Dietary carbohydrate digestion and fermentation
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RESULTS AND DISCUSSION

1. The second meal effects

The effects of an evening meal on the metabolism of the subsequent breakfast were investigated (Appendix 1). Compared with white bread evening meal (WB), the barley kernel evening meal (BA) improved glucose tolerance in healthy young men the next morning. The plasma glucose response after the glucose drink was 29% lower after the BA evening meal ($P = 0.019$). The change of insulin response was not statistically significant. The diminished postprandial glucose response and unchanged insulin response after BA was in line with results of other authors (17;18;21;22).

The blood glucose kinetics during oral glucose tolerance test (OGTT) was monitored using the double-label stable isotope technique. This technique enable the measurement of systemic rate of appearance of total glucose (RaT), endogenous glucose production (EGP), systemic rate of appearance of exogenous glucose (RaE), and glucose clearance rate (GCR), which reflect the total influx rate of glucose into blood circulation, the glucose influx from liver, the glucose influx from intestinal absorption, and the rate that glucose is cleared from blood circulation, respectively. It was found that the GCR after BA was significantly higher (23%, $P = 0.016$) than that of WB, but RaT, EGP, and RaE were the same. The net blood glucose was determined by the influx and the clearance. As the influx, i.e., RaT, was the same, whereas the clearance, i.e., GCR, was higher for BA than that of BW, the net blood glucose should be lower for BA than that of WB. This was exactly what was observed by the measurement of blood glucose.

Because the insulin response was the same for BA and WB, the increased GCR could be the outcome of improved insulin sensitivity, or the outcome of other factors which can play an insulin-like role. It was proposed that insulin
sensitivity can be measured during an OGTT (24). According to this approach we calculated the insulin sensitivity index (ISI) after BA and WB. We found a higher ISI for BA compared with that of WB.

It has been reported that the second meal effect of a barley evening meal compared with a white bread evening meal was mediated by suppressed free fatty acids (FFA) (20-23;25) and delayed gastric emptying after the barley meal (20). We didn’t find different FFA for the two evening meals, and the gastric emptying was not expected to be different because we observed the same RaE for both BA and WB. Also we observed a higher plasma butyrate for BA (103 % higher 0-2 h incremental area under the curve (iAUC), \( P = 0.041 \)) which indicated a higher colonic fermentation, as also shown by higher breath hydrogen for BA. Therefore, the improved GCR might be induced by butyrate. We could not conclude from our results a direct causal relationship between butyrate and the observed beneficial effect. As SCFAs are the main products of colonic fermentation, they were the focus of this thesis, but other colonic fermentation related factors could also be possible mediators, e.g. angiopoietin-like protein 4 (also know as fasting-induced adipose factor), lipopolysaccharide (LPS), incretin hormones (26), and transformation of dietary compounds into bioactive products (27).

Furthermore, moderation of plasma pro-inflammatory cytokines was seen for BA. After the WB, the postprandial mean IL 6 (WB vs. BA: 19.7 ± 5.1 vs. 5.1 ± 0.7 pg/mL, \( P = 0.024 \)) and tumor necrosis TNF-α (WB vs. BA: 7.8 ± 2.1 vs. 5.3 ± 1.6 pg/mL, \( P = 0.008 \)) levels were significantly higher compared with the BA, with the values after the BA almost the same as the baseline values. Pro-inflammatory cytokines can deteriorate the insulin sensitivity (28). This should not be the case in our study since the significant difference of both IL 6 and TNF-α occurred during postprandial 0-4 h rather than 0-2 h, although the change of the pro-inflammatory profile in our study might have relevance to the insulin sensitivity in the long run. In contrast, except for glucose, the significant differences for butyrate, and GCR occurred during postprandial 0-2 h. The iAUC
for glucose remained significantly different after 2 h. It was shown that high blood glucose can induce pro-inflammatory cytokines secretion (29). Therefore, the higher pro-inflammatory cytokines could be resulted from the higher blood glucose, whereas the moderated pro-inflammation might be due to butyrate or other fermentation related factors.

