Excretion of starch and esterified short-chain fatty acids by ileostomy subjects after the ingestion of acylated starches[1–3]

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ABSTRACT

Background: Short-chain fatty acids (SCFAs) have a role in maintaining bowel health and can assist in the prevention and treatment of colonic disease. The ability of acylated starches to deliver SCFAs to the large bowel has been shown in animal studies but has not been established in humans.

Objective: The aim was to determine whether cooked, highly acylated starches were resistant to small intestinal digestion in ileostomy volunteers.

Design: Volunteers consumed single doses of custards containing 20 g cooked acetylated, propionylated, or butyrylated high-amylose maize starches (HAMSA, HAMSP, and HAMSB, respectively) on each collection day. The amounts of starch and of esterified SCFAs ingested and subsequently excreted in the stoma effluent were measured. Custards containing unacylated high-amylose maize starch (Hylon VII, HAMS) and low-amylose maize starch (3401C, LAMS) were consumed as controls.

Results: Between 73% and 76% of the esterified SCFAs survived small intestinal digestion, which showed the potential of acylated starches to deliver specific SCFAs to the large bowel. The resistance of starches to small intestinal digestion as measured by ileal excretion was significantly greater for HAMSA, HAMSP, HAMSB, and HAMS than for LAMS ($P < 0.001$). The concentration of acetate in stoma digesta was higher than expected in all groups; this additional acid may have been derived from endogenous sources.

Conclusions: Acylated starches are a potentially effective method of delivering significant quantities of specific SCFAs to the colon in humans. These products have potential application in the treatment and prevention of bowel disorders amenable to modulation by SCFAs. *Am J Clin Nutr* 2007;86:1146–51.

KEY WORDS Resistant starch, acylation, short-chain fatty acids, ileostomates, butyrate

INTRODUCTION

Short-chain fatty acids (SCFAs), principally acetate, propionate, and butyrate, are produced during the bacterial fermentation of carbohydrates in the human colon and are important for the normal function of the viscera. The actions of SCFAs have potential in the treatment and prevention of disorders of the colon, including constipation, diarrhea, colorectal cancer, and ulcerative colitis, and may assist in the acute recovery from chemotherapy and surgery (1). Of the major SCFAs, butyrate is of particular interest because it has an important role as a metabolic fuel and regulator of the cells that line the large bowel and has been shown to suppress the growth of cancer cells in vitro (2).

There are practical limitations with the current strategies to increase the levels of specific SCFAs in the colon. The consumption of resistant starch (RS) increases SCFAs in the large bowel; however, the use of RS is limited because the microflora of some individuals cannot ferment certain types of RS (3). Acylating starches with specific SCFAs to resist small intestinal amylolysis has been shown to be effective in rat studies (4–6). The SCFAs are liberated by bacterial enzymes and are available for absorption and utilization by colonocytes or gut microbes. Acylating starches offers a degree of specificity in SCFA delivery because the greatest rise is in the acid that has been esterified, whereas the residual starch is also available for additional fermentation to SCFAs. To date, no studies have been conducted to investigate whether esterified SCFAs survive the transit through the small intestine of humans.

The present trial was designed to determine whether acylated starches were an effective vehicle for the delivery of SCFAs to the human colon. The starches were cooked (by heating with water) because it was anticipated that they will be used in processed food products, and cooking is known to lower the resistance of starches to amylolysis (6). Known amounts of cooked test starches were fed to ileostomy volunteers, and the stoma digesta were collected. The resistance of the starches to small intestinal digestion and the proportion of esterified SCFAs in the stoma digesta were measured to determine whether the esterified SCFAs were available for release in the large intestine.

SUBJECTS AND METHODS

Subjects

All experimentation was approved by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Human

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[2] Supported by the Preventative Health National Research Flagship and Commonwealth Scientific and Industrial Research Organisation Human Nutrition (a Centre of Food Science Australia). The National Starch Food Innovation and Ian Brown provided the acylated and high-amylose starches.

