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Physiological and Metabolic Properties of a Digestion-Resistant Maltodextrin, Classified as Type 3 Retrograded Resistant Starch

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There is a growing interest in highly fermentable dietary fibers having the potential to reduce risks of disease through the production of short-chain fatty acids (SCFA). Recently a digestion-resistant retrograded maltodextrin (RRM), classified as type 3 resistant starch was developed. Systematic work to determine its molecular and physiological properties was carried out to determine (1) the fraction resistant to digestion in vitro and in vivo, (2) its postconsumption effect on blood glucose in healthy volunteers, and (3) its in vitro fermentation pattern, at different ages, by use of pooled fresh human fecal inoculum. Results: The digestion resistant fraction obtained in vivo from ileostomy patients (59.4%) is similar to that obtained by the AOAC method for measuring retrograded resistant starch (59.7%). The relative glycemic response after consumption of 50 g of RRM was 58.5% compared to glucose set as 100%. When exposed to colonic microbiota, in vitro obtained indigestible fractions behave similarly to those obtained in vivo in ileostomy patients. Fermentation of RRM and production of butyric acid is negligible during the first months of life but develops subsequently during weaning. In adults, RRM fermentation results in a high yield of SCFA, with butyrate representing 21−31 mol % of total SCFA. The high yield of SCFA during colonic fermentation, observed from weaning age on, as well as the potential to help reduce glycemic load may be of benefit to a number of health-related functions in the host. Further study on clear clinical end points is warranted.

KEYWORDS: Resistant starch; resistant maltodextrin; retrogradation; fermentation; microbiota; glycemia; ileostomy; butyrate

INTRODUCTION

Although recent meta-analysis of epidemiological data has shown that dietary fibers consisting of non-starch polysaccharides may not be protective for colon cancer, other data are accumulating that certain classes of highly fermentable dietary fibers do have the potential to reduce risks of colon disease through the production of short-chain fatty acids. Especially the production of butyric acid has attracted significant interest in this respect. For example, Cassidy et al. (*1*) did not observe a correlation between fiber consumption and the occurrence of colon cancer but did find a significant negative correlation between the consumption of starch and colorectal cancer incidence. In their study the authors assessed the starch consumption of populations in 12 countries as measured in individual surveys. A positive correlation with fat and protein consumption was observed but only a weak and nonsignificant correlation with non-starch polysaccharide intake. There was, however, an inverse correlation between colorectal cancer incidence and starch intake ($r = -0.70$) and this association was maintained after controlling for partial correction due to meat and fat consumption. Assuming a resistant starch (RS) fraction of 5%, within total starch consumed, the suggestion was put forward that the observed beneficial effect may be related to butyrate being produced during its fermentation in the large bowel.

Resistant starch is generally defined as starch or starch degradation products not absorbed in the small intestine of healthy individuals. Originally resistant starch is classified into three types: RS type I (RS1) is starch that is physically inaccessible for digestive enzymes because it is "packed" in

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fiber material, for example, grains and seeds. RS type II (RS2) is raw starch granules, as present in raw potato and green banana. RS type III (RS3) is recrystallized (retrograded) amylose and amylopectin as present in starch after heating and subsequent cooling. This type is mainly present in cooked and subsequently cooled potato, in bread, and in corn flakes. The resistance to digestion of the three RS types is attributed to particular physical structures, while molecular parameters of the starch (D-glucose units linked by α-1,4/α-1,6 glucosidic bonds) remain substantially unchanged.

Recently some papers mention an RS type IV, covering some chemically modified starches. In this case the resistance to digestion is due to structural changes in the starch backbone. Not only the amount of RS reaching the colon seems to be important. It is thought that especially its molecular and physical structure may impact on the accessibility to the gut microbiota and thus determines its prebiotic and butyrogenic properties. The latter correlates well with the scientific consensus that the products of bacterial fermentation, especially propionate and butyrate, rather than the presence of dietary fiber itself determine its physiological impact in the large intestine on health. Butyrate is known to have a beneficial impact on inflammatory responses and on several steps in the cell cycle known to affect proliferation, apoptosis, and neoplasm development. Consequently, stable butyrate production is assumed to potentially reduce the risks of certain intestinal disease states (*2*−*5*, *18*, *29*, *30*).

