A Cereal-Based Evening Meal Rich in Indigestible Carbohydrates Increases Plasma Butyrate the Next Morning\textsuperscript{1,2}

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\textbf{Abstract}

Epidemiological studies have shown an inverse relation between a whole grain consumption and risk of type-2 diabetes and cardiovascular disease. One tentative mechanism relates to colonic metabolism of indigestible carbohydrates. In a previous study, we reported a positive relation between colonic fermentation and improved glucose tolerance. This work can be seen as an extension of that study, focusing on the tentative role of specific colonic metabolites, i.e. SCFA. Plasma concentrations of acetate, propionate, and butyrate were determined in the morning in healthy participants (5 women and 10 men, mean ± SD: 25.9 ± 3.2 y, BMI < 25) following 8 different cereal-based evening meals (50 g available starch) varying in content of indigestible carbohydrates. Each participant consumed all test meals in a random order on separate evenings. At a standardized breakfast following evening test meals, the postprandial glucose response (incremental area under the curve, 0–120 min) was inversely related to plasma butyrate (r = −0.26; P < 0.01) and acetate (r = −0.20; P < 0.05) concentrations. Evening meals composed of high-amylose barley kernels or high-\textbeta-glucan barley kernels resulted in higher plasma butyrate concentrations the following morning compared with an evening meal with white wheat bread (P < 0.05). The results support the view that cereal products rich in indigestible carbohydrates may improve glucose tolerance through a mechanism involving colonic fermentation and generation of SCFA, where in particular butyric acid may be involved. This mechanism may be one explanation by which whole grain is protective against type 2 diabetes and cardiovascular disease. J. Nutr. 140: 1932–1936, 2010.

\textbf{Introduction}

Food with a low glycemic index (GI)\textsuperscript{6} (1–5) and whole grain diets (6–10) have proven beneficial in the prevention and/or treatment of diabetes, cardiovascular disease, and metabolic syndrome. Furthermore, a low-GI diet combined with high intake of cereal fiber appears to be particularly advantageous in preventing diabetes type 2 in both women (11) and men (12). In addition to lowering the acute blood glucose response, low-GI foods also may reduce the blood glucose response following a standardized “second meal” consumed after 4 h (e.g. from breakfast to lunch) (13). Some cereal foods that are rich in indigestible carbohydrates may, compared with a white wheat bread (WWB), also lower the blood glucose response to a meal in a 10-h perspective (e.g. from an evening meal to a subsequent breakfast) (14–16). Colonic fermentation of carbohydrates [dietary fiber (DF) and resistant starch (RS)] results in formation of metabolites, particularly SCFA (mainly acetic, propionic, and butyric acids) and gases (e.g. hydrogen) (17,18). Although a substantial part of the SCFA formed is extracted during passage of the liver (19), a fraction may enter the systemic circulation and can be determined in the peripheral blood (20). It has been suggested that certain SCFA may be involved in the modulation of glucose metabolism (14,16,21) and lipid metabolism (22) and it can be hypothesized that this may in fact be one possible mechanism whereby whole grain foods exert beneficial metabolic effects as seen in observational studies. However, whole grain foods are, in addition to being rich in DF, also rich in other potentially bioactive phytochemicals such as minerals, plant stanols and sterols, phenolic antioxidants, and vitamins (10,23). Consequently, the mechanisms for the beneficial effects of a whole grain diet may be multi-factorial and remain to be elucidated.

Previously, we reported that cereal-based evening test meals containing high amounts of whole grain constituents (barley DF and RS) were capable of improving glucose excursion in healthy...
Materials and Methods

Participants. Seventeen healthy volunteers, 6 women and 11 men, aged 22–32 y (mean ± SD; 25.9 ± 3.2 y) with normal BMI (mean ± SD; 22.5 ± 2.1 kg/m²) were included in the study. They were carefully informed that they could quit the experiment at any time without giving any explanation. One man was excluded due to poor compliance with the bread intake and 1 woman dropped out due to gastrointestinal discomfort. Thus, 15 participants completed the study. Approval of the study was given by the Regional Ethical Review Board in Lund, Sweden.

