

Original Paper

Breath Hydrogen and Methane Responses of Men and Women to Breads Made with White Flour or Whole Wheat Flours of Different Particle Sizes

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Key words: whole grains, fiber, wheat, methane, hydrogen, fermentation, particle size

Objective: While it is well known that consumption of whole grain foods results in beneficial health effects, the great majority of Americans prefer bread made with white flour. Consumption of whole grain foods in high fiber menus may cause undesirable intestinal responses. The purpose of this study was to determine whether consumption of bread made with ultra fine ground whole wheat flour retained beneficial effects while reducing undesirable effects.

Methods: Twenty-six men and women (31 to 55 years of age) consumed glucose solutions or bread made with white, whole wheat or fine ground whole wheat flour (1 g carbohydrate/kg body weight) in a Latin square design after two days of controlled diet. Breath methane and hydrogen were determined over the 24 hours after consumption of test foods.

Results: Hydrogen and methane responses of men and women were similar. There were no significant differences in methane responses to the different treatments, but hydrogen response was increased by all breads ($p < 0.0001$). Although the overall mean response values were similar for all three breads, the patterns of hydrogen response differed (treatment*time, $p < 0.003$). Gastrointestinal symptoms were not associated with fiber content or particle size of bread; however, subjects reporting symptoms tended to have higher methane responses ($0.05 < p < 0.10$).

Conclusion: Neither fiber content of bread nor particle size of whole wheat flour substantially affected breath hydrogen or methane responses or gastrointestinal symptoms. Fine ground whole wheat breads may provide a more acceptable food choice than standard whole wheat bread without sacrificing the beneficial health effects of higher fiber.

INTRODUCTION

Evolutionary research suggests that fiber intake in paleolithic man exceeded 100 grams a day [1]. Present day consumption in the United States is approximately one fifth of this amount [2]. Most health organizations and government agencies involved in the health of the nation recommend an increase in dietary fiber [3–8]. Consumption of diets high in dietary fiber have been reported to result in a number of beneficial health effects including reduced risk of heart disease [9], hypertension [10], colon cancer [11,12], diabetes [13] and obesity [14,15]. Replacing white bread with whole grain breads is usually mentioned as one way to accomplish an increase in

dietary fiber. Consumption of carbohydrate from white bread is five times that of whole wheat, rye and other dark breads [16]. Adding intake of complex carbohydrate from doughnuts, cookies, and cakes increases the ratio of white flour to whole grain flour to seven to one. Although there has been some recent increase in the consumption of whole grain flours and as people get older they consume more whole grain foods [17], Americans prefer white flour. Many factors influence the food choices people make [18]. One deterrent to whole grain food consumption is the perception that diets high in dietary fiber cause undesirable gastrointestinal side effects [19], especially in susceptible people [20]. Diarrhea and flatulence may occur [21,22]. Expiration of breath hydrogen is a measure of the

Presented in part at the annual meeting of the American College of Nutrition, New York, September 27, 1997.

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Journal of the American College of Nutrition, Vol. 18, No. 4, 296–302 (1999)
Published by the American College of Nutrition

fermentation in the large intestine of carbohydrate which has escaped digestion in the small intestine [23]. This measure therefore can quantify the undigested amount of carbohydrate. Particle size of foods is inversely related to glucose and insulin responses [24]; but the effect on breath hydrogen has not been examined. Breath methane is a metabolite of hydrogen and an indicator of colonic fermentation [25]. The goal is to maximize the beneficial effects of whole grain foods, while minimizing undesirable side effects. The purpose of this study is to compare the effects of white bread and whole grain breads made with standard whole wheat flour and fine ground whole wheat flour to determine whether particle size affects the digestibility as measured by expiration of breath hydrogen.

