Maldigestion and colonic fermentation of wheat bread in humans and the influence of dietary fat\textsuperscript{1-4}

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**ABSTRACT** A fraction of wheat bread is malabsorbed in healthy humans. The malabsorbed fraction is bigger than what can be accounted for by in vitro measurements of dietary fibers and resistant starch. To determine whether it is a specific fraction defined by the structure of the starch molecule or a variable amount—which depends on the individual, the amount ingested, and other components of the meal—we performed a dose-response study on wheat bread in healthy human volunteers. Malabsorption was evaluated by using the breath-hydrogen test. Test meals were as follows: 20 g wheat bran mixed in 100 mL water; bread made from 25, 75, 100, 150, and 200 g white wheat flour (WWF); bread made from 180 g WWF and 20 g wheat bran; and bread made from 100 g WWF served with 11 or 26 g butter, corresponding to 20\% or 35\% of energy from fat in the meals. Three of seven volunteers malabsorbed a fraction of the bread made from 25 g WWF and five of seven a fraction of the bread made from 75 g WWF. All volunteers malabsorbed a fraction of the 100-g WWF bread. Bread made from 180 g WWF and 20 g wheat bran resulted in a breath-hydrogen response of the same magnitude as that from bread made from 200 g WWF alone. The 100-g WWF bread + 11 g butter resulted in a significantly higher breath-hydrogen response than did the bread alone, whereas the 100-g WWF bread + 26 g butter resulted in an average response of the same magnitude as that from bread alone. We conclude that the malabsorbed fraction of wheat bread was dependent on the amount ingested, the composition of the meal, and individual gastrointestinal handling. Fermentation of wheat bran resulted in a very low breath-hydrogen response compared with lactulose or wheat bread. Addition of 11 g butter to the bread seemed to increase the malabsorbed fraction of the starch, an effect that was abolished when the amount of butter was increased to 26 g. *Am J Clin Nutr* 1997;66:62-6.

**KEY WORDS** Breath tests, colon, fermentation, hydrogen, ileal brake, starch, maldigestion, wheat bread, white wheat flour, humans

**INTRODUCTION**

It is well established that a fraction of bread made from wheat flour is malabsorbed in healthy humans (1-3). Whether this is a definite fraction, determined by the structure of the starch in the bread, ie, resistant starch (4), or a variable amount—which depends on the individual, the amount ingested, and other components of the meal—is unknown, and trials to quantitate the amount malabsorbed have produced different results depending on the methods used (for an overview, see reference 5). The interest in starch malabsorption is mainly due to the growing awareness of the role of bacterial fermentation in the production of short-chain fatty acids (SCFAs).

It has been estimated that 60–70 g carbohydrate/d (starch as well as nonstarch polysaccharides, NSP) are necessary to maintain the average load of colonic bacterial flora (6). The standard Western diet contains 17–22 g NSP and 2–3 g resistant starch. The gap between the estimated 60–70 g carbohydrate and the 25 g obvious substrate for fermentation must be accounted for by other sources, most likely from digestible carbohydrates in the food. We found it of interest to study whether the amount of carbohydrate in a meal or the composition of the total meal affected the fraction of carbohydrate malabsorbed. As a basic test meal, we chose bread made from white wheat flour (WWF) because there have been divergent results in different studies concerning the fraction malabsorbed, as mentioned previously. We performed a dose-response study to elucidate whether the malabsorbed fraction is dose-dependent. Further, to examine whether the malabsorbed fraction depends on the amount of energy or on the composition of the meal, we compared the malabsorbed fraction after ingestion of 100 g bread alone, 150 g bread, and 100 g bread + 11 g butter or 26 g butter. The 150-g bread was chosen as a nearly isoenergetic meal compared with the 100-g bread + 26 g butter. Eleven and 26 g butter were chosen because these amounts represent 20\% and 35\% of energy from fat in the total meal, equivalent to variations in the usual diet.

Finally, we compared the breath-hydrogen response of a bread in which 20 g flour (10\% by wt) was replaced by 20 g wheat bran because wheat bran has been proven to reduce the orocecal transit time (3), which might affect the fraction of the bread that is malabsorbed.

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SUBJECTS AND METHODS

Subjects

Fifteen healthy volunteers aged 22–29 y (10 males and 5 females) participated in the study after providing written, informed consent. They had no history of diabetes or gastrointestinal or pulmonary disease, and had not used antibiotics recently. All volunteers had a body mass index (BMI; in kg/m\(^2\)) < 25. The study was approved by the Ethical Committee of Copenhagen County and was carried out in accordance with the Helsinki Declaration II.