Evening test meals and the standardized breakfast. Eight cereal-based evening test meals with different GI and contents of indigestible carbohydrates were included in the study. Details regarding composition and glycemic characteristics of the test meals are described elsewhere (24). Briefly, the test meals consisted of bread with the main proportion of carbohydrates derived from: 1) white wheat flour (WWB, reference product); 2) ordinary barley kernels (OB; nonspecified ordinary Swedish barley provided by Lantmännen Food); 3) OB that were cut 1–2 times (cutOB); 4) barley kernels from a variety with elevated amounts of amylose, yielding increased levels of RS in the finished product (HAB; 31% RS, starch basis, Karmosé, Svalöf Weibull); 5) barley kernels from a variety with elevated amounts of β-glucans (HBB; 14–16% β-glucans on dry basis, mutant 13, Svalöf Weibull); 6) WWB+RS; WWB with added RS (RS2) from a high-amylase corn starch (Hi-maize 1043, Biomin) to match the RS content in OB; or 7) WWB+RS+HBB, WWB with added similar amounts of RS and also DF from barley (Lyckeby Stärkelsen) to match the content of indigestible carbohydrates in the OB bread product.

To investigate a possible dose-response effect, 1 test meal consisted of one-half a portion of OB bread (1/2OB bread). The size of all test meals (except for the half portion) corresponded to 50 g available carbohydrates, calculated by subtracting RS (25) from the total starch content (26). White wheat flour was added to the kernel-based breads in proportions (weight percent) of 10/90 white wheat flour/kernels. Water (unlimited amounts) was consumed with the test evening meals.

The standardized breakfast consisted of 116.7 g WWB corresponding to 50 g available starch. Water (250 mL) was served with the standardized breakfast meal.

Research design. The study had a randomized crossover design of 8 single evening meal treatments (7 test meals and 1 reference meal. The test meals were consumed in the evenings and the test markers were collected in the next morning. At 2130 h in the evening prior to the experimental day, the test participants consumed 1 of the 8 test bread meals in random order on 8 occasions separated by ~1 wk. On the experimental days, volunteers attended the research department at 0745 h after an overnight fast (~10 h). When arriving on the experimental days, an i.v. cannula (BD Venflon, Becton Dickinson) was inserted into an antecubital vein to be used for blood sampling. The standardized breakfast (see the section above) was consumed at 0800 and the participants were told to consume the breakfast within 10–12 min.

The participants were encouraged to standardize their meal pattern and avoid foods rich in DF the day prior to each experimental day. Furthermore, they should avoid alcohol and excessive physical exercise in the evening and should not have consumed antibiotics or probiotics during the previous 2 wk.

Chemical analyses and GI characteristics of the test products. The test products were analyzed for total starch, RS, DF (soluble and insoluble, excluding RS), and GI characteristics. The available starch content was calculated by subtracting RS from total starch. The methods used and the results are presented elsewhere (24).

Determination of plasma SCFA. Venous blood was collected into tubes containing K2EDTA. The blood samples were withdrawn at fasting immediately prior to the standardized breakfast and at 30 min after the start of the breakfast. The tubes were centrifuged and plasma was separated and stored in a freezer (<−20°C) until analyzed. SCFA (acetate, propionate, and butyrate) were analyzed essentially as described in (27) using 2-ethyl butyrate (Fluka No. 03190; Sigma Aldrich) as an internal standard rather than iso-valeric acid.

Calculations and statistical methods. The study design was a within-subjects design. SCFA concentrations were collected at time = 0 and at time = 30 min. Differences in SCFA between the products at different time points (time = 0 and time = 30 min) were analyzed by using a mixed model (PROC MIXED in SAS release 8.01; SAS Institute) with repeated measures and an autoregressive covariance structure. If nothing else is stated in the “Results” section, further statistical calculations regarding acetate, propionate, and butyrate were based on the means of the 2 time points (time = 0 and time = 30 min). Significant differences in SCFA concentrations dependent of the test meals were assessed using ANOVA followed by Dunnett’s simultaneous test comparison method for comparing test means to the WWB control by using MINITAB Statistical Software (release 13.32; Minitab). Transformed data (Box Cox transformation in MINITAB) were used in the SAS and MINITAB calculations. Generally, calculations were based on n = 15, 1 exception being the calculations of results following the HBB evening meal (n = 14). The results are expressed as means ± SEM. Spearman rank correlation was used to study relations between results of plasma SCFA and results of relevant metabolic parameters reported previously (plasma glucose, serum insulin, serum FFA, plasma GLP-1, breath hydrogen, satiety, and GER) (24) and between plasma SCFA in the morning and amounts of indigestible carbohydrates (DF and RS) included in the test evening meals. Each participant obtained results emanating from 8 separate test meals, with 1 exception (7 test meals; see above). For the correlation calculations, a correlation for each participant was calculated and from these values the mean of Spearman correlation coefficient was obtained. To determine the P-value, a permutation test was performed using MATLAB with the null hypothesis that no correlations existed (the alternative hypothesis was that the data were correlated). Differences of P < 0.05 were considered significant.