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TABLE 1
Sequence of ingestion of custards containing test starches[1]

	Period 1			Period 2		
Volunteer	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1	HAMSB	LAMS	HAMSA	HAMSP	HAMS	LAMS
2	LAMS	HAMSA	HAMSB	HAMS	LAMS	HAMSP
3	HAMSA	HAMSB	LAMS	LAMS	HAMSP	HAMS
4	HAMS	HAMSP	LAMS	HAMSA	LAMS	HAMSB
5	HAMSP	LAMS	HAMS	LAMS	HAMSB	HAMSA
6	LAMS	HAMS	HAMSP	HAMSB	HAMSA	LAMS
7	HAMSB	LAMS	HAMSA	HAMSP	HAMS	LAMS

[1] $n = 7$ except low-amylose maize starch (LAMS), $n = 14$. HAMS, high-amlyose maize starch; HAMSA, acetylated HAMS; HAMSP, propionylated HAMS; HAMSB, butyrylated HAMS.

Nutrition Human Ethics Committee. Volunteers signed informed consent forms after being provided oral and written information about the aims and methods of the study. Seven ileostomy subjects volunteered for the study, 6 women and 1 man with a mean age of 56 y (range: 37–81). All volunteers had well-established ileostomies and were in good health, without symptoms or signs of small intestinal inflammation or dysfunction. Five subjects had been proctocolectomized for ulcerative colitis and two for Crohn disease, which was limited to the large bowel. The major criteria used to exclude unsuitable volunteers included the use of any form of drug therapy, medication, or supplements on a regular basis that may interfere with bowel function, and the definite or suspected personal history of adverse events or intolerance of starchy or other foods, which may be tested in this study. An individual was included in the study only if, according to the volunteer, he or she had <10 cm of the distal ileum removed at surgery.

Study design

The trial was divided into 2 test periods, each of which consisted of 3 consecutive collection days (total of 6 d; **Table 1**). There were a total of 5 treatments, and the treatment sequence was balanced to ensure equal numbers of each acylated starch. To allow for possible carryover effects from day to day, the treatments were arranged so that each sequence of the diets occurred in each possible order for approximately the same number of times. The study was designed so that each volunteer consumed the custard containing low-amylose maize starch (LAMS) twice (total $n = 14$) and consumed the custards containing the other starches once (total $n = 7$ for each custard).

During the two 3-d treatment periods and for 24 h before each test period, the volunteers consumed a diet consisting of foods commonly consumed in a Western diet, which contained low levels of starch. The CSIRO provided the volunteers with all the foods consumed during these two 4-d periods of dietary restriction. The test custards were eaten by the volunteers in addition to the low-starch diet. To minimize the bacterial degradation of the stoma effluent, the volunteers emptied their stoma bags every 2 h until 2100 on the test days, and the contents were placed in portable freezers (−20 °C). The final collection for each test starch was made at 0700 on the day after the starch was ingested so that the stoma were collected for 24 h after consumption.

Test products

The trial involved the consumption of 20 g of 3 acylated high-amylose maize starches (acetylated, HAMSA; propionylated, HAMSP; butyrylated, HAMSB) and 2 control treatments: LAMS (3401C; National Starch and Chemical Company, Bridgewater, NJ), which is a low-amylose maize starch that was included as a control to enable the baseline intake of RS to be determined; and HAMS (Hylon VII; National Starch and Chemical Company), which is a high-amylose maize starch containing ≈70% amylose. HAMS was the base used to manufacture the acylated starches used in the present study. The degree of substitution, which is the number of hydroxyl groups on each D-glucopyranosyl unit derivatized by substituent groups, was determined by the National Starch and Chemical Company by use of ^{13}C-NMR spectroscopy (DRX-500 spectrometer; Bruker, Billerica, MA), by using the resolution of the 6 glucose carbons as assigned by Dais and Perlin (7); the degrees of substitution were 0.23, 0.24, and 0.25 respectively, for HAMSA, HAMSP, and HAMSB.

The starches were consumed as milk-based chocolate custards that were baked at 170 °C until set and were refrigerated for a maximum of 72 h before being consumed. Each serving of custard contained 20 g starch, 100 mL Pura classic chocolate milk (National Foods Ltd, Melbourne, Australia), 12 g Capilano chocolate topping (Capilano Honey Ltd, Richlands, Australia), and vanilla essence. The custards for one volunteer were made with lactose-free milk. Four batches of custards were made for analysis and for bacterial screening.