Scientific studies support the view that retrograded resistant starch, when being fermented, is the most powerful butyrate-supplying substrate (*4*). Interestingly, a study by Martin et al. (*6*) in pigs, which are close to humans in terms of intestinal physiology, suggested that butyrate produced from RS3 is also more distally fermented in the colon and therefore could be a more efficacious type of RS for the distal colon than raw potato starch (RS2). Recently a number of detailed reviews have highlighted many functional and physiological properties of RS. Although most of the effects observed indicate potential reductions in gut disease-associated risk factors, some studies did not observe such effects and warrant the need for further research (*4*, *8*, *9*, *30*, *36*). Here we report on a number of systematic study steps to determine the molecular and physiological properties of a retrograded resistant maltodextrin (C*ActistarRM, Cargill, Vilvoorde, Belgium. It is equivalent to type III resistant starch that is formed in many staple starchy foods in which retrogradation happens as a result of traditional culinary practices, as acknowledged in 2003 by AFSSA, the French food safety agency. It is obtained from partially hydrolyzed starch (tapioca or potato) by enzymatic debranching with isoamylase, followed by subsequent retrogradation (*10*). It consists of linear α-D-glucans of which more than 50% have a polymerization degree between 10 and 35 in the resistant fraction. The indigestible retograded resistant maltodextrin (RRM) fraction is quantified appropriately by the 2002.02 AOAC method for measuring resistant starch. In the present study, mainly tapioca-based retrograded resistant maltodextrins (RRM) was used.

Aims of the present study are as follows: (1) to quantify the fraction of truly digestion-resistant matter of a RRM-containing product in vivo in healthy ileostomy patients (study 1; Sahlgrenska Hospital, Göteborg University, Göteborg, Sweden); (2) to determine the glycemic response of the RRM-containing product in vivo in healthy volunteers (study 2; NutriScience BV, University of Maastricht Holding, Maastricht, The Netherlands); (3) to determine the fermentation pattern in vitro of the separated RRM fractions in fresh human fecal matter obtained from individuals of different age groups (study 3; Institute of Food Science and Nutrition, ETH Zurich, Zurich, Switzerland).

EXPERIMENTAL PROCEDURES

Study 1: Quantification of Digestion-Resistant Fraction of RRM in Vivo in Ileostomy Patients. Digestive behavior and physiological effects of RRM should preferably be measured in vivo in humans. The ileostomy model is considered to be the gold standard for the measurement of the degree of digestibility and absorption in humans, allowing for quantitative total collection of ileal effluents from the distal ileum. Studies in ileostomy subjects have been performed since the 1940s in order to investigate the excretion of nutrients from the small bowel. Since the introduction of strict inclusion criteria (minimal resections of the distal ileum) in combination with short sampling periods of ileostomy effluents (≤2 h) and immediate deep-freezing (*20*), the model has been used by several groups; for a review see ref *19*. It has been shown that when a test product, RS, is given in the morning together with a plant polysaccharide-free diet, the product is totally excreted from the small bowel within 12 h after ingestion (*11*) The ileostomy model directly measures the amount of starch not digested and absorbed in the small intestine and is considered to be one of the most reliable methods to analyze RS in vivo. There is, however, a difference in the microbial population, with 10^5-10^6 bacteria/g in a normal distal ileum compared to 10^7-10^8 bacteria/g in the terminal ileum in ileostomy subjects−− (*16*), although these numbers are small compared to the numbers usually found in cecum, amounting to 10^{12}/g. If the bags are changed every second hour and deep-frozen, the bacterial degradation of NSP and RS is small (*20*, *21*). In the study by Englyst and Cummings (*21*), low amounts of short-chain fatty acids (SCFA) were found in the ileal effluents, and treatment with antibiotics did not change the amounts of carbohydrates recovered. In a recent study, however, somewhat higher amounts of SCFA and lactic acid in the ileal effluents have been measured on the addition of 10 or 30 g of inulin to the diet in ileostomy subjects (*22*). The amounts of SCFA and lactic acid found were still estimated to correspond to only around 2−3 g of fermented carbohydrate per day. This means that the model may give a slight underestimation of the amount of carbohydrate recovered, especially of easily fermented carbohydrates such as oligosaccharides. Another indicator of only a relatively small presence of bacteria is that only minimal amounts of secondary bile acids have been found in ileostomy effluents (*23*), and bile acid excretion in ileostomy studies has been found to be similar to that of normal subjects (*23*). It can be concluded that a small degradation of carbohydrates takes place during the passage of the effluents through the end of the small intestine and also in the ileostomy bag. Accordingly, obtained results should be corrected.

Methods. Eight subjects (mean age 62.5 years, BMI 24.7 kg/m^2, four males and four females) were included in the study. The study protocol was approved by the Ethical Committee of the University of Götenborg and written informed consent was obtained from each study participant. Inclusion criteria were conventional ileostomy; less than 10 cm of the ileum removed; operated due to ulcerous colitis; more than 6 months after operation; normal volumes of ileal effluents, less than 1000 mL/day; and normal amounts of bile acids in the ileal effluents, less than 1000 mg/day.