Results

Plasma SCFA concentrations. Plasma concentrations of butyrate in the morning were affected by the test products consumed the previous evening (P = 0.022). The evening test meals affected the plasma butyrate concentrations at fasting and 30 min after commencing the standardized breakfast (P < 0.05). Those composed of HAB and HBB resulted in higher concentrations the following morning than those with WWB (P < 0.05; Table 1). Plasma butyrate and propionate concentrations were higher at 30 min after the standardized breakfast (2.7 ±
0.11 μmol/L and 8.0 ± 0.17 μmol/L, respectively) than at time = 0 (2.1 ± 0.08 μmol/L and 7.6 ± 0.18 μmol/L, respectively) (P < 0.01).

The concentrations of plasma SCFA in the morning were positively correlated with the contents of indigestible carbohydrates included in the evening test meals (Table 2). Most pronounced was the relation between indigestible carbohydrates and plasma butyrate, but plasma acetate was also positively correlated with the content of indigestible carbohydrates.

**Relations between SCFA and metabolic test markers.** The results of plasma butyrate and acetate concentrations in the mornings following the 8 different evening test meals were inversely related to the postprandial glucose response [incremental area under the curve (iAUC) 0–120 min; r = −0.26, P < 0.01 and r = −0.20, P < 0.05, respectively] (24). Additionally, the plasma butyrate concentrations at fasting were inversely related to the serum insulin response (iAUC 0–120 min) (r = −0.19; P < 0.05). Plasma butyrate at 30 min and fasting concentrations of serum FFA tended to be inversely correlated (r = −0.22; P = 0.056).

Plasma butyrate (mean of 0 min and 30 min) was positively related to the concentrations of breath hydrogen (mean excretion 0–120 min after breakfast) (r = 0.30; P < 0.01).

**Discussion**

In this study, we report an increase in plasma butyrate concentrations in the morning following an evening meal composed of barley kernel-based bread. As has been reported previously, with the exception of the WWB + RS bread and the 1/2OB bread, all evening test meals resulted in lower glucose response (iAUC 0–120 min) at the following standardized breakfast compared with the WWB evening meal (24). Due to inverse relations in the previous study between breath hydrogen (breath hydrogen was determined as a marker of colonic fermentation) and postprandial glucose response (r = −0.25; P < 0.05) and GER (r = −0.24; P < 0.05), and a positive relation between breath hydrogen and satiety (r = 0.27; P < 0.01), it was put forward that benefits to metabolism can be mediated through colonic fermentation of indigestible carbohydrates. The plasma butyrate concentrations determined in this study were significantly correlated to breath hydrogen excretion obtained previously (r = −0.31; P < 0.01).

Although the evidence is indirect, SCFA produced during colonic fermentation of indigestible carbohydrates are suggested to have beneficial effects on glucose metabolism (14,21,28). The results obtained from the present study, showing an inverse relation between postprandial glucose response and plasma butyrate, support this concept.

The HAB and HBB evening meal breads resulted in significantly increased concentrations of plasma butyrate in the morning compared with a WWB evening meal. The HAB product was characterized by high levels of RS (22 g/portion) and soluble DF (5.2 g/portion, mostly β-glucans). Previous observations suggest that β-glucans, but also RS, promote butyric acid production upon colonic fermentation (15,29); thus, the elevated plasma butyrate concentrations after HAB bread intake is in accordance with previous studies. However, although the ingested amount of barley DF and RS was considerably higher in the HBB compared with all the other test products, this bread did not result in higher concentrations of SCFA compared with the HAB. One explanation for this discrepancy between the amount of indigestible carbohydrates consumed in the evening and SCFA concentrations in plasma in the morning could be a reduced transit time from mouth to cecum caused by the high amounts of viscous DF in the HBB (30,31). This makes it plausible that only a part of the indigestible carbohydrates in the test meal had reached the colon at the time of the standardized breakfast. Accordingly, there was an increase in plasma levels of butyrate and propionate from the fasted state to 30 min supporting an ongoing fermentation process, suggesting that a time factor is important and that higher concentrations of plasma SCFA levels might have been reached later in the postprandial phase, as has been observed in pigs fed wheat-, oat-, or rye-based bread (32,33).

A limitation in this study may be that the high amounts of indigestible carbohydrates in the HBB resulted in a considerably larger serving of this evening test meal compared with the other meals. One of the participants failed to eat this meal and another 3 finished only ~75% of the serving. Two of the participants reported mild stomachache and 2 participants reported mild nausea after the HBB.

