Positive Methane-Producing Status Associated With Increased Serum Cholesterol in Subjects With Impaired Glucose Tolerance

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OBJECTIVE — To determine if those who produce methane (i.e., have presence of methane in breath) have higher serum cholesterol than those who do not produce methane in subjects with impaired glucose tolerance (IGT).

RESEARCH DESIGN AND METHODS — We measured breath gases and fasting serum total and high-density lipoprotein (HDL) cholesterol and triglyceride (TG) levels in 21 subjects with IGT.

RESULTS — The 11 methane-producers were well matched to the 10 non-methane-producers for age, sex, and body mass index. Methane-producers had higher fasting serum total (6.5 ± 0.3 vs. 5.5 ± 0.2 mmol/l; $P < 0.02$) and low-density lipoprotein (4.3 ± 0.3 vs. 3.4 ± 0.2 mmol/l; $P < 0.05$) cholesterol concentrations with no difference in TG or HDL levels.

CONCLUSIONS — The results suggest that in subjects with IGT, positive methane-producing status may be associated with increased serum cholesterol levels.

Unabsorbed carbohydrates are fermented by colonic bacteria with the production of hydrogen gas and the short chain fatty acids (SCFAs) acetate, propionate, and butyrate in the approximate molar ratio of 60:20:20, respectively. SCFAs are rapidly absorbed from the human colon, and a conservative estimate is that 100–450 mmol are produced daily (1). SCFA may influence systemic carbohydrate and lipid metabolism, depending on the amounts and types produced (2). SCFA production is influenced by many factors including dietary carbohydrate and the nature of colonic bacterial flora. About half of the population excrete methane in their breath, indicating the presence of methanogenic bacteria in the colon (3,4). Since methane-producing status may influence serum acetate (5) and acetate is the primary substrate for hepatic lipid synthesis, we hypothesized that methane-producers have higher serum cholesterol than non-methane-producers.

RESEARCH DESIGN AND METHODS

We prospectively studied 21 subjects with impaired glucose tolerance (IGT) and 8 control subjects in the morning after a 12-h overnight fast, using procedures approved by the human subjects review committee of the University of Toronto. Subjects took a liquid test meal (Enrich, Ross Laboratories, Montreal, Canada) (450 kcal, 55% carbohydrate, 30.5% fat, 14.5% protein) and remained seated for the next 2 h. Alveolar breath samples were obtained from IGT subjects only, using a modified Haldane-Priestly tube, both during fasting and at 15-min intervals for 1 h after the start of the test meal. Venous blood samples were obtained at fasting and 2 h after starting the test meal. Fasting serum total and high-density lipoprotein (HDL) cholesterol and triglyceride (TG) levels were measured by the University of Toronto Core Lipid Laboratory. Low-density lipoprotein (LDL) cholesterol was calculated as follows: LDL = total cholesterol − HDL − TG/2.2. Methane (CH_4) and hydrogen (H_2) were measured by gas chromatography (Quintron Microlyzer, Model DP, Milwaukee, WI) in breath samples and simultaneously obtained room air. IGT was diagnosed according to World Health

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BMI, body mass index; HDL, high-density lipoprotein; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; SCFA, short-chain fatty acid; TG, triglyceride.

Organization criteria (6) (fasting plasma glucose <7.8 mmol/l and plasma glucose >7.7 and <11.1 mmol/l 2 h after 75 g oral glucose). Methane-producing status was determined before the blood lipid results were known. Methane-producers were defined as having a mean breath CH_4 concentration at least 1 ppm over that of room air (3,4). Results are expressed as means ± SE with the significance of differences assessed by analysis of variance.

RESULTS — The control subjects had the same body mass index (BMI) as the IGT subjects, but their average age was 9 years less than IGT subjects' ($P < 0.05$). Control subjects had significantly lower plasma glucose 2 h after the test meal, but the differences in fasting glucose, insulin, and lipid levels did not reach significance (Table 1). Of the 21 IGT subjects, 11 were methane-producers, and they were well matched to the non-methane-producers for sex, age, BMI, and plasma glucose and insulin concentrations (Table 1). Breath H_2 and CH_4 did not change significantly over the 1-h measurement period. As expected (5), methane-producers had significantly lower mean breath H_2 than non-methane-producers (Table 1). Mean serum total and LDL cholesterol concentrations in methane producers were significantly greater than in non-methane-producers by 19 and 26%, respectively, with no difference in TG or HDL cholesterol concentrations (Table 1).

