 μL) was dried/fixed using a centrifugal evaporator and mixed with 20 μL of 3 N HCl in n-Butanol (12 N HCl: n-Butanol =3:1). The solution was further dried/fixed using a centrifugal evaporator and mixed with 1.0 mL of 50% acetonitrile and 0.1% formic acid solution. After vortexing, 195 μL of 0.1% formic acid solution was added to 5 μL of the sample, which was then used as the sample solution. The LC-MS/MS measurement conditions are presented in **Table 2**.

Statistical analyses

Given that none of the results showed normality, the Mann-Whitney U test was used to determine significant differences in gastrointestinal hormones between the Bz-Tyr-Ala and control groups and between the L-[1-^{13}C] alanine and control groups as well as compare the results between both groups. All analyses were performed using JMP Pro version 14 (SAS Institute Inc., North Carolina, USA).

Results

Bz-Tyr-Ala breath test and changes in plasma Bz-Tyr-Ala and Bz-Tyr levels

During the Bz-Tyr-Ala breath test, the $\Delta^{13}CO_2$ value (‰) reached the maximum at 20 min after administration but successively decreased thereafter, showing a plateau at ≥50 min (**Figure 1A**).

Changes in plasma Bz-Tyr-Ala levels were similar to those in expired air $\Delta^{13}CO_2$ values where-

Table 2. Measurement conditions of alanine and tyrosine

Chromatographic conditions	Manufacturer	Waters
	Model	Acquity UPLC
	Column	ACQUITY UPLC BEH Phenyl 1.8 μm, 2.1 × 50 mm
	Column temperature	40°C
	Mobile Phase A	Water and 0.1% formic acid
	Mobile phase B	Acetonitrile and 0.1% formic acid
	Gradient elution	B: 5% (0.2 min), 50% (6.2 min), 98% (6.3 min), 5% (7.2 min)
	Flow rate	0.3 ml/min
	Injection volume	5 μL
Mass spectrometric conditions	Manufacturer	Waters
	Model	Xevo TQ-S micro
	Electrospray ionization	Positive
	Monitor ion	
	Alanine	146>89, Precursor ion > Product ion
	Tyrosine	238>135, Precursor ion > Product ion

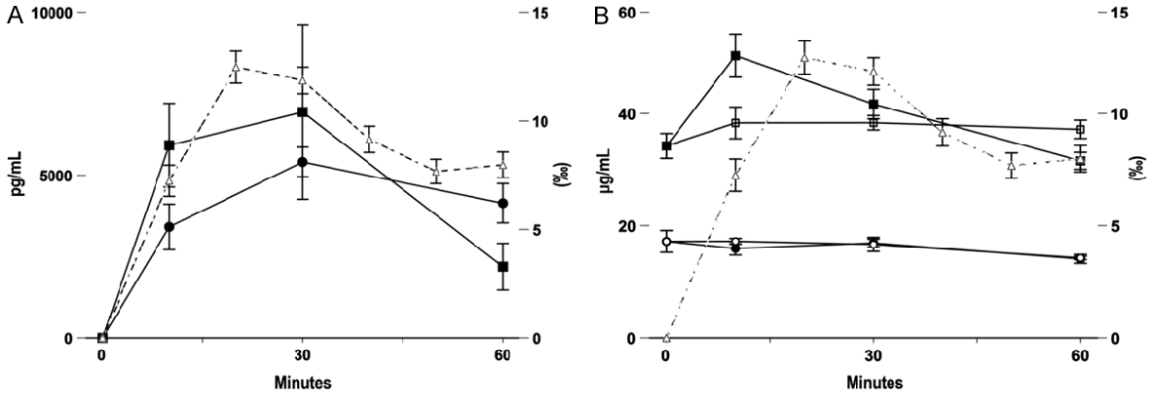

Figure 1. Changes in plasma N-benzoyl-L-tyrosyL-[1-^{13}C] alanine (Bz-Tyr-Ala), N-benzoyl-L-tyrosine (Bz-Try), alanine, and tyrosine after Bz-Tyr-Al administration. A: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-■: changes in plasma Bz-Tyr-Ala, ●: changes in plasma Bz-Try. B: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-■: changes in plasma alanine after Bz-Tyr-Ala administration, □: changes in plasma alanine in control, ●: changes in plasma tyrosine after Bz-Tyr-Ala administration, ○: changes in plasma tyrosine in controls.

in the maximum was reached at 30 min but decreased thereafter. Changes in plasma Bz-Tyr levels were similar to those in plasma Bz-Tyr-Ala levels until 30 min after administration, but the plasma levels of the former were greater than those of the latter at 60 min (**Figure 1A**).