Even though previous results show conflicting results, e.g., concerning effects of different SCFA, SCFA produced during colonic fermentation of indigestible carbohydrates are suggested to have beneficial effects on glucose metabolism (15,16,21,28). In contrast, others studies have failed to show benefits of SCFA on glucose metabolism in healthy participants following rectal (34) or gastric (35) infusion of propionate, acetate, or a

**TABLE 1** Plasma concentrations of SCFA, mean of fasting and at 30 min after start of the standardized breakfast, following intake of different test meals the previous evening1,2

<table>
<thead>
<tr>
<th>Test meals</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWB</td>
<td>1.48 ± 0.72</td>
<td>7.4 ± 0.26</td>
<td>2.0 ± 0.18</td>
</tr>
<tr>
<td>OB</td>
<td>1.58 ± 0.29</td>
<td>7.7 ± 0.50</td>
<td>2.5 ± 0.26</td>
</tr>
<tr>
<td>1/2OB</td>
<td>1.53 ± 0.55</td>
<td>7.9 ± 0.43</td>
<td>2.3 ± 0.21</td>
</tr>
<tr>
<td>cutOB</td>
<td>1.64 ± 0.55</td>
<td>8.5 ± 0.49</td>
<td>2.3 ± 0.17</td>
</tr>
<tr>
<td>HAB</td>
<td>1.63 ± 10.2</td>
<td>7.7 ± 0.33</td>
<td>2.8 ± 0.29**</td>
</tr>
<tr>
<td>HBB</td>
<td>1.70 ± 0.7</td>
<td>8.3 ± 0.72</td>
<td>2.6 ± 0.29*</td>
</tr>
<tr>
<td>WWB+RS</td>
<td>1.47 ± 0.84</td>
<td>7.3 ± 0.39</td>
<td>2.2 ± 0.26</td>
</tr>
<tr>
<td>WWB+RS+DF</td>
<td>1.52 ± 0.73</td>
<td>7.8 ± 0.34</td>
<td>2.6 ± 0.31</td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM, n = 15 except HBB; n = 14. Asterisks indicate different from WBB: *P < 0.05, **P < 0.01.
2 The results are based on means of concentrations at baseline and at 30 min.

**TABLE 2** Spearman rank correlation coefficients for the relations between plasma SCFA concentrations in the morning and contents of indigestible carbohydrates in the previous evening’s test meals1,2

<table>
<thead>
<tr>
<th></th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insoluble DF</td>
<td>0.33*</td>
<td>0.21</td>
<td>0.34**</td>
</tr>
<tr>
<td>Soluble DF</td>
<td>0.23</td>
<td>0.08</td>
<td>0.34**</td>
</tr>
<tr>
<td>Total DF</td>
<td>0.32*</td>
<td>0.12</td>
<td>0.34**</td>
</tr>
<tr>
<td>RS</td>
<td>0.28*</td>
<td>0.06</td>
<td>0.32**</td>
</tr>
<tr>
<td>Total DF+RS</td>
<td>0.30*</td>
<td>0.09</td>
<td>0.32**</td>
</tr>
</tbody>
</table>

1 Asterisks denote significant correlations: *P < 0.05, **P < 0.01.
2 SCFA concentrations used in the statistical calculations are the means of times 0 and 30 min.

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combination of these. The mechanisms whereby colonic fermentation of indigestible carbohydrates and formation of SCFA may positively influence metabolic parameters are not clear, but several possible mechanisms can be suggested. It is known that circulating FFA is strongly connected to insulin sensitivity (36) and glucose tolerance (16,24,37). Interestingly, our results show a tendency ($r = -0.22; P = 0.056$) toward an inverse relation between plasma butyrate concentrations at 30 min after the standardized breakfast and fasting concentrations of serum FFA [FFA determined in the previous study (24)]. Although not observed in the present study, other possible mechanisms whereby colonic fermentation of SCFA may improve glucose tolerance are through a reduced motility in the gastrointestinal tract and a lowered GER (38) or through a mechanism including increased release of incretins (GLP-1) (39).

In conclusion, the results of this study show that it is possible to increase the colonic production of SCFA in a semiacute perspective (i.e. from an evening meal to the following morning) by choice of cereal foods rich in barley DF and RS. Both the quantity and the quality of the indigestible carbohydrates play a role in the amounts and pattern of SCFA formed and delivered to the circulation. As judged from the benefits to glucose metabolism and related parameters in an overnight perspective, we put forward that colonic production SCFA, and in particular production of butyric acid, may be one mechanism by which whole grain is protective against type 2 diabetes and cardiovascular disease. Tailoring of prebiotics to reach different patterns of colically derived SCFA may therefore be important for the design of food products with added benefits on both glucose and lipid metabolism. More research in this area is important to clarify relations between colonic fermentation, SCFA production, and metabolic benefits.

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Literature Cited