Association between small intestinal bacterial overgrowth and beta-cell function of type 2 diabetes

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Abstract

Aims: Previous studies suggest that small intestinal bacterial overgrowth (SIBO) is associated with type 2 diabetes. However, few studies have evaluated the association between SIBO and beta-cell function in type 2 diabetes. The aim of this study was to evaluate whether beta-cell function was associated with SIBO.

Materials and methods: One hundred four patients with type 2 diabetes were included in this study. Based on the presence of SIBO, the patients were divided into SIBO-positive and SIBO-negative groups. Oral glucose tolerance tests were performed. Insulin sensitivity was measured using 1/homeostasis model assessment of insulin resistance (1/HOMA-IR) and the insulin sensitivity index (ISIM). Insulin release was calculated by HOMA-β, early-phase insulin secretion index $InsAUC_{30}/GluAUC_{30}$, and total-phase insulin secretion index $InsAUC_{120}/GluAUC_{120}$.

Results: Compared with the SIBO-negative group, patients in the SIBO-positive group showed a higher glucose level at 120 minutes, HbA1c, 1/HOMA-IR, and ISIM and a lower HOMA-β level, early-phase $InsAUC_{30}/GluAUC_{30}$, and total-phase $InsAUC_{120}/GluAUC_{120}$. Multiple linear regression analysis showed that body mass index, glucose at 0 minutes, and SIBO were independently associated with the early-phase and total-phase insulin secretion.

Conclusion: SIBO may be involved in lower levels of insulin release and worse glycemic control.

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Introduction

Small intestinal bacterial overgrowth (SIBO) is a condition that is defined as excessive colonization of Gram-negative aerobic and anaerobic bacteria in the proximal small bowel. A jejunal aspiration culture is considered to be the gold standard diagnostic test for SIBO. However, the H_2/CH_4 breath test is more readily available, safe, inexpensive, and noninvasive compared with the jejunal aspiration culture for the diagnosis of SIBO. Therefore, the H_2/CH_4 breath test is currently used in clinical practice. SIBO and intestinal microbiota have been associated with various diseases, such as Crohn's disease, irritable bowel syndrome,[1,2] functional gastrointestinal disorders,[3] deep venous thrombosis,[4] nonalcoholic fatty liver disease,[5–7] and diabetes.[8,9] Type 2 diabetes (T2DM) is a metabolic disease that is characterized by decreased insulin secretion and variable degrees of peripheral insulin resistance, which leads to hyperglycemia. Recently, many studies focused on a new mechanism where SIBO is involved in the development of T2DM. Most studies suggest that diabetic patients have a higher incidence of SIBO, especially in patients with T2DM combined with diabetic peripheral neuropathy.[9,10] SIBO has been associated with an increased risk of diabetic complications and T2DM severity. Gastrointestinal complications are common in longstanding T2DM patients.[11,12] Marked hyperglycemia decreases the motility index and propagation of duodenal and jejunal waves and slows small-intestinal transit.[13–15] Previous studies established that gastrointestinal symptoms in SIBO-positive patients with chronic abdominal pain or diarrhea and weak blood glycemic control in T2DM patients may be improved after treatment of SIBO.[16,17]

Multiple mechanisms, including glucotoxicity, lipotoxicity, oxidative stress, endoplasmic reticulum stress, and amyloid deposits in the islets,[18–20] are involved in the impaired insulin function in T2DM, which are strongly associated with glycemic control and the severity of T2DM. There is a lack of studies that have evaluated the association between beta-cell function of T2DM and SIBO. We hypothesized that SIBO is associated with insulin secretion. Therefore, the aim of the study was to evaluate whether beta-cell function was associated with SIBO.