In order to understand the underlying mechanisms in relation to the fermentation processes we addressed the following questions:

-a. What was the fermentable substrate when given barley kernels?
-b. What was the fermentation site?
-c. Why did the fermentation increase following the ingestion of the breakfast?

a. **What was the fermentable substrate when given barley kernels?**

We have to realize that the oral-cecal transit time (OCTT) plays in important role in answering this question. The OCTT for similar barley kernel test meals was 4 h (Appendix 3). In this study, the evening meal was ingested for 10 h when the subsequent breakfast was ingested. But during sleep, the OCTT was possibly slower than when subjects are active. We didn’t know how slow it was in this experimental situation since we didn’t monitor the OCTT to avoid disturbing sleeping, but an OCTT of 10 h is possible. In addition, it was found that barley is rich in soluble dietary fiber and RS, and the relative intact physical structure of barley kernel may reduce the availability of the substrate to the digestive enzymes, subsequently increased the undigested starch reaching colon (30). This implies that the carbohydrate fraction reaching the colon to be fermented might be more than that estimated based on the RS and dietary fiber (DF) measurement.

Furthermore, most of the methods for OCTT measurement reflect only the time when the head of the digesta reaches caecum. It takes longer time for all
RESULTS AND DISCUSSION

the digesta to arrive at the caecum. The realistic situation could be that there is always some digesta at the end of the ileum 10 h after intake of the evening meal. In addition, the way the digesta transfers from the terminal ileum to caecum might imply that there is still some digesta waiting at the end of the ileum to be propelled to the caecum. The ileocolonic transit increased postprandially and slowed down during fasting (31). Therefore, the fermentable substrate in the morning is possibly still from the evening test meal. This should be proven in a future study by applying stable isotope labeled evening meal and measuring the OCTT of the evening meal with feasible methods, for example, a smartpill (32), a wireless device which monitors luminal pH and pressure.

*b. What was the fermentation site?*

Upon the arrival of the digesta at the colon, most of the fermentation occurs at the ascending colon. It was found that 12 h after intake of a solid meal, most of the ingested meal was in the ascending colon (33). The digesta takes about 13 h (34) to pass the ascending colon. In addition, the movement of the colon could be activated by eating. The colon movement would push digesta to distal sites of the colon. The microbiota located in different colon sections is different. The digesta composition disfavored by microbiota in caecum might be the favorable substrate for microbiota residing in the transverse colon and beyond. There was also cross-feeding between microbiota which is especially important for the production of butyrate (35-37). It was therefore reasonable to assume that during the OCTT in the morning, fermentation was increased not only in the caecum, but also distally, possibly in transverse colon.

c. *Why did the colonic fermentation increase following the ingestion of the breakfast?*

The movement of the colon is achieved by three different types of contractions
RESULTS AND DISCUSSION

(38): the individual phasic contractions that include the short- and long-duration contraction, organized groups of contractions that include the migrating and nonmigrating motor complexes, and special propulsive contractions (giant migrating contractions). These contractions are controlled by myogenic, neural, and chemical mechanisms. It has been shown that eating triggers ileal evacuation and the movement of the colon(39;40). These eating triggered movements might result in the transit of digesta and make the substrate available for fermentation by pushing and stirring the digesta.

The study in appendix 1 showed that colonic fermentation factors were related to the improved insulin sensitivity in peripheral tissues. We hypothesize that SCFAs, as the main products of colonic fermentation non-digestible carbohydrates are the possible mediators.

2. Relevance of SCFA production for insulin sensitivity

The association of SCFAs to insulin sensitivity, the possible mechanisms involved and the possibility to prevent and control insulin resistance through dietary manipulation of colonic fermentation were discussed in Appendix 2. The colonic fermentation is a complicated process because fermentation rate, site and pattern are all related to their physiological effects. Evidences exists that SCFAs have insulin sensitizing effects. The underlying mechanisms include suppression of pro-inflammation, improvement of FFA concentrations, and the direct effect of SCFAs on glucose metabolism. The available data suggested that the effect of SCFAs may depend on the SCFAs profile. Therefore, an optimal SCFAs profile is needed for the colonic fermentation to have an insulin sensitizing effect. Data from both in vivo and in vitro experiments showed that the colonic production of SCFAs could be manipulated by using different substrates, changing transit time, and applying enzyme inhibitors. It seemed that manipulation of colonic fermentation could be an effective approach to prevent insulin resistance and insulin resistance related diseases.
RESULTS AND DISCUSSION

The availability of the fermentation substrate is dependent mainly on the amount and type of dietary carbohydrate. The caecum has the most abundant substrate for fermentation due to its anatomic advantage. The caecum has also the greatest number of microbiota. Therefore, fermentation is the most active in caecum (41;42). SCFAs production and absorption in caecum are also higher than that of the distal parts of the colon. It was believed that the production of the SCFAs is the determinant of the plasma SCFAs concentrations because the absorption capacity of the colon is far more than the colonic SCFAs concentrations that can be reached physiologically (43).