Sampling and analysis procedures

The stoma digesta samples from each volunteer collected during the 24 h after the ingestion of test custards were defrosted, pooled, homogenized, and subsampled for analysis. The custard and stoma digesta samples were analyzed for unesterified and total SCFAs. The latter were measured in custard and starch samples after they were hydrolyzed by vortexing for 2 h with 12.5 times the sample volume of 0.45 mol NaOH/L and neutralized with 10% H_3PO_4 before distillation. The stoma digesta samples were freeze-dried and hydrolyzed before distillation and were analyzed for total SCFAs. The amounts of SCFAs in the hydrolyzed freeze-dried samples were compared with SCFA analyses of wet digesta samples to ensure that unesterified SCFAs were not lost during the freeze-drying of the digesta (data not shown).

The SCFA analysis and dry matter determinations were undertaken as previously described (6). Duplicate samples of 4 batches of each custard were distilled for SCFA analyses.

To our knowledge, there is no standard or approved method to measure total starch in acylated starches; consequently, the custards and stoma digesta were analyzed by using a modification of a method (8) in which the starches were dispersed with 10 times the sample volume in 4.0 mol KOH/L. To determine whether the dispersal step resulted in complete hydrolysis of esterified SCFAs, the starch analysis of the custards was repeated by using the same method but with the incorporation of a pretreatment hydrolysis step in which the samples were vortexed for 2 h with 12.5 times the sample volume of 0.45 mol NaOH/L. This is the hydrolysis step used in a method to determine the acetyl content of acetylated starches (9).

Calculations and statistical analyses

GRAPHPAD PRISM version 4.0 for WINDOWS computer software (Graph Pad Software, San Diego, CA) was used for statistical analyses and for the generation of the graphs. When appropriate, data were compared by using 1-factor analyses of variance with Tukey's post hoc test for multiple comparisons; a nonparametric Kruskal-Wallis test was used to compare the proportion of bound SCFAs that survived small intestinal digestion. To correct for the differences in variation between esterified SCFAs in the custards, a single estimate of variation was used for the comparisons of the levels of each acid, and the df were reduced when necessary; this was noted when undertaken. Data are expressed as means ± SEMs, with statistical significance indicated when $P < 0.05$.

To check for differences between periods, LAMS values were compared, and no significant differences were found. The LAMS values for each volunteer were averaged, and $n = 7$ measures were used for these analyses. The amounts of prehydrolysis (unesterified SCFAs) SCFAs were deducted from the posthydrolysis values (total SCFAs) to calculate the amount of esterified SCFAs excreted in the stoma digesta and ingested in the custards.

RESULTS

Stoma digesta measures

There were no significant differences in stoma digesta output, pH, or dry matter content (%) between treatment groups (**Table 2**). All the pH values were above neutrality, which suggested minimal acid production through starch fermentation by bacterial activity in the terminal ileum or stoma bags.

There were no significant differences in the amount of any of the unesterified (nonhydrolyzed) SCFAs (mmol) excreted in the stoma digesta (**Table 3**) between treatment groups. The amounts of unesterified butyrate and propionate were low, but the amounts of unesterified acetate were high in all treatment groups; even ingestion of LAMS resulted in 17.6 ± 3.6 mmol unesterified acetate excreted in the stoma digesta.

Hydrolysis of the starch in stoma digesta released substantial amounts of SCFAs, which indicated that ingestion of acylated starches results in the passage of large amounts of esterified SCFAs through the small intestine (31.6 ± 2.5, 19.5 ± 0.9, and 20.9 ± 1.1 mmol for acetate, propionate, and butyrate in HAMSA, HAMSP, and HAMSB, respectively). The quantity of esterified SCFAs for the relevant acylated starch was significantly larger than the amount of the same acid in all other treatment groups (**Figures 1, 2,** and **3**; all $P < 0.001$). The amount of esterified acetate excreted in the stoma digesta was higher than expected in all treatment groups (average for all groups excluding HAMSA was 13.1 ± 1.2 mmol). The quantity of acetate released from the digesta exceeded the quantity of esterified acetate ingested for each treatment, which suggests that an endogenous source of esterified acetate was present in the stoma effluent.