Materials and methods

This is an observational study. From April 2016 to August 2018, 104 patients with T2DM from Tianjin Medical University Chu Hsien-I Memorial Hospital were included in this study. Data were collected retrospectively. Patients with T2DM were treated with insulin or with oral antidiabetic drugs before they were recruited into this study. This study was approved by the ethics committee of Tianjin Medical University Chu Hsien-I Memorial Hospital, and the study was conducted in accordance with the provisions of the Declaration of Helsinki. All participants provided written informed consent before they were included in this study.

Inclusion and exclusion criteria

The inclusion criteria were as follows: patients with 1) glucose H_2/CH_4 breath test for evaluation of small intestinal bacterial overgrowth; and 2) oral glucose tolerance test (OGTT) that was performed at our institution. Glucose-lowering agents that may have affected the results were discontinued before OGTT. The exclusion criteria were as follows: previous diagnosis of type 1 diabetes mellitus and special types of diabetes mellitus; previous gastrointestinal tract surgery; associated diseases that might influence intestinal microbiota; use of medications that are known to influence intestinal microbiota; and use of antibiotics during the 2 months before being enrolled into the study.

Study and control groups

One hundred four patients who had an OGTT before or after the glucose H_2/CH_4 breath test were included in the study. The study group comprised patients with SIBO and the control group included those without SIBO. We identified the sample size of subjects in this study using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

SIBO diagnostic criteria

SIBO was diagnosed using the glucose H_2/CH_4 breath test. Study participants followed a standard protocol. One day before the test, the subjects were advised to avoid high-fiber foods, butter, margarine, and sodas and asked to fast for 12 hours before the test, consuming no food except water. Subjects were required not to smoke, sleep, or exercise vigorously up to 30 minutes before or at any time during the test. The H_2/CH_4 breath concentration was expressed in parts per million (p.p.m.) and measured by gas chromatography after the administration of an oral loading dose of lactulose (20 g in 30 mL of sterile water). The test was considered to be positive if it showed one or more of the following:[21] (1) a baseline breath concentration of >10 ppm for hydrogen; or (2) an increase within 90 minutes (small intestine) that was followed by a larger peak (colonic), which indicated a positive study result (with a decrease of at least 5 ppm following the first peak). The first increase had to have one of the following to be considered positive: (1) an increase of at least 12 ppm of methane over the baseline by 90 minutes; or (2) if producing hydrogen only, an increase of at least 20 ppm of hydrogen over the baseline by 90 minutes. All breath tests were evaluated by a single experienced reader who was blinded to the treatment regimen.

Clinical measurements

All patients provided their medical history and underwent a physical examination. Clinical variables were age, sex, and duration of disease. Laboratory measurements included fasting blood glucose, HbA1c, and OGTT results. Height and weight were measured to the nearest 0.05 cm and 0.01 kg, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

Oral glucose tolerance test

A 2-hour OGTT (75 g of glucose) was performed, and samples for postprandial glucose and insulin were recorded at 0, 30, 60, 120, and 180 minutes. Patients were diagnosed with type 2 diabetes based on the OGTT results as follows: OGTT 0-minute glucose ≥ 7.0 mmol/L and/or OGTT 120-minute glucose ≥ 11.1 mmol/L.[22] Insulin sensitivity was measured using 1/homeostasis model assessment of insulin resistance (1/HOMA-IR) and the Mastuda insulin sensitivity index (ISI_M). Insulin release was calculated using the basal homeostasis model assessment-β

(HOMA-β), the early-phase insulin secretion index InsAUC$_{30}$/GluAUC$_{30}$ (where Ins is insulin, glu is glucose, and AUC30 is area under the curve at 30 minutes), and the total-phase InsAUC$_{120}$/GluAUC$_{120}$ (where AUC 120 is the area under the curve at 120 minutes). The trapezoidal method was used to calculate the glucose AUC and insulin AUC during the OGTT. Surrogate indexes of insulin sensitivity and insulin secretion were calculated based on published formulas (Table 3),[23] using glucose and insulin concentrations at 0, 30, 60, and 120 minutes.

