Intestinal Methane Production in Obese Individuals Is Associated with a Higher Body Mass Index

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Abstract: *Background:* Obesity is an epidemic that affects 1 in 3 individuals in the United States, and recent evidence suggests that enteric microbiota may play a significant role in the development of obesity. This study evaluated the association between methanogenic archaea and obesity in human subjects. *Methods:* Subjects with a body mass index (BMI) of 30 kg/m^2 or higher were prospectively recruited from the weight loss program of a tertiary care medical center. Subjects who met the study's inclusion criteria were asked to complete a questionnaire that included a series of visual analogue scores for bowel symptom severities. Subjects then provided a single end-expiratory breath sample to quantitate methane levels. Bivariate and multivariate analyses were used to determine associations with BMI. *Results:* A total of 58 patients qualified for enrollment. Twenty percent of patients (n=12) had breath test results that were positive for methane (>3 parts per million [ppm]), with a mean breath methane concentration of 12.2±3.1 ppm. BMI was significantly higher in methane-positive subjects (45.2±2.3 kg/m^2) than in methane-negative subjects (38.5±0.8 kg/m^2; P=.001). Methane-positive subjects also had a greater severity of constipation than methane-negative subjects (21.3±6.4 vs 9.5±2.4; P=.043). Multiple regression analysis illustrated a significant association between BMI and methane, constipation, and antidepressant use. However, methane remained an independent predictor of elevated BMI when controlling for antidepressant use (P<.001) and when controlling for both constipation and antidepressant use (6.55 kg/m^2 greater BMI; P=.003). *Conclusion:* This is the first human study to demonstrate that a higher concentration of methane detected by breath testing is a predictor of significantly greater obesity in overweight subjects.

Keywords
Obesity, nutrition, secretion, absorption, motility, methane

Obesity is a complex, multifactorial disease that contributes significantly to major health problems such as heart disease, type 2 diabetes, and certain types of cancer.[1-3] A large National Health and Nutrition Examination Survey found a

9% increase in overweight individuals (body mass index [BMI] ≥25 kg/m^2), an 8% increase in obese individuals (BMI ≥30 kg/m^2), and an almost 2-fold increase in extreme obesity (BMI ≥40 kg/m^2) in the United States between 1994 and 2000.[4] Currently, the age-adjusted prevalence of obesity in the United States is 33.8%, and the combined prevalence of obese and overweight individuals is 68%.[5] The potential benefits of reducing obesity levels are considerable, as 6% of the US healthcare budget is spent on treating obesity.[6] Major contributors to the increasing prevalence of obesity include genetic predisposition, metabolic disorders, and changes in physical activity and diet.[7]

Increasing evidence supports an association between the composition of gut microflora and the development of obesity. Indirect evidence for this association comes from data showing that obese human subjects have increased breath ethanol concentrations.[8] This increase in breath ethanol is believed to be related to gut microflora, as earlier animal studies revealed higher breath ethanol concentrations in obese versus lean mice; these concentrations decreased following administration of oral antibiotics.[9] More recent animal studies have shown that the composition and quantity of gut microflora are altered in obese mice.[10]

One particular alteration in gut microflora that is associated with increased weight gain in animal models is the presence of methanogenic archaea, specifically *Methanobrevibacter smithii*.[11] Methanogens are common in normal human enteric flora, and *M. smithii* is the most common methanogenic colonizer in humans.[12,13] Methanogens have been shown to affect caloric harvest by increasing the capacity of polysaccharide-eating bacteria to digest polyfructose-containing glycans, which leads to increased weight gain in mice.[14] Further, previous studies by our group have demonstrated that methane gas slows proximal small intestinal transit by 59% in an in vivo model.[15] This slowing of proximal small intestinal transit may contribute to increased weight gain by increasing the total gut microbiome load or the amount of time during which energy is harvested from meals. Given the associations between methanogens and weight gain in animal models, coupled with the finding of an association between methane and delayed transit, this study hypothesized that human subjects with increased concentrations of methane on breath testing might exhibit increased levels of obesity compared to individuals without elevated methane concentrations. To test this hypothesis, this study tested for associations between obesity, altered bowel symptoms, and the presence or absence of methane in breath samples in human subjects.