Study Design. This present experiment was a part of a larger, double-blind and completely randomized study in which the subjects consumed various types of indigestible carbohydrates, along with a plant polysaccharide-free diet during a 3-day period (the first day was used as a run-in day; the second and third day were the test days) (Table 1). For this paper we report here the results related to the consumption of the tapioca-based RRM. After an overnight fast, the subjects came to the department for a standard breakfast, including 40 g/day RRM corresponding to 36.4 g/day dry matter (DM) mixed in 300 mL of water. The subjects were then provided with the food and drinks for the rest of the day and took it home with them for consumption. During both test days the subjects changed their ileostomy bags every second hour between 8 am and 8 pm. The ileostomy bags were immediately deep-frozen on CO_2-ice in a Dewar vessel and brought to the laboratory the following morning for analysis and quantification of undigested matter.

Table 1. Composition of the Menu for the Plant Polysaccharide-free Diet (2000 kcal) and Time of Consumption

08:00	breakfast	yogurt, sugar, orange drink, egg
10:00	snack	soft drink
12:00	lunch	chicken with cream, ice cream with chocolate sauce, soft drink
15:00	snack	coffee, meringues
18:00	dinner	fillet of pork, white bread with margarine, mineral water
20:00	evening meal	white bread, margarine, cheese, ham

Sample Treatment. The frozen ileostomy bags (−20 °C) were pooled according to 12-h periods of sampling and subsequently homogenized. Pooled samples were put in a freeze-dryer (Lyovac GT2) at −20 °C, vacuum was established, 5×10^{-1} mbar, and the samples were freeze-dried for a period of 7 days.

In order to quantify undigested matter, total α-D-glucans, including free glucose, maltose, and maltooligosaccharides in ileal effluents were determined by a method adapted as described by Faisant et al. (*11*). In short, water (5 mL) was added to 50 mg of sample. After gelatinization at 100 °C for 30 min, samples were cooled to 0 °C in ice and 5 mL of KOH (4 M) was added. After 30 min under mixing at 0 °C, 1 mL of the mixture was added to 10 mL of acetic acid (0.5 M) containing $CaCl_2$ (4 mM). Amyloglucosidase (AMG; EC 3.2.1.3; 400 AGU, Novo Nordisk Bioindustries, Bagsvaerd, Denmark) was added before hydrolysis was performed at 70 °C for 30 min. After 10 min at 100 °C and subsequent cooling, samples were neutralized by 0.6 mL of KOH (4 M). Total glucose was determined by use of the glucose oxidase (GOD-PAP) reagent after centrifugation (1000*g*, 5 min). The small intestine of ileostomy patients does contain low amounts of microbiota that may cause some degree of fermentation that may lead to a small overestimation of digested matter. On the basis of measurements of substrate degradation and SCFA production in freshly obtained ileostomy effluent, kept at a constant temperature of 37 °C and spiked with resistant starch (results not shown), it was calculated that the amount of RS fermented while being stored in the ileostomy bags before being deep-frozen ranges between 1.5 and 2.0 g/day. Accordingly, a correction was made by adding 1.75 g/day to the analyzed mean amount of undigested starch excreted with the small bowel effluent.

Statistics. Results are presented as mean and SEM. A computer package (SYSTAT 7.0) was used for calculation.

Study 2: Determination of Relative Glycemic Response Value of RRM-Containing Carbohydrate Source in Vivo. *Methods.* Twelve healthy lean subjects recruited from the university campus were included in the study, seven males and five females. The study protocol was approved by the Medical Ethical Committee of Maastricht University and a written informed consent was obtained from each study participant. Testing of the relative glycemic response index was performed by use of inclusion and exclusion criteria and by applying test criteria for glycemia testing as recently recommended by an international group of experts convened by the International Life Sciences Institute (ILSI Europe, Brussels) (*12*). The design of the study was a single-blind crossover study.

Test Procedures. On the morning of a test session, the subjects reported to the laboratory in a fasting state at 8:00 am (from 10:00 pm the day before the test day, no foods and drinks were allowed, only 100−200 mL of water. The investigator first checked that each subject was feeling well and had complied with the preceding experimental conditions. Subsequently, hemoglobin was measured after collection of a drop of blood via finger prick, using the Hemocue-device (HemoCue AB, Ängelholm, Sweden). If the hemoglobin value had fallen below 7.0 mmol/L, the subject was scheduled to wait 1 week before the next test day. If on the second occasion the hemoglobin value had fallen again below 7.0 mmol/L, the subject was excluded from further participation in the study.

After body weight of the subjects was determined for control during the study, an intravenous catheter was placed into an antecubital vein. A fasting blood sample was taken (0 min) and then the subject started eating one of the test products. Other blood samples were collected at $t = 15$, 30, 45, 60, 90, and 120 min after ingestion of the test products. Between two blood samples, the intravenous catheter was flushed with 1 mL of saline (physiological salt solution) containing 1% heparin to prevent the catheter from clotting. The catheter was removed after the last blood sample was taken. After completing an experimental session, the subjects received a cup of coffee or tea and could choose a small snack. All subjects received the test and control products in random order on different test days. Subjects consumed 50 g [dry (DM) and corrected for crystal water content] of the test and control carbohydrate in 250 mL of cold water. The solution was stirred just before consumption. After consumption, 50 mL of cold water was added to the empty bottle and stirred again. The remaining 50 mL was consumed as well.