MATERIALS AND METHODS

Subjects

Twenty-six subjects were selected for the study after clinical blood and urine screening and a medical evaluation (Table 1). Subjects were excluded for disease or if they were hypertensive or taking prescription drugs. Subjects discontinued any vitamin or mineral supplements for the duration of the study. All subjects completed the study. The study was approved by the Institutional Review Board of The Johns Hopkins School of Public Health and the US Department of Agriculture Human Studies Committee. Medical supervision was provided by Dr. Benjamin Caballero, Division of Human Nutrition, The Johns Hopkins University School of Public Health.

Study Design

After selection, subjects consumed a controlled menu on four different Thursdays, Fridays and Saturdays. The menu was identical during each of the four periods (Table 2). Each subject consumed the same energy level during all four periods. Nutrient content of the menu was similar to dietary recommendations [26] (Table 3). Subjects came to the Beltsville Human Nutrition Research Center's Human Diet Facility before breakfast to be weighed, eat breakfast and pick up lunch and dinner each day. They were required to consume all foods and beverages given and nothing else unless they were approved by the principal investigator. Any additional foods such as water, noncaloric beverages, salt and pepper were recorded daily. On

Table 1. Subject Characteristics¹

	Men	Women
n	13	13
Age (range)	45.6 (32–55)	40.5 (31–52)
Height (M)	1.76 ± 0.02	1.65 ± 0.01
Weight (kg)	81.0 ± 4.2	72.5 ± 4.0
BMI	25.9 ± 0.9	26.7 ± 1.4

¹ Height, weight and body mass index (BMI) are mean ± SEM.

Table 2. Study Menus¹

Thursday	Friday	Saturday
Breakfast		
Orange juice	Orange juice	Grapefruit juice
Egg substitute	Bran cereal	Waffles
Milk	Milk	Strawberries
Margarine	Margarine	Milk
Corn muffin	Blueberry muffin	Margarine
Lunch		
Tomato soup	Cheese	Tuna
Peanut butter	Chicken	Lettuce
Pudding	Lettuce, tomato	Tomato
Celery	Green pepper	Ranch dressing
Cucumber	French dressing	Applesauce
Ranch dressing	Cracked wheat bread	Vanilla wafers
Fruit cocktail	Chocolate chip cookie	
White bread		
Dinner		
Cherries	Peaches	Turkey, gravy
Yogurt	Roll	Sweet potatoes
Roast beef, gravy	Flounder, rice	Green beans
Mashed potatoes	Lemon juice	Lettuce
Roll	Spinach	Celery
Lettuce	Cucumber	French dressing
French dressing	Lettuce, tomato	Roll
	Italian dressing	Blueberries
	Brownie	Yellow cake

¹ All foods containing milk used lactose-hydrolyzed 2% milk.

Table 3. Mean Daily Nutrient Content of 3-Day Menu for 8.33 Mj (2000 kcal)

	Amount
Protein (g)	76
Carbohydrate (g) ¹	262
Fat (g)	76
P/S Ratio	1.1
Cholesterol (mg)	248
Dietary fiber	16

¹ Including the average glucose solution amount for day 3.

Friday evenings subjects were required to consume all food by 8 p.m. The following Saturdays, subjects arrived at the Center between 6 and 8 a.m. Fasting blood and breath samples were collected. Subjects then consumed either a glucose solution or a test bread (Table 4) containing an equal amount of carbohydrate (one gram of carbohydrate/kg body weight based on the mean of Thursday and Friday weights). Blood samples were collected at one-half, one, two and three hours after consumption of the test food. Blood samples were drawn by a certified phlebotomist. These blood samples were collected so that blood glucose and glucoregulatory hormone responses could be measured. These responses are being reported elsewhere [27]. Additional breath samples were collected at 2, 4, 5, 6, 7, 8, 9, 10 and 24 hours after the solution or foods were consumed. After the three hour blood sample was drawn, subjects consumed breakfast at the Center and took the remaining breath collection bags and lunch and dinner for the rest of the day with them.

