J Nutr Sci Vitaminol, **55**, 242–251, 2009

Mannitol Lowers Fat Digestibility and Body Fat Accumulation in Both Normal and Cecectomized Rats

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(Received November 12, 2008)

Summary Mannitol is a six-carbon sugar alcohol that is widely distributed in plants. Sugar alcohols are widely used in various food products because of their numerous beneficial health effects. The present study investigated the effects of mannitol consumption on digestion, large gut fermentation and nutrient metabolism in normal and cecectomized male Wistar rats. After 28 d of feeding with three kinds of diet containing 0, 4 or 8% mannitol, mannitol consumption dose-dependently lowered the digestibilities of crude fat and crude protein, the ratio of body fat accumulation to energy absorbed and the hepatic and serum triglyceride levels in normal rats. After 24 d of feeding with three kinds of diet comprising a control diet, a 5% mannitol-containing diet and a 5% fructooligosaccharides (FOS)-containing diet, mannitol lowered the digestibility of fat and the ratio of body fat accumulation to energy consumed and absorbed in cecectomized rats. On the other hand, FOS consumption had no effect on the accumulation of body fat, but lowered the digestibility of fat. FOS consumption greatly improved the accumulation of body ash in cecectomized rats. These results suggest that mannitol has a lowering effect on body fat accumulation, and further indicate that the cecum is not essential for the appearance of effects of mannitol on digestion, absorption and metabolism.

Key Words mannitol, digestion, fat accumulation, rat, cecum

Excessive accumulation of body fat is considered to be a major risk factor for several chronic disease conditions such as coronary heart disease, cancer, diabetes and hypertension. Fatness is also a specific reason for the persistence of chronic fatty liver, representing one of the major causes of hepatic diseases such as acute steatohepatitis and chronic liver failure (*1*). Proper weight management is one of the key components of a healthy lifestyle that avoids the onset of these diseases.

Mannitol is a naturally-occurring six-carbon sugar alcohol or polyol that is widely distributed in plants, including algae, onions, grasses, olives and pumpkins. It is used as an osmotic diuretic and cleared by the kidneys, in a similar manner to inulin (*2*). Mannitol is not easily digested in the small intestine, but is fermentable in the large intestine (*3*, *4*). In a previous study, up to 50% of uniformly labeled ^{14}C-mannitol orally administered to humans was recovered in urine and stool samples after 48 h and as much as 18% of a dose was recovered as expired CO_2, whereas little metabolism was observed after intravenous administration (*5*). In another human study, the blood glucose level and respiratory quotient remained unaffected within 2 h after administration of mannitol (*6*). These findings suggest that part of the ingested mannitol is utilized as an energy source, possibly after being metabolized to organic acids by intestinal microflora and absorbed from the large intestine, although mannitol can be used as a sweetener by people with diabetes.

Non-digestible but fermentable oligosaccharides stimulate the growth of bifidobacteria (*7*) and increase the intestinal production of short-chain fatty acids (SCFAs), which are thought to have various physiological functions including stimulatory effects on intestinal cell proliferation (*8*), gut motility (*9*) and absorption of minerals and water in the large intestine (*10*). SCFAs produced in the large intestine may also modulate hepatic lipid metabolism (*11*). These findings suggest that fermentable sugar alcohols also affect lipid and energy metabolism.

In a previous study, we reported the possibility of using cecectomized rats as a model for studying the physiological effects of sugar alcohols in humans. In the same study, we showed that cecectomy modified the effects of sugar alcohols, sorbitol and lactitol on digesta retention and gut fermentation in rats (*12*). Some of these physiological and metabolic effects may be caused by metabolites produced through microbial fermentation in the large intestine, since SCFAs have many important functions in lipid and energy metabolism (*13*, *14*). Rats have a large cecum, which provides an appropriate environment for fermentation of sugar alcohols. Although many authors have reported the effects of sugar alcohols in normal rats, the effects of sugar alcohols on physiological aspects and nutrient

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metabolisms in cecectomized rats and the actual participation of the cecum in sugar alcohol metabolism and utilization remain unknown. This information must be relevant for estimating the physiological and nutritional effects of sugar alcohols. If the cecum plays a role in the appearance of physiological and nutritional effects of sugar alcohol feeding, different results must be obtained between experiments in normal or cecectomized rats.

The purpose of the present study was to identify the effects of mannitol in normal and cecectomized rats and confirm the actual participation of the cecum in sugar alcohol metabolism and utilization by animals. To achieve these aims, we investigated the effects of mannitol on lipid and nitrogen metabolisms in rats with or without their cecum.

MATERIALS AND METHODS

Animals and diets.

