Energy Utilization of Sorbose in Comparison with Maltitol in Growing Rats

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Energy utilization of L-sorbose was investigated in two experiments. In experiment 1, growing rats were fed a control diet containing cornstarch as a carbohydrate or glucose, sucrose, sorbose, and maltitol for 6 weeks at 10% level. Dietary sorbose appeared to be an inefficient source of energy compared with other dietary carbohydrate sources except for maltitol, because there were significant decreases in values for body weight gain, food efficiency, body lipid accumulated, and total energy accumulated. In experiment 2, $[U^{-14}C]$ sorbose was administered (200 mg/kg of body weight) to sorbose-adapted and -unadapted rats, and $[U^{-14}C]$ maltitol was administered to maltitol-adapted rats. Both adaptation levels were 900-1000 mg/kg of body weight and were done for 1 week. The recovery of radioactivity of expired CO_2 in sorbose-adapted rats was significantly lower and 60% of that in maltitol-adapted counterparts. Both urinary and fecal excretions of radioactivity were significantly increased in $[U^{-14}C]$ sorboseadministered rats compared to in $[U^{-14}C]$ maltitol-administered animals. Fecal recovery of radioactivity in sorbose-adapted rats was less than in sorbose-unadapted rats.

INTRODUCTION

L-Sorbose is an isomer of D-fructose and is obtained in nature from the berries of the mountain ash, Sorbus aucuparia (family Rosaceae). It can be produced industrially from sorbitol by fermentation with acetic acid forming bacteria (Beshkov et al., 1970). It appears to be noncariogenic (Mühlemann, 1976; Mühlemann and Schneider, 1976; Takeuchi et al., 1989). In physiological characteristics, the absorption rate of sorbose was slow (Verzár and Laszt, 1935; Annegers, 1964) and the plasma insulin level was lowered by the supplementation of sorbose (Siebert et al., 1980); consequently, sorbose can be proposed as a sugar substitute characterized with a sweet, noncariogenic, and highly inefficient source of energy.

Maltitol (4-O- α -D-glucopyranosyl-D-sorbitol) is a sugar alcohol of disaccharide maltose. It is readily available from maltose by catalytic hydrogenation (Abdel-Akher et al., 1951) and is produced industrially. Some studies on the metabolism in the mouse (Kamoi et al., 1972) and the rat (Inoue et al., 1970; Yoshizawa et al., 1975) suggested that energy utilization of maltitol was low. Rennhard and Bianchine (1976) suggested, however, that maltitol was degraded by the gut microflora to volatile fatty acids which were easily absorbed and utilized by host animals, and Zunft et al. (1983) concluded that maltitol would be digested and utilized by human beings, rats, and rabbits.

Previously, we reported that dietary sorbose lowered fat deposition and energy utilization in growing rats without reducing protein utilization (Furuse et al., 1989). The present study was first conducted to investigate the effect of dietary sorbose on energy utilization of growing rats in comparison with other dietary carbohydrate sources, especially maltitol. The second experiment was done to investigate metabolic studies in rats using L-[U-1⁴C]sorbose as a tracer compared with [U-1⁴C]maltitol.

MATERIALS AND METHODS

Experiment 1. Animals and Diets. Male weanling (3 weeks of age) Wistar rats (ST strain) were purchased from the Shi-

Table I. Composition of a Control Diet

ingredient	g/kg	ingredient	g/kg	
cornstarch	650	corn oil	50	
casein	200	mineral mixture ^a	35	
DL-methionine	3	vitamin mixture ^b	10	
cellulose	50	choline bitartrate	2	

zuoka Experimental Animals and Agricultural Cooperative, Hamamatsu, Shizuoka, Japan. The animals were individually housed in wire-mesh cages in a constant-temperature room (24 °C) with a 12-h light cycle (7:00 a.m. to 7:00 p.m.). Twenty-five rats were initially fed a purified control diet (Table I) ad libitum. After 5 days, they were ranked by body weight to equalize initial body weight as much as possible among groups and divided into five dietary groups of four rats each and one group of five rats for determining initial body composition. The control group continued to eat the control diet ad libitum for the following 6 weeks. The other four groups were fed one of four respective diets ad libitum for the following 6 weeks: a diet containing 100 g of sucrose/kg; a diet containing 100 g of glucose/kg; a diet containing 100 g of maltitol/kg; a diet containing 100 g of sorbose/ kg. These experimental diets were made at the expense of corn starch in the control diet.