Statistical analysis

Continuous variables are presented as mean values with the standard deviation (SD) for variables with a normal distribution or as median values with the interquartile range (IQR; 25th–75th percentiles) for variables without a normal distribution. Categorical variables are presented as a percentage. Differences in demographic and clinical variables between groups were compared using the χ^2 analysis for categorical variables. For variables with a normal distribution, a Student's *t*-test for continuous variables was used. For variables without a normal distribution, the nonparametric Mann–Whitney U-test was used. Multiple linear regression analysis was performed to assess the risk factors that are associated with beta cell function in T2DM patients. All results were analyzed by SPSS version 19.0 (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered to be statistically significant.

Results

Clinical and anthropometric characteristics of T2DM patients

Clinical characteristics and laboratory data from T2DM patients are summarized in Table 1. There was no significant difference between the SIBO-negative and SIBO-positive patients regarding the proportion of insulin or secretagogues. The SIBO was positive among 56 (53.85%) T2DM patients. There was no significant difference between the SIBO-negative and SIBO-positive patients regarding age (53.69±8.39 years vs. 53.52±10.54 years), gender (% male: 58.33% vs. 60.71%), duration of diabetes (9.00 [5.00, 12.75] years vs. 8.50 [4.25, 13.00] years), height (166.25± 7.60 cm vs. 167.38±8.31 cm), and weight (79.83±15.37 kg vs. 75.10±14.16 kg). Compared with SIBO-negative patients, SIBO-positive patients showed a lower BMI (26.67±3.85 kg/m^2 vs. 28.67±3.94 kg/m^2, P=0.011) (Table 1).

Comparison of glucose level, circulating insulin concentration, insulin sensitivity, and insulin release between SIBO-positive and SIBO-negative patients

As shown in Table 2, there was no significant difference between SIBO-negative and SIBO-positive patients regarding 0-minute glucose (8.32±1.87 mmol/L vs. 9.05±2.41 mmol/L), 30-minute glucose (13.66±3.02 mmol/L vs. 14.47±3.31 mmol/L), 60-minute glucose (17.06±4.04 mmol/L vs. 18.56±3.63 mmol/L), or 180-minute glucose (13.99±5.04 mmol/L vs. 15.78± 4.33 mmol/L). Compared with SIBO-negative patients, SIBO-positive patients had a higher level of HbA1c (8.70 [7.28, 10.20]% vs. 7.40 [6.83, 8.60]%; P=0.014) and 120-minute glucose (19.53 [17.15, 22.54] mmol/L vs. 18.72 [14.24, 21.80] mmol/L; $P = 0.049$) (Table 2). However, compared with SIBO-negative patients, SIBO-positive patients displayed a lower level of 0-minute insulin (8.71 [5.65, 12.44] mIU/L vs. 12.72 [7.72, 18.53] mIU/L; P=0.001), 30-minute insulin (17.91 [11.15, 25.68] mIU/L vs. 30.72 [18.42, 52.96] mIU/L; $P < 0.001$), 60-minute insulin (24.51 [17.49, 37.20] mIU/L vs. 43.20

Table 1. Clinical and anthropometric characteristics of T2DM patients.

Variables	SIBO(−) (n = 48)	SIBO(+) (n = 56)	P
Age (years)	53.69±8.39	53.52±10.54	0.929
Gender (male%)	58.33%	60.71%	0.805
Duration of T2DM (years)	9.00 (5.00, 12.75)	8.50 (4.25, 13.00)	0.953
Height (cm)	166.25±7.60	167.38±8.31	0.476
Weight (cm)	79.83±15.37	75.10±14.16	0.105
BMI (kg/m²)	28.67±3.94	26.67±3.85*	0.011

Data are expressed as the mean ± standard deviation for variables with normal distribution or as the median and interquartile range (25th–75th percentiles) for variables without a normal distribution.
*P<0.05 vs. SIBO-negative group.
SIBO, small intestinal bacterial overgrowth; T2DM, type 2 diabetes mellitus; BMI, body mass index.

