Reduction in fat storage during 4^G-β-D-galactosylsucrose (lactosucrose) treatment in mice fed a high-fat diet

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Abstract

Lactosucrose (4^G-β-D-galactosylsucrose) was found to inhibit the absorption of 2-mono-oleoylglycerol by rat brush border membrane vesicles, and to reduce plasma triacylglycerol levels previously elevated by oral administration of a lipid emulsion containing corn oil. These results suggest that lactosucrose may inhibit the intestinal absorption of dietary fat. Based on these experimental results, the anti-obesity activity of lactosucrose was then examined. Lactosucrose was found to cause reductions in perimetrial adipose tissue weight and hepatic triacylglycerol levels, which had been elevated by oral administration of a high-fat diet (containing 40 % of beef tallow) to female mice. The mechanism underlying the anti-obesity activity of lactosucrose in discussed.

Key words lactosucrose, obesity, high-fat diet, perimetrial adipose tissue weight, hepatic triacylglycerol level.

Introduction

Lactosucrose (4^G-β-D-galactosylsucrose) is produced from lactose and sucrose by the action of β-fructofuranosidase and is an oligosaccharide consisting of galactose, glucose and fructose. Lactosucrose is virtually undigested by the small intestine and is metabolized by intestinal flora in the large intestine. Ogata *et al.*[1] reported that oral administration of lactosucrose to healthy volunteers caused an increase in bifidobacteria and decreased levels of lecithinase-positive clostridia, sulfide and ammonia in feces. Lactosucrose has also been reported to improve various clinical features and fecal characteristics in elderly patients with constipation.[2] Because of these attributes, lactosucrose in now widely used as a sweetener, and has 30 % of the sweetness of sucrose.

We have recently been investigating substances that inhibit the intestinal absorption of carbohydrates and fat.[3-7] In a preliminary experiment, we found that lactosucrose might inhibit the absorption of 2-monoacylglycerol into the brush border membrane vesicles and significantly reduced the plasma triacylglycerol level after elevation by oral administration of a lipid emulsion containing corn oil. Based on these facts, we investigated the effect of lactosucrose in mice by the oral administration of a high-fat diet for ten weeks.

Materials and Methods

Materials : [1-^{14}C] Palmitic acid (specific activity: 2.1 Gbq/mmol) and [9,10-^{3}H] trioleoylglycerol (specific activity : 991.6 Gbq/mmol) were obtained from Du Pont NEN. [^{3}H] 2-Monooleoylglycerol was prepared from [^{3}H] trioleoylglycerol by treatment with pancreatic lipase.[8] 4^G-β-D-galactosylsucrose (lactosucrose) was kindly donated by Fuji-Bio. (Shizuoka, Japan). The triglyceride E test kit was

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purchased from Wako Pure Chemical Co. (Osaka, Japan).

Animals : Male Wistar king strain rats (7 weeks old) and female ICR strain mice (3 weeks old) were purchased from CLEA Japan Inc. (Osaka, Japan), respectively.

Lipid absorption by brush border membrane vesicles : Brush border membrane vesicles were prepared from the jejunum portion of rat small intestine according to the method of Kessler *et al.*[9] Donor vesicles composed of egg PC/2-monooleoylglycerol (93 : 7 mole ratio) and a trace of [^{3}H] 2-monooleoylglycerol were made by sonicating 1.0 ml of the mixed lipid dispersion in 10 mM Hepes-Tris buffer, pH7.5, containing 100 mM mannitol (buffer A) for 5 min at 4 °C. The assay mixture for investigating lipid absorption consisted of 0.24 ml buffer A containing 5.9 nmol 2-monooleoylglycerol (34,000 dpm) and the brush border membrane vesicles (48 μg protein). Incubation was carried out for 30 min at 20°C. After incubation, 0.1 ml of the incubation medium was diluted with 1 ml ice-cold buffer A, and this solution was immediately filtered through 0.45 μm cellulose nitrate filters and washed four times with 1 ml ice-cold buffer A. The filters were then dissolved in composition of supplier ACS , and their redioactivity was measured. For the palmitic acid absorption test, donor vesicles composed of PC/palmitic acid (93 : 7 molar ratio) and a trace of [1-^{14}C] palmitic acid were made by sonicating these substances in buffer A. The donor vesicles (29 nmol palmitic acid ; 160,000 dpm) were incubated with the brush border membrane vesicles (48 μg protein) for 30 min at 20°C, and, then the donor vesicles were separated from the brush border membrane by filtration as described above.