Experimental Procedure. Food consumption was measured weekly. The feces and urine from each rat were collected during the last 3 days of the experimental period. Feces were sprayed with 0.5 N HCl to avoid the evaporation of NH₃, air-dried at 55 °C for 48 h, and ground for analysis. Urine was frozen at -20 °C until analysis. At the final day of the experimental period, rats were killed by decapitation and blood samples were collected. The gastrointestinal tract, liver, kidneys, and heart were removed immediately. The gastrointestinal tract was divided into the stomach, small intestine, colon, and cecum. The segment was cut lengthwise, washed with physiological saline solution, blotted, and weighed. Empty body weight was obtained by subtracting the weight of the gastrointestinal content from the body weight. Organ weight was expressed per 100 g of empty body weight. Food efficiency was calculated by dividing body weight gain by food consumption. All parts of the carcass were put together for each body and frozen at -20 °C. The frozen carcass was minced with a meat grinder. The mince was frozen again with liquid N_2 , minced for a second time, and dried at 55 °C for 48 h. Protein content $(N \times 6.25)$ in the diet and carcass was determined by a Kjeldahl procedure. Lipid content in the carcass was extracted

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Table II. Time Course of Body Weight Change in Rats Fed Diet Containing Different Carbohydrate Sources^e

feeding	dietary carbohydrate source, 100 g/kg of diet				
period (week)	control (starch), 4 rats	sucrose, 3 rats ^b	glucose, 4 rats	maltitol, 4 rats	sorbose, 4 rats
initial	76 ± 5.0	78 ± 4.2	77 ± 5.5	76 ± 5.2	77 ± 4.4
1	133 ± 3.8	137 ± 5.1	128 ± 10.8	127 ± 8.4	118 ± 8.0
2	$182^{AB} \pm 6.6$	$193^{A} \pm 6.4$	$178^{ABC} \pm 9.4$	$171^{BC} \pm 11.3$	$165^{\circ} \pm 11.9$
3	$235^{AB} \pm 11.2$	$249^{A} \pm 7.0$	$232^{ABC} \pm 7.1$	$222^{BC} \pm 15.3$	$215^{\circ} \pm 12.5$
4	$282^{AB} \pm 5.0$	$303^{A} \pm 9.5$	$278^{ABC} \pm 3.9$	$269^{BC} \pm 19.7$	$259^{\circ} \pm 11.6$
5	$323^{B} \pm 17.3$	349^ ● 9 .1	$318^{BC} \pm 6.4$	$310^{BC} \pm 19.2$	$296^{\circ} \pm 13.6$
6	$350^{B} \pm 25.5$	$381^{+} \pm 8.7$	$338^{BC} \pm 12.3$	$335^{BC} \pm 20.0$	$318^{\circ} \pm 15.6$

^a Mean \pm SD. Means not sharing a common superscript letter are significantly different at p < 0.05 in the same row. ^b One missing value due to an outlier.

Table III. Body Weight Gain, Food Consumption, Food Efficiency, and Tissue Weight of Rats Fed Diets Containing Different Carbohydrate Sources⁴