Experiment 1: A total of 24 growing male Wistar rats (3 wk of age) were purchased from Japan SLC, Inc. (Shizuoka, Japan) at 2 wk before the experiment and housed individually in wire-mesh steel cages in an air-conditioned room maintained at 23±1˚C with a constant 12-h light (07:00–19:00) and 12-h dark cycle. At the beginning of the experiment, the mean weight (±SD) of the rats was 184.0±17.6 g. The three rats with the lowest body weights and three rats with the highest body weights were euthanized by exsanguination under diethyl ether anesthesia. The body energy contents of these animals were analyzed as the initial energy contents at the beginning of the feeding trial.

The remaining 18 rats were divided into 3 groups of 6 rats, and fed one of the following three different experimental diets for 28 d: control diet (C); 4% mannitol-containing diet (4M); or 8% mannitol-containing diet (8M). All rats were given free access to food and water. The ingredients of the experimental diets are shown in Table 1. During the experimental period, food residues were collected every day and subtracted from the amount provided to calculate the daily intake. Body weights were measured on day 1 and day 28 of the experiment, and feces were collected daily for 7 d from day 20 to estimate the digestibility. At the end of the experimental period, the rats were anesthetized with diethyl ether after fasting for 12 h. Blood samples were taken from the inferior vena cava and the cecum and liver were collected from each rat. The digestive tracts without the cecum were opened, washed with physiological saline and replaced in the carcass. The serum samples, cecums, livers and carcasses were stored at −30˚C until analysis.

Experiment 2: A total of 26 growing male Wistar rats (5 wk of age; approximately 150 g in body weight) were maintained individually in wire-mesh steel cages in the same room as the rats for Experiment 1. The rats were anesthetized with 0.10–0.15 mL of pentobarbital (50 mg/mL; Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) prior to undergoing a cecectomy.

Table 1. Compositions of the experimental diets (g/kg).

Ingredients	Experiment 1			Experiment 2		
	C	4M	8M	C	5M	5FOS
α-Corn starch	562	562	562	362	362	362
Sucrose	100	60	20	300	250	250
Casein	200	200	200	200	200	200
L-Cystine	3	3	3	3	3	3
Soybean oil	70	70	70	—	—	—
Lard	—	—	—	70	70	70
Cellulose powder	20	20	20	20	20	20
Vitamin mix[1]	10	10	10	10	10	10
Mineral mix[1]	35	35	35	35	35	35
D-Mannitol	—	40	80	—	50	—
Fructooligosaccharide	—	—	—	—	—	50
Gross energy (kcal/g)[2]	4.59	4.59	4.59	4.59	4.59	4.59

C: control diet; 4M: 40 g/kg mannitol-containing diet; 8M: 80 g/kg mannitol-containing diet; 5M: 50 g/kg mannitol-containing diet; 5FOS: 50 g/kg fructooligosaccharides-containing diet.

[1] AIN-93G (Reeves PG et al. (*42*)).

[2] Carbohydrate, protein and fat were considered to be 4.1, 5.6 and 9.4 kcal/g, respectively.

The cecectomy was carried out as follows. A 20-mm incision was made on the midline, starting at 20 mm caudal to the last rib. The cecum was pulled through the incision with tweezers, and a ligature was placed around the junction between the cecum and the colon at the end of the ileum. An incision was made laterally to this ligature to remove the cecum. The incision in the cecum was washed with povidone iodine solution (Meiji Seika Ltd., Tokyo, Japan), and a few drops of penicillin solution were placed into the abdominal cavity after the ligature was returned. The musculature and skin were separately sutured with ligatures.

All rats were maintained for about 10 day to allow recovery from the cecectomy and supplied with a commercial diet. On day 11 after the cecectomy, the rats were divided into the following 3 groups: 8 rats fed a control diet (C); 9 rats fed a 5% mannitol-containing diet (5MN); and 9 rats fed a 5% fructooligosaccharides (FOS)-containing diet (5FOS). FOS, which are frequently used as functional sugars, were employed as a reference. The 3 groups had similar average body weights at the beginning of the experiment. The animals had free access to the experimental diets and water for 24 d. The ingredients of the experimental diets are shown in Table 1.

During the experimental period, food residues were collected every day and subtracted from the amount provided to calculate the daily intake. Body weights were measured on day 1 and day 24 of the experiment, and feces were collected daily for 7 d from day 15 to estimate the digestibility. At the end of the experimental period, the rats were anesthetized with diethyl ether after fasting for 12 h. Blood samples were collected from the inferior aorta of each rat. The digestive tracts were opened, washed with physiological saline and replaced

Table 2. Body weights, food intakes, feeding efficiencies,[1] cecal weights, fecal weights and apparent digestibilities[2] of food components in rats fed three experimental diets for 28 d (Experiment 1).