Table 2. Comparison of glucose level, circulating insulin concentration, insulin sensitivity, and insulin release in different groups.

Variables	SIBO(−) (n=48)	SIBO(+) (n=56)	P
HbA1c (%)	7.40 (6.83, 8.60)	8.70 (7.28, 10.20)*	0.014
Glu0min (mmol/L)	8.32±1.87	9.05±2.41	0.100
Glu30min (mmol/L)	13.66±3.02	14.47±3.31	0.214
Glu60min (mmol/L)	17.06±4.04	18.56±3.63	0.055
Glu120min (mmol/L)	18.72 (14.24,21.80)	19.53 (17.15,22.54)*	0.049
Glu180min (mmol/L)	13.99±5.04	15.78±4.33	0.066
Ins0min (mIU/L)	12.72 (7.72,18.53)	8.71 (5.65,12.44)*	0.001
Ins30min (mIU/L)	30.72 (18.42,52.96)	17.91 (11.15,25.68)*	<0.001
Ins60min (mIU/L)	43.20 (26.74,66.16)	24.51 (17.49,37.20)*	<0.001
Ins120min (mIU/L)	46.84 (27.37,72.79)	30.73 (20.61,44.13)*	0.005
Ins180min (mIU/L)	33.20 (20.78,46.51)	23.07 (16.80,33.81)*	0.035
1/HOMA-IR	0.21 (0.12,0.35)	0.30 (0.19,0.49)*	0.015
ISIM	2.36 (1.66,3.87)	3.55 (2.39,5.69)*	0.006
HOMA-β	60.84 (36.61,90.53)	35.49 (18.22,64.13)*	0.002
InsAUC$_{30}$/GluAUC$_{30}$	13.19 (8.60,24.78)	7.51 (5.31,13.75)*	<0.001
InsAUC$_{120}$/GluAUC$_{120}$	17.29 (9.54,28.76)	9.16 (6.46,16.64)*	<0.001

Data are expressed as the mean ±standard deviation for variables with normal distribution or as the median and interquartile range (25th–75th percentiles) for variables without a normal distribution.
*P<0.05 vs. SIBO negative group
InsAUC$_{30}$/GluAUC$_{30}$ = (insulin at 0 minutes + insulin at 30 minutes of an OGTT)/(glucose at 0 minutes + glucose at 30 minutes of an OGTT).
InsAUC$_{120}$/GluAUC$_{120}$ = (insulin at 0 minutes + 2 × insulin at 30 minutes + 3 × insulin at 60 minutes + 2 × insulin at 120 minutes of an OGTT)/(glucose at 0 minutes + 2 × glucose at 30 minutes + 3 × glucose at 60 minutes + 2 × glucose at 120 minutes of an OGTT).
SIBO, small intestinal bacterial overgrowth; 1/HOMA-IR, 1/homeostasis model assessment of insulin resistance; ISI$_M$, Matsuda insulin sensitivity index; OGTT, oral glucose tolerance test; Glu, glucose; Ins, insulin.

[26.74, 66.16]) mIU/L; P<0.001), 120-minute insulin (30.73 [20.61, 44.13] mIU/L vs. 46.84 [27.37, 72.79] mIU/L; P=0.005), and 180 minute insulin (23.07 [16.80, 33.81] mIU/L vs. 33.20 [20.78, 46.51] mIU/L; P=0.035).

Insulin sensitivity was measured using the 1/HOMA-IR and the ISI$_M$. Compared

Table 3. Multiple linear regression analysis for early-phase insulin secretion in T2DM patients.

Variables	B	SE	β	P
SIBO	−4.675	2.285	−0.197	0.044
Glu0min	−1.287	0.545	−0.238	0.020
BMI	0.837	0.294	0.281	0.005

Note: Variables also included in the multiple linear regression analysis were age, sex, duration of T2DM, SIBO, glucose at 0 minutes, and BMI.
B, unstandardized beta; SE, standard error; β, standardized beta; T2DM, type 2 diabetes mellitus; SIBO, small intestinal bacterial overgrowth; glu, glucose; Glu0min, glucose at 0 minutes; BMI, body mass index.