Clinical Chemical Measurements. Glucose concentrations were measured with the Gluco-quant glucose/hexokinase test kit of Roche Diagnostics (Mannheim, Germany) according to the manufacturer's instructions and using a Hitachi 917 clinical chemistry analyzer (Roche Diagnostics, Basel, Switzerland.) Intra-assay precision was 3.0%, whereas inter-assay precision was 1.2%.

Insulin concentrations were measured with an immunoassay, using the Immulite chemiluminescence system (Diagnostic Products Corp., Los Angeles, CA) according to manufacturer's instructions. Intra-assay precision was 3.8%, and inter-assay precision was 5.6%.

Statistics. The incremental area under the curve (iAUC) was determined for blood glucose as well as blood insulin response, using the computerized GraphPad Prism for Windows statistical package (GraphPad Software, Inc, San Diego, CA). Any area under baseline was ignored. The glycemic response index (GRI) is determined by the following equation:

$$\mathrm{GRI} = \frac{\text{individual iAUC of test product}}{\text{individual iAUC of control product}} \times 100$$

Glucose served as control and was tested twice. The average iAUC of the control was used as iAUC control in the above-mentioned equation. The GRI was compared to 100 (control) by Student's *t*-test. The insulin response index (IRI) was calculated similarly as specified above for the GRI. The IRI was compared to 100 (control) by Student's *t*-test. All statistical analyses were performed with GraphPad Prism. Differences were considered significant at a two-tailed p value of 0.05 or below.

Study 3: Determination of in Vitro Fermentation Pattern of RRM fractions Obtained after in Vitro and in Vivo Digestion from Human Inoculate of Various Age Groups. *Methods.* In vitro fermentation was carried out by a batch technique with a mixture of fresh fecal material from three healthy nonmethanogenic humans, under strictly anaerobic conditions as described previously (*14*). For preparation of fermentation substrates, prior to the in vitro fermentation study the digestible fraction of the product containing RRM was removed by an in vitro model. This model simulates digestion in mouth, stomach, and small intestine and has been described in detail (*13*). In short, degradation products were removed by dialysis and retentates were freeze-dried. Additionally, in vivo digestion residues of RS were obtained as freeze-dried ileostomy effluents from study 1 described above. The study protocol was approved by the Ethical Board of the University Hospital of Zurich.

In Vitro Fermentation. Substrates were tested within one single fermentation experiment together with lactulose and blank (without any substrate) samples as controls. Duplicates were taken after 0, 2, 4, 6, 8, and 24 h to follow the fermentation kinetics. Fermentation was stopped by the addition of 0.1 mL of a saturated $HgCl_2$ solution. Fermentability was established by measuring pH, production of total gas, hydrogen, and short-chain fatty acids (SCFA) (*14*). Starch degradation was determined by measuring the decrease in total starch as analyzed in the fermentation residues. In one experiment we addressed the question as to whether RRM produced from either tapioca or potato starch would differ in fermentation behavior as well as whether our RRM behave differently compared to another type of indigestible maltodextrins (Fibersol, Matsutani Chemical Industry Co, Ltd, Japan). Fibersol is an indigestible dextrin produced by pyrodextrination and subsequent enzymatic treatment and is considered to be a novel food. In an additional fermentation trial we addressed the question whether in vivo digestion residues of RS as obtained from freeze-dried ileostomy

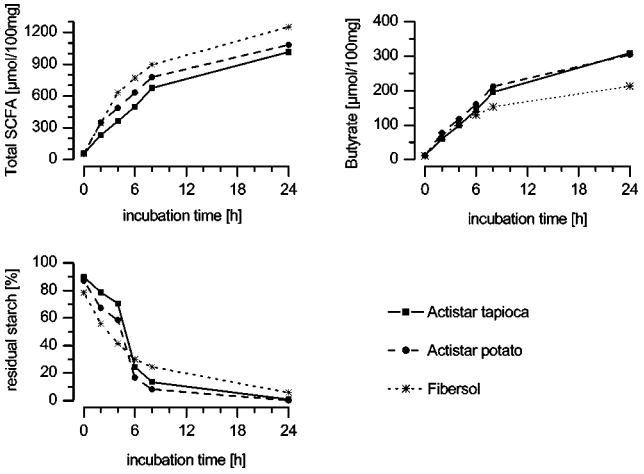

Figure 1. In vitro fermentation patterns of RM produced from tapioca and potato in comparison with Fibersol. Data are mean values of duplicates; rel SD < 5% (except for substrate degradation in Fibersol: rel SD < 8%).