Table 4. Nutrient Content of Breads (Amount/100 g Bread)

Nutrient	White	Whole wheat	Fine whole wheat
Energy (kcal)	238	231	225
Protein (g)	8.3	10.6	8.4
Carbohydrate (g)	46.5	41.0	40.8
Sugar (g)	4.0	4.7	4.6
Dietary Fiber (g)	2.0	6.6	6.5
Fat (g)	2.2	3.0	3.2
Water (g)	40.4	39.2	38.7
Soluble fiber (g)	0.7	1.0	1.0
Insoluble fiber (g)	1.1	5.3	5.3

¹ Breads supplied by Glen Weaver, ConAgra, Omaha, Nebraska 68103. All breads were made with hard white winter wheat (Platte variety). Particle size distribution of fine ground whole wheat flour: 100% <150 μm, 90% <73 μm, 53% <42 μm, 45% <37 μm; standard whole wheat flour: 100% <850 μm, 95% <425 μm, 90% <250 μm, 80% <180 μm, 50% <150 μm; white bread: 100% <150 μm.

Breakfast was consumed three hours after consumption of the glucose solution or test bread, lunch was consumed six hours after consumption of the glucose or test bread and dinner was consumed ten hours after consumption of the glucose or test bread. The last breath sample was collected when subjects arose on Sunday morning and collection bags were returned to the Center on Monday mornings. Order of treatment was in a Latin square design.

Sample Collection and Analyses

Subjects were shown the proper procedure for collection of breath samples as well as given written directions on each collection day. Alveolar air samples were obtained after subjects exhaled through a mouthpiece connected to a dual-bag system by a three-way valve. Subjects were instructed to consume lunch after the five-hour collection. The menus contained no known hydrogen producers such as lactose in milk products. Subjects were not permitted to exercise during collection. Breath samples were analyzed for hydrogen, methane and carbon dioxide by gas chromatography (MicroLyzer Model SC, Quintron Instrument Co., Inc., Milwaukee, WI). The instrument was calibrated with a reference standard containing measured amounts of hydrogen, methane and carbon dioxide. Duplicate readings were taken for each sample. Breath hydrogen and methane readings were corrected for carbon dioxide content. Data for breath samples containing carbon dioxide levels below those predicted for alveolar air were excluded from analyses [28].

Subjects recorded any gastrointestinal symptoms such as diarrhea or flatulence on the day of each tolerance.

Statistical Analyses

Differences in initial characteristics between men and women were tested by using Student’s *t* test. Differences in corrected breath hydrogen and methane values were analyzed

by the mixed procedure [29], including subject (gender), period, subject *times* treatment *times* gender *times* period as covariance parameters and gender, treatment (bread or glucose), gender *times* treatment, time, treatment *times* time, gender *times* time, and treatment *times* gender *times* time as fixed effects. Mean differences were considered statistically significant if *p*<0.05.

RESULTS

Subjects were asked to record any adverse intestinal symptoms on the day of each tolerance test. This information was returned with breath samples on Mondays. Two women and four men reported symptoms. One man and one woman reported diarrhea after consumption of glucose. Two men reported diarrhea and one reported gas after consuming the white bread. One man reported diarrhea and gas after fine ground whole wheat bread. One woman reported an upset stomach after consumption of the standard whole wheat bread. One of these men reported symptoms after two of the treatments. No clear-cut differences in reported symptoms could be attributed to specific treatments.

Mixed analysis procedure found significant time (*p*<0.0001), treatment (*p*<0.02) and treatment**time* (*p*<0.003) effects for breath hydrogen (Table 5). Breath responses of men and women did not differ. Baseline values were subtracted from corrected hydrogen levels to adjust for the slight initial differences. Breath hydrogen levels were higher after consumption of all three of the breads than after glucose. For glucose at no time was the breath hydrogen significantly higher than at the fasting level. Fasting, two hour and four hour levels did not vary with treatment. At five hours after consumption of fine whole wheat flour bread there was an elevation above the two-hour value for fine whole wheat in corrected breath hydrogen which remained elevated at 24 hours. This five-hour value was also significantly higher than the five-hour value for glucose, though the values for the other two breads were intermediate and different neither from the corresponding five-hour value for glucose or fine ground whole wheat bread. For white bread and standard whole wheat bread, significant elevation occurred at six hours, remaining elevated for white bread at 24 hours, but only to ten hours for standard whole wheat bread. At five and six hours, the responses to the fine bread were higher than the responses to glucose, while levels of the white and standard whole wheat bread were intermediate. The sum of the responses between breakfast and lunch (four to six hours) were 1.0, 5.9, 1.8, and 16.3 for the glucose, white bread, standard, and fine whole wheat bread, respectively. At seven and eight hours the responses to all breads were higher than to glucose. At seven hours the response to white bread was higher than to standard whole wheat bread, while the response to fine bread was intermediate. At nine hours, the response to white bread was higher than to standard whole wheat, to which the response was