	C	4M	8M
Body weight			
Initial (g)	183.4±23.7	184.8±18.8	184.1±18.3
Final (g)	354.3±10.6	344.5±19.5	327.3±18.9
Daily gain (g/d)	5.85±0.66	5.64±0.72	5.06±0.32
Food intake (g/d)	17.9±0.09	18.0±0.11	17.9±0.28
Feed efficiency[1]	0.34±0.04	0.32±0.04	0.29±0.02
Fecal weight (g)	5.71±0.46[a]	6.47±0.47[ab]	8.08±1.04[b]
Apparent digestibility[2]			
Dry matter	0.96±0.003[a]	0.95±0.003[ab]	0.94±0.007[b]
Crude fat	0.97±0.008[a]	0.96±0.008[ab]	0.92±0.031[b]
Crude protein	0.96±0.005[a]	0.94±0.006[b]	0.92±0.015[b]
Crude ash	0.72±0.026	0.74±0.029	0.71±0.051
Carbohydrate[3]	0.97±0.004	0.97±0.004	0.96±0.003
Gross energy[4]	0.97±0.003[a]	0.96±0.003[ab]	0.94±0.091[b]

Abbreviations: See footnotes to Table 1.
[1] Weight gain/feed intake.
[2] (Intake−fecal excretion)/intake.
[3] Dry matter−(crude ash+crude fat+crude protein).
[4] Calculated as 4.1 kcal/g for carbohydrate, 5.6 kcal/g for protein and 9.4 kcal/g for fat.
[a,b] Mean values within a row not sharing a common superscript letter differ significantly at $p<0.05$ by the Steel-Dwass test. Values are shown as means±SD ($n=6$).

in the carcass. The carcasses were stored at −30°C until analysis.

Analytical methods. Pooled fecal samples from the feeding trials were oven-dried at 60°C for 24 h and then powdered with a ball mill. The fecal and diet samples were analyzed in duplicate for moisture, nitrogen, diethyl ether extract components and crude ash, as previously described (*15*). The carbohydrate content was estimated by the difference between the dry matter content and the total crude protein, crude fat and crude ash content. The carcasses were homogenized using a mincer and analyzed for moisture, nitrogen, diethyl ether extract components and crude ash as described above.

Serum total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride and non-esterified fatty acid (NEFA) levels were determined using commercial kits (Cholesterol C-test, HDL-cholesterol-test, Triglyceride E-test and NEFA C-test, respectively; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Hepatic lipids were extracted using a previously validated procedure (*16*). Total cholesterol and triglyceride in serum were analyzed as previously described (*17*). Hepatic cholesterol and triglyceride levels were analyzed with the same kits used for the serum analyses.

The cecums were weighed before their contents were collected, and the cecal tissues were weighed after removal of the contents. For analysis of the organic acid concentrations, homogenized cecal contents were prepared as previously described (*18*) and analyzed using high performance liquid chromatography (Column: Shodex kc-811; Detector: 4000 UV Detector; Hitachi Ltd., Tokyo, Japan). The pH values were determined using a TWIN pH meter (Horiba Ltd., Kyoto, Japan).

Calculations. The protein accumulation, crude ash, fat energy and total energy in the body were estimated by the differences between the corresponding amounts at the beginning and end of each experiment. The content of each component at the beginning of the experiment was calculated by multiplying the mean concentration (g/g body weight) in the 6 rats euthanized at the beginning of the experiment by the body weight (g) at the beginning of the experiment.

Statistical analysis. All data are presented as means±SD, and were analyzed by one-factorial (control, 4M and 8M in Experiment 1; control, 5MN and 5FOS in Experiment 2) ANOVA. The significance of differences among values was analyzed by the Tukey-Kramer multiple comparison test for data regarded as having a normal distribution or the Steel-Dwass test for data not regarded as having a normal distribution. All statistical analyses were carried out using SPSS 13.0J software (SPSS Inc., Chicago, IL). Differences were considered significant at $p<0.05$.

Ethics. All animal experiments were performed according to the rules and regulations for animal experiments of Okayama University.

RESULTS

Body weights, food intakes and apparent digestibilities in Experiment 1

Consumption of the 8% mannitol diet induced a slight diarrhea; however, after 2 or 3 d of feeding all rats recovered. There were no significant differences in the body weights, food intakes, feeding efficiencies or liver weights among the experimental groups. The fecal weights were higher in the 8M group than in the C group. The digestibilities of dry matter, crude fat, crude

Table 3. Concentrations of serum and hepatic lipids in rats fed three experimental diets for 28 d (Experiment 1).

	C	4M	8M
Serum			
Total cholesterol (mmol/L)	1.81±0.26	1.69±0.30	1.68±0.30
HDL-cholesterol (mmol/L)	0.62±0.08	0.60±0.10	0.59±0.08
Triglyceride (mmol/L)	0.63±0.05[a]	0.49±0.11[b]	0.35±0.03[c]
NEFA[1] (mEq/L)	0.48±0.14	0.39±0.10	0.40±0.11
Liver			
Weight (g)	12.4±1.74	12.9±2.08	11.6±1.16
(g/100 g BW)	3.50±0.52	3.72±0.40	3.53±0.18
Total cholesterol (mmol/L)	0.58±0.08	0.52±0.08	0.57±0.06
Triglyceride (mmol/L)	0.77±0.16[a]	0.48±0.11[b]	0.49±0.11[b]

Abbreviations: See footnotes to Table 1.
[1] Non-esterified fatty acids.
[a,b,c] Mean values within a row not sharing a common superscript letter differ significantly at $p<0.05$ by the Tukey-Kramer test for NEFA and the Steel-Dwass test for HDL-cholesterol and triglyceride.
Values are shown as means±SD ($n=6$).