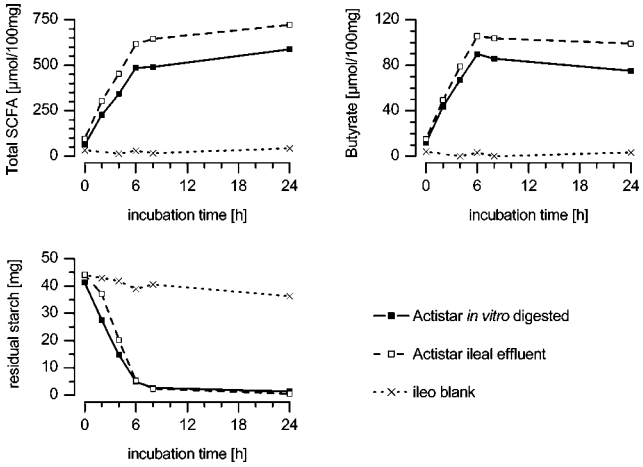

Figure 2. In vitro fermentation patterns of in vivo and in vitro digested RRM. Data are mean values of duplicates; rel SD < 5%.

effluents (from study 1 described above) would show different fermentation behavior compared to the in vitro-obtained digestion residue as described by Lebet et al. (*13*). In a third experiment, the ability of microbiota of different age groups to degrade RS was compared. Fecal mixtures of formula-fed infants, weaning infants, and adults were used to ferment in vitro digestion residues of tapioca-based RRM in comparison with blanks (endogenous fermentation capacity of inoculum only) and lactulose (*31*).

Statistics. Data are presented as calculated means based on duplicate fermentation experiments and subsequent analysis.

RESULTS

Study 1 Results. After ingestion of 40 g of RRM powder per day, the mean excretion of undigested starch was 19.8 ± 0.57 g/day with a range of 17.02−21.76 g/day. The mean wet weight was 335.9 ± 38 g/day and the mean dry weight was 43.4 ± 1.5 g/dat. The mean amount of undigested (resistant) starch was 19.83 g/day. After correction for potential fermentation as described above, this resulted in a mean daily digestion resistant matter recovery of 21.58 g. The subjects were given a total of 40 g/day RRM, which equals 36.4 g/day DM. On the basis of this calculation, the truly resistant matter content of RRM, observed to be excreted from the small intestine, amounted to 59.4%.

Study 2 Results. Compared to glucose control, AUC, set as 100, postconsumption elevation of blood glucose and insulin were significantly reduced after consumption of RRM, respectively 58.52% ± 7.33% ($p < 0.05$) and 24.81% ± 10.12% ($p < 0.01$) (mean ± SEM).

Study 3 Results. The fraction of undigested residue obtained after in vitro digestion procedures corresponded to 59.5%, which was very well comparable to the result obtained by the newly established AOAC method for measuring (type 3) retrograded resistant starch, by McCleary and Monaghan (*15*), 59.7%, as well as the result from our ileostomy data in study 1, 59.4%.

Figure 1 shows the total SCFA and butyrate production as well as substrate degradation/disappearance for the in vitro-obtained digestion residue of either potato and tapioca starch-based retrograded maltodextrins or pyrodextrinated maltodextrins. Slightly lower total amounts of SCFA were produced from the RRM preparations than from the pyrodextrinated maltodextrins. However, butyrate production turned out to be clearly higher, confirming the butyrogenicity of RRM. Tapioca- and potato-based RRM led to similar substrate degradation and fermentation profiles, showing a rather slow decrease during the first 4 h of fermentation, followed by a fast and nearly complete disappearance during the intermediate phase. Conversely, pyrodextrinated maltodextrins were metabolized faster during the first 4 h but showed a lower degradation rate afterward.

Data from comparison of fermentation behavior of the RRM fraction as obtained from ileostomy patients or as obtained by the in vitro digestion procedure are presented in **Figure 2**. Total SCFA and butyric acid production is somewhat higher for the ileal effluent-obtained RS fraction than for the in vitro-obtained RS residue. "Ileo-blank", a sample without addition of fecal material, was included to estimate the endogenous fermentative capacity of the ileal effluents, which are known to contain a certain amount of bacteria (*16*). The production of both total SCFA and butyrate during the 24 h incubation was found to be very small and can be neglected if compared to the fermentation blank containing inoculum only (*17*). A complete metabolism of starch by colonic microbiota within 6 h of fermentation was observed for both in vitro- and in vivo-obtained RRM fractions. However, a tendency for a moderate retardation during the first 4 h was observed for the RRM fraction obtained from ileal effluent.