The SCFAs profile is not only determined by the type of non-digestible carbohydrate, but also the whole gut transit. The OCTT may change SCFAs profile by influencing the availability of fermentation substrate, while the colonic transit may determine the SCFAs by changing the fermentation site. Different colon sites are dominated by different microbiota which may favor different substrate and produce a different SCFA profile. The fermentation occurring in colon beyond caecum may be important as colon cancer and ulcerative colitis are mainly occurred in the distal colon. On the one hand, the fermentation will determine the local SCFAs production which can influence the local colon health and have a systemic effect after absorption. On the other hand, spatial change of the fermentation site from proximal colon to distal colon also means temporal change of SCFAs production which may influence the SCFAs profile in the blood circulation. How much the host benefits from the colonic fermentation is therefore influenced by various factors affecting the SCFAs profile. Current available evidence may not be enough to define an optimal SCFAs profile. A higher total SCFAs concentration with higher butyrate proportion, as seen with the plasma profile of SCFAs derived from barley kernel (44), could be an optimal SCFAs profile on the basis of metabolic effects. The relation between the SCFAs profile and insulin sensitivity should be further studied. Results from this type of study may be not only important to the prevention of insulin resistance and its related diseases, but also important towards other aspects of colonic fermentation, for
example, extraction of energy (45).

3. Monitoring the ratio of digestion vs. fermentation of substrates

In order to be able to manipulate colonic fermentation, it is important to know the fermentable part of the substrate. We developed a mathematical model to evaluate the degree of digestion and fermentation for a carbohydrate food (Appendix 3).

Boiled barley kernel containing 50 g available carbohydrate was given to 17 young volunteers. The breath samples were collected for 12 h to measure hydrogen and $^{13}$CO$_2$. Four hours after intake of barley, breath hydrogen started to increase significantly which was regarded as a sign of colonic fermentation. This suggested that the digesta of the test meal reached the caecum and started to ferment about 4 h after intake of the test meal.

The $^{13}$CO$_2$ (%dose/h) increased and reached peak values 4 h after intake of the barley test meal. It decreased after the peak value, but was still higher than the baseline value 12 h later. The barley was labeled with $^{13}$C during growth which implies that both the digestible part and non-digestible part of the barley kernel were labeled and could produce $^{13}$CO$_2$. Before the digesta reached the caecum, all the $^{13}$CO$_2$ came from the small intestinal digestion process, after that, $^{13}$CO$_2$ was also produced by colonic fermentation. With the $^{13}$CO$_2$ data derived from the digestion process a best fit curve was constructed, which subsequently could be used to estimate the total $^{13}$CO$_2$ derived from the digestion process. The difference between the fitted curve and the observed curve reflected the contribution of $^{13}$CO$_2$ from colonic fermentation. The results showed that 18-19% of the recovered $^{13}$CO$_2$ was from colonic fermentation. The samples were only collected for 12 h; the information about digestion and fermentation after 12 h could be estimated using the fitted and observed curve. By doing this, we found that the $^{13}$CO$_2$ levels would return to baseline after 24 h, and estimated that 24-25% of $^{13}$C was contributed by colonic fermentation.
RESULTS AND DISCUSSION

It was suggested the energy value of dietary fiber and RS is 2 kcal/g (46). According to the content of dietary fiber and RS in the test meal, the energy contribution can be estimated to be 14.8%. The barley was ingested as boiled kernel. The relative intact physical and chemical structure made it possible that more carbohydrate may escape the small intestinal digestion and reach the colon for fermentation (30). In this sense, it’s reasonable that we derived a higher value (24-25% vs. 14.8%).