Table 4. Ratios of crude protein and energy accumulated[1] to these consumed[2] or absorbed[3] in rats fed three experimental diets for 28 d (Experiment 1).

		C	4M	8M
Crude protein	Consumed[2]	0.284±0.02	0.276±0.05	0.251±0.02
	Absorbed[3]	0.295±0.02	0.294±0.05	0.273±0.03
Crude ash	Consumed[2]	0.318±0.08[a]	0.404±0.08[b]	0.287±0.03[a]
	Absorbed[3]	0.403±0.11[a]	0.539±0.09[b]	0.404±0.04[a]
Crude fat energy[4]	Consumed[2]	0.105±0.03[a]	0.086±0.02[ab]	0.067±0.02[b]
	Absorbed[3]	0.108±0.04[a]	0.090±0.02[ab]	0.071±0.02[b]
Gross energy[5]	Consumed[2]	0.172±0.04[a]	0.154±0.02[ab]	0.127±0.01[b]
	Absorbed[3]	0.178±0.04[a]	0.161±0.02[ab]	0.134±0.01[b]
Modified efficiency of fat energy accumulation[6]	Consumed[2]	0.105±0.03[a]	0.089±0.02[ab]	0.072±0.02[b]
	Absorbed[3]	0.108±0.04[a]	0.093±0.02[ab]	0.076±0.02[b]

Abbreviations: See footnotes to Table 1.
[1] Difference between the amount in the carcass and the amount in the body at the beginning of the feeding period. For details, see the main text.
[2] Amount consumed during the feeding period.
[3] Digestible amount calculated using the amount consumed during the feeding period and the digestibility in Table 2.
[4] Ratio of fat energy accumulated to the energy consumed or absorbed.
[5] Calculated as 4.1 kcal/g for carbohydrate, 5.6 kcal/g for protein and 9.4 kcal/g for fat.
[6] Ratio of fat energy accumulation to the intake of energy except for mannitol consumed or absorbed.
[a,b] Mean values within a row not sharing a common superscript letter differ significantly at $p<0.05$ by the Steel-Dwass test.
Values are shown as means±SD ($n=6$).

protein and energy were significantly lower in the 8M group than in the C group (Table 2).

Serum and hepatic lipid levels in Experiment 1

Mannitol consumption lowered the serum and hepatic triglyceride levels. The level of serum triglyceride was lowest in the 8M group. The serum and hepatic total cholesterol levels did not differ significantly among the experimental groups (Table 3).

Accumulation rate of body components in Experiment 1

There were no significant differences in the ratios of protein accumulation in the body relative to the amounts of protein consumed or absorbed among the experimental groups. The ratios of accumulated ash relative to the amount consumed or absorbed were significantly increased after consumption of 4% mannitol, while consumption of 8% mannitol had no effect. On the other hand, the ratios of accumulated fat or total energy to the total energy consumed or absorbed were significantly decreased following consumption of mannitol (Table 4).

Cecal weights and pH values in Experiment 1

The cecal tissue weights, cecal content weights and amounts of total organic acids were significantly higher after consumption of mannitol. There were no significant differences in the dry matter contents, pH values or organic acid concentrations of the cecal contents among the experimental groups (Table 5).

Food intakes, body weight gains, feeding efficiencies and digestibilities in Experiment 2

The rats fed mannitol or FOS suffered from mild diar-

Table 5. Cecal weights and amounts of cecal organic acids in rats fed three experimental diets for 28 d (Experiment 1).

	C	4M	8M
Cecal weight			
Tissue (g/100 g BW)	0.17±0.01[a]	0.30±0.04[b]	0.52±0.07[c]
Contents (g/100 g BW)	0.53±0.13[a]	1.10±0.42[b]	1.55±0.36[c]
Dry matter content (g/g contents)	0.24±0.03	0.24±0.04	0.23±0.04
pH of contents	7.3±0.17	7.2±0.15	7.2±0.10
Amount of total organic acids (μmol/cecum)	135.7±48.31[a]	268.2±176.85[ab]	517.6±155.91[b]
Concentration of organic acids (μmol/g contents)			
Succinate	ND	ND	ND
DL-Lactate	4.86±6.52	ND	ND
Formate	ND	ND	ND
Acetate	47.36±15.46	43.26±15.94	60.84±13.37
Propionate	11.26±2.75	14.68±5.09	15.45±2.11
i-Butyrate	ND	0.22±0.55	ND
n-Butyrate	4.62±2.78	5.74±2.39	4.21±1.54
i-Valerate	ND	ND	ND
n-Valerate	ND	ND	ND
Total	75.47±30.65	63.91±22.57	80.20±38.02

Abbreviations: See footnotes to Table 1.
[a,b,c] Mean values within a row not sharing a common superscript letter differ significantly at $p<0.05$ by the Steel-Dwass test for the weights of the tissues and contents.
ND: not detected.
Values are shown as means±SD ($n=6$).

