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Abstract

Although the associations between a patient's body mass index (BMI) and metabolic diseases, as well as their breath test results, have been studied, the relationship between breath hydrogen/methane levels and metabolic diseases needs to be further clarified. We aimed to investigate how the composition of exhaled breath gases relates to metabolic disorders, such as diabetes mellitus, dyslipidemia, hypertension, and nonalcoholic fatty liver disease (NAFLD), and their key risk factors. An analysis was performed using the medical records, including the lactulose breath test (LBT) data of patients who visited the Ajou University Medical Center, Suwon, Republic of Korea, between January 2016 and December 2021. The patients were grouped according to four different criteria for LBT hydrogen and methane levels. Of 441 patients, 325 (72.1%) had positive results for methane only (hydrogen $<$ 20 parts per million [ppm] and methane $\geqslant$ 3 ppm). BMIs and NAFLD prevalence were higher in patients with only methane positivity than in patients with hydrogen and methane positivity (hydrogen $\geqslant$ 20 ppm and methane $\geqslant$ 3 ppm). According to a multivariate analysis, the odds ratio of only methane positivity was 2.002 (95% confidence interval [CI]: 1.244–3.221, $P = 0.004$) for NAFLD. Our results demonstrate that breath methane positivity is related to NAFLD and suggest that increased methane gas on the breath tests has the potential to be an easily measurable biomarker for NAFLD diagnosis.

1. Introduction

The prevalence of raised body mass indexes (BMIs) has significantly increased in many countries over several decades [1], requiring continued efforts to address this problem. A high BMI is a significant risk factor for metabolic diseases, including nonalcoholic fatty liver disease (NAFLD), diabetes mellitus, dyslipidemia, and hypertension [2–4]. The increasing prevalence of NAFLD, which progresses to liver fibrosis, cirrhosis [5–7], and even hepatocellular carcinoma, has become a global health issue in particular.

The human intestine contains approximately 40 trillion intestinal microorganisms of approximately 1000 species, including archaea, bacteria, and eukaryotic cells [8, 9]. They maintain human health by interacting normally with the body. However, an imbalance in normal intestinal microorganisms contributes to the development of functional digestive diseases, inflammatory bowel diseases (IBDs), and metabolic diseases, such as NAFLD, obesity, and diabetes mellitus [10–14]. Emerging evidence has revealed a relationship between microbial imbalances in the small intestine and metabolic diseases. Small intestinal bacterial overgrowth (SIBO), a small intestinal microbial imbalance, is a heterogeneous condition characterized by the overgrowth of specific microorganisms in the small intestine. SIBO is

associated with obesity [15] and contributes to the development of NAFLD through the gut-liver axis via microbe-derived metabolites, bile salt deconjugation, and increased intestinal permeability [16]. However, previous studies have shown that the gut microbiota's effect on obesity which is associated with NAFLD, may vary depending on the specific type of gas produced by microorganisms in the small intestine. For example, an increase in hydrogen-producing bacteria is associated with weight loss [17], and an increase in methane-producing micro-organisms is associated with weight gain [18]. However, these studies had limitations in that a sufficient number of patients were not analyzed. Therefore, using a sufficient number of patients, we examined whether there is a relationship between metabolic diseases, such as obesity and NAFLD, depending on the type of gas produced in the small intestine.

The lactulose breath test (LBT) is widely used to detect and diagnose hydrogen-producing bacteria or methanogens by measuring the concentrations of breath hydrogen and methane produced by the intestinal microbiota [19]. In this study, we investigated the association between hydrogen and methane levels in the LBT and metabolic diseases, including NAFLD, diabetes mellitus, dyslipidemia, and hypertension.