TABLE 2
Output and pH of the stoma digesta of ileostomy volunteers after ingestion of 20 g cooked test starches[1]

Treatment	Digesta pH	Digesta wet weight	Digesta dry weight
		g/d	*g/d*
LAMS	7.40 ± 0.06	936 ± 134	60 ± 6
HAMS	7.40 ± 0.06	882 ± 98	64 ± 5
HAMSA	7.40 ± 0.06	1145 ± 214	66 ± 6
HAMSP	7.50 ± 0.04	879 ± 123	65 ± 7
HAMSB	7.40 ± 0.05	936 ± 133	63 ± 5

[1] All values are $\bar{x}$ ± SEM. $n = 7$. HAMSA, HAMSP, and HAMSB, acetylated, propionylated, and butyrylated high-amylose maize starches (HAMS); LAMS, low-amylose maize starch. There were no significant differences between groups for any marker when compared by using 1-factor ANOVA.

Ileal excretion and digestibility

Digesta starch output and digestibility are shown in **Table 4**. Starch output was significantly lower after the volunteers ingested cooked LAMS than after ingestion of the other starches; there were no significant differences in starch output between consumption of HAMS and the acylated starches. The resistance to digestion in the small intestine of the LAMS starch in the custard was also significantly lower than that of the cooked HAMS and acylated starches; this lower resistance was reflected in a significantly higher starch digestibility for LAMS.

Custard measures

SCFA content of custards

Compared with the HAMS and LAMS custards, the custards cooked with acylated starches contained significantly larger

TABLE 3
Unesterified short-chain fatty acids in the stoma digesta of ileostomy volunteers after ingestion of 20 g cooked test starches[1]

	Acetate	Propionate	Butyrate
	mmol	*mmol*	*mmol*
LAMS	17.6 ± 3.6	1.3 ± 0.2	1.3 ± 0.2
HAMS	15.3 ± 3.4	1.4 ± 0.3	1.1 ± 0.2
HAMSA	17.9 ± 3.4	1.3 ± 0.2	1.5 ± 0.1
HAMSP	13.1 ± 2.5	1.9 ± 0.2	1.4 ± 0.5
HAMSB	9.9 ± 1.9	1.0 ± 0.1	1.4 ± 0.1

[1] All values are $\bar{x}$ ± SEM. $n = 7$. HAMSA, HAMSP, and HAMSB, acetylated, propionylated, and butyrylated high-amylose maize starches (HAMS); LAMS, low-amylose maize starch. There were no significant differences between groups for any acid when compared by using 1-factor ANOVA.

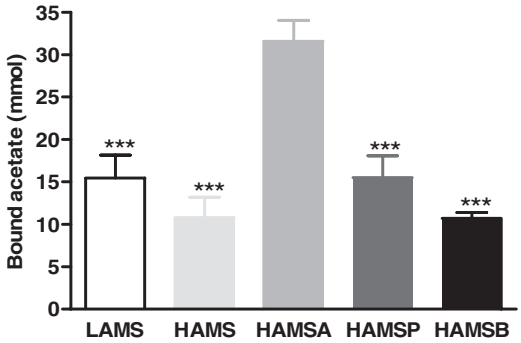

FIGURE 1. Mean (±SEM) estimated amount of esterified acetate released by the hydrolysis of stoma digesta of volunteers ingesting cooked starches ($n = 7$). ***Significantly different from the relevant acylated starch [acetylated high-amylose maize starch (HAMSA)] by use of 1-factor ANOVA with reduced df ($P < 0.001$). LAMS, low-amylose maize starch; HAMS, high-amylose maize starch; HAMS, high-amylose maize starch; HAMSP, propionylated high-amylose maize starch; HAMSB, butyrylated high-amlyose maize starch.

amounts of the respective unesterified acids (4.7 ± 0.2, 2.5 ± 0.04, and 1.1 ± 0.03 mmol for acetate, propionate, and butyrate in HAMSA, HAMSP, and HAMSB, $P < 0.001$). The amounts of unesterified acetate, propionate, and butyrate for LAMS were 0.6 ± 0.02, 0.03 ± 0.02, and 0.02 ± 0.01 mmol, respectively, and were 0.6 ± 0.02, 0.01 ± 0.01, and 0.02 ± 0.01 mmol for HAMS, respectively. This suggests that unesterified fatty acids remained in the starches after acylation or that esterified fatty acids were released during the cooking of the starches. The custards made with the acylated starches contained large amounts of esterified SCFAs (**Table 5**), whereas the LAMS and HAMS custards contained negligible amounts of any esterified SCFAs.