Table 6. Body weight gains, food intakes, feeding efficiencies[1] and apparent digestibilities[2] of food components in cecectomized rats fed three experimental diets for 24 d (Experiment 2).

	C	5M	5FOS
Food intake (g/d)	19.9±0.76	19.9±1.23	21.1±1.74
Body weight gain (g/d)	7.25±0.90[a]	6.33±0.77[b]	7.90±0.83[a]
Feed efficiency[1]	0.38±0.039[a]	0.32±0.030[b]	0.37±0.028[a]
Apparent digestibility[2]			
Dry matter	0.95±0.003[a]	0.94±0.006[b]	0.94±0.008[ab]
Crude fat	0.96±0.010[a]	0.90±0.038[b]	0.92±0.039[b]
Crude protein	0.96±0.005[a]	0.91±0.010[b]	0.91±0.015[b]
Crude ash	0.79±0.026[a]	0.83±0.014[b]	0.80±0.040[ab]
Carbohydrate[3]	0.95±0.003	0.96±0.003	0.95±0.006

Abbreviations: See footnotes to Table 1.
[1] Weight gain/feed intake.
[2] (Intake−fecal excretion)/intake.
[3] Dry matter−(crude ash+crude fat+crude protein).
[a,b] Mean values within a row not sharing a common superscript letter differ significantly at $p<0.05$ by the Steel-Dwass test.
Values are shown as means±SD ($n=8$ for C; $n=9$ for 5M and 5FOS).

rhea from the 2nd day of feeding. After 3 or 4 d the rats recovered. However, fecal pellets of the rats fed mannitol or FOS were softer compared to those of control rats during the experimental period. There were no significant differences in the food intakes among the experimental groups. Mannitol consumption significantly lowered the body weight gains and feeding efficiencies were lower in rats fed mannitol than in rats fed the control and FOS diets, while FOS had no effects on the body weight gains or feeding efficiencies. The digestibilities of crude protein and crude fat were significantly lowered by consumption of mannitol or FOS. Mannitol significantly increased the digestibility of crude ash compared to the control diet (Table 6).

Compositions and accumulation rates of body components in Experiment 2

Mannitol consumption significantly decreased the concentration of crude fat and slightly increased the concentration of crude protein in the carcass. FOS did not affect the concentrations of crude protein or crude fat. The concentration of ash was increased by consumption of FOS but not by consumption of mannitol.

There were no significant differences in the ratios of protein accumulation in the body relative to the amount of protein consumed among the experimental groups. However, mannitol addition increased the ratio

Table 7. Chemical compositions (g/g) of carcasses and ratios of protein, ash and energy accumulated[1] to those consumed[2] or absorbed[3] in rats fed three experimental diets for 24 d (Experiment 2).

		C	5M	5FOS
Chemical composition (g/g)				
Moisture		0.594±0.025	0.615±0.025	0.601±0.030
Crude protein		0.195±0.006[a]	0.202±0.003[b]	0.194±0.005[a]
Crude fat		0.177±0.021[a]	0.152±0.025[b]	0.171±0.024[a]
Crude ash		0.025±0.002[a]	0.028±0.003[ab]	0.029±0.003[b]
Carbohydrate[4]		0.009±0.002[a]	0.004±0.003[b]	0.005±0.004[b]
Ratio of accumulation to consumption or absorption				
Crude protein	Consumed[2]	0.35±0.050	0.39±0.035	0.36±0.030
	Absorbed[3]	0.37±0.053[a]	0.43±0.035[b]	0.40±0.041[ab]
Crude fat energy[5]	Consumed[2]	0.20±0.032[a]	0.16±0.032[b]	0.19±0.045[ab]
	Absorbed[3]	0.21±0.035[a]	0.17±0.034[b]	0.20±0.048[ab]
Crude ash	Consumed[2]	0.18±0.061[a]	0.26±0.070[b]	0.31±0.068[b]
	Absorbed[3]	0.23±0.081[a]	0.31±0.089[ab]	0.39±0.099[b]
Gross energy[6]	Consumed[2]	0.26±0.037	0.22±0.044	0.25±0.055
	Absorbed[3]	0.27±0.039	0.23±0.046	0.26±0.058

Abbreviations: See footnotes to Table 1.
[1] Difference between the amount in the carcass and the amount in the body at the beginning of the feeding period. For details, see the main text.
[2] Amount consumed during the feeding period.
[3] Digestible amount calculated using the amount consumed during the feeding period and the digestibility in Table 6.
[4] Dry matter−(crude ash+crude fat+crude protein).
[5] Ratio of fat energy accumulated to the energy consumed or absorbed.
[6] Calculated as 4.1 kcal/g for carbohydrate, 5.6 kcal/g for protein and 9.4 kcal/g for fat.
[a,b] Mean values within a row not sharing a common superscript letter differ significantly at $p<0.05$ by the Steel-Dwass test.
Values are shown as means±SD ($n=8$ for C; $n=9$ for 5M and 5FOS).