2. Materials and methods

2.1. Patients and design

The patients in this study were selected from patients who underwent an LBT among those who visited the Department of Family Medicine and the Health Promotion Center at Ajou University Medical Center, Suwon, Republic of Korea, between January 2016 and December 2021 due to abdominal discomfort, pain, abnormal bowel habit changes, and indigestion. Figure 1 shows a flowchart of the selection of the patients for this study. Of the initial 1,476 patients identified, 1025 patients who met any one of the following criteria were excluded: (1) taking dietary supplements or medications such as probiotics, taking antibiotics that could alter the composition of the intestinal microbiome, or taking any other medication that might have an impact on bowel function for 1 month before this study; (2) a history of gastrointestinal disorders such as peptic ulcer disease or IBD and prior intestinal surgeries (excluding appendectomies); (3) heavy drinking (consuming $>$210 g of alcohol per week for males and $>$140 g per week for females); and (4) missing data in the records. A total of 451 patients were reviewed, of which the data of 441 were analyzed in this study.

2.2. Anthropometry and data collection

The BMIs were determined by dividing an individual's weight by the square of their height (kg m^{-2}). Alcohol consumption data collected using a self-report questionnaire were transformed into weekly measurements of alcohol intake and expressed as grams of ethanol per week. The conversion was conducted using a graduated-frequency method [20]. A diagnosis of diabetes mellitus was defined by the administration of insulin or oral hypoglycemic drugs or by fasting blood glucose levels $\geqslant$126 mg dl^{-1}.

Dyslipidemia was defined as the presence of a previously diagnosed hyperlipidemic condition or use of cholesterol-lowering medication during the study period. Hypertension was defined by verifying antihypertensive medication use or evaluating blood pressures (BPs) (systolic BP $\geqslant$140 mmHg, diastolic BP $\geqslant$90 mmHg). The presence of a fatty liver was verified through an abdominal ultrasonography scan using a 3.5 MHz transducer. Abdominal ultrasound was performed by one of three experienced radiologists. The diagnosis of NAFLD was based on standard criteria, which included the evaluation of parenchymal brightness, bright vessel walls, beam attenuation, and liver-renal contrast [21]. We employed the following criteria to identify cases of NAFLD: indistinct presentation of the intrahepatic stromal structure, mild to moderate hepatomegaly with blunt borders, reduced intrahepatic blood flow signal with normal blood flow distribution, and increased liver brightness compared to the renal parenchyma, where fatty changes are less likely to occur. We considered the presence of a fatty liver, irrespective of the degree of fat accumulation, as an indicator of NAFLD.

2.3. LBT

All patients were requested to comply with the following restrictions to minimize basal hydrogen and methane levels on the LBT tests: (1) a carbohydrate-restricted diet 1 d before the breath test, and (2) avoid engaging in physical exercises and smoking within 2 h before and during the 2 h test. Before collecting the breath gas samples for the baseline analysis of hydrogen and methane, participants were asked to wash their mouths with 20 ml of 1% chlorhexidine solution. Before the following breath sample collections, participants consumed a syrup mixed with 10 g of lactulose concentrate (Duphalac® manufactured by JW Pharmaceutical, South Korea) and 200 ml of water.

Subsequently, exhaled breath samples were collected for 2 h (every 20 min within the initial hour, followed by 15 min intervals for the next hour). The collected hydrogen and methane samples were analyzed using a gas chromatograph (Breath Tracker SC Analyzer, QuinTron, Wisconsin, USA).

In this study, patients with hydrogen (H_2) and methane (CH_4) breath tests were classified into the following four groups: (1) H+ (hydrogen only positive: $CH_4 < 3$ parts per million [ppm] at any time and $\geqslant$20 ppm increase in H_2 over baseline within