CONCLUSIONS — The results support our hypothesis that positive methane-producing status is associated with increased serum cholesterol in subjects with IGT. The presence of methanogenic bacteria in the colon may influence blood lipids by altering systemic availability of acetate and propionate produced during carbohydrate fermentation.

There is evidence that methane-producers have higher fasting and postprandial serum acetate concentrations than nonproducers (5). The presence of methanogenic bacteria increases acetate and reduces propionate production from other ruminant bacterial species in vitro (7). In addition, in vitro fermentation of cornstarch by fecal bacteria from human methane-producers resulted in a greater proportion of acetate-to-propionate than that from nonproducers (8). When healthy volunteers consumed 20 g of the unabsorbed sugar lactulose twice daily for 7 days, fecal pH fell in non-methane-producers but not in methane-producers. Since there was no difference in total acid production during fermentation of lactulose by fecal bacteria from the two groups, it was suggested that methane-producers absorb SCFA more avidly than non-methane-producers (9).

There is evidence that colonic SCFAs influence lipid metabolism, but the exact mechanisms of action are unknown. Rectally infused acetate raises serum cholesterol, and this effect is blocked by adding propionate (2). In addition, consuming 25 g/day lactulose, which yields primarily acetate during in vitro fermentation (10), raises serum cholesterol in humans (11). Acetate is the primary substrate for cholesterol synthesis, and propionate inhibits its incorporation. However, neither acetate nor propionate affect the overall rate of cholesterol synthesis in hepatocytes as measured by tritiated water (12).

Since we could study only 21 subjects with IGT, there is a chance that our results may not be reproducible, and further studies are needed before firm conclusions can be drawn. However, if the same phenomenon occurs in subjects with normal glucose tolerance, the effect may be smaller than in subjects with IGT, who tend to have high plasma insulin concentrations, which may enhance the effect of increased substrate availability on cholesterol synthesis.

We conclude that events within the colon may influence systemic lipid metabolism in humans. Further studies are required to confirm and explain the effects we observed.

Table 1—*Mean age, BMI, breath gases, and plasma glucose, insulin, and serum lipid concentrations in control subjects and IGT subjects according to methane-producing status*

	IGT subjects		
	Methane-producers	Non-methane-producers	Control subjects
n (M/F)	7:4	6:4	4:4
Age (years)	55.9 ± 2.5	53.0 ± 2.9	45.4 ± 3.2*
BMI (kg/m^2)	30.0 ± 0.8	29.9 ± 1.9	29.7 ± 1.8
Mean breath hydrogen (ppm)	3 ± 1	12 ± 3§	—
Mean breath methane (ppm)	12 ± 4	0§	—
Glucose (mmol/l)			
Fasting	4.96 ± 0.17	5.29 ± 0.29	4.44 ± 0.18
2-h postprandial	7.07 ± 0.59	6.61 ± 0.46	4.24 ± 0.44†
Insulin (pmol/l)			
Fasting	181 ± 44	134 ± 17	117 ± 20
2-h postprandial	833 ± 104	1080 ± 214	579 ± 141
Total cholesterol (mmol/l)	6.49 ± 0.28	5.47 ± 0.23§	5.94 ± 0.14
TGs (mmol/l)	2.72 ± 0.45	2.72 ± 0.83	1.78 ± 0.30
HDL cholesterol (mmol/l)	1.05 ± 0.11	1.04 ± 0.11	—
LDL cholesterol (mmol/l)	4.32 ± 0.31	3.44 ± 0.23‡	—

Data are means ± SE. Differences between control and IGT subjects: *$P < 0.05$; †$P < 0.01$. Differences between methane-producers and nonproducers: ‡$P < 0.05$; §$P < 0.02$.

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