Table 8. Colon weights and amounts of colonic organic acids in rats fed three experimental diets for 24 d (Experiment 2).

	C	5M	5FOS
Colonic weight (g)			
Tissue (g/100 g BW)	0.31±0.05	0.38±0.04	0.42±0.09
Contents (g/100 g BW)	0.39±0.16	0.53±0.29	0.48±0.13
Dry matter content (g/g contents)	0.22±0.04	0.19±0.05	0.20±0.01
pH of contents	7.36±0.22	6.73±0.47	7.00±0.48
Amount of total organic acids (μmol/colon)	29.16±11.80[a]	50.79±28.00[ab]	68.80±23.22[b]
Concentration of organic acids (μmol/g contents)			
Succinate	4.52±5.50	5.54±4.30	8.06±4.81
DL-Lactate	3.48±2.56	11.62±14.03	14.11±11.54
Formate	1.19±0.96[a]	0.97±0.42[a]	4.78±3.44[b]
Acetate	16.90±6.65	12.54±6.55	18.83±5.49
Propionate	4.12±2.24	6.55±6.81	2.73±0.76
i-Butyrate	0.21±0.20	0.10±0.18	0.07±0.14
n-Butyrate	2.93±1.80	2.02±1.20	1.56±1.11
i-Valerate	0.27±0.32	0.21±0.30	0.22±0.26
n-Valerate	ND	ND	ND
Total	33.63±11.88	39.54±20.84	50.37±18.39

Abbreviations: See footnotes to Table 1.
[a,b] Mean values within a row not sharing a common superscript letter differ significantly at $p<0.05$ by the Steel-Dwass test.
ND: not detected.
Values are shown as means±SD ($n=8$ for C; $n=9$ for 5M and 5FOS).

of protein accumulation relative to the amount absorbed. On the other hand, the ratio of accumulated fat or total energy to the total energy consumed or absorbed was significantly decreased following consumption of mannitol. FOS had no effect on fat accumulation, whereas the ratio of accumulated ash relative to the amount consumed or absorbed was significantly increased after consumption of FOS. Mannitol consumption significantly increased the ratio of accumulated ash relative to the amount consumed (Table 7).

Colonic weights and pH values in Experiment 2

There were no significant differences in the colonic tissue weights, colonic content weights, colonic dry matter contents or pH values among the experimental groups. Consumption of mannitol had no significant effect on the amount or concentration of organic acids of the colonic contents. However, the amount of total organic acids in the colon and the formate concentration in the colonic contents were significantly higher after consumption of FOS (Table 8).

DISCUSSION

Digestibilities

Mannitol consumption lowered the digestibilities of crude fat, crude protein and gross energy in a dose-dependent manner in Experiment 1. Mannitol increased the weights of the cecal tissues and contents. Such increments in the weights of the cecal tissues and contents should be related to the stimulation of microbial multiplication, which would increase the excretion of microbial proteins, thereby resulting in a lowering of the apparent digestibility of protein. It is therefore suggested that some of the consumed mannitol was utilized as a substrate for microbial fermentation and multiplication. The lowering effect of mannitol on the digestibility of fat may also have partially resulted from the increased microbial multiplication stimulated by mannitol, since almost all fat excreted into the feces is considered to originate from the gut epithelium and microbes (*19*).

Tetens et al. (*20*) reported that fermentable dietary fibers increased fecal nitrogen excretion and decreased urinary nitrogen excretion dose-dependently, suggesting that the shift in nitrogen excretion from urine to feces can be mainly explained by the degree of microbial fermentation in the large intestine caused by the addition of dietary fiber and emphasizing the modifying role that certain dietary fiber supplements may have on the enterohepatic cycle of nitrogen. In a previous study using different types of FOS, namely 1-kestose, nystose or a mixture of these substances, FOS increased fecal excretion of nitrogen, but there was no clear dose-dependence and the extent of the increase in fecal excretion of nitrogen was similar for each FOS consumed (*21*). A lowering effect of FOS on the apparent digestibility of nitrogen accompanied by increases in cecal volume and organic acids was also reported in rats (*22*). The increase in fecal nitrogen was thought to be dependent on the proliferation of intestinal bacteria, which is stimulated by feeding of indigestible sugars.