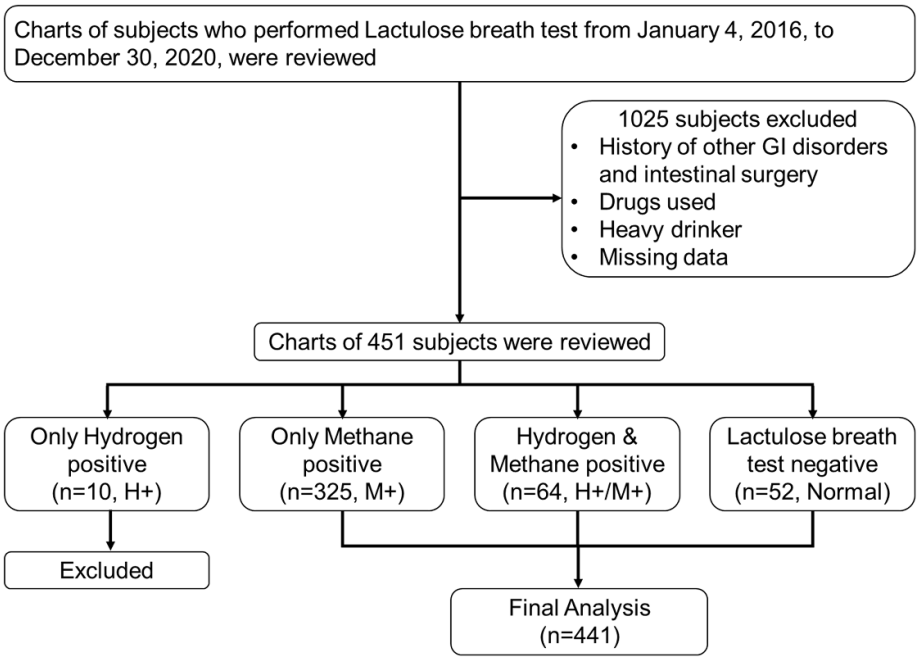

Figure 1. Flow chart showing the selection of study subjects. LBT = lactulose breath test.

90 min); (2) M+ (methane only positive: CH4 $\geqslant$ 3 ppm at any time and <20 ppm increase in H_2 over baseline within 90 min); (3) H+/M+ (hydrogen and methane positive: CH_4 $\geqslant$ 3 ppm at any time and $\geqslant$20 ppm increase in H_2 over baseline within 90 min); (4) normal (CH_4 < 3 ppm at any time and <20 ppm increase in H_2 over baseline within 90 min) [18, 22]. As the primary objective of this study was to examine the association between breath hydrogen/methane gas formed by intestinal microbiota and metabolic diseases, we applied the criterion used in previous studies on the relationship between methane gas and BMI as a methane gas positivity criterion [18, 22].

On the other hands, although lactose intolerance is common in Asians, the amount of lactose required to diagnose this condition is more than 10 g [23, 24], so the amount of lactose contained in Duphalac®, which consists of a syrup mixed with 10 g of lactulose, is not enough to raise concern about false positives. Therefore, false positives due to hidden lactose intolerance patients can be ignored.

2.4. Data analysis

SPSS Statistics 26.0 software (IBM) was used for the statistical analysis of the data. Continuous variables were expressed as means ± standard deviations, and categorical variables were presented using numbers and percentages.

To analyze continuous variables, independent *t*-tests and one-way analysis of variance with Scheffé's post-hoc test were used for the mean comparison of two and three groups, respectively. The chi-squared test was used to compare categorical variables between the groups.

Logistic regression analyses were performed to investigate the relationship between metabolic diseases and breath test results, considering potential confounding factors, including age, sex, obesity, and alcohol consumption. Statistical significance was set at $P < 0.05$.

2.5. Ethics statement

This study followed the ethical principles of medical research as stated in the Declaration of Helsinki. Approval was obtained from the Institutional Review Board (IRB) of Ajou University Medical Center, Suwon-si, Republic of Korea (IRB No. AJOUIRB-DB-2023-158). The IRB waived the requirement for informed consent.

3. Results

Of the 451 patients enrolled in this study, 325 (72.1%) tested positive for methane only (M+), 10 (2.2%) tested positive for hydrogen only (H+), 64 (14.2%) tested positive for both hydrogen and methane (H+/M+), and 52 (11.5%) tested negative for both hydrogen and methane (normal) (table 1). Patients with H+ results were excluded from further analyses because the number of patients in this group was not statistically significant.

No significant differences were found in the general characteristics, such as sex, height, and alcohol consumption, among the three groups of patients when considering the hydrogen or methane breath test results. However, patients with M+ results had a higher mean age, body weight, BMI, and NAFLD prevalence than those with H+/M+ results. Conversely,

Table 1. Comparisons between methane and hydrogen positive groups (n = 441).