Data obtained from experiments using human fecal microbiota from formula-fed babies, infants during weaning, and adults were compared. They were tested for endogenous fermentation (blank values) as well as for fermentation of RRM and lactulose (a easy fermentable substrate used as standard control). Results in **Figure 3** are given as SCFA production and substrate degradation. Microbiota from formula-fed babies showed a rather high endogenous activity, as can be seen from considerable SCFA production in the blank sample (**Figure 3**). Only a small increase in SCFA production was observed when RRM was present as a substrate, pointing to negligible fermentation of RRM, even over a 24-h fermentation period. Virtually no butyric acid was produced. Lactulose, however, turned out to be rapidly and completely metabolized by formula-fed babies' microbiota. During weaning, higher SCFA production, starting production of butyric acid and faster substrate degradation were observed. However, the rate of metabolism at this age was still lower than that observed in adults

DISCUSSION

The possibility of large-scale industrial production of retrograded resistant starch and maltodextrins makes the addition to

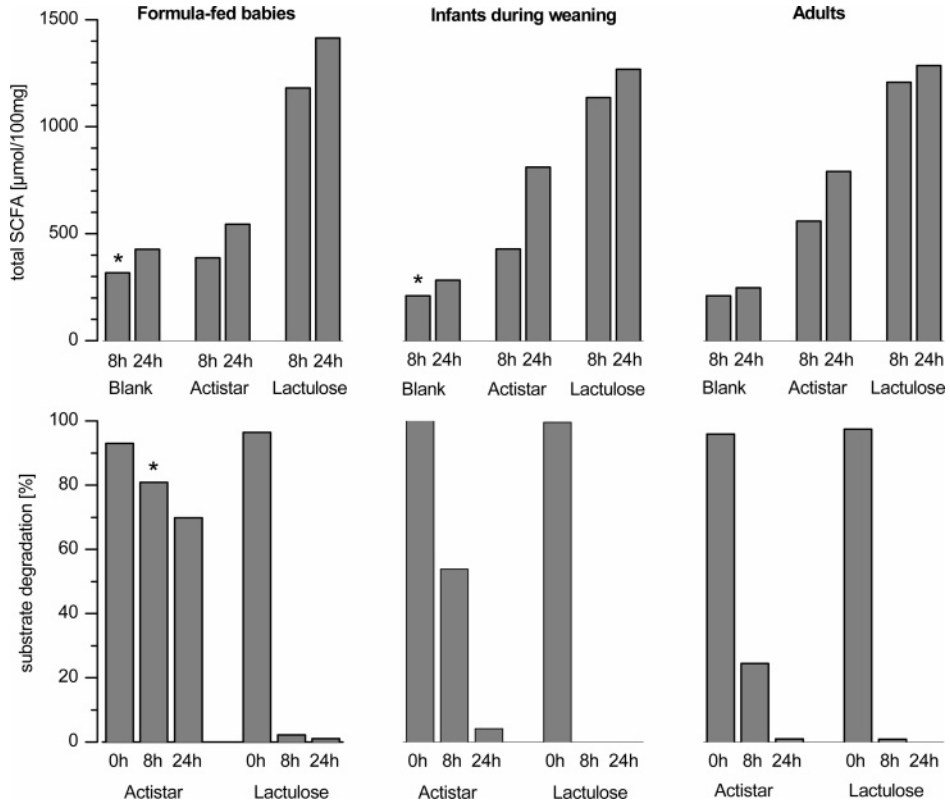

Figure 3. In vitro fermentation patterns of RRM and lactulose from human fecal flora of individuals of different age groups. Data are mean values of duplicates; relative SD < 5% (except where marked by asterisk: rel SD < 9%).

various food applications possible. Its inclusion in foods, replacing available carbohydrate, potentially may help reduce glycemic load and lower postingestion glycemia. Additionally, its inclusion will result in a substantial postconsumption substrate availability for colonic fermentation. The experiments presented here help shed light on the digestive and metabolic behavior of resistant maltodextrins, classified as type 3 resistant starch.

Study 1: RS Recovery from Ileostomy Effluents. From earlier observations and studies (*20*−*23*) it can be assumed that the quantity of resistant starch that is fermented by microbiota present in the small intestine and ileal excretes of ileostomy patients ranges from 1.5 to 2.0 g/day. Based on the results obtained from spiking fresh ileostomy effluent with RRM (unpublished observations), this figure is representative for the present study as well. The subjects were given 40 g/day of RRM, which equals 36.4 g/day DM. The digestion-resistant fraction of RRM, recovered from the ileostomy effleuents and corrected for fermentation was 59.4%, which is in good agreement with the resistant matter content of 59.7% as measured by a specific in vitro procedure for the measurement of retrograded resistant starch (*15*). These results show that the retrogradation process does not cause all maltodextrins to be incorporated in indigestible matter, leaving about 40% to be available carbohydrate.