On the other hand, since several indigestible materials are known to inhibit the in vitro activities of trypsin and lipase (*23*), it is possible that mannitol has inhibitory effects on protein and fat digestions. Mannitol consumption also decreased the digestibilities of crude fat and crude protein in cecectomized rats. In cecectomized rats, microbial multiplication and fermentation in the large intestine are very low and no differences were observed in colonic fermentation after feeding of fermentable sugar alcohols, sorbitol or lactitol (*12*). Therefore, we considered that the observed increases in microbial protein and fat excretion in the feces after mannitol feeding were low in cecectomized rats. These findings suggest that the lowered digestibilities of fat and protein may be due to the inhibitory effects of mannitol on lipase activity and/or micelle formation.

It is well known that the retention and transit times of digesta, as well as intestinal or gastric emptying, are important determinants of the degrees of intestinal digestion and absorption of dietary components. The small intestinal transit time of lactitol (*24*, *25*) and gastrointestinal transit times of sorbitol and maltitol (*26*) in normal rats are reduced in dose-dependent manners. Furthermore, significantly shorter mean retention and transit times of digesta in cecectomized rats fed sorbitol- and lactitol-containing diets compared to those in normal rats have been reported (*12*). Therefore, the lowered digestibilities of fat and protein observed in rats fed mannitol and FOS may be at least partially due to faster transit of digesta in the digestive tract, or inhibitory effects of mannitol on lipase activity and/or micelle formation and a stimulatory effect on microbial proliferation in the large intestine.

Lower digestibilities of protein and fat compared to the control group were also clearly observed after FOS intake in cecectomized rats. It is considered that FOS intake also has lowering effects on the digestion rates of nutrients in the small intestine.

Concentration and accumulation of body components

In the present study, mannitol consumption lowered the serum and hepatic levels of triglyceride in rats, suggesting that triglyceride synthesis was lowered by mannitol intake, whereas the serum and hepatic cholesterol levels were not significantly affected. It was reported that consumption of the indigestible oligosaccharide inulin lowered plasma cholesterol and triacylglycerol levels and altered the biliary bile acid profile in hamsters fed a high-fat diet with added cholesterol (*27*). Since the rats in the present study were fed a diet with no hypercholesterolemic effects, the lack of effects of mannitol on cholesterol is not surprising.

There were no significant differences in the ratios of protein accumulation in the body relative to the amount of protein consumed or absorbed, as evaluated by the multiple range test in Experiment 1. On the other hand, mannitol consumption increased the concentration of body protein and the protein accumulation rate based on the amount of protein absorbed in cecectomized rats, despite the fact that it lowered the digestibility of protein. Based on these results, it can be considered that there were no negative effects of the lowered digestibility of protein induced by mannitol feeding on nitrogen metabolism and accumulation, although the mechanism of action by which mannitol consumption increases the protein accumulation rate in cecectomized rats remains unclear.

The results of Experiment 1 also revealed that mannitol-containing diets decreased the levels of serum triglyceride and body fat accumulation in dose-dependent manners. Addition of sugar alcohols, xylitol or sorbitol

decreased food efficiency and total body weight gain as well as the plasma triglyceride and cholesterol levels in rats regardless of the type of sugar alcohol fed, suggesting that the decreases in body weights associated with consumption of sugar alcohols are due to decreased fat tissue weights (*28*). This corresponds with our findings in rats fed mannitol. A lowering effect of mannitol on body fat accumulation was also found in cecectomized rats. The ratios of accumulated fat to the total energy consumed or absorbed were decreased following consumption of mannitol in both normal and cecectomized rats. These observations suggest that mannitol lowered the food energy utilization, which is connected to the digestibility of energy sources and responses of the body energy metabolism after mannitol consumption.

The ratios of ash accumulation to that consumed or absorbed were significantly increased in rats after consumption of 4% mannitol, whereas 8% mannitol had no effect. In cecectomized rats, the digestibility of crude ash and ratio of ash accumulation to the amount consumed were increased after consumption of a 5% mannitol- or 5% FOS-containing diet. Stimulatory effects of indigestible sugars, such as FOS, on mineral absorption and utilization in the body have been reported (*29–32*), although the stimulated absorption of minerals did not always result in increased retention of minerals (*30*). The effects of fermentable sugars on the absorption of minerals differ depending on the type of mineral and region of the intestine. Stimulation of calcium and magnesium absorption by FOS occurs in both the small and large intestine (*32*). It is thought that the stimulatory effects of indigestible sugars on mineral absorption occur via a transepithelial diffusion route in the small intestine (*33*) and solubilization of minerals (*29*) and/or activation of absorptive cells (*34*) by SCFAs produced from fermentable sugars in the large intestine. It is therefore suggested that the stimulatory effects of mannitol on the absorption and retention of minerals in both normal and cecectomized rats in this experiment were caused by the actions of mannitol in the small intestine, independently of the production of organic acids in the cecum. In fact, the amount and concentration of organic acids in the cecum or proximal colon were not increased by mannitol in the rats fed 4% mannitol in Experiment 1 or 5% mannitol in Experiment 2. On the other hand, FOS significantly increased colonic organic acids and increased the retention of minerals in Experiment 2. It was reported that the stimulatory effects of FOS on the absorption of minerals were lowered by cecectomy (*31*). Therefore, the increased retention of minerals by FOS observed in Experiment 2 may be related to the production of organic acids. The results of the present study reveal that mannitol also has stimulatory effects on the utilization of minerals, although the doses associated with these stimulatory effects are limited.