Variables	M + ($n = 325$)[a]	H + /M + ($n = 64$)[b]	Normal ($n = 52$)[c]	P value	Post Hoc
Age (yr)	51.35 ± 9.04	48.03 ± 9.89	49.35 ± 7.85	0.015*	$a > b$
Gender (male, %)				0.554†	
Man	291 (89.54)	56 (87.50)	44 (84.62)		
Woman	34 (10.46)	8 (12.50)	8 (15.38)		
Alcohol (g/week)	45.88 ± 55.48	48.95 ± 58.42	41.03 ± 49.35	0.743*	
Height (cm)	169.95 ± 6.76	169.36 ± 6.57	170.00 ± 6.20	0.803*	
Weight (kg)	73.90 ± 11.13	70.30 ± 10.09	71.33 ± 10.75	0.027*	$a > b$
BMI (kg m^{-2})	25.47 ± 3.11	24.39 ± 2.62	24.55 ± 2.88	0.008*	$a > b$
Hypertension (%)	68 (20.92)	6 (9.38)	7 (13.46)	0.058†	
Diabetes mellites (%)	43 (13.23)	4 (6.25)	4 (7.69)	0.182†	
Dyslipidemia (%)	55 (16.92)	12 (18.75)	4 (7.69)	0.200†	
Non-alcoholic fatty liver (%)	225 (69.23)	27 (42.19)	31 (59.62)	<0.001†	$a > b$

Data are expressed as mean ± SD or number (percentage), as appropriate.

BMI = body mass index, SD = standard deviation.

* P value was calculated using One-way analysis of variance. † P value was calculated using χ^2 test.

Table 2. Hydrogen gas values over time in the hydrogen gas positive, methane gas positive, hydrogen/methane positive, and normal group in the breath test.

Variables	M + ($n = 325$)[a]	H + /M + ($n = 64$)[b]	Normal ($n = 52$)[c]	P value*	Post Hoc
H_2_Baseline (ppm)	9.10 ± 12.26	6.88 ± 7.95	10.58 ± 12.36	0.221	
H_2_20 min (ppm)	8.35 ± 10.62	8.09 ± 10.02	11.27 ± 8.55	0.152	
H_2_40 min (ppm)	7.55 ± 9.60	9.88 ± 12.62	10.70 ± 9.44	0.045	
H_2_60 min (ppm)	7.45 ± 9.60	17.91 ± 15.61	9.34 ± 8.36	<0.001	$b > a, c$
H_2_75 min (ppm)	8.22 ± 10.12	30.70 ± 19.31	11.13 ± 9.84	<0.001	$b > a, c$
H_2_90 min (ppm)	9.47 ± 10.75	43.66 ± 19.16	13.54 ± 8.92	<0.001	$b > a, c$
H_2_Min (ppm)	5.31 ± 8.00	5.58 ± 7.11	6.96 ± 7.13	0.358	
H_2_Max (ppm)	12.83 ± 12.61	44.11 ± 19.25	17.06 ± 11.59	<0.001	$b > a, c$

* P value was calculated using One-way analysis of variance.

Table 3. Methane gas values over time in the hydrogen gas positive, methane gas positive, hydrogen/methane positive and normal group in the breath test.

Variables	M + ($n = 325$)[a]	H + /M + ($n = 64$)[b]	Normal ($n = 52$)[c]	P value*	Post Hoc
CH_4_Baseline (ppm)	8.89 ± 8.17	7.42 ± 11.03	0.54 ± 0.60	<0.001	$a, b > c$
CH_4_20 min (ppm)	8.45 ± 7.03	7.58 ± 11.35	0.75 ± 0.62	<0.001	$a, b > c$
CH_4_40 min (ppm)	8.34 ± 6.64	7.69 ± 9.55	0.73 ± 0.59	<0.001	$a, b > c$
CH_4_60 min (ppm)	8.30 ± 6.50	8.78 ± 9.96	0.56 ± 0.57	<0.001	$a, b > c$
CH_4_75 min (ppm)	8.58 ± 6.82	10.45 ± 11.57	0.67 ± 0.64	<0.001	$a, b > c$
CH_4_90 min (ppm)	8.74 ± 6.39	11.55 ± 11.92	0.92 ± 0.58	<0.001	$a, b > c$
CH_4_Min (ppm)	7.14 ± 5.69	6.28 ± 8.53	0.27 ± 0.48	<0.001	$a, b > c$
CH_4_Max (ppm)	10.24 ± 8.26	11.94 ± 12.94	1.27 ± 0.44	<0.001	$a, b > c$

* P value was calculated using One-way analysis of variance.

no notable association was observed between breath test results and other metabolic diseases such as hypertension, diabetes mellitus, or dyslipidemia.