Plasma triacylglycerol after oral administration of lipid emulsion : Male Wistar King strain rats (7 weeks old) weighing 230~250 g were fasted overnight and orally administered 4ml of lipid emulsion consisting of corn oil (6 ml), cholic acid (80 mg), cholesteryloleate (2 g) and saline solution (6 ml) with or without lactosucrose (375 mg). Blood was collected from tail vein before and after the oral administration and subjected to measurement of plasma triacylglycerol using triglyceride E-Test Wako (Wako Pure Chemical Industries Ltd. Osaka, Japan.)

Experiment in mice with high-fat diet-induced obesity : Female ICR mice (3 weeks old) were maintained in a 12h/12h light/dark cycle in a temperature- and humidity-controlled room. The animals were fed with laboratory pellet chow (CLEA Japan Inc. ; protein 24 %, lipid 3.5 %, carbohydrate 60.5 %) and given water *ad libitum*. One hundred and five mice were divided into seven groups (each n=15), with each group matched for body weight, after one week of feeding. The control mice continued to be fed laboratory pellet chow *ad libitum*. The basic composition of the experimental diet was as follows (wt %) ; beef tallow 40 %, corn starch 10 %, sugar 9 %, vitamin mixture (AIN-76TM, Oriental Yeast, Co., Ltd. Tokyo, Japan.) 1 % and mineral mixture (AIN - 76TM, Oriental Yeast, Co., Ltd. Tokyo, Japan.) 4 %. The composition of the diet for each experimental group was as follows ; high-fat group: casein 36 % and basic components ; lactosucrose plus high-fat group: different amounts of casein(18.5 %. 23.5 %, 29.75 %, 33 % and 34.5 %) and lactosucrose (17.5 %, 12.5 %, 6.25 %, 3 % and 1.5 %). In preliminary experiment, we found that methyl cellulose showed slight anti-obesity action and reduction of casein in the high fat diet from 36 % to 21 % did not affect both body weight and perimetrial adipose tissue weight.[7] On the other hand, lactosucrose had no effect on protein absorption from small intestine (data not shown). Based on these facts, we added lactosucrose to the high fat diet instead of casein. To avoid auto-oxidation of their fat contents, the feeds were stored at -30°C and freshly prepared each day.

After 10 weeks of feeding on the appropriate experimental diet, blood from each mouse was taken from the venous puncture under anesthetization with diethylether, and then the monse was killed with an overdose of diethylether. The livers and perimetrial white adipose tissues were quickly removed and weighed, and the liver tissues were stored at -80°C until analysis. The liver triacylglycerol contents were measured as follows: a portion (0.5 g) of the liver tissue was homogenized in Krebs Ringer phosphate buffer (pH7.4, 4.5 ml) and the homogenate (0.2 ml) was extracted with chloroform-methanol (2 : 1, v/v, 4 ml) and the extract was concentrated under a nitrogen stream. The residue was determined by Triglycer-

ide E-Test Wako kit.

Each mouse was weighed once a week and the weight recorded. The total amount of food intake by each mouse was recorded at least three times a week.

Statistical analysis : Results are expressed as means and standard error (s.e.m.). Statistical analysis was performed by the Fisher's Protected LSD test to determine significance ($P<0.05$) using Super ANOVA Software. The statistical analysis for body weight was performed using Super ANOVA, based on the data of body weight measured weekly. The statistical analysis for plasma triacylglycerol was performed with Student's t-test.