Table 4. Multiple linear regression analysis for total-phase insulin secretion in T2DM patients.

Variables	B	SE	β	P
SIBO	−6.402	2.980	−0.201	0.034
Glu0min	−2.611	0.711	−0.359	<0.001
BMI	0.765	0.383	0.191	0.049

Note: Variables also included in the multiple linear regression analysis were age, sex, duration of T2DM, SIBO, glucose at 0 minutes, and BMI.
B, unstandardized beta; SE, standard error; β, standardized beta; T2DM, type 2 diabetes mellitus; SIBO, small intestinal bacterial overgrowth; glu, glucose; Glu0min, glucose at 0 minutes; BMI, body mass index.

with SIBO-negative patients, SIBO-positive patients showed a higher level of 1/HOMA-IR (0.30 [0.19, 0.49] vs. 0.21 [0.12, 0.35]; $P=0.015$) and ISI_M (3.55 [2.39, 5.69] vs. 2.36 [1.66, 3.87]; $P=0.006$), as shown in Table 3. Insulin release was calculated using basal HOMA-β, early-phase $InsAUC_{30}/GluAUC_{30}$, and total-phase $InsAUC_{120}/GluAUC_{120}$. Compared with SIBO-negative patients, SIBO-positive patients showed a lower level of HOMA-β (35.49 [18.22, 64.13] vs. 60.84 [36.61, 90.53], $P=0.002$), early-phase $InsAUC_{30}/GluAUC_{30}$ (7.51 [5.31, 13.75] vs. 13.19 [8.60, 24.78], $P<0.001$), and total-phase $InsAUC_{120}/GluAUC_{120}$ (9.16 [6.46, 16.64] vs. 17.29 [9.54, 28.76], $P<0.001$), as shown in Table 2.

Multiple linear regression analysis of risk factors associated with beta-cell function in T2DM

We further assessed the association between the aforementioned variables to identify the beta-cell function and the risk factors using multiple linear regression analysis. Early-phase insulin secretion and total-phase insulin secretion were considered to be dependent variables, and age, sex, duration of T2DM, SIBO, glucose at 0 minutes, and BMI were independent variables. The results revealed that SIBO ($P=0.044$), glucose at 0 minutes ($P=0.020$), and BMI ($P=0.005$) were significantly correlated with the early phase insulin secretion index $InsAUC_{30}/GluAUC_{30}$ (Table 3). SIBO ($P=0.034$), 0-minute glucose ($P<0.001$), and BMI ($P=0.049$) were also significantly correlated with the total-phase insulin secretion index $InsAUC_{120}/GluAUC_{120}$ (Table 4).

Discussion

It has previously been demonstrated that SIBO was related to diabetes gastrointestinal symptoms[9–12,24,25] and autonomous neuropathy.[26,27] However, little is known about the relationship between SIBO and beta-cell function. This study demonstrated that T2DM combined with SIBO patients showed the worst glycemic control and a lower level of insulin release compared with patients without SIBO. This suggests that the presence of SIBO in T2DM subjects could be associated with the beta-cell function.

Previous studies have shown a high prevalence of SIBO in patients with T2DM. Rana et al.[9] reported that SIBO was observed in 14.8% T2DM patients and in 2.8% of healthy controls. Rana et al.[25] reported that the glucose hydrogen breath

test was suggestive of SIBO in 15.5% of patients with T2DM mellitus, but only in one (2.2%) of the controls. Zetiz et al.[26] reported that SIBO was observed in 34.0% of diabetic patients. In accordance with previous studies, our results revealed that the prevalence of SIBO in patients with T2DM was up to 53.85%. However, there was a significant difference in the prevalence of SIBO. The discrepancies in these studies may be a result of the different diagnosis criteria and the different study population. In most of the studies, SIBO was diagnosed using the glucose H_2/CH_4 breath test, but the gold standard for diagnosing SIBO is duodenal or jejunal aspirate culture. Despite these differences, the data in all the above studies and our study support a higher rates of SIBO in patients with T2DM.