Table 5. Levels of Breath Hydrogen above Baseline (ppm) Corrected for Carbon Dioxide after Consumption of Glucose and Breads¹

Time	Glucose	White bread	Whole wheat	Fine whole wheat
2-hour Breakfast	-2.2	-3.0 ^w	-2.5 ^w	-2.7 ^w
4-hour	1.7	0 ^{wx}	-2.6 ^w	1.6 ^{wx}
5-hour	-3.4 ^a	-0.2 ^{abwx}	-0.2 ^{abwx}	5.3 ^{bxy}
6-hour Lunch	0.7 ^a	6.1 ^{abxy}	4.6 ^{abxy}	9.4 ^{by}
7-hour	3.5 ^a	19.0 ^{cz}	12.2 ^{bz}	15.1 ^{bcz}
8-hour	1.7 ^a	16.1 ^{bz}	11.2 ^{byz}	11.9 ^{bz}
9-hour	2.6 ^a	15.4 ^{cz}	7.5 ^{abxy}	9.8 ^{bcyz}
10-hour Dinner	2.5 ^a	10.5 ^{byz}	6.1 ^{abxy}	8.3 ^{abyz}
24-hour	4.8	8.5 ^y	3.8 ^{wxy}	4.2 ^{xy}
Treatment	1.2 ^a	7.3 ^b	4.0 ^{ab}	6.3 ^b

¹ Results of mixed procedure: Treatment, $p < 0.02$; Time, $p < 0.0001$; Treatment*Time, $p < 0.003$. Gender, Treatment*Gender, Gender*Time, Treatment*Gender*Time all NS. Covariance parameters: Subject (gender), period, subject*gender*treatment*period. Two sets of superscripts are used to show differences. One set denotes differences among treatments, i.e. values within a row (time) are different (among treatments) if they do not share one of these common superscripts ^{a-c}. The other set denotes differences within treatment among different times, i.e. values within a column (treatment) are different (for different times) if they do not share one of these common superscripts ^{w-z}. Pooled SEM=3.5; $p < 0.05$. The final row (treatment) shows differences among the mean treatment values above baseline of hydrogen in ppm.

higher than to glucose. The response to fine bread was higher than to glucose. At ten hours only the response to white bread was higher than to glucose, and the responses to whole wheat bread were intermediate. By the 24-hour collection there was no difference in treatment response levels, but levels were elevated above initial fasting levels for the white and fine whole wheat breads. Mean response levels for white bread and fine whole wheat bread were higher than for glucose, but the mean response to standard whole wheat bread was intermediate.

Corrected hydrogen responses of subjects (S) who reported gastrointestinal symptoms ($n=6$) were compared to those (N) who reported no symptoms (Fig. 1). There were no significant differences in responses of the two groups. In both groups hydrogen responses after all three breads were higher than after glucose. If anything, average hydrogen responses were slightly higher in the group reporting no symptoms; however, the