Test samples

Bread was made according to the following recipe: 500 g commercially purchased WWF (50 g protein, 355 g carbohydrate, 5 g fat, and 1420 kJ), 5 g dry yeast, 5 g sugar, 7 g salt, and 300 mL water. Bread for all experiments was made from the same batch of WWF and dry yeast. All ingredients were at room temperature before being mixed. The dough was mixed in a kneading machine for 3 min, placed at 34–37 °C for 60 min to rise, kneaded by hand, left to rise for another 30 min, kneaded again, put into a tin, left to rise for 60 min, then baked at 230 °C for 35 min.

Each bread made from 500 g WWF was weighed, the crust removed, and the rest cut into pieces corresponding to either 25, 75, 100, 150, or 200 g WWF and stored at −20 °C until 12 h before ingestion. A bread made from 100 g WWF weighed 136 g. In one sample 50 g per 500 g WWF was replaced by wheat bran to make a fiber-enriched bread; the wheat bran was commercially purchased (48% dietary fiber). In another sample the water was replaced by the same amount of skimmed milk (< 1% fat and 3.4% protein) and 11 g butter (fat content > 80% saturated fatty acids) per 100 g wheat flour; ∼20% of the energy in the meal was fat.

Test meals

Ten test meals were served in randomized order: 1) 20 g wheat bran mixed in 100 mL water; bread made from 2) 25 g; 3) 75 g; 4) 100 g; 5) 150 g; 6) 200 g WWF; 7) wheat bran–enriched bread made from 180 g WWF and 20 g wheat bran; 8) bread made from 100 g WWF served with 11 g butter; 9) 26 g butter, corresponding to meals with either 20% or 35% of the energy as fat; and 10) bread made from 100 g WWF, skimmed milk, and butter.

Study design

After fasting for 12 h, the volunteers met at 0730 in the laboratory on different days, each separated by ≥ 7 d. Seven volunteers participated in studies 1, 2, 3, 6, and 7; 11 volunteers participated in studies 4, 8, and 9; and 5 volunteers participated in studies 5 and 10. Test meals were served in the laboratory, followed by 200 mL tap water.

All subjects were initially challenged with 10 g lactulose (SAD, Copenhagen) in 100 mL tap water. All were able to generate a significant sustained rise in hydrogen in end expiratory air of > 10 ppm (7). Before ingestion of the test meal, a breath test [after a mouthwash with a 0.1% solution of chlorhexidine (8, 9)] was performed to obtain a fasting end expiratory breath-hydrogen concentration. If the concentration was > 20 ppm, the test was repeated after 30 min; if it was still > 20 ppm, the experiment was postponed to another day. The test meal was served at 0800 and the volunteers fasted for the next 10 h; however, tap water was allowed ad libitum. End expiratory hydrogen was measured every 30 min during these 10 h; the volunteers were seated comfortably and smoking, sleeping, and exercise were not permitted.

End expiratory breath samples were obtained by expiration through a T-piece connected to a 20-mL plastic syringe (Once; Maersk Medical, Nakskov, Denmark) (10). Breath-hydrogen excretion (in ppm) was measured as the mean of duplicate samples with a model DP microlyzer (Quintron Instruments Co, Milwaukee).

Calculations

As the basal hydrogen value, we used the lowest hydrogen concentration before the initial sustained rise (10). The cumulated excessive end expiratory hydrogen response (ppm/h) was calculated as $\Sigma H_2x - H_2b$, from the start of a sustained rise in end expiratory hydrogen to the end of the observation period; $H_2b$ is the basal value and $H_2x$ is the actual hydrogen value. Results are presented as means ± SEMs. For statistical analysis we used Student’s paired t test, and the Wilcoxon signed-rank test for paired data (11). P values < 0.05 were considered statistically significant. We made statistical calculations based on experiments with 7 or 11 participants.

RESULTS

Ingestion of bread made from 25 g WWF elicited a significant increase in end expiratory hydrogen in three of seven volunteers (Figure 1), indicating that a small fraction of the bread was undigested and fermented in these volunteers. Of the four volunteers with no response to the 25-g WWF bread, two did not respond to a bread made from 75 g WWF whereas all

![FIGURE 1. Mean (± SEM) end expiratory hydrogen in three volunteers with an increase (●) and in four volunteers with no increase (——) after ingestion of bread made from 25 g white wheat flour.](https://academic.oup.com/ajcn/article-abstract/66/1/62/4655570/6265570)
volunteers had an increase in end expiratory hydrogen after consuming bread made from 100 g WWF. Thus, all volunteers had a colonic bacterial flora able to ferment undigested residues of the bread. Further, we found that the mean hydrogen response was as follows: 100 g bread > 75 g bread > 25 g bread.