	dietary carbohydrate source, 100 g/kg of diet				
	control (starch), 4 rats	sucrose, 3 rats ^b	glucose, 4 rats	maltitol, 4 rats	sorbose, 4 rats
body wt gain, g/6 weeks food consumption, g/6 weeks food efficiency, % empty body wt, g rel organ wt, g/100 g of empty body wt	$273^{B} \pm 21.0$ $770^{AB} \pm 40.7$ $35.4^{AB} \pm 1.22$ $345^{B} \pm 25.3$	$303^{A} \pm 5.0$ $811^{A} \pm 24.8$ $37.3^{A} \pm 0.59$ $376^{A} \pm 8.7$	$\begin{array}{c} 262^{\rm BC}\pm14.3\\ 776^{\rm AB}\pm12.8\\ 33.7^{\rm B}\pm1.80\\ 334^{\rm BC}\pm12.2 \end{array}$	$\begin{array}{c} 259^{\rm BC}\pm 16.5 \\ 728^{\rm BC}\pm 50.1 \\ 35.6^{\rm AB}\pm 0.19 \\ 329^{\rm BC}\pm 20.7 \end{array}$	$\begin{array}{c} 242^{\rm C} \pm 11.5 \\ 704^{\rm C} \pm 44.3 \\ 34.3^{\rm B} \pm 1.40 \\ 313^{\rm C} \pm 15.3 \end{array}$
stomach small intestine cecum colon	$\begin{array}{c} 0.36 \pm 0.087 \\ 1.61^{\rm B} \pm 0.175 \\ 0.30^{\rm BC} \pm 0.069 \\ 0.34 \pm 0.054 \end{array}$	$\begin{array}{c} 0.37 \pm 0.015 \\ 1.55^{\rm BC} \pm 0.090 \\ 0.23^{\rm C} \pm 0.072 \\ 0.32 \pm 0.050 \end{array}$	$\begin{array}{c} 0.35 \pm 0.039 \\ 1.38^{\rm C} \pm 0.022 \\ 0.21^{\rm C} \pm 0.034 \\ 0.31 \pm 0.024 \end{array}$	$\begin{array}{c} 0.36 \pm 0.005 \\ 1.68^{AB} \pm 0.093 \\ 0.43^{A} \pm 0.047 \\ 0.32 \pm 0.040 \end{array}$	0.36 ± 0.015 $1.83^{A} \pm 0.109$ $0.34^{AB} \pm 0.078$ 0.34 ± 0.107

^a Means \pm SD. Means not sharing a common superscript letter are significantly different at $p \leq 0.05$ in the same row. ^b One missing value due to an outlier.

overnight (about 16 h) with diethyl ether by using a Soxhlet apparatus and determined gravimetrically. Gains in lipid, protein, and energy over the experimental period were determined by subtracting the initial values of body composition from the final ones. Energy content of the rat was calculated by using the values 39.12 and 23.68 kJ/g for lipid and protein in the body, respectively. The energy contents of diets, feces, and urine were measured with an automatic bomb calorimeter (Shimadzu CA-4, Shimadzu Co. Ltd., Kyoto, Japan). Both digestible and metabolizable energy values of sorbose and maltitol were calculated according to the method of Cooley and Livesey (1987). The serum total cholesterol level was determined by using a kit with cholesterol esterase (Cholescolor Ace, Ono Chemical Industry Co. Ltd., Osaka, Japan).

Experiment 2. Animals and Diets. Fifteen male weanling (4 weeks of age) Wistar rats (ST strain) were divided into three dietary groups of five rats each to equalize initial body weight (113–137 g, mean 125 g) among groups. The control group was fed the control diet (Table I) ad libitum for 7 days before the administration of $[U^{-14}C]$ sorbose. The second and third groups were adapted to sorbose and maltitol, respectively, by receiving respective diets where 10 g/kg of corn starch in the control diet was replaced by sorbose and maltitol, respectively, for 7 days before the administration of the respective ¹⁴C-labeled materials.

Experimental Procedure. Both [U-14C]sorbose and [U-14C]maltitol, having 98% radiochemical purity, were purchased from Amersham Japan, Tokyo, Japan. [U-14C]Sorbose diluted with unlabeled sorbose solution (20%) was administered with a gastric sound to rats fed the control and sorbose diets. [U-14C]Maltitol diluted with unlabeled maltitol solution (20%) was administered similarly to rats fed the maltitol diet. Seventy-four kilobecquerels in 0.1 mL/100 g of body weight was administered in each rat (200 mg of sorbose or maltitol/kg of body weight). Immediately after administration, each rat was transferred to a glass metabolic cage (Metabolica, Sugiyamagen Co., Tokyo, Japan) kept at 25 °C. Each rat was fed the respective experimental diet and allowed free access to water. The expired ${}^{14}CO_2$ was trapped with 500 mL of monoethanolamine (Tokunaga et al., 1981), and the sample was obtained at 2, 4, 6, 8, 10, 12, and 24 h. Urine and feces were collected separately in bottles over 24 h. The feces were weighed and homogenized with about 5 parts of distilled water and centrifuged at 3800 rpm for 20 min. Radioactivities of the expired ¹⁴CO₂ trapped with monoethanolamine, urine, and the supernatant of fecal homogenate were determined by a liquid scintillation counter (Model LS-3801, Beckmann, Palo Alto, CA),