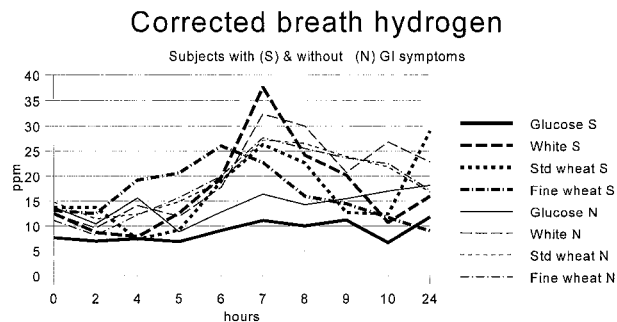


Fig. 1. Comparison of corrected hydrogen responses to glucose and white, standard whole wheat and fine whole wheat flour breads of subjects (S) reporting gastrointestinal symptoms ($n=6$) and those (N) who did not ($n=20$). Mixed procedure analysis: Treatment $p < 0.01$; Time $p < 0.0001$; Treatment*time $p < 0.01$; Glucose < all breads; Group NS.

highest mean value did occur in the subjects reporting symptoms seven hours after consuming the white bread.

Corrected breath methane levels did not vary as a result of treatment for the entire group. Only time tended to be different ($p=0.05$) for breath methane (data not shown). Methane responses tended to be higher after glucose and the whole wheat breads in the six subjects reporting gastrointestinal symptoms, but this finding was not statistically significant ($0.05 < p < 0.10$) (Fig. 2).

Eight subjects were classified as methane producers (corrected methane response > 10 ppm above fasting value) (Fig. 3). Two of the subjects selected above as reporting gastrointestinal symptoms were methane-producers. Subject selection is demonstrated by the higher responses of these subjects (P) in comparison to non-producers (N) ($p < 0.01$). Though there was no significant mean treatment effect among methane producers; peak levels occurred at different times for the different treatments: glucose at four hours; white bread at seven hours; standard whole wheat at six hours; and fine bread at five hours.

Comparison of the hydrogen responses of methane-producers (P) with subjects who did not produce methane (N) showed that both groups had higher responses to all breads (Treatment $p < 0.005$), but that the methane-producers also produced somewhat more hydrogen (Glucose: P=12.5, N=12.3; White bread: P=22.1, N=17.8; Standard wheat: P=23.7; N=15.6; Fine wheat: P=19.7; N=16.3). (Fig. 4).

DISCUSSION

Fermentation of undigested carbohydrate to short chain fatty acids has been proposed as the protective mechanism against colon cancer in those who consume diets high in fiber

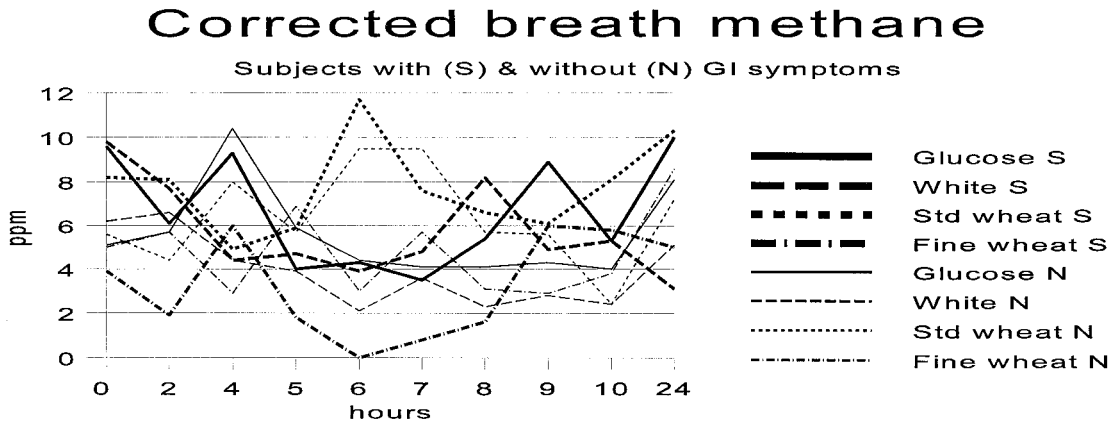


Fig. 2. Comparison of corrected methane responses to glucose and white, standard whole wheat and fine whole wheat flour breads of subjects (S) reporting gastrointestinal symptoms (n=6) and those (N) who did not (n=20). Group $0.05 < p < 0.10$.