Patients with M+ results had a statistically significant higher mean BMI (25.47 ± 3.11 for M+ vs. 24.39 ± 2.62 for H+/M+, $P = 0.009$) based on the t-test between the M+ and H+/M+ groups. They also had a significantly higher mean BMI than the normal group ($P = 0.046$). Patients with M+ results also had a higher prevalence of hypertension (20.92% for M+ vs. 9.38% for H+/M+, $P = 0.031$) and NAFLD (69.23% for M+ and 42.19% for H+/M+, $P < 0.001$) than patients with H+/M+ according to the chi-squared test for intergroup comparison.

Table 2 shows the differences in hydrogen gas concentrations between the groups over time. Exhaled hydrogen concentration values measured at baseline and 20 min after ingesting lactulose showed no differences between the groups. However, the exhaled hydrogen concentration values measured at and 40, 60, 75, and 90 min after lactulose administration were different between the groups. Table 3 shows the differences in methane gas concentration values between the groups over time. The methane gas concentration

Table 4. Multiple Logistic Regression Analysis of M+ as an Independent Variable and Metabolic disease as a Dependent Variable.

Variables	Univariate analysis		Multivariate analysis*	
	Crude OR (95%CI)	*P* value	Adjusted OR (95%CI)	*P* value
Hypertension	1.958 (1.073–3.574)	0.029	1.546 (0.819–2.919)	0.179
Diabetes mellites	1.982 (0.936–4.197)	0.074	1.423 (0.647–3.129)	0.380
Dyslipidemia	1.147 (0.650–2.024)	0.635	0.905 (0.500–1.637)	0.742
Non-alcoholic fatty liver	2.180 (1.430–3.322)	<0.001	2.002 (1.244–3.221)	0.004

CI = confidence interval, Obesity = BMI equal to or above 25.

* Multivariate analysis was performed using binary logistic regression analysis.

Adjusted for gender, age, obesity, and alcohol consumption.

values differed between the groups in all breath tests over time.

To validate the relationship between metabolic diseases and M+ results, a logistic regression analysis was conducted. Table 4 shows the relationship between metabolic diseases and M+ results. According to the univariate analysis, hypertension and NAFLD were related to M+ results in the patients in this study. The odds ratio (OR) for M+ results was 1.958 ($P = 0.029$) in patients with hypertension and 2.180 ($P < 0.001$) in those with NAFLD. After adjusting for potential confounding factors, only NAFLD was significantly associated with M+ results (OR: 2.002, $P = 0.004$).

4. Discussion

This retrospective study found an association between methane positivity (M+), high BMIs, and NAFLD. The criterion for methane positivity (3 ppm) used in this study differed from the consensus methane level (10 ppm) generally used for the diagnosis of intestinal methane overgrowth (IMO) [25]. However, different methane level criteria have been used in previous reports [26]. The association between BMI and breath methane levels has previously been demonstrated using a methane level criterion of 3 ppm [18, 22]. Participants with high methane and low hydrogen levels (M+) showed higher prevalences of metabolic diseases, including hypertension and NAFLD, than those with H+/M+ positivity.

The relationship between the BMI and methane concentration measured using the breath test was first demonstrated by Basseri *et al* [18]. In their study, patients with methane-positive results had higher BMIs than those with methane-negative results. Methanogens in the gut have been suggested to have the potential to increase energy extraction efficiency from food, thus contributing to obesity. Through the anaerobic fermentation of dietary fibers, such as polysaccharides, methanogens can generate more short-chain fatty acids, which are absorbed in the intestines [26, 27] and become additional energy sources. The relationship between higher BMIs and higher breath methane concentrations may also be due to constipation [28–30]. Slowing of colonic transit due to constipation increases the duration for absorption of nutrients [31, 32].