using 10 mL of a scintillator (toluene/Triton X-100 (2:1 v/v) with 4.0 g/L Omniflour (New England Nuclear, Boston, MA)) in addition to 5 mL of methanol. Methanol avoids separation of the sample solution from the scintillator, and a clear mixture is obtained. This treatment had little effect on the counting efficiency (Muramatsu et al., 1985). Urine and the supernatant of fecal homogenate were separated with thin-layer chromatography on a silica gel plate (Kiesel Gel 60, Merk and Co., Rahway, NJ). Development was carried out two times with the solvent of butanol/acetic acid/distilled water (8:1:1 v/v). The gel of the spot area of sorbose (0.27 as the R_f value obtained) was scraped and its radioactivity was measured by using the scintillation cocktail.

Statistical Procedure. Data were subjected to analysis of variance, and significance of difference between means was determined by Duncan's multiple range test using a commercially available statistical package (SAS, 1985). According to analysis of variance, one outlier in the sucrose diet group in experiment 1 was detected and omitted.

RESULTS

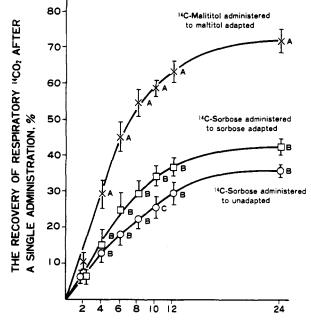
Experiment 1. The time course of body weight change of rats fed diet containing different carbohydrate sources is shown in Table II. The body weight gain in the sorbose diet tended to be the smallest among the diets and was significantly less than those in the control and sucrose diets after 2 weeks.

Body weight gain, food consumption, food efficiency, and tissue weight of rats fed diets containing different carbohydrate sources for 6 weeks are given in Table III. The values for body weight gain, food consumption, and empty body weight in the sorbose diet were the lowest and significantly reduced compared with those in the control and sucrose diets. Food efficiency in the sorbose diet was the lowest and significantly less than that in the sucrose diet. No significant differences between the sorbose and maltitol diets were observed in these four parameters. There were no significant differences among diets in relative weights of stomach, colon, liver (3.8-4.6 g/100 g)of empty body weight), kidneys (0.60-0.67 g/100 g) of empty body weight), and heart (0.31-0.36 g/100 g) of empty body weight), though the weight of the small intestine in the

Table IV. Lipid, Protein, and Energy Accumulations of Rats Fed Diets Containing Different Carbohydrate Sources^a

	dietary carbohydrate source, 100 g/kg of diet				
	control (starch), 4 rats	sucrose, 3 rats ^b	glucose, 4 rats	maltitol, 4 rats	sorbose, 4 rats
body lipid content, %	19.1 ± 2.14	18.1 ± 2.63	21.3 ± 3.22	16.7 ± 2.01	16.1 ± 1.76
lipid accumulated, g/6 weeks	$58.6^{AB} \pm 9.8$	$60.6^{AB} \pm 10.7$	$63.6^{A} \pm 9.9$	$47.6^{BC} \pm 9.8$	$42.7^{\circ} \pm 5.5$
energy accumulated as lipid, kJ/6 weeks	2292 ^{AB} ± 383	$2370^{AB} \pm 418$	$2487^{A} \pm 386$	$1863^{BC} \pm 382$	$1670^{\circ} \pm 216$
body protein content, %	19.2 ± 0.60	19.4 ± 0.64	19.2 ± 0.88	19.5 ± 0.68	19.9 ± 0.48
protein accumulated, g/6 weeks	$52.9^{B} \pm 4.46$	$59.2^{A} \pm 1.86$	$51.0^{B} \pm 3.32$	$50.7^{B} \pm 1.95$	$48.8^{B} \pm 2.80$
total energy accumulated, kJ/6 weeks	3544 ^{AB} ± 465	$3772^{A} \pm 385$	3694 ^A ± 303	$3065^{BC} \pm 423$	$2826^{\circ} \pm 195$