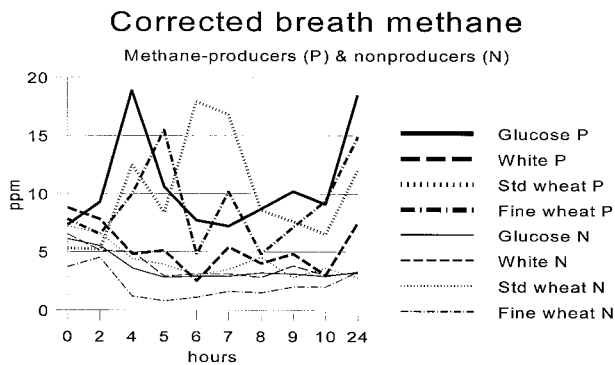


Fig. 3. Comparison of corrected methane responses to glucose and white, standard whole wheat and fine whole wheat flour breads of methane-producers [subjects (P) with methane responses >10 ppm above fasting value (n=8) and those (N) who did not produce methane (n=18)]. Group < 0.01 .

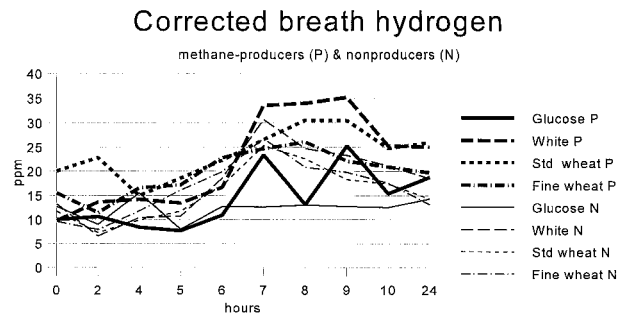


Fig. 4. Comparison of corrected hydrogen responses to glucose and white, standard whole wheat, and fine whole wheat flour breads of methane-producers [subjects (P) with methane responses >10 ppm above fasting value (n=8) and those (N) who did not produce methane (n=18)]. Mixed procedure: Treatment $p < 0.005$; Time $p < 0.0001$; Treatment*time $p < 0.002$; Group NS.

[30], although a recent report questions the role of fiber in prevention of colon cancer [31]. Breath hydrogen and its metabolite methane are measures of fermentation [32]. Previous studies have reported that consumption of foods with larger particles results in more substantial reductions in glucose and insulin responses than more finely ground or chopped foods [24]. Reductions in glucose responses were attributed to incomplete digestion of ingested carbohydrates in the small intestine, resulting in a greater amount of fermentation in the large intestine which would result in increased breath hydrogen. Results of the present study show that particle size of whole wheat flour had little effect on the cecal fermentation of undigested carbohydrate. Both standard and fine breads resulted in significant increases in hydrogen excretion compared to glucose. Even fiber content did not appear to have a significant effect on hydrogen excretion since white bread evoked similar average values.

Mixed results have been reported on the effect of various carbohydrate sources on hydrogen excretion. Van Munster *et al.* [33] reported a 35% increase in breath hydrogen in 19

healthy volunteers who consumed 15 grams of amylo maize three times per day for seven days in a crossover design compared to dextrin given as a placebo. Eastwood *et al.* [34] found no effect of gum tragacanth or wheat bran on hydrogen excretion, while gum arabic and pectin increased breath-hydrogen excretion. Levitt *et al.* [35] reported increases in hydrogen excretion after consumption of flours made from wheat, oats, potatoes and corn, but not rice. Behall and Howe [36] reported increased breath hydrogen after both acute and chronic consumption of diets high in amylose versus diets high in amylopectin. Rosado *et al.* [37] measured digestibility and breath-hydrogen in 16 Mexican women who consumed typical rural and urban Mexican diets. Although digestibilities of carbohydrate from the high fiber rural diets were significantly lower than from the low fiber urban diets, there were no differences in hydrogen excretion. The authors concluded that breath-hydrogen was a poor measure of comparison of carbohydrate fermentation from mixed diets. Since diets in the present study were controlled with only the bread being different, interpretation of our results do not suffer from this limitation. Studies

of subjects with ileostomies have been used to verify the amount of carbohydrate, which passes to the cecum undigested [38]. Soy fiber was given in five test doses (1.0–16.5 g/day for two days) to eight men and women. There was a clear dose response in the amount of carbohydrate which survived its passage to the ileostomy.