Methods

Study Subjects

Subjects were prospectively recruited from the weight management program of a tertiary care medical center. Individuals were eligible to participate if they were between 18 and 65 years of age and had a BMI of at least 30 kg/m^2 (which is the clinical definition of obesity) but no more than 60 kg/m^2. Subjects were excluded if they had a history of a known gastrointestinal motility disorder, gastrointestinal surgery (except for cholecystectomy and appendectomy), clinically significant abdominal adhesions, collagen vascular disease, HIV infection, uncontrolled hypo/hyperthyroidism, or uncontrolled diabetes. Subjects were also excluded if they had utilized oral antibiotics or medications that affect gastrointestinal motility (including prokinetics, antikinetics, narcotics, or metformin) within 2 months. The study was approved by the Institutional Review Board at Cedars-Sinai Medical Center.

Study Design

Informed written consent was obtained from subjects who met the eligibility criteria for this study. Subjects were then asked to complete a questionnaire that collected demographic and bowel symptom information. The presence and degree of bowel symptoms were determined based on a visual analogue scale (VAS).[16] The VAS scores were scaled from 0 to 100, with 100 mm denoting maximum severity. Bowel symptoms included constipation, diarrhea, bloating, excess gas, incomplete evacuation, abdominal pain, urgency, straining, and excessive mucous secretion from the rectum. Height and weight were recorded to determine the patient's current BMI. Data were also collected regarding current medications, medical history, and medical comorbidities (eg, diabetes mellitus, hypertension, hyperlipidemia, and fatty liver disease).

After completing the questionnaire, subjects were asked to provide a breath sample that could be assessed for the presence of methane. Specifically, subjects were asked to provide an end-expiratory breath sample using the Quintron dual bag collection system (Quintron Instrument Company). The breath sample was then analyzed using a Quintron SC gas chromatograph (Quintron Instrument Company) to determine the presence of methane. Subjects were considered to be positive for methane if methane was detected at a level of 3 parts per million (ppm) or above.[17,18]

Data Analysis

Bivariate and multivariate analyses were utilized to assess for associations between the presence of methane on breath testing and BMI. First, methane-positive and methane-negative groups were compared in terms of demographics and bowel symptom variables. T-tests were performed to compare the mean methane concentrations for each of these continuous variables. Second, Pearson product-moment correlations of continuous demographic and bowel symptom variables and BMI were calculated to determine how strongly each of these predictors correlated with BMI. A correlation matrix was also produced to determine how strongly bowel symptom variables and BMI intercorrelated. Third, independent sample t-tests were conducted to compare the mean BMI values for 2 independent groups of dichotomous predictor variables (eg, gender, presence of any diagnoses or conditions, and use of medication currently or within the last 2 months). Fourth, multivariate regression models were used to identify the association between each candidate predictor retained from bivariate analyses (independent variables) and BMI (the dependent variable), controlling for potential confounding variables. As the primary hypothesis was tested in the multivariate analyses and pairwise comparisons were used in bivariate analysis only for selection of predictors to build the regression model, *P*-values were not adjusted for multiple comparisons. A Huber-White standard error estimator was used to obtain a more conservative estimate of the *P*-value.[19] For all analyses, $P<.05$ was considered to be statistically significant.

Results

Subject Demographics

Fifty-eight obese subjects (43 female and 15 male) were enrolled in this study. All subjects completed a VAS survey to describe and rate the severity of their bowel symptoms and provided an end-expiratory breath sample for methane breath testing. The average age of the enrolled subjects was 41.8 years (range, 22–64 years), and the average BMI was 40.0 kg/m^2 (range, 30.3–57.2 kg/m^2).

Bivariate Analyses

Of the 58 obese subjects, 12 subjects (20.7%) were categorized as methane-positive and had an average breath methane concentration of 12.2±3.1 ppm. On bivariate analysis, methane-positive subjects had a greater average BMI than methane-negative subjects (6.7 kg/m^2; P=.001; Table 1). Methane-positive subjects also had a significantly greater average VAS score for constipation compared to methane-negative subjects (11.79 mm; P=.043).