Study 2: Blood Glucose Response. The obtained data show that ingestion of 50 g of the RRM-containing carbohydrate (which apart from 59.4% resistant matter also contains 40.7% digestible maltodextrins) significantly lowers its postingestion blood glucose and insulin response compared to 50 g of glucose. However, large interindividual glycemic responses were observed. The highest GI value of 91.7 versus the lowest value of 14.3 on glycemic response raises questions about possible outliers. Some researchers use a difference of 2 times the standard deviation as a cutoff for inclusion/exclusion. However, the present values did not fit such criteria. Accordingly, there was no reason not to accept these data. In another study (unpublished data), in which 75 g of RRM was compared to 75 g of glucose in 12 healthy volunteers, similar values were obtained. The mean-relative glycemic response value for RRM in this study was 54.3 with a standard deviation of 25.2. The highest response observed was 92.4 and the lowest was 11.7. Individual differences in gastric emptying and subsequent digestion and transit as well as insulin response and insulin sensitivity may explain such large differences.

Generally it is supposed that substitution of resistant starch for available carbohydrate in a food or beverage will reduce glycemic load and 24 h glycemia. The latter may be beneficial for health, especially in individuals with overweight, reduced insulin sensitivity, and impaired blood glucose regulation. A recent crossover study (*24*) determined the effect of RRM-containing bread on postingestion blood glucose concentrations in 9 male and 11 female adults having a fasting blood glucose concentration of 100−140 mg/dL. The bread supplied 6 g of RS in each serving of two slices and was compared to normal bread not containing RS. The subjects were stratified into a group that had an above-normal fasting blood glucose concentration, indicating some degree of impaired insulin sensitivity, referred to as a "borderline group" (>110 mg/dL), and a normal group (<111 mg/dL) with the upper limit of a normal blood glucose concentration determined as 110 mg/dL. Postprandial blood glucose and insulin increases were significantly reduced in subjects of the borderline group ($p < 0.01$). These results show that the physiological effect of adding RS to a starchy food, in exchange for available starch (flour), is exaggerated in individuals who are insulin-insensitive and may develop into type 2 diabetes. Since 60−80% of overweight individuals develop some degree of insulin resistance, this finding is of significant importance for reducing diabetes risks.

Alternatively, apart from affecting glycemia through a reduction in the amount of bioavailable glucose, there are data that support the concept that SCFA obtained from RS fermentation influence carbohydrate and fat metabolism beneficially. For example, Robertson et al. (*7*) showed that RS consumption leads to improved insulin sensitivity and induced better glucose disposal. This effect was explained by a significant uptake of acetic and propionic acid by the adipocytes, influencing substrate uptake, storage, and oxidation. Another recent study (*25*) examined the relationship between the RS content of a meal and postprandial/postabsorptive fat oxidation. The data obtained from indirect calorimetry and oxidation of [1-^{14}C]triolein to $^{14}CO_2$ showed that addition of 5.4% RS to the diet significantly increased fat oxidation. In fact, postprandial oxidation of [1-^{14}C]-triolein was 23% higher with the 5.4% RS meal than the 0% RS meal ($p = 0.0062$). These data indicate that replacement of 5.4% of total dietary carbohydrate with RS significantly increased postprandial lipid oxidation and therefore could decrease fat accumulation in the long term. Thus, apart from impacting colonic fermentation and metabolism, circulating SCFA derived from fiber fermentation can influence cellular substrate uptake, storage, and metabolism in a way that it potentially may help reduce the risk of developing insulin resistance in overweight individuals.

Study 3: RRM Fermentation Characteristics. The molar proportion of SCFA produced during fermentation depends on the extent to which the resistant matter is accessible to the microbiota. Retrograded resistant starch has been shown to be an easily fermentable substrate that may more than double the luminal concentrations of butyrate and propionate (*26*). An early in vitro fermentation assay of RRM was performed in a batch system with human feces according to the methodology described by ref *35*. Its fermentation pattern was shown to be complete and rapid, resulting in a dose-dependent increase of the total SCFA and a concomitant drop in pH. Butyrate represented 21−28 mol % of total SCFA proportions in these experiments. However, it should be noticed that butyrate production is influenced to a considerable extent by microbiota differences, as five repetitive incubations of RRM have shown (*17*). On the basis of analysis of the study variables, it was concluded that a donor-related effect may have occurred, since clearly lower butyrate concentrations could be attributed to the microbiota of one specific subject. Large donor-specific variations in butyrate production from RS have also been reported elsewhere (*27*). New microbiology techniques such as 16S rRNA sequencing have uncovered a remarkable diversity of butyrate-producing bacteria (*6*), suggesting that butyrogenic substrates such as RS may be handled in a donor-specific way. Therefore, it may be useful to include such techniques to uncover the remarkable diversity of butyrate-producing bacteria between individuals on one hand and to follow substrate-induced changes during in vitro experiments on the other hand. Moreover, the butyrogenic effect of RS may vary considerably between individuals. Arrigoni et al. (*28*) used an in vitro batch system with predigested substrate, as described by ref *13*. They compared various types of RS and oligosaccharides. Compared to indigestible short-chain oligosaccharides (fructo-oligosaccharides, xylo-oligosaccharides), the fermentation of RS is fairly slow. The accumulation of hydrogen after RS intake is lower and the production of butyrate was significantly higher. According to the authors, this may be due to the specific presence of partially crystalline retrograded amylose in the product. When compared to retrograded debranched high-amylose corn starch (Cristalean, Opta Foods, Denmark) and thermally modified granular high-amylose corn starch (Novelose 240, type 2RS, National Starch), RRM resulted in a significantly faster substrate disappearance and higher total SCFA and butyrate production at 8 h of incubation (*28*).