Results and Discussion

Lactosucrose did not affect the hydrolysis of triolein, emulsified with cholic acid and lecithin, by pancreatic lipase and failed to inhibit the absorption of palmitic acid by small intestinal brush border membrane vesicles. On the other hand, the absorption of 2-monooleylglycerol by the brush border membrane vesicles was inhibited dose-dependently by lactosucrose (Fig. 1). This suggests that lactosucrose may reduce the intestinal absorption of dietary fat by inhibiting the absorption of 2-monoacylglycerol, one

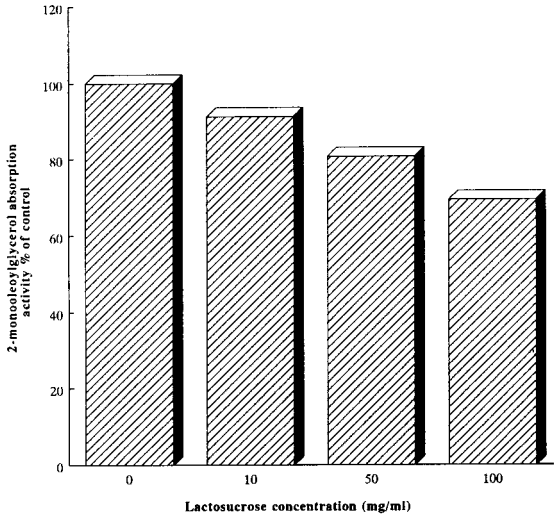

Fig. 1 Effect of lactosucrose on 2-monooleoylglycerol absorption by brush border membrane vesicles.
The procedure was as described in Materials and Methods. The rate of absorption was expressed as percentage activity of that in the absence of lactosucrose.

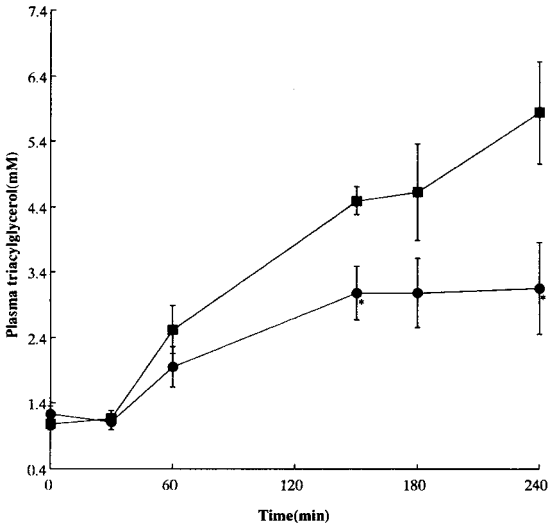

Fig. 2 Effect of lactosucrose on rat plasma triacylglycerol levels after oral administration of lipid emulsion.
The procedure was as described in Materials and Methods. ■ : Lipid emulsion alone. ● : Lipid emulsion and lactosucrose (500mg/kg body weight). Each point represents the mean±s.e.m of four rats. * : Statistical analysis was analyzed using Student's t test, $p<0.05$.

of the hydrolysis of dietary fat, and was confirmed in the later *in vivo* experiment. Lactosucrose was also found to significantly reduce the plasma triglyceride level after elevation by oral administration of a lipid emulsion containing corn oil (Fig. 2). Lactosucrose is known not to be hydrolyzed by mammalian digestive enzymes, but is readily utilized by the Bifidobacterium and Bactericides species that predominate in the human large intestine.[10] These results suggest that lactosucrose may not directly affect the metabolism of plasma triacylglycerols and, thus, the reduction of triacylglycerol levels by lactosucrose may be mediated through inhibition of the intestinal absorption of 2-monoacylglycerol produced by the hydrolysis of corn oil. Although the precise mechanism underlying the lactosucrose - induced reduction of plasma triacylglycerols remains unknown, an *in vivo* experiment was designed to examine its effect on fat-induced obesity. The mean food consumption per week per mouse during the whole experimental period was significantly different between the laboratory chow and high-fat diet groups, being 383.5±28.0 KJ (Food intake ; 25.5±1.9 g) in laboratory chow and 634.8±45.8 KJ (Food intake ; 26.2±1.9 g) in high-fat diet group,

Table I The average body weight (g) of mice fed various diets.