Changes in plasma alanine and tyrosine levels after Bz-Tyr-Ala administration

Plasma alanine levels were significantly higher than control values 10 min after Bz-Tyr-Ala administration. However, no significant differences were observed at 30 or 60 min. Further, no increase in plasma tyrosine levels was noted at any point during measurement, with changes being similar to those in controls (**Figure 1B**).

Changes in plasma hormone levels during the Bz-Tyr-Ala breath test

The results of hormone measurement after Bz-Tyr-Ala administration are detailed in **Figure 2** and **Table 3**.

Gastrin: Plasma gastrin levels increased until 30 min after Bz-Tyr-Ala administration but decreased thereafter. Meanwhile, control values showed a successive and gradual decrease. The Bz-Tyr-Ala group showed significantly higher

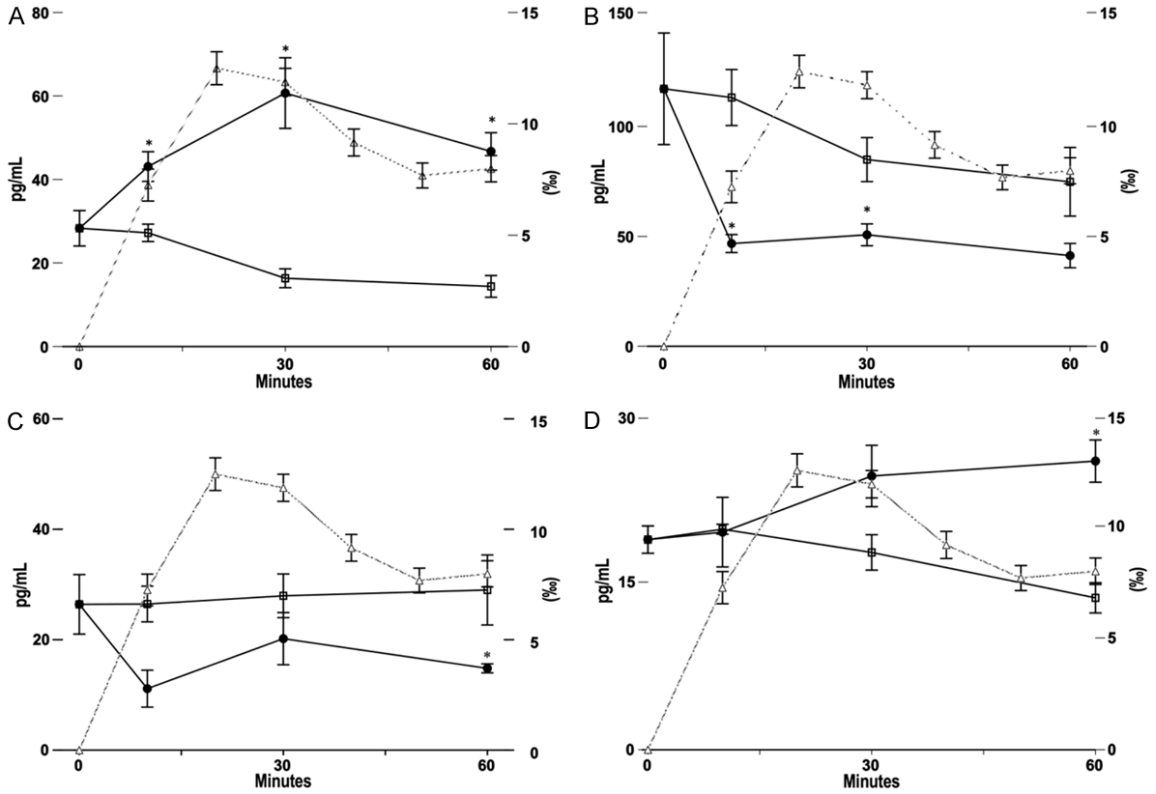

Figure 2. Changes in plasma gastrin, cholecystokinin (CCK), secretin, and somatostatin after N-benzoyl-L-tyrosyL-[1-^{13}C] alanine (Bz-Tyr-Ala) administration. A: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-●: changes in plasma gastrin after Bz-Tyr-Ala administration, □: changes in plasma gastrin in controls. B: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-●: changes in plasma CCK after Bz-Tyr-Ala administration, □: changes in plasma CCK in controls. C: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-●: changes in plasma secretin after Bz-Tyr-Ala administration, □: changes in plasma secretin in controls. D: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-●: changes in plasma somatostatin after Bz-Tyr-Ala administration, □: changes in plasma somatostatin in controls. *P<0.05.