In **Figure 1**, total SCFA and butyrate production as well as substrate disappearance are shown, resulting from fermentation of RRM tapioca, RRM potato, and Fibersol, respectively. From the RRM preparations, slightly lower total amounts of SCFA were produced compared to Fibersol. However, butyrate production of RRM tapioca and potato turned out to be clearly higher, confirming the butyrogenicity of RRM: respectively 309 and 304 versus 213 μmol/100 mg of substrate, equivalent to 31, 28, and 17 mol %. Tapioca and potato starch-based resistant maltodextrins led to similar final substrate degradation with only very small differences in time. RRM was completely degraded at the end of the fermentation period, whereas approximately 6% of Fibersol remained undegraded. The latter stands in contrast to the earlier obtained results of a single administration test in rats, where the fecal excretion rate was found to be 38% (*34*). The differences in degradation patterns can be explained by differences in structure. Despite its highly branched molecular structure (*34*), a small fraction of Fibersol seems to be more easily susceptible to the human colonic microbiota than RRM. However, in the second phase of fermentation its degradation slows down, pointing to more complex structures, that resist bacterial attack to a greater extent.

Comparison of in vitro and in vivo (ileal effluent) digestion residues of RRM showed that total SCFA and butyric acid production is somewhat higher for the ileal effluent than for the in vitro digestion residue (**Figure 2**). This is due to the fact that slightly lower amounts of undigested starch were present in the latter. Moreover, ileal effluents contained 11.0% fiber matters [results not shown], despite the fact that ileostomy subjects consumed a predetermined fiber-free diet (**Table 1**). Ileo blank, a sample without addition of fecal material, was included to estimate the endogeneous fermentative capacity of the ileal effluents, which are known to contain small amounts of bacteria (*16*).

On the basis of the present observations, it can be concluded that in vitro digestion residues of RRM behave similarly to ileal effluents obtained from the same starting material when they are subjected to colonic microbiota at identical starch concentrations. So far it has only been shown that in vitro-digested RRM obtained by another method is structurally similar to its corresponding in vivo residue (*33*). Supply of easily fermentable carbohydrates to either help grow microbiota beneficially or obtain an elevated supply of SCFA to the gut epithelium and other tissues has received considerable interest over the past decade (*29, 30*).

Gut colonization in humans starts immediately after birth, but major diversification takes place during weaning (*32*). Therefore, it has been of interest to test at which stage infants develop the ability to ferment RS. For this purpose, fermentative characteristics of fecal microbiota obtained from formula-fed babies, infants during weaning, and adults were compared. They were tested for endogenous fermentation (blank values), as well as on RRM and on lactulose (easily fermentable control substrate). The results showed that the fermentation of the supplied substrates clearly depends on the degree of development of the intestinal microbiota. Whereas lactulose was completely fermented, RRM was only degraded to a negligible amount by formula-fed infants, indicative of the fact that the microbiota still lacked species that are able to ferment RS. Very recent observations (*31*) showed this also to be the case for

breast-fed infants. In contrast, during weaning the ability to ferment RS is developing, as the higher SCFA production and the faster substrate degradation show. However, the rate of metabolism is still somewhat retarded compared to that of adults. Accordingly, it has been shown that during weaning the production of butyrate starts to be relevant (*31*). Thus, the ability to degrade RRM, and presumably also retrograded resistant starch, is only being established during weaning, that is, at the stage at which microbiota diversify considerably to finally approach the composition of that of adults. In adults, RS3 and RRM fermentation is known to result in relatively high concentrations of butyric acid, which is known to favor epithelial energy supply and growth as well as to reduce a number of risk factors associated with intestinal disease. Therefore, the inclusion of retrograded maltodextrins (type 3 RS) in the diet of weaning infants in order to stimulate butyric acid-producing bacteria may be attractive and warrants further research.

ABBREVIATIONS USED

DM, dry matter; GRI, glycemic response index; RRM, retrograded resistant maltodextrins; RS1. type 1 entrapped resistant starch; RS2, type 2 raw resistant starch; RS3, type 3 retrograded resistant starch; RS, resistant starch; SCFA, short-chain fatty acids.

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