Weeks	Lab. chow diet	High-fat diet	High-fat diet +1.5 % LC	High-fat diet +3 % LC	High-fat diet +6.25 % LC	High-fat diet +12.5 % LC	High-fat diet +17.5 % LC
Baseline	20.8±0.4	20.8±0.4	20.8±0.4	20.8±0.4	20.8±0.4	20.8±0.4	20.8±0.4
1	25.2±0.3	24.7±0.6	26.0±0.5	25.2±0.6	25.4±0.6	25.1±0.4	22.2±0.6*
2	26.6±0.4	26.6±0.5	28.2±0.5	26.7±0.6	27.3±0.6	27.0±0.5	20.7±0.8*
3	28.6±0.5	29.7±0.7	31.3±0.7	29.5±0.8	30.5±0.7	30.1±0.5	23.5±1.3*
4	30.1±0.6	31.7±0.8	32.6±0.7	31.0±0.8	31.5±0.8	31.0±0.5	26.2±1.6*
5	31.3±0.6	32.8±0.7	34.0±0.8	31.8±0.8	33.0±0.9	32.6±0.5	29.7±1.6*
6	32.0±0.6	33.8±1.0	33.7±0.7	32.8±0.9	34.0±1.2	33.0±0.5	30.8±1.1*
7	32.6±0.8	35.0±0.9	35.2±0.7	34.1±1.0	34.2±1.0	34.3±0.5	32.5±1.0*
8	33.2±0.7*	36.4±1.0	36.7±0.9	35.1±0.9	35.9±1.2	34.7±0.6	33.3±0.8*
9	33.9±0.7*	38.3±1.0	37.9±0.8	35.6±1.0*	38.0±1.3	36.4±0.8	33.7±1.0*
10	34.2±0.7*	39.0±1.0	39.7±1.1	36.1±1.1*	37.6±1.3	36.4±0.7	34.4±0.9*

Each value represents the mean±s.e.m. of 15 mice.* : Significantly different from high-fat diet treated group, $p<0.05$. LC: Lactosucrose.

but not significantly different between the high-fat and high-fat plus 1.5 %, 3 %, 6.25 %, 12.5 %, 17.5 % lactosucrose diet groups, being 634.8±45.8 KJ (high-fat diet), 621.9±60.0 KJ (Food intake ; 25.9±2.5 g) (1.5 % lactosucrose diet), 570.9±44.3 KJ (Food intake ; 24.0±1.9 g) (3 % lactosucrose diet), 542.9±30.9 KJ (Food intake ; 23.4±1.3 g) (6.25 % lactosucrose diet), 541.2±20.8 KJ (Food intake ; 24.4±0.9 g) (12.5 % lactosucrose diet), 571.0±46.8 KJ (Food intake ; 26.8 ±2.2 g) (17.5 % lactosucrose diet), respectively. Table I shows the changes in body weights of the groups during the experiments. Feeding a high-fat diet containing 40 % beef tallow for 10 weeks caused significant increases in body weight at 8-10 weeks and in the final perimetrial adipose tissue weight as compared to the normal diet group (laboratory pellet chow) (Table I and Fig. 3). Feeding a high-fat diet containing 3 % or 17.5 % lactosucrose significantly reduced the increase and feeding a high-fat diet containing 6.25 % or 12.5 % lactosucrose shows a downward tendency in body weight as compared to feeding a high-fat diet (Table I). In addition, feeding a high-fat diet containing 1.5 % lactosucrose did not affect in body weight as compared to feeding a high-fat diet (Table I). The perimetrial adipose tissue weight increased by feeding a high-fat diet was significantly reduced by feeding a high-fat diet containing 3 %, 6.25 %, 12.5 % or 17.5 % lactosucrose as compared to feeding a high-fat diet (Fig. 3). Feeding a high-fat diet containing 1.5 % lactosucrose failed to reduce the perimetrial adipose tissue weight increased by feeding a high-fat diet (Fig. 3). Feeding a high-fat diet containing 3 %, 6.25 %, 12.5 % or 17.5 % lactosucrose significantly reduced the hepatic triacylglycerol content elevated by feeding a high-fat diet (Fig. 4) (except feeding a high-fat diet