^a Means \pm SD. Means not sharing a common superscript letter are significantly different at p < 0.05 in the same row. ^b One missing value due to an outlier.



HOURS AFTER ADMINISTRATION OF HC

Figure 1. Recovery of respiratory ¹⁴CO₂ after a single administration of uniformly ¹⁴C labeled materials to rats.

sorbose diet was the highest and significantly higher than those in the other diets except for the maltitol diet and the value for the relative weight of the cecum in the maltitol diet was significantly higher than those in the other diets but for the sorbose diet.

Table IV shows the values for lipid, protein, and energy accumulations of rats fed diets containing different carbohydrate sources. Although no significant difference in lipid content was observed (p = 0.0545), the value in the sorbose diet was the lowest among the diets. The lipid accumulated and energy accumulated as lipid in the sorbose diet were the lowest and significantly less than those of the other diets except for the maltitol diet, and these parameters in the sorbose diet reflected not only its smaller body weight gain and food consumption but also its lower lipid content and food efficiency. There was no significant difference in the values for body protein content and efficiency of protein utilization among diets. The value for total energy accumulated in the sorbose diet was the lowest and significantly less than the other diets except for the maltitol diet.

The values for serum total cholesterol in the sorbose and maltitol groups (110 and 118 mg/100 mL, respectively) were significantly lower than those in the sucrose and glucose groups (156 and 159 mg/100 mL, respectively). The value for dry feces/food intake in 3 days in the sorbose group (90 mg/g) was significantly higher than those in the control, sucrose, and glucose groups (77, 78, and 76 mg/g, respectively), although there was no significant difference compared with the maltitol group (85 mg/g).

Table V. Recovery of Radioactivity in Respiratory CO₂, Urine, and Feces over 24 h after Oral Administration of [U-¹⁴C]Sorbose and [U-¹⁴C]Maltitol to Rats Fed for 1 Week (Percent)⁴

¹⁴ C-labeled material: diet: no. of rats:	sorbose control (starch) 5	sorbose 1% sorbose 5	maltitol 1% maltitol 5
CO2	35.9 ^B ± 3.7	$42.5^{B} \pm 4.8$	71.6 ^A ± 6.9
urine	$18.4^{\text{A}} \pm 2.1$	$16.2^{A} \pm 2.4$	$4.0^{B} \pm 0.2$
as sorbose in urine	13.6 ± 1.8	12.7 ± 2.3	
feces	$22.2^{A} \pm 3.4$	$18.3^{B} \pm 2.5$	$3.4^{\circ} \pm 0.4$
as sorbose in feces	$6.8^{\text{A}} \pm 2.4$	$2.8^{B} \pm 0.8$	
total (CO ₂ + urine + feces)	76.6 ± 4.8	76.9 ± 4.9	79.0 ± 6.9

^a Values are means (%) \pm SD. Means not sharing a common superscript letter are significantly different at p < 0.05 in the same row. Seventy four kilobecquerels in 0.1 mL/100 g of body weight was administered to each rat (200 mg of sorbose or maltitol/kg of body weight). Body weights of rats fed the control, sorbose, and maltitol diets were 172-186 (mean 178), 158-190 (mean 177), and 162-196 g (mean 179 g), respectively.