General

Sucrose in the control diets was replaced with mannitol or FOS in the experimental diets in this study. Increases in liver and blood triacylglycerol levels by feeding large amounts of fructose are well-established phenomena. However, higher body fat accumulation is not always observed in rats fed fructose compared with rats fed glucose in their diet (*35, 36*). In rats fed 20% fructose or glucose in the diet, there were no differences in the liver or blood triacylglycerol levels or body fat accumulation between the sugars (*37*). Fructose caused increases in plasma triacylglycerol, but no changes in body weight or the triacylglycerol levels in the liver or muscle after 4 wk of fructose ingestion at 1.5 g/kg body weight/d (*38*). The fructose levels in the diets in the present study were 5% in Experiment 1 and 15% in Experiment 2. In addition, the differences in the dietary fructose levels among the treatment groups were 4% at maximum. Therefore, the differences in the fructose contents in the diets used in the present study are unlikely to have influenced our estimations of the effects of mannitol feeding.

Lard was used as a source of dietary fat in Experiment 2, whereas soybean oil was used in Experiment 1. In Experiment 1, the feed intake did not increase as expected. Since feed intake must increase sufficiently to obtain a lot of body fat in the present experimental model, lard and a higher level of sucrose were used to achieve a good appetite in Experiment 2.

In a previous study, consumption of 7% sugar alcohol induced diarrhea in rats, and this diarrhea was reduced by a diet containing 5% sorbitol in cecectomized rats (*39*). Therefore, we considered 5% sugar alcohol in the diet to be the maximum proportion of sugar alcohol that could be expected to reveal the effects of mannitol consumption under normal conditions without diarrhea in cecectomized rats. This was the reason why a dose of 5% mannitol was selected for Experiment 2, although doses of 4% and 8% mannitol were used for Experiment 1.

In the present study, the cecal weight and amount of organic acids were increased by mannitol consumption. These findings demonstrate that mannitol flowing into the cecum was fermented to organic acids, mainly SCFAs. These SCFAs can be used by the host animal as energy sources and body components. However, some of the energy generated from mannitol is lost during fermentation as gases, as well as microbial components. Furthermore, although mannitol is not easily absorbed, a proportion of ingested mannitol is absorbed in the small intestine. Absorbed mannitol is not metabolized, and becomes excreted in the urine (*40*). Since glucose is not produced from mannitol, secretion of insulin is not stimulated by mannitol feeding (*41*). These observations indicate that mannitol absorbed in the small intestine is not utilized as an energy source. Accordingly, the extent of energy utilization may be limited owing to a decrease in the metabolizable energy intake in rats fed mannitol-containing diets, and this may be associated with the lower rate of fat accumulation. To further examine this aspect, we calculated the net accumulation rate of body fat energy as the ratio of energy accumulated in the body fat to the energy intake except for mannitol. This calculation revealed that the rate of

energy accumulation was lower in rats fed mannitol than in control rats. This finding indicates that the low metabolizable energy of mannitol alone is not a cause of the decreased fat energy accumulation rate in rats fed mannitol.

The effects of mannitol feeding on the colonic tissue weights, colonic content weights, colonic dry matter contents, pH values, and amounts and concentrations of the colonic total organic acids did not differ significantly in the cecectomized rats. These findings may indicate that incomplete fermentation of mannitol results in deficient availability or utilization of organic acids. The very limited fluid reservoir of the colon causes faster transit times and shorter retention times for digesta, which may be the reason why mannitol cannot be completely fermented in the colon of cecectomized rats. However, similar results for the effects of mannitol consumption on the digestibilities of protein and fat as well as body fat accumulation were obtained in the normal and cecectomized rats. Therefore, it appears that the function of dietary mannitol in the present study was independent of the cecum.

In conclusion, the results of the present study reveal that mannitol has lowering effects on serum and hepatic triglyceride as well as body fat accumulation, possibly by decreasing the digestibility of fat and the lower available energy of mannitol compared with glucose, thereby lowering the energy utilization for adiposity. These findings contribute to a clear description of the mechanisms by which mannitol feeding lowers adiposity and suggest that mannitol may represent a potent food additive for preventing adiposity.

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