Starch content of custards

The starch content (≈130 g/custard serving) of the custards is shown in Table 5. The values for the custards analyzed by using potassium hydroxide as the dispersing agent were similar to those hydrolyzed with sodium hydroxide, which indicated that the dispersing agent hydrolyzed the esterifed SCFAs. The starch results were within the range of expected values; ie, the acylated products contained between 1 and 2 g less starch than did HAMS.

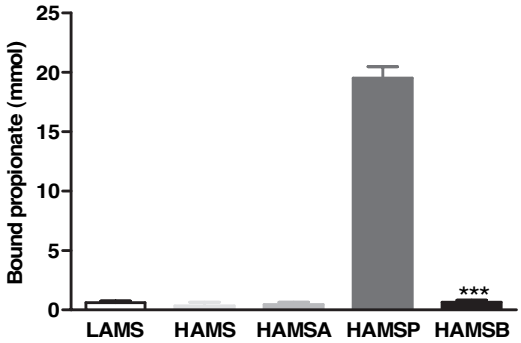

FIGURE 2. Mean (±SEM) estimated amount of esterified propionate released by the hydrolysis of stoma digesta of volunteers ingesting cooked starches ($n = 7$). ***Significantly different from the relevant acylated starch [propionylated high-amylose maize starch (HAMSP)] by use of 1-factor ANOVA with reduced df ($P < 0.001$)]. LAMS, low-amylose maize starch; HAMS, high-amylose maize starch; HAMSA, acetylated high-amylose maize starch; HAMSB, butyrylated high-amlyose maize starch.

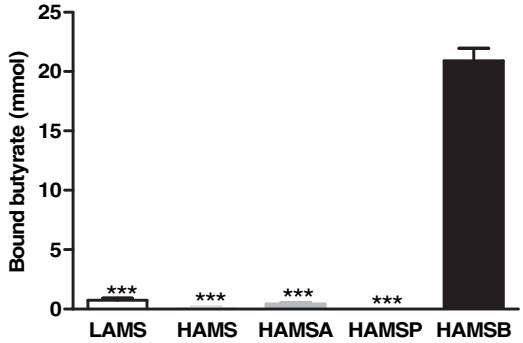

FIGURE 3. Mean (±SEM) estimated amount of esterified butyrate released by the hydrolysis of stoma digesta of volunteers ($n = 7$) ingesting cooked starches (mmol). ***Significantly different from the relevant acylated starch [butyrylated high-amylose maize starch (HAMSB)] by use of 1-factor ANOVA with reduced df ($P < 0.001$). LAMS, low-amylose maize starch; HAMS, high-amylose maize starch; HAMSA, acetylated high-amylose maize starch; HAMSP, propionylated high-amlyose maize starch.

Balance calculations for SCFAs

The proportion of ingested esterified SCFAs that survived passage through the small intestine was calculated by deducting the average amount of esterified SCFAs in the stoma digesta from the average amount of esterified SCFAs in a serving of each of the custards. To correct for the amount of esterified acids derived from custard ingredients or endogenous secretions, the amount of esterified SCFAs in the digesta was corrected for the amount of esterified SCFAs present in the stoma waste when volunteers ingested starches other than those containing the relevant acylated starch. The amount of esterified SCFAs in the custards containing nonacylated starches was low, and the amount deducted was only substantial for acetic acid. In conclusion, the proportion of SCFAs esterified to cooked acylated starches that survived digestion in the small intestine was between 73% and 76% (**Table 6**).

DISCUSSION

The present study shows for the first time in humans that when highly acylated starches (degree of substitution: 0.23–0.25) are

TABLE 4
Starch output and digestibility in the stoma digesta of ileostomy volunteers after ingestion of 20 g cooked test starches[1]

Treatment	Starch output	Starch digestibility[2]
	g/d	*%*
LAMS	1.0 ± 0.1[3]	94.6 ± 0.5[3]
HAMS	5.8 ± 0.3	72.9 ± 1.4
HAMSA	5.6 ± 0.4	71.6 ± 2.0
HAMSP	5.5 ± 0.3	72.4 ± 1.5
HAMSB	6.5 ± 0.4	67.9 ± 2.2

[1] All values are $\bar{x}$ ± SEM. $n = 7$.