The values for the digestible energy (DE) in 100 g of sorbose/kg and 100 g of maltitol/kg diets were calculated as 15.77 (SE 0.026) and 15.85 (SE 0.028) kJ/g, respectively, and were not significantly different from that in the control diet (mean 15.87 kJ/g, SE 0.059). The values for the metabolizable energy (ME) in 100 g of sorbose/kg and 100 g of maltitol/kg diets were calculated as 14.80 (SE 0.105) and 15.03 (SE 0.049) kJ/g, respectively, and both were significantly lower (p < 0.05) than that in the control diet (mean 15.35 kJ/g, SE 0.054). By use of the published values of 16.58 kJ/g for both DE and ME of cornstarch in rats (Metta and Mitchell, 1954), DE and ME for sorbose were calculated as 15.6 and 12.1 kJ/g and for maltitol 16.4 and 13.3 kJ/g, respectively.

Experiment 2. The time course of recovery of radioactivity in expired CO_2 is shown in Figure 1. The values in [U-14C]maltitol-administered rats were significantly higher than those in [U-14C]sorbose-administered rats, irrespective of adapted or unadapted to sorbose. Between the sorbose-adapted and -unadapted groups, the recovery of radioactivity in expired CO_2 did not differ significantly, although the value for adapted rats tended to be higher. The recovery of radioactivity over 24 h is shown in Table V. Both urinary and fecal recoveries of radioactivity in [U-14C]maltitol-administered rats were small. Recovery of radioactivity both in urine and in sorbose excreted in urine did not differ significantly between adapted and unadapted rats. The value for fecal recovery of radioactivity in unadapted rats was significantly higher than that in adpated rats, and the fecal recovery of radioactivity as sorbose also showed the same tendency.

DISCUSSION

In experiment 1, by use of the level of 100 g/kg of diet, which was set to be large enough to clear the differences among various carbohydrate sources, it was shown that dietary sorbose and maltitol were inefficient sources of

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energy compared with cornstarch, glucose, and sucrose and that there was no significant difference between dietary sorbose and maltitol in body weight gain, food efficiency, body lipid content, and energy accumulated in total and as lipid (Tables III and IV). Therefore, in experiment 2 we investigated the difference of the energy utilization in more detail between the two sugar substitutes by using uniformly ¹⁴C labeled tracers. In this experiment, we used the level of 10 g/kg of diet for the adaptation level of sorbose or maltitol to reflect the current lifestyle in man. At 10 g/kg either sorbose or maltitol diet, rats consumed daily about 900-1000 mg/kg of body weight. Since daily sucrose consumption in Japan during 1983-1986 was about 62 g per capita (Food Chemicals Newspaper Co., 1989) and body weight in man is standardized as 60-70 kg, the consumed sucrose could be replaced by 10 g/kgeither sorbose or maltitol. For uniformly ¹⁴C labeled tracer administration 200 mg/kg of body weight was set to meet the proper amount in a meal.

The recoveries of radioactivity both in feces and in sorbose excreted in feces were lowered by the adaptation. It might be likely that hind-gut microbes ferment sorbose to a greater extent by adaptation, although the recovery of ¹⁴CO₂ between unadapted and adapted rats did not differ significantly. The value for the total recovery of $^{14}CO_2$ in [U-14C]maltitol-administered rats reached 72% over 24 h, which was higher than the value (46%) reported by Rennhard and Bianchine (1976). The higher value, 72%, can be explained, not entirely but partly, by adaptation, since Rennhard and Bianchine used unadapted rats. Thus, it is possible that maltitol is more affected by adaptation compared with sorbose. From the recovery of radioactivity in expired CO_2 over 24 h in sorbose-adapted and maltitoladapted rats, the apparent energy utilization of a single dose of sorbose was estimated to be about 60% that of maltitol, since recoveries of radioactivity in expired CO_2 were 43 and 72% in sorbose and maltitol diets, respectively. The large difference in energy utilization rate between sorbose and maltitol is reflected in the higher excretion of sorbose in urine and also in feces (Table V). The latter fact implies that sorbose is less fermentable in the gut than maltitol.

Prolonged intake of dietary fiber has been shown to lower serum total cholesterol and to increase the relative cecum and fecal weights (Oku et al., 1981; Cooley and Livesey, 1987). In the present experiment those effects of dietary fiber were also observed for dietary sorbose or maltitol feeding, even though sorbose and maltitol have smaller molecular weights.

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