Nobigrot *et al.* [39] found increased breath hydrogen in children consuming juices which had sorbitol or more fructose than glucose. Hydrogen excretion was highest in those subjects who reported diarrhea. A breath hydrogen response of over 40 ppm was the best predictor of diarrhea. Van Munster *et al.* [33] found increased flatulence in 19 men after consumption of resistant starch compared to dextrans, but no other gastrointestinal symptoms were different, even though hydrogen was significantly increased. Reported gastrointestinal symptoms in our study do not reflect any increase in intestinal distress resulting from either whole wheat bread. In fact, the most [3] reports of distress occurred after consumption of white bread. Gastrointestinal symptoms did not appear to be related to the amount of fermentation occurring in the cecum. In the Mexican study [37], subjects reported more gastrointestinal symptoms when they consumed the rural high fiber diets even though there was no difference in hydrogen responses.

Methane is produced as a metabolite of hydrogen in the large intestine in those people who have intestinal bacteria capable of metabolizing hydrogen in a ratio of four hydrogen molecules/molecule of methane produced [40]. Although approximately 50% of adults are reported to be methane producers, in this study only eight subjects had methane responses 10 ppm above the fasting levels. When methane producers and non-producers were compared, both hydrogen and methane responses were higher in the methane-producers. Van Munster *et al.* [33] found similar results in eleven methane-producers of 19 men after consumption of amylo maize. A subject was classified as a methane excreter if methane concentration exceeded 3 ppm in two of four samples collected on two separate days. Since all mean values of methane excreters for both maltodextrin and amylo maize were well above 3 ppm, apparently both diets induced methane production. Our criterion for classification as a methane producer was more stringent, requiring an increase of greater than 10 ppm methane above fasting value. While methane appeared to be a less sensitive measure for dietary differences in the whole group, those subjects who produce methane were affected by treatment.

CONCLUSION

Subjects consuming breads made with white and either standard or fine ground whole wheat flour had increased hydrogen excretion in breath in comparison to a glucose solution. Neither particle size of the whole wheat flour or fiber content of the bread significantly affected the average hydrogen response. Reports of gastrointestinal symptoms were minimal

and not related to any of the four treatments or to hydrogen or methane production. If hydrogen production is inversely related to risk for colon cancer [41], then these results suggest that whole wheat or white bread produces similar average amounts; however, whole wheat bread provides other beneficial effects such as reduction of glucose, insulin and blood lipids; these effects do not occur after consumption of white bread. Use of fine ground whole wheat flour did affect fermentation of carbohydrate in the colon as compared to standard whole wheat flour or white flour. Consumption of whole grain foods has been demonstrated to have beneficial effects on health parameters, but the American population has overwhelmingly chosen breads made with fine white flour. Since the bread made with fine ground whole wheat flour much more closely resembles white bread, it may provide both beneficial health effects and be more acceptable to the typical American than standard whole wheat bread.

ACKNOWLEDGMENTS

The authors would like to thank Evelyn Lashley, Chief Dietitian of the Human Study Facility, for her conscientious supervision of the HSF; the research cooks Linda Lynch and Sue Burns for excellent food preparation and cheerful interaction with subjects; Daniel J. Scholfield for study coordination; Willa Mae Clark and Anna van der Sluijs for analysis of the breath samples; Elisa Armero for painless phlebotomy, and all subjects who participated in the study.

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Received April 1998; revision accepted March 1999.