[2] The digestibility of starches was calculated by using the following formula: digestibility = {[(grams of ingested starch) − (grams of starch output)]/(grams of ingested starch)} × 100.

[3] Significantly different from high-amylose maize starch (HAMS), acetylated HAMS (HAMSA), propionylated HAMS (HAMSP), and butyrylated (HAMSB) by using 1-factor ANOVA with Tukey's post hoc tests ($P < 0.001$).

TABLE 5
Esterified short-chain fatty acid (SCFA) and starch contents of a single serving (≈130 g) of each of the cooked test custards ingested by ileostomy volunteers[1]

	Esterified SCFAs			Starch	
Treatment	Acetic	Propionic	Butyric	Standard method[2]	Hydrolyzed with NaOH[3]
	mmol			*g*	
LAMS	0.08 ± 0.03	0 ± 0.02	0.11 ± 0.01	21.7 ± 0.1	20.7 ± 0.3
HAMS	0.13 ± 0.03	0.0 ± 0.01	0.14 ± 0.01	21.1 ± 0.2	21.4 ± 0.1
HAMSA	25.1 ± 0.4[4]	0.02 ± 0.02	0.11 ± 0.02	19.9 ± 0.7	19.8 ± 0.2
HAMSP	0.46 ± 0.09	24.9 ± 0.6[4]	0.11 ± 0.01	21.1 ± 0.3	19.9 ± 0.5
HAMSB	0.30 ± 0.02	0.15 ± 0.06	28.2 ± 0.2[4]	20.2 ± 0.3	20.1 ± 0.2

[1] All values are $\bar{x}$ ± SEM. $n = 4$. HAMSA, HAMSP, and HAMSB, acetylated, propionylated, and butyrylated high-amylose maize starches (HAMS); LAMS, low-amylose maize starch. Analyses were undertaken in duplicate on samples from 4 batches of starches.
[2] Values were analyzed by using a modification of a standard method (8) involving dispersal with 4.0 mmol KOH/L.
[3] Samples were hydrolyzed with 0.45 mmol NaOH/L before analysis; *see Sampling and analysis procedures* in Subjects and Methods.
[4] Significantly different from other starches by using 1-factor ANOVA with Tukey's post hoc tests ($P < 0.001$). Because of the high values and larger variances with acylated starches, these statistical tests were based on one estimate of variation.

ingested, significant fractions of the esterified SCFAs (73–76%) survive digestion in the small intestine. This finding confirms those of animal studies in which these starches were shown to resist small intestinal amylolysis and to increase large bowel SCFA levels (4–6). The data in the current study suggest that significant amounts of each of the esterified SCFAs (19–21 mmol acetate, propionate, or butyrate) would reach the colon in humans and, if released by bacterial enzymes, would be available to exert a potential health benefit.

On the basis of a reported study (10) in which raw starches were fed to colectomized rats, it was anticipated that nearly one-half the acylated starches would be digested in the small intestine, whereas nearly two-thirds of the HAMS would be digested. However, in the present study, the small intestinal digestibilities of the cooked acylated HAMS were similar to those of cooked HAMS, which was the base used to manufacture the acylated starches. The small intestinal digestibilities of these starches were very similar to the digestibility of cooked high-amylose cornstarch reported in vitro (6) and when measured by using endoscopic retrograde bowel insertion methodology (34%; 11). Other workers (12) using a combined $^{13}CO_2/H_2$ breath test found that cooking high-amylose starches reduces the resistance to small intestinal digestion from 64% to 28%, which is also similar to the level reported in this study. In the current study, LAMS was nearly completely digested, which is comparable with the digestibility of standard maize starch in rats (10).