Table 1. Subject Characteristics Stratified by Presence of Methane

	Total group (n=58)	Methane not detected (n=46)	Methane present (n=12)	
Subject characteristics	**Mean±SE**	**Mean±SE**	**Mean±SE**	***P*-value***
Demographics				
Age (years)	41.8±1.4	41.9±1.6	41.6±3.3	.933
Height (in)	66.7±0.6	66.5±0.7	67.7±1.4	.373
Weight (lbs)	255.0±8.0	242.3±7.1	299.0±22.7	.002
BMI (kg/m^2)	40.0±0.9	38.5±0.8	45.2±2.3	.001
Bowel symptoms (VAS)				
Bloating	22.5±3.7	23.2±4.3	20.3±7.7	.756
Gas	29.5±3.6	30.1±4.1	27.3±7.2	.752
Incomplete evacuation	14.2±2.7	12.0±2.6	21.6±7.9	.137
Abdominal pain	10.3±2.5	10.2±2.6	10.4±7.0	.973
Constipation	12.2±2.4	9.5±2.4	21.3±6.4	.043
Diarrhea	13.9±3.3	14.2±3.5	13.1±8.4	.898
Urgency	12.5±2.8	13.0±3.0	10.7±7.2	.738
Mucous	4.0±1.8	2.4±1.0	10.0±7.7	.084
Straining	13.1±2.6	12.8±2.9	14.4±6.0	.806

**P*-value is comparing methane-producing obese subjects to non-methane–producing obese subjects.

BMI=body mass index; SE=standard error; VAS=visual analogue scale.

Table 2. Bivariate Correlations with Body Mass Index for Continuous Variables

	Correlation coefficient	*P*-value
Demographics		
Age	–0.16	.230
Height	0.10	.467
Bowel symptoms		
Bloating	–0.11	.407
Gas	–0.12	.362
Incomplete evacuation	0.35	.007
Abdominal pain	–0.07	.605
Constipation	0.34	.008
Diarrhea	–0.17	.198
Urgency	–0.10	.457
Mucous	0.00	.997
Straining	0.29	.027

Pearson correlation coefficients were calculated for continuous predictor variables and BMI. Incomplete evacuation (r=0.35), constipation (r=0.34), and straining (r=0.29) had the highest correlations with BMI (Table 2). These symptoms also strongly correlated with each other (r=0.64) in each pairwise comparison (results not shown). As these bowel symptoms were highly intercorrelated, constipation was chosen as the proxy to encompass incomplete evacuation and straining.

T-tests of dichotomous predictor variables indicated that observed differences in mean BMI were significant for comorbidity with depression and antidepressant use (Table 3). As the depression and antidepressant use variables were highly correlated (r=0.79; *P*<.001), antidepressant use was selected as the proxy for depression, since antidepressant use is a tangible variable, while the self-reported diagnosis of depression is more subjective. Mean BMI was 5.40 kg/m^2 lower in subjects who were currently taking antidepressant medications compared to subjects who were not taking antidepressants (*P*=.017).

Multivariate Analysis of Predictors for Body Mass Index

For the multivariate analysis, significant predictors retained from the bivariate analyses were included to build the regression model (Table 4). Since methane has been associated with constipation in existing literature and because the motor changes induced by methane could contribute to constipation, one possibility is that methane and constipation are collinear.[18,20-22] Thus, the regression analysis was conducted using the following approach: First, when only antidepressant use (binary variable) and a positive methane breath test result (binary variable) were entered into the regression model (Model 1), both variables were significantly associated with BMI. The expected BMI was 7.45 kg/m^2 higher in subjects who had a positive methane breath test result than in methane-negative subjects (*P*=.002); conversely, the expected BMI was 4.25 kg/m^2 lower in subjects who were currently on antidepressants (*P*=.009). The overall model was statistically significant (F=10.76; *P*<.001).

Interestingly, this association persisted after adjusting for constipation. After constipation (continuous variable) was added into the model (Model 2), methane and antidepressant use remained significant correlates of BMI (Table 4). Further, constipation was not significantly correlated with antidepressant use (r=–0.14). Subjects who had a positive methane breath test result had a BMI that was 6.55 kg/m^2 higher than the BMI of methane-negative subjects (*P*=.003), and subjects who were currently on antidepressant medications had a BMI that was 3.91 kg/m^2 lower than that of subjects who were not taking antidepressants (*P*=.009). In this model, constipation was not a statistically significant correlate of BMI at the *P*<.05 level; however, the overall model remained significant (F=6.96; *P*<.001).