Mean (± SEM) breath-hydrogen concentrations of seven volunteers after ingestion of bread made from 25, 100, and 200 g WWF are shown in Figure 2. The increase in end expiratory hydrogen after ingestion of 20 g wheat bran, bread made from 200 g WWF, and bread made from 180 g WWF and 20 g wheat bran is shown in Figure 3. Addition of bran reduced the orocecal transit time (P < 0.05), defined as the initial significant rise in end expiratory hydrogen, whereas the total incremental increase in end expiratory hydrogen was unaffected by the wheat bran. The increase in end expiratory hydrogen after 10 g lactulose is shown in Figure 4. Comparisons between the areas under the curve (AUCs) after lactulose, wheat bran, and bread showed that the pattern of fermentation, or the amounts of fermentable substrate, were very different among the three substrates.

The dough was made from WWF, skimmed milk, and butter; the fat content was ≈ 20% of energy. This change (added fat) resulted in a higher breath-hydrogen response (cumulated as well as peak response) than that from the bread prepared from the same amount of wheat flour, without added fat (Figure 5). We also measured the effect of adding fat to the bread, serving 11 g butter to the bread made from 100 g WWF and water. We found a significantly higher breath-hydrogen response when the fat was added than when the bread was served alone (Figure 5). Bread served with 26 g butter, with a composition similar to that of the usual diet (35% of energy from fat), resulted in variable breath-hydrogen responses compared with bread alone, but an average increase in end expiratory hydrogen of

The same magnitude as that from the bread alone (Figure 5 and Figure 6). The AUC after bread alone was 199 ± 53 ppm/h and that after bread + 11 g butter was 284 ± 53 ppm/h. Ten of 11 volunteers had a higher AUC after bread + 11 g butter than after bread alone (P < 0.005). The AUC after bread + 26 g butter was 227 ± 58 ppm/h. Six volunteers had a higher AUC and five had a lower AUC after bread + 26 g butter than after bread alone (P > 0.05). Thus, the apparent increase in the

FIGURE 2. Mean (± SEM) end expiratory hydrogen after ingestion of bread made from 25 (—), 100 (▲), and 200 (●) g white wheat flour. n = 7.

FIGURE 3. Mean (± SEM) end expiratory hydrogen after ingestion of 20 g wheat bran (●), bread made from 200 g white wheat flour (●), and bread made from 180 g white wheat flour and 20 g wheat bran (—). n = 7.

FIGURE 4. Mean (± SEM) end expiratory hydrogen after ingestion of 10 g lactulose. n = 7.
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FIGURE 5. Mean (± SEM) end expiratory hydrogen after ingestion of bread made from 100 g white wheat flour (▲; n = 7); bread made from 100 g white wheat flour, 11 g butter, and skimmed milk (--; n = 5); and bread made from 100 g white wheat flour served with 11 g butter (●; n = 7).

fraction of maldigested bread provoked by the addition of 20% of energy from fat to the bread was neutralized when the fat content of the meal was increased to 35%.

The energy content of the meal consisting of bread made from 150 g WWF corresponded fairly well to the meal consisting of bread made from 100 g WWF + 26 g butter. There was a trend that the 100-g bread + 11 g butter resulted in a higher cumulated breath-hydrogen response than even the 150-g bread (Figure 6).

DISCUSSION

It is well established that a fraction of bread made from wheat flour is malabsorbed. Several studies have contributed estimates of the total amounts of malabsorbed carbohydrate—indirectly [by comparison with lactulose (2)] and directly [by measuring ileostomy outputs (12, 13)] and in vitro studies (4). The results are somewhat conflicting, ranging from <1% to 13% starch malabsorbed. In the present study the fraction of malabsorbed carbohydrate, as determined by using the breath-hydrogen test, depended on several factors; it was not a definite fraction of the bread. First, we found that the malabsorbed fraction depended on the total amount of ingested starch, i.e., only three of seven volunteers malabsorbed a measurable fraction of 25 g and five of seven malabsorbed a fraction of 75 g, whereas all malabsorbed a fraction of bread made from 100 g WWF. It might be questioned whether the breath-hydrogen test is sufficiently sensitive to measure a small fraction of the 25 g. In a previous study we proved that the breath-hydrogen test is able to measure an increase in end expiratory hydrogen after ingestion of 5 g raw potato starch, corresponding to 2.7 g slowly fermentable resistant starch (14).