Both HAMS and acylated HAMS supply similar levels of RS as fermentable substrate to the large bowel. The average RS intake for Australians is between 3.4 and 9.4 g/d (13), which is similar to the amount of cooked HAMS that escaped small intestinal digestion in the present study (5.8 g). On the basis of the work of others (14), it is estimated that colonic fermentation of 5.8 g cooked HAMS would provide 40, 7, and 9 mmol acetic, propionic, and butyric acids, respectively. These amounts compare with the totals of 59, 26, and 30 mmol acetic, propionic, and butyric acids either delivered (19–21 mmol) or fermented (40–9 mmol) from 20 g ingested cooked HAMSA, HAMSP, or HAMSB. The production of acylated starches provides a method of tailoring the delivery of significant amounts of specific SCFAs to the large bowel to concentrations in excess of those normally produced by colonic fermentation of RS. Because SCFAs are absorbed from the human gut in a concentration-dependent manner (15), increasing the concentration of SCFAs in the large bowel by the the ingestion of acylated starches results in greater uptake than when similar quantities of HAMS or the typical Australian diet are ingested. Acylated starches, therefore, have

TABLE 6
Percentage of ingested esterified acetic, propionic, and butyric acid in the stoma digesta of ileostomy volunteers after ingestion of 130 g custard[1]

	Amount of relevant esterified acid ingested[2]	Amount of relevant esterified acid in stoma digesta	Amount of relevant esterified acid in stoma of nonacylated starch groups[3]	Ingested esterified acid in stoma digesta
	mmol	*mmol*	*mmol*	*%*
HAMSA	25.1 ± 0.4	31.6 ± 2.5	13.1 ± 1.2	73.9 ± 9.8
HAMSP	24.9 ± 0.6	19.5 ± 0.9	0.5 ± 0.1	76.2 ± 3.8
HAMSB	28.2 ± 0.2	20.9 ± 1.1	0.3 ± 0.1	73.1 ± 3.7

[1] All values are $\bar{x}$ ± SEM.
[2] Analyses were undertaken in duplicate on samples from 4 batches of starches.
[3] Average amount of each esterified acid in stoma digesta of groups not ingesting the relevant esterified short-chain fatty acids [eg, average of low-amylose maize starch, high-amylose maize starch (HAMS), propionylated HAMS (HAMSP), and butyrylated HAMS (HAMSB) for acetylated HAMS (HAMSA)]. There were no significant differences in the percentage of esterified acids surviving passage through the small intestine when compared by using a nonparametric Kruskal-Wallis test.

potential application in conditions amenable to improvement by increasing SCFAs in the large bowel. For example, SCFA enemas have been used to treat distal ulcerative colitis (16), whereas ingestion of acylated starches may be a more acceptable strategy.

The use of ileostomy subjects to study small intestinal excretion is considered an accurate, direct, and quantitative method (17) of measuring the amount of starch resistant to digestion. Potential criticisms are that small intestinal function may be modified by the surgery, or the terminal ileum may be colonized with bacteria, which could ferment starch. However, the colonization effect is likely to be small, and the ileostomy model appears to be a reliable in vivo method of measuring RS (18). The consistently high pH of digesta stoma from the volunteers in the present study supports the hypothesis that minimal SCFA release or starch fermentation occurred in the small intestine of the volunteers.

The concentrations and amount of acetic acid (both unesterified and esterified) in the stoma digesta were disproportionally high when compared with the other acids, and this additional acid may have been derived from endogenous sources. For example, the highly glycosylated mucin secretions of the duodenal Brunner's glands contain high concentrations of *N*-acetyl-galactosamine and *N*-acetyl-glucosamine in New World primates (19), although the concentrations may be lower in the mucus of humans (20). Acetate has been reported previously in the stoma digesta from ileostomy subjects (21), although the concentrations in the earlier study were tenfold those of the unesterified acetate concentrations found in the current study. The difference in acetate concentration may have been due to the method of analysis (22), which may have released esterified endogenous acetate in the earlier study.

In conclusion, we showed for the first time that when highly acylated starches are ingested by humans, 73–76% of esterified SCFAs resist digestion in the small intestine. The data confirm that acylated starches are a potentially effective method of delivering significant quantities of specific SCFAs to the colon, and hence the potential for these products to have application in the treatment and prevention of bowel disorders is considerable.

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The authors' responsibilities were as follows—JMC: participated in the design and conduct of the experiment, the evaluation and interpretation of the data, and the preparation of the manuscript; LC: was involved with the conception and design of the experiment, interpretation of the data, and preparation of the manuscript; ARB: was involved with the design and conduct of the experiment, the interpretation of the data, and the preparation of the manuscript; and DLT: participated in the conception and design of the experiment and preparation of the manuscript. DLT and ARB are inventors on patents WO 01/02016 A1 and WO 02/02102 A1. JMC and LC had no personal or financial conflicts of interest.