Discussion

This study is the first to demonstrate a significant association between the presence of methane on breath testing and the degree of obesity. In a bivariate analysis, methane-positive obese subjects had a BMI that was 6.7 kg/m^2 higher than the BMI of methane-negative obese subjects. In multivariate analysis, methane status remained significant after controlling for constipation and other variables.

Obesity is a growing epidemic in the United States; currently, 1 in 3 Americans over the age of 20 years are obese, and 2 in 3 Americans are overweight.[23,24] The healthcare burden of obesity is extremely high, as obesity is associated with type 2 diabetes mellitus, coronary artery disease, hypertension, cerebral vascular accidents, numerous malignancies, and other diseases that lead to considerable morbidity and mortality.[25,26] The economic cost of these comorbidities is threatening an already inundated healthcare system.[1-3] During the past 3 decades, caloric consumption has significantly increased in concert with a considerable reduction in physical activity, which together have contributed greatly to the high prevalence of obesity.[27]

The human gut is an intricate microbial ecosystem populated by approximately 10^{14} bacteria, alterations to which may contribute to obesity through increasing dietary energy harvest and adipose deposition.[28] Researchers' understanding of the microbial composition of the gut is improving as newer technologies enable better identification and classification of

Table 3. Observed Differences in Mean Body Mass Index (BMI) for Dichotomous Predictor Variables

Predictor variables	N	Percent	Group differences in BMI (kg/m^2)	*P*-value
Demographics				
Female gender	43	74.1	–2.56	.216
Prior diagnosis and conditions				
Irritable bowel syndrome	4	6.9	–2.52	.483
Diabetes	8	13.8	1.14	.665
Hypertension	23	39.7	–1.40	.454
Cholesterol	19	32.8	0.96	.621
Fatty liver disease	8	13.8	–0.49	.852
Depression	13	22.4	–4.21	.050
Thyroid disease	9	15.5	–1.27	.613
Bowel surgery	2	3.4	–1.49	.766
Other medical problems	21	36.2	–2.06	.274
Current medications				
Narcotics	2	3.4	4.76	.347
Antidepressants	11	19.0	–5.40	.017
Medications within the last 2 months				
Narcotics	2	3.4	–3.76	.452
Acid reflux medications	9	15.5	–0.21	.932

Table 4. Regression Coefficients Relating Body Mass Index to Predictor Variables

	Model 1			Model 2		
Variable*	**Coefficient**	**SE**	***P*-value**	**Coefficient**	**SE**	***P*-value**
Methane	7.45	2.245	.002	6.55	2.120	.003
Antidepressant use	–4.25	1.571	.009	–3.91	1.450	.009
Constipation	—	—	—	0.07	0.036	.053
	R^2=0.300 (F=10.76; *P*<.001)			R^2=0.335 (F=6.96; *P*<.001)		

*Methane and antidepressant use are binary variables. Constipation is a continuous variable.

SE=standard error.

enteric flora.[29-31] For example, the metagenome of the gut microbiome has recently been cataloged.[32] An individual's indigenous gut flora is established within the first year of life and is progressively modified throughout adulthood by endogenous and exogenous factors, including dietary intake and genetic predisposition.[33-38]

While obesity generally results from an imbalance between energy consumption (eating) and energy expenditure (physical activity and catabolism), an increase in the efficiency with which an individual's gut flora can extract energy from food may also contribute to obesity.[39] Bäckhed and colleagues showed that germ-free mice weighed significantly less than mice with normal gut flora, illustrating the significant role of gut microbiota in nutrient metabolism.[40] Further, colonization of the distal gut of germ-free mice with flora from their conventionally raised, obese counterparts resulted in excessive weight gain. Germ-free lean mice colonized with the microbiome of obese mice experienced significant increases in body fat compared to mice colonized with a conventional microbiome.[14] These data demonstrate that gut flora can play a significant role in the development of obesity.