Further, we found that minor modifications of substrate (e.g., substitution of 20 g WWF to 20 g wheat bran) resulted in nearly identical responses in end expiratory hydrogen within the same group of volunteers (Figure 3). The orocecal transit time was slightly reduced with the bran-enriched bread whereas the total AUCs were nearly identical, indicating that the malabsorbed fraction of bread was unaffected by the addition of bran. Hamberg et al (3) also found a reduction in orocecal transit time after adding wheat bran to a bread made of wheat starch, but contrary to the present results they found that addition of bran increased the fraction of starch malabsorbed. Hamberg et al added 36 g wheat bran to 100 g starch whereas we added 20 g wheat bran to 180 g WWF because we wanted the bread to be similar to that in a realistic meal. The different results may have been due to the effect of the bran on the orocecal transit time. Chapman et al (12) showed that slowing the transit time increased starch digestibility and speeding it up reduced digestibility.

The lactulose and the wheat bran test meals contained approximately the same amounts of potentially fermentable substrates. It is well established that lactulose is rapidly fermentable whereas wheat bran is slowly and incompletely fermented (15, 16), and this is mirrored in the very different AUCs after the two test meals. Although wheat bran may be incompletely fermented, we must assume that >10% is fermented (mean AUC for lactulose = 420 ppm/h; mean AUC for wheat bran = 45 ppm/h). This implies that a lactulose standard is not suitable for estimating amounts of fermented wheat bran. Hydrogen generation differs depending on the fermentation substrate (17, 18), and hydrogen is only one of the end products for fermentation (19). We are unable to say whether the undigested residues of the bread are fermented in a pattern resembling that of lactulose or wheat bran. Anderson et al (1) found that

FIGURE 6. Mean (± SEM) end expiratory hydrogen after ingestion of bread made from 150 g white wheat flour (▼; n = 5), bread made from 100 g white wheat flour served with 11 g butter (●; n = 7), and bread made from 100 g white wheat flour served with 26 g butter (--; n = 7).
low-gluten wheat bread caused no increase in breath hydrogen, adding gluten to the low-gluten flour yielded no increase in hydrogen, whereas bread made from common wheat flour resulted in increases in hydrogen similar to those in the present study. Thus, we assume that the malabsorbed fraction of the bread was not simple carbohydrates, and therefore the pattern of fermentation was not necessarily comparable with that of lactulose. This implies that we were unable to estimate the amount of malabsorbed carbohydrate from the bread by the breath-hydrogen test.

We showed that the composition of the meal, in this case the amount of added fat, affects the fraction of carbohydrate malabsorbed. When 20% of the energy content of the meal was fat, the malabsorbed fraction was significantly greater than that when the meal contained no fat or 35% fat. Because of the large inter- and intridual variability of the breath-hydrogen test (10) it is questionable whether this test is sensitive enough to draw such a conclusion. We found that four of five volunteers had a higher integrated hydrogen response (AUC) after bread made from butter, skimmed milk, and WWF than after bread made from WWF and water. Six of seven volunteers had a higher AUC after bread + 11 g butter than after bread alone, and we brought another four volunteers into the study and found that four of these four had a higher AUC after bread + 11 g butter than after bread alone. When the amount of butter was increased to 26 g, 5 of 11 volunteers had a lower response and 6 of 11 had a higher response than after bread alone. Small bowel transit is prolonged in patients with steatorrhea (20). Chronic nonspecific diarrhea in children frequently responds to added dietary fat (21). Infusion of fat into the ileum delays the passage of a meal through the stomach and small intestine (22), and the associated increase in small intestinal residence is accompanied by enhanced absorption of a carbohydrate meal (23). Thus, we had expected enhanced carbohydrate absorption in the meals containing fat, reflected in a smaller integrated end expiratory hydrogen concentration.

Because we assumed that the source of fermentable substrate was the ingested bread and principally the same whether it was served with butter or alone, we concluded that the significantly higher cumulated hydrogen response after the bread served with 11 g butter must have been due to an increased fractional malabsorption of the bread caused by the presence of the fat in the meal. This malabsorption-promoting effect of the added 20% fat was neutralized if the fraction of fat in the test meal was increased to 35%.

We have not been able to find results similar to ours in the literature; therefore, an explanation of these findings requires further study. A possible explanation could be that fat in the form of the 11 g butter in the meal was insufficient to provoke the ileal brake—the slowing of intestinal transit during fat perfusion of the distal small intestine (22, 24)—whereas a sufficient proportion of the 26 g butter traversed the small intestine unabsorbed to reach the ileum and provoke this response. It is very interesting that even minor changes to an ordinary diet are able to influence the pattern of absorption and malabsorption of ordinary food items.

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REFERENCES