REFERENCES

1. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev 2001;81:1031–64.
2. Whitehead RH, Young GP, Bhathal PS. Effects of short chain fatty acids on a new human colon carcinoma cell line (LIM1215). Gut 1986;27:1457–63.
3. Cummings JH, Beatty ER, Kingman SM, Bingham SA, Englyst HN. Digestion and physiological properties of resistant starch in the human large bowel. Br J Nutr 1996;75:733–47.
4. Annison G, Illman RJ, Topping DL. Acetylated, propionylated or butyrylated starches raise large bowel short-chain fatty acids preferentially when fed to rats. J Nutr 2003;133:3523–8.
5. Bird AR, Brown IL, Topping DL. Low and high amylose maize starches acetylated by a commercial or a laboratory process both deliver acetate to the large bowel of rats. Food Hydrocolloids 2006;20:1135–40.
6. Bajka BH, Topping DL, Cobiac L, Clarke JM. Butyrylated starch is less susceptible to enzymic hydrolysis and increases large-bowel butyrate more than high-amylose maize starch in the rat. Br J Nutr 2006;96:276–82.
7. Dais P, Perlin AS. High-field, ^{13}C-N.M.R. spectroscopy of beta-d-glucans, amylopectin, and glycogen. Carbohydrate Res 1982;100:103–16.
8. Champ M. Determination of resistant starch in foods and food products: interlaboratory study. Eur J Clin Nutr 1992;46(suppl 2):S51–62.
9. International Starch Institute. SPAD ISI 14-2e determination of acetyl content. Science Park Aarhus, Denmark: International Starch Institute, 1999. Internet: http://www.starch.dk/isi/methods/14acet.htm.
10. Morita T, Kasaoka S, Kiriyama S, Brown IL, Topping DL. Comparative effects of acetylated and unmodified high-amylose maize starch in rats. Starch-Starke 2005;57:246–53.
11. Danjo K, Nakaji S, Fukuda S, Shimoyama T, Sakamoto J, Sugawara K. The resistant starch level of heat moisture-treated high amylose cornstarch is much lower when measured in the human terminal ileum than when estimated in vitro. J Nutr 2003;133:2218–21.
12. Symonds EL, Kritas S, Omari TI, Butler RN. A combined $^{13}CO_2/H_2$ breath test can be used to assess starch digestion and fermentation in humans. J Nutr 2004;134:1193–6.
13. Roberts J, Jones GP, Rutishauser IHE, Birkett A, Gibbons C. Resistant starch in the Australian diet. Nutr Diet 2004;61:98–104.
14. Cummings JH. The large intestine in nutrition and disease. Brussels, Belgium: Danone Institute of Belgium, 1997.
15. Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt MG. Absorption of short-chain fatty acids by the colon. Gastroenterology 1980;78:1500–7.
16. Scheppach W, Sommer H, Kirchner T, et al. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 1992;103:51–6.
17. Langkilde AM, Champ M, Andersson H. Effects of high-resistant-starch banana flour (RS_2) on in vitro fermentation and the small-bowel excretion of energy, nutrients, and sterols: an ileostomy study. Am J Clin Nutr 2002;75:104–11.
18. Englyst HN, Kingman SM, Hudson GJ, Cummings JH. Measurement of resistant starch in vitro and in vivo. Br J Nutr 1996;75:749–55.
19. Posel P, Schumacher U, Frick H, Welsch U. Histochemistry of mucosubstances in Brunner's-gland cells and duodenal goblet cells of 2 New World monkey species (*Saimiri-Sciureus* and *Saguinus-Fuscicollis*). Histochemistry 1988;88:327–32.
20. Smits HL, Kramer MF. Human duodenal gland (Brunner's gland) mucus glycoprotein analysis. Arch Biochem Biophys 1984;228:64–70.
21. Mitchell BL, Lawson MJ, Davies M, et al. Volatile fatty acids in the human intestine: studies in surgical patients. Nutr Res 1985;5:1089–92.
22. Storer GB, Trimble RP, Illman RJ, Snoswell AM, Topping DL. Effects of dietary oat bran and diabetes on plasma and caecal volatile fatty acids in the rat. Nutr Res 1983;3:519–26.