In humans, methane-producing archaea (methanogens) produce methane through anaerobic fermentation; the most common methanogen in the human gut is *M. smithii*, which is found in 70% of human subjects.[30] Analysis of expiratory methane by lactulose breath testing can serve as an indirect measure of methane production.[17,41,42] A minority of subjects (15%) produce large quantities of methane early in the breath test, suggesting a greater methane potential, and increased methane production as measured by breath testing correlates with increased levels of *M. smithii* in stool, as determined by quantitative polymerase chain reaction assay.[13,43,44]

Methanogens remove hydrogen atoms and accelerate the fermentation of polysaccharides and carbohydrates, thus increasing the production of short-chain fatty acids that are subsequently absorbed in the intestines and serve as an additional source of energy for the human host.[45] This more efficient energy extraction may lead to weight gain and may ultimately contribute to obesity.[46] A study by Zhang and colleagues that utilized a different modality for methane measurement (stool assays) also demonstrated a promising association between methane and obesity in human subjects.[47]

Besides alterations in luminal metabolic processing, methane gas itself may influence motility. Recently, our group demonstrated that infusion of methane gas into the small intestine resulted in a slowing of small intestinal transit by 59% in an in vivo animal model.[15] The slowing effects of methane on intestinal transit could have 2 possible consequences: First, slowing of intestinal transit could increase the duration of nutrient absorption in the postprandial state. Second, slowing of transit could result in higher levels of gut microflora. Both of these effects could lead to increased weight gain and the development of obesity.

The current study demonstrates that humans with methane detectable via breath testing have a significantly higher BMI than methane-negative controls. This finding was remarkable because all subjects in this study were obese, per the study's inclusion criteria. This result remained significant when controlling for other factors, including constipation, which is an indicator of slowed transit. This result may be due to the collinearity of constipation and BMI. Although it remains unclear why methane was significant even when controlling for the clinical manifestation of transit (ie, constipation), the results of a recent animal study may help to explain this observation. In a study that has been submitted for publication, our group found for the first time that colonization of the rat gut with the methanogen *M. smithii* is not limited to the large bowel but rather extends to the small bowel, including the ileum, jejunum, and duodenum. Therefore, obese human subjects may have increased numbers of methanogens in the small bowel, rather than in the colon, thus exerting slowing effects in the small bowel while preserving colonic transit.

Another interesting finding in this study was that subjects who were currently taking antidepressant medications had a BMI that was 3.91 kg/m^2 lower than the BMI of subjects who were not taking antidepressants. While specific antidepressant medications have been shown to produce weight gain, obesity is also associated with depression, and overeating can be a sign of depression. Thus, one possible explanation for the observed data is that depression leads to a sedentary lifestyle and self-destructive behaviors such as overeating in some subjects. By treating depression with antidepressant medications, perhaps the provocation for these eating behaviors is decreased and the desire to exercise or engage in other physical activities is increased. In addition, tricyclic antidepressants have anticholiergic side effects; these medications can, therefore, lead to suppression of appetite due to delayed gastric emptying. Further studies with larger numbers of subjects would be required to test this association.

This study clearly demonstrates a relationship between intestinal methane production and BMI. However, there are some limitations to the study's data. First, this is a preliminary study that was intended to evaluate a novel relationship; thus, the sample size was relatively small, and the study was performed at a single center. The observed lack of statistical significance for some comparisons may therefore be related to the small sample size in the methane-positive group, although the multivariate analysis found that methane remained an independent predictor of elevated BMI when controlling for antidepressant use ($P<.001$) and when controlling for both constipation and antidepressant use (6.55 kg/m^2 greater BMI; $P=.003$). Second, the subjects in this study were all seeking assistance for surgical or medical weight loss, and such patients may be different from obese individuals who are not actively trying to lose weight. Therefore, larger studies will be needed to confirm our findings. However, our data are supported by recent findings in gnotobiotic animal studies; Samuel and coauthors found that *Bacteroides thetaiotaomicron–M. smithii* co-colonization produced a significant increase in host adiposity compared to monoassociated animals or *B. thetaiotaomicron–Desulfovibrio piger* biassociated animals.[45] As *M. smithii* is the most common methanogen colonizing the human gut, the increased breath methane concentration associated with greater BMI in this study also likely results from increased *M. smithii* colonization.[13,48,49]

In conclusion, this study demonstrates that the presence of methane is associated with higher BMI among obese subjects. This finding further supports the role of gut flora in obesity. Moreover, this information may expand on the evolving data in animal models, which support a specific association between methanogenic archaea and obesity. While the mechanism of this association remains unknown (slowed transit vs metabolic interactions of gut microflora), these intriguing results lay the foundation for further research in this area.

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