Compared with SIBO-negative patients, SIBO-positive patients showed a lower BMI. These results were consistent with those a previous study that showed that subjects with SIBO had significantly lower BMI and waist circumference compared with subjects without SIBO in non-constipation irritable bowel syndrome.[28] Although it is difficult to provide definite clarification between BMI and SIBO in T2DM in this study, there are several potential explanations for this association, based on previous studies. SIBO may be accompanied by malabsorption, which can lead to a variety of nutrient deficiencies and weight loss.[29] The nutritional consequences of intestinal bacterial overgrowth include vitamin deficiencies, fat malabsorption, and malnutrition. Similar results were reported in another study in T2DM, which found that SIBO could lead to vitamin deficiencies, fat malabsorption, and under-nutrition.[9] Therefore, these findings suggest that there is an inverse association between SIBO and BMI.

In previous studies, SIBO was associated with poor glycemic control and probiotics that might improve glycemic control.[16,30] Sajjad et al.[30] found that patients with non-alcoholic steatohepatitis combined with SIBO showed a higher prevalence of impaired glucose tolerance compared with nonalcoholic fatty liver disease patients without SIBO. A meta-analysis suggests that probiotic supplementation might improve, at least to some extent, metabolic control in subjects with T2DM.[16] However, these studies did not further elucidate the mechanisms of the improvement in metabolic control. Because beta-cell function is closely related to blood glucose control, we evaluated the association among blood glucose control, beta-cell function, and SIBO in our study. As shown in our results, compared with SIBO-negative patients, SIBO-positive patients showed higher OGTT 120-minute glucose and HbA1c levels. To further clarify the association between SIBO and glycemic control, we analyzed the beta-cell function and SIBO. We found that both early-phase and total insulin release were substantially lower in SIBO-positive patient compared with SIBO-negative patients. These data demonstrated that SIBO may influence insulin secretion in T2DM. Previous studies have not assessed the relationship between SIBO and insulin secretion in T2DM. However, in a previous study in non-alcoholic steatohepatitis patients, fasting insulin increased after, compared with before, ciprofloxacin. These changes in fasting insulin following ciprofloxacin suggest that these parameters may be influenced by small intestinal bacterial activity.[30] The mechanisms leading to a decrease in insulin secretion remain unclear. Recent evidence from nonalcoholic fatty liver disease patients showed that compared with those without SIBO, patients with SIBO showed significantly higher endotoxin levels and higher CD14 mRNA, nuclear factor (NF)-κB mRNA, and TLR4 protein expression.[31] Additionally, activation of inflammatory

pathways reduced insulin secretion by islets cells.[32] Therefore, we conclude that inflammation may be associated with decreased insulin secretion in SIBO-positive patients. Further studies are needed to confirm the precise mechanisms.

There are some limitations in our study. First, the number of patients included in this study was small, which might have compromised the effect of this study. Second, this study did not include healthy controls, and we could not determine the causal relationship between SIBO and T2DM. Third, this study was performed at a tertiary center, which may produce selection and referral bias. In addition, this was a cross-sectional analysis, and it was not possible to evaluate the causal relationship between SIBO and beta-cell function. Future prospective studies are needed to confirm a cause-and-effect association between SIBO and beta-cell function.

Conclusions

Despite these limitations, the present results suggest that T2DM combined with SIBO is inversely associated with insulin secretion and worse glycemic control. Further studies are necessary to confirm SIBO as a related factor for beta-cell function and to establish if treatment for SIBO improves beta-cell function and glycemic control in this population. Our study may provide a new view of T2DM and new evidence for the association between SIBO and T2DM.

Author contributions

All the authors were involved in the data collection and analysis.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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