Our results with the curve fitting model showed promising results which might be applied with other foods as well. With $^{13}$C labeled foods available, we may obtain a specific fermentation index (FI) for each carbohydrate food or diet. The fermentation index together with the GI would be able to provide comprehensive information about the biological characteristics of carbohydrate rich foods.

4. SCFAs profile in serum and urine

A fermentation index would be able to reflect the fermentation characteristic in general, but cannot provide information about the SCFAs profile. As was discussed above, the SCFAs profile could be the critical determinant for its effect. We therefore developed a method to monitor the SCFAs profile in plasma and urine derived from colonic fermentation. The possibility of manipulating the SCFAs by using different non-digestible carbohydrates sources was investigated (Appendix 4).

After ingestion of barley kernel or barley porridge test meals which provide 50 g available carbohydrates, breath samples were collected for 6 h, blood samples 14 h, and urine for 24 h. The OCTT estimated from breath hydrogen and $^{14}$CO$_2$ was the same for both test meals, barley porridge (360 ± 47 min for H$_2$-measurements and 365 ± 55 min for $^{14}$CO$_2$-measurements) and barley kernels (372 ± 18 min for H$_2$-measurements and 400 ± 0 min for $^{14}$CO$_2$-measurements) ($P = 0.705$ for H$_2$-measurements and $P = 0.285$ for $^{14}$CO$_2$-measurements).

An increase of $^{13}$C-acetate was observed early after ingestion of the meals (<
RESULTS AND DISCUSSION

6 h) for both test meals. A rise in $^{13}$C-propionate in the fermentation phase could only be detected after the porridge and not after the kernels meal. An increase in $^{13}$C-butyrate was found for both test meals in the fermentation phase and was higher after the barley kernels. Urine $^{13}$C-SCFAs data were consistent with these observations.

This study showed that plasma and urine SCFAs profiles of different non-digestible carbohydrates can be monitored. The application of $^{13}$C labeled substrate made it possible that the observed SCFAs could be linked to the specific substrate. This technique would help to identify the causal relationship between SCFAs and its effect, e.g. improvement of insulin sensitivity.

The study also provided evidence that colonic fermentation can be manipulated by dietary carbohydrates. It was known that soluble fiber is fermentable and completely used by colonic microbiota, whereas the insoluble fiber is less fermentable and contributes to the bulk of the feces. But the fermentation of soluble fiber may be affected by the presence of insoluble fiber and RS (47). By selecting different substrates, or combining different substrates, SCFAs profile of colonic fermentation can be manipulated.

Due to the limitation to access colonic fermentation, information about colonic fermentation has not been widely available. Although it is critical for biological studies on effects of colonic fermentation, the quantitative evaluation of fermentation is still very difficult. Most of the studies concerning colonic fermentation measured SCFAs profile in faeces. The SCFAs profile in faeces may not be the same as that in caecum, and obviously not the same as in plasma. On the one hand, it was shown that the SCFAs concentration decreased in the order of caecum, ascending colon, transverse colon and rectum, although the proportion of the individual SCFA may keep stable (41). On the other hand, we don’t know whether the higher faeces SCFAs concentrations are the outcome of higher production or lower absorption. Before we have information about this, it would be reasonable to refer to the SCFAs of a known food for the optimal SCFAs.
RESULTS AND DISCUSSION

Whole grain barley has been used in our studies and in the studies of others. Based on these results (18;21-23;48), we hypothesize that a food or diet which has a favorable whole-gut transit and produce a SCFAs profile similar to that of barley kernel are potent in prevention of T2DM.

In conclusion, the results in this thesis showed evidence that colonic metabolism of fermentable carbohydrates was related to insulin sensitivity and anti-inflammatory effects, which is considered as highly relevant for the prevention of Type 2 Diabetes. This preventive effect might be dependent on the quantity and quality of the SCFA profile. The quantity of the fermentation can be monitored by the Fermentation Index, derived from a mathematical model developed by us. Furthermore we were able to demonstrate that fermentation and thereby the SCFAs profile can be manipulated by the choice of substrates. Manipulating the colonic fermentation with dietary carbohydrates to produce the optimal SCFAs profile could play an important role in prevention of chronic diseases associated with decreased insulin sensitivity including T2DM and cardiovascular diseases.
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