Table 3. Changes in gastrointestinal hormones induced by N-benzoyl-L-tyrosyl-[1-^{13}C] alanine (Bz-Tyr-Ala) administration

Hormone	Administration	10 min	P	30 min	P	60 min	P
Gastrin (pg/mL)	Bz-Try-Ala	43.1 (±3.5)	0.02*	60.6 (±8.4)	0.02*	46.7 (±4.4)	0.02*
	control	27.2 (±2.1)		16.3 (±2.2)		14.3 (±2.6)	
CCK (pg/mL)	Bz-Try-Ala	46.7 (±4.1)	0.02*	50.6 (±4.9)	0.03*	41.2 (±5.5)	0.06
	control	113.1 (±12.7)		84.8 (±10.0)		74.7 (±15.6)	
Secretin (pg/mL)	Bz-Try-Ala	11.1 (±3.3)	0.06	20.1 (±4.7)	0.31	14.8 (±0.8)	0.02*
	control	26.4 (±3.3)		27.9 (±3.9)		29.0 (±6.3)	
Somatostatin (pg/mL)	Bz-Try-Ala	19.4 (±3.1)	0.39	24.4 (±2.7)	0.06	25.8 (±1.8)	0.02*
	control	19.7 (±0.4)		17.6 (±1.5)		13.5 (±1.3)	

(± number): means ± standard error of the mean. *P<0.05.

plasma gastrin levels than the control group value at all points between 10 and 60 min after Bz-Tyr-Ala administration (**Figure 2A**).

CCK: Plasma CCK levels at 10 and 30 min during the Bz-Tyr-Ala breath test were significantly lower than control values. No increase was observed during measurement. Control values gradually decreased during measurement compared with that before the start of the test (**Figure 2B**).

Secretin: Plasma secretin levels 10 and 30 min after Bz-Tyr-Ala administration were lower

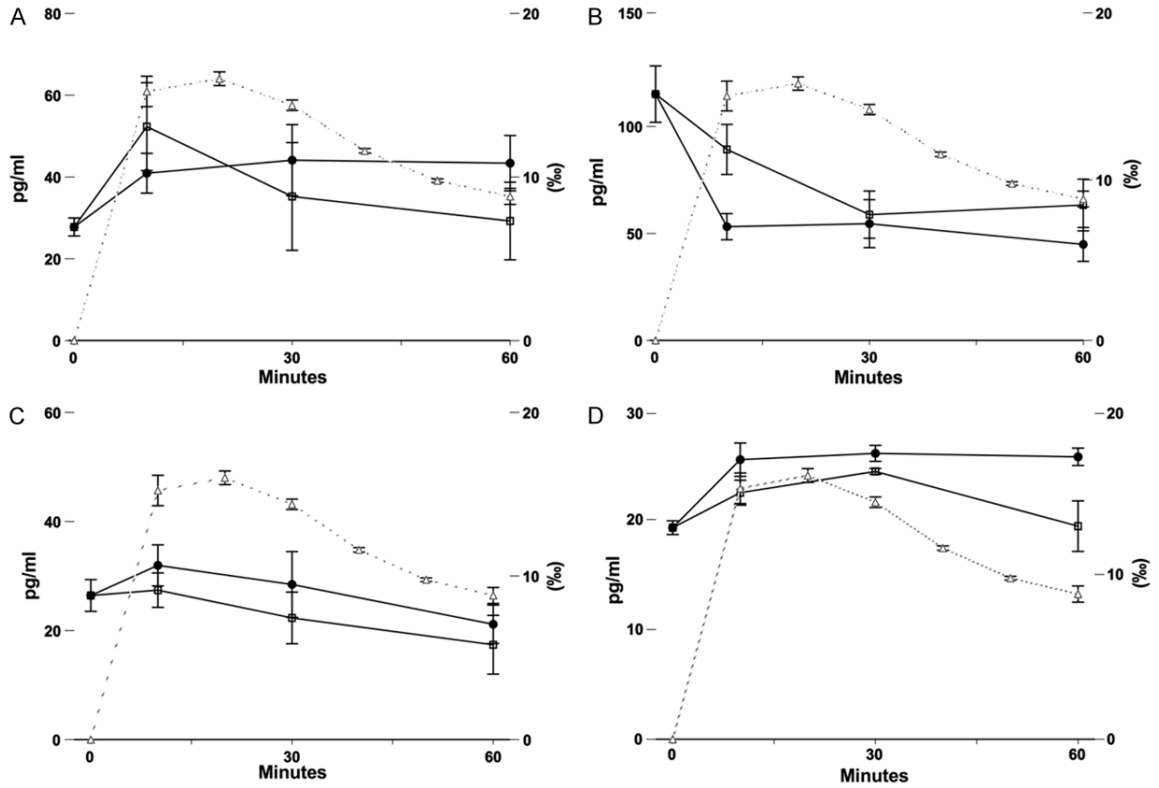

Figure 3. Changes in plasma gastrin, cholecystokinin (CCK), secretin, and somatostatin after ^{13}C-alanine administration. A: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-●: changes in plasma gastrin after ^{13}C-alanine administration, □: changes in plasma gastrin in controls. B: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-●: changes in plasma CCK after ^{13}C-alanine administration, □: changes in plasma CCK in controls. C: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-●: changes in plasma secretin after ^{13}C-alanine administration, □: changes in plasma secretin in controls. D: Left axis-Δ: $\Delta^{13}CO_2$. Right axis-●: changes in plasma somatostatin after ^{13}C-alanine administration, □: changes in plasma somatostatin in controls.

than control values, although no significant differences were noted. However, plasma secretin levels 60 min after Bz-Tyr-Ala administration were significantly lower than control values. There were no significant changes in the control value during measurement (**Figure 2C**).

Somatostatin: Plasma somatostatin levels after Bz-Tyr-Ala administration were slightly higher than control values. At 60 min, plasma somatostatin after Bz-Tyr-Ala administration were significantly higher than control values (**Figure 2D**).

^{13}C-ABT and changes in plasma hormone levels

As plasma alanine levels increased 10 min after Bz-Tyr-Ala administration, an equimolar amount of ^{13}C-alanine present in the Bz-Tyr-Ala dose was orally administered to examine changes in plasma hormone levels. The results of plasma hormone measurements after alanine administration are shown in **Figure 3** and **Table 4**.

Gastrin: Although both the alanine-treated and control rats showed an increase in plasma gastrin levels at 10 min, no significant differences were observed between the two groups during measurement (**Figure 3A**).

CCK: Although both alanine-treated and control rats showed a slight decrease in plasma CCK levels, no significant differences were noted between the groups during measurement (**Figure 3B**).

Secretin: Both alanine-treated and control rats showed a slight increase in plasma secretin levels at 10 min. However, no significant differences were observed between the groups during measurement (**Figure 3C**).

Table 4. Changes in gastrointestinal hormones induced by ^{13}C-alanine administration

Hormone	Administration	10 min	P	30 min	P	60 min	P
Gastrin (pg/mL)	^{13}C-alanine	40.9 (±4.8)	0.93	44.1 (±8.6)	0.12	43.3 (±6.7)	0.20
	control	52.3 (±10.7)		35.2 (±13.1)		29.2 (±9.5)	
CCK (pg/mL)	^{13}C-alanine	53.1 (±6.1)	0.07	54.5 (±11.2)	0.94	44.8 (±7.9)	0.55
	control	89.2 (±11.6)		58.7 (±11.0)		63.2 (±12.0)	
Secretin (pg/mL)	^{13}C-alanine	31.9 (±3.7)	0.67	28.4 (±6.0)	0.51	21.1 (±3.5)	0.44
	control	27.4 (±3.1)		22.3 (±4.7)		17.3 (±5.3)	
Somatostatin (pg/mL)	^{13}C-alanine	25.4 (±1.5)	0.14	25.4 (±0.6)	0.06	25.7 (±0.8)	0.07
	control	22.4 (±1.1)		23.8 (±0.7)		19.4 (±2.3)	

(± number): means ± standard error of the mean.

Somatostatin: Both alanine-treated and control rats showed a slight increase in plasma somatostatin levels at 10 min. At 30 min, the treated group showed higher values than the control group. However, no significant differences were noted between the groups during measurement (**Figure 3D**).

Discussion

Several studies have reported that the Bz-Tyr-Ala breath test facilitates the rapid diagnosis of pancreatic exocrine dysfunction (20 or 30 min) in patients with chronic pancreatitis or those undergoing pancreatectomy [22, 23]. However, no study has examined the relationship between the Bz-Tyr-Ala breath test and gastrointestinal hormones, which are important for pancreatic exocrine function and closely related to the regulation of extra-pancreatic secretion. Hence, the current study investigated the secretion of gastrointestinal hormones during the Bz-Tyr-Ala breath test in rats.

After Bz-Tyr-Ala administration, plasma Bz-Tyr-Ala and Bz-Tyr levels increased together with expired air $^{13}CO_2$ levels. At the same time, alanine levels transiently increased at 10 min but decreased thereafter (**Figure 1A** and **1B**), suggesting that carboxypeptidase in pancreatic fluid promotes alanine release via Bz-Tyr-Ala, absorption/metabolism, and expired air $^{13}CO_2$ excretion over a short period. Moreover, Bz-Tyr levels were higher than Bz-Tyr-Ala levels at 60 min, with no increase or decrease in tyrosine levels during measurement. This indicated that Bz-Tyr remained in the plasma in its unchanged form without releasing tyrosine.

Conditions for increased gastrin secretion include an influx of amino acids and proteins/peptides into the stomach. After Bz-Tyr-Ala administration, gastrin levels were higher than control values at all measurement time points (**Figure 2A**; **Table 3**). To clarify whether changes in gastrin levels were associated with Bz-Tyr-Ala/Bz-Tyr or a transient increase in alanine levels, a ^{13}C-ABT was performed, which subsequently found no significant changes in gastrin levels (**Figure 3A**; **Table 4**). Of note, McArthur et al. reported that alanine administration did not induce gastrin secretion [27], suggesting that Bz-Tyr-Ala stimulated gastrin secretion.

An experiment in dogs and humans showed that phenylalanine or tryptophan potently secreted CCK, even in fatty acids, proteins, peptides, and amino acids [28]. Moreover, several studies have indicated that these pancreatic exocrine function-promoting actions are mediated by the vagus nerve [29, 30]. After Bz-Tyr-Ala administration, plasma Bz-Tyr-Ala and Bz-Tyr levels increased, whereas plasma CCK levels decreased (**Figure 2B**; **Table 3**). However, no changes in CCK levels were observed during ABT (**Figure 3B**; **Table 4**). Stephen et al. reported that alanine administration (80 mM) did not promote CCK release [31]. Given that the alanine dose for ABT used herein was 7 mM, our findings suggesting no changes in CCK levels are consistent with those of previous studies. Considering that 7 mM alanine was present in Bz-Tyr-Ala, the temporary increase in plasma alanine may not have affected CCK secretion. Therefore, the decrease in CCK during oral Bz-Tyr-Ala administration could be attributed to Bz-Tyr-Ala or Bz-Tyr or increased somatostatin secretion. This result suggests that the Bz-Tyr-Ala breath test was performed under conditions insufficient to induce basic CCK secretion. However, this matter, including the mechanism of action via the vagus nerve, requires further investigation.

Gastric acid flow in the duodenum has been considered a physiological factor for secretin release. Furthermore, an intra-duodenal pH of ≤4.5 has been found to trigger secretin release, suggesting that secretin release depends on the pH strength of gastric acid. Secretin promotes pancreatic bicarbonate secretion, which neutralizes the intra-duodenal cavity and adjusts the pH for optimal pancreatic enzyme activity. After Bz-Tyr-Ala administration, secretin levels slightly decreased and were significantly lower than control values at 60 min (**Figure 2C**; **Table 3**). However, ABT showed no significant differences between the control and treatment groups across all measurement time points (**Figure 3C**; **Table 4**). This suggests that no change in secretin secretion occurred during ABT and that the increase in plasma alanine levels after Bz-Tyr-Ala administration did not affect secretin secretion. McArthur et al. reported that 100-mM alanine administration did not induce gastric acid secretion [27], indicating no promotion in gastric acid secretion even when gastrin levels increased after Bz-Tyr-Ala administration. This may have been caused by unsuitable intra-duodenal pH conditions for promoting secretin secretion. The decrease in secretin 60 min after Bz-Tyr-Ala administration may have been caused by the effects of increased Bz-Tyr concentrations and somatostatin. Future studies are needed to examine secretin secretion through the oral administration of Bz-Tyr over time.

Somatostatin levels were slightly increased at 30 min after Bz-Tyr-Ala administration and remained significantly higher than control values at 60 min. However, no similar phenomenon was observed during ABT, suggesting that changes in plasma alanine levels did not affect somatostatin.

As somatostatin inhibits gastrin/CCK/secretin secretion, the significant increase in gastrin levels associated with Bz-Tyr-Ala administration during measurement may have increased somatostatin levels. Furthermore, increased gastrin levels may have increased somatostatin and suppressed CCK and secretin secretion. Of note, the present findings showed that somatostatin was elevated when the plasma levels of both Bz-Tyr-Ala and Bz-Tyr peaked, suggesting that the direct stimulation of the pancreas by Bz-Tyr-Ala and Bz-Tyr cannot be ruled out.

In a study using rats, Kohno et al. indicated that Bz-Tyr-Ala was stable under acidic conditions and that it promoted ^{13}C-alanine release under alkaline conditions [21]. Moreover, they reported that pancreatic exocrine-insufficient rats had significantly lower $\Delta^{13}CO_2$ during the Bz-Tyr-Ala breath test performed at 20 min than control rats. Ishii et al. reported that the 20- or 30-min values of the Bz-Tyr-Ala breath test were highly sensitive and specific for evaluating pancreatic exocrine secretion [22]. Pancreatic exocrine function may be evaluated using the 30-min Bz-Tyr-Ala breath test under the conditions of increased gastrin secretion, decreased CCK levels, and basal secretin/somatostatin secretion. As such, there is a need to examine whether this remains true in humans under similar conditions.

Our results showed that gastrin secretion increased, CCK secretion decreased, secretin secretion remained at basal levels, and somatostatin secretion remained at basal levels or higher upon the evaluation of exocrine pancreatic secretion using Bz-Tyr-Ala. Future studies should examine whether a similar gastrointestinal hormonal environment is present among healthy adult humans when measuring exocrine pancreatic secretion using Bz-Tyr-Ala.

Acknowledgements

We would like to thank all Medical Research Support Center members at Nihon University School of Medicine for their support with LC/MS/MS analysis. Special thanks to Mr. Hiroshi Watanabe for his help.

Disclosure of conflict of interest

None.

Address correspondence to: Yukimoto Ishii, Division of Research Planning and Development, Medical Research Support Center, NihonUniversity School of Medicine, Ohyaguchi 30-1, Itabashi-ku, Tokyo 173-8610, Japan. Tel: +81-3-3972-8111; E-mail: ishii.yukimoto@nihon-u.ac.jp

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