Although the exact mechanism that leads to the relationship between methane gas-producing microbiota and NAFLD is unknown, an increase in methane-producing microbiota indicates gut dysbiosis [33]. This concept can be explained as follows. Damage to the intestinal barrier due to increased methanogen levels in the gut results in increased intestinal permeability, accumulation of lipopolysaccharides, and increased endogenous ethanol production, which may contribute to fat accumulation in the liver [33, 34]. It also affects energy recovery from food and choline and bile acid metabolism, contributing to the development of NAFLD [18, 33, 34]. Another possible mechanism involves the relationship between methanogens, butyrate, and fatty liver. A previous study used human feces to examine the relationship between butyric acid and the presence and abundance of methanogenic archaea. In this study, researchers found that most of the methanogenic archaea were *Methanobrevibacter smithii* and that there was an inverse relationship between the mean fecal butyrate concentration and methanogen abundance [35]. In contrast, *in vivo* supplementation with butyrate or butyrate-producing microbiota alleviates the occurrence of metabolic diseases such as NAFLD and liver fibrosis [36, 37]. This is because butyrates can act as regulators that promote fatty acid oxidation and reduce lipogenesis [36, 38]. Therefore, reduced butyrate production by methanogenic microbiota may contribute to NAFLD formation by inhibiting fatty acid oxidation and increasing lipogenesis in the liver.

Meanwhile, our study results showed that the prevalence of methane positivity in patients was higher (72%) than previous knowledge. According to previously published literature [39, 40], approximately 30%–60% of the population can be expected to be methane-positive. The difference between our findings and those of other studies could be because the methane-positivity criteria applied in this study was >3 ppm, which is lower than the 10 ppm applied in the IMO diagnostic criteria [25].

This study had several potential limitations. First, a causal relationship could not be established because

of this study's cross-sectional design. Second, more reliable results should be obtained through histological evidence or other imaging tests, such as elastography, to diagnose NAFLD. Thus, the fact that it was diagnosed using an easily measurable ultrasound should be recognized as a major limitation. Third, patients with hydrogen positivity (H+) were excluded because of the small number of individuals (2.2% of the total patients), which limited their representation in the study. Fourth, although the LBT is widely used to detect the concentrations of breath hydrogen and methane produced by intestinal bacteria, LBT results are an indirect way to assess the profile of intestinal bacteria. Additional investigations are needed to evaluate the role of the microbiome in the development of NAFLD and other metabolic diseases, as well as to determine the significance of LBT test results through the application of molecular diagnostics, such as next-generation sequencing [41, 42]. Finally, the observed power of our data was 0.57, as the event per variable for some metabolic diseases (dyslipidemia and diabetes mellitus) was less than 10, and the stability of the logistic model may be low. Therefore, future research is needed with a sample size that includes a sufficient prevalence of these metabolic diseases.

Despite these limitations, this study had several strengths. We used a cross-sectional sample of 451 Korean patients. This is the first study to examine the association between breath gases, elevated BMIs, and metabolic diseases. In addition, while previous studies investigated methane only [22] or concluded a positive association when patients produced both methane and hydrogen [18], the findings of this study demonstrated significant associations between M+ patients, higher BMIs, and NAFLD. Patients positive for hydrogen and methane had a lower mean BMI and lower prevalence of NAFLD.

In summary, we demonstrated an association between methane positivity on breath tests and NAFLD. This study also found that patients with a hydrogen-producing enteric condition in addition to a methane-producing condition might have a low BMI and low prevalence of NAFLD. Future studies on patients with a balanced proportion of confounding variables, such as sex, BMI, and methane and hydrogen positivity, are needed to develop a breath test as a novel diagnostic tool for obesity and NAFLD.

Data availability statement

The data cannot be made publicly available upon publication because they contain sensitive personal information. The data that support the findings of this study are available upon reasonable request from the authors.

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Conflict of interest

The authors declare no conflict of interest.

Contributors

All authors contributed to the conduct of the clinical trial, interpretation, drafting, and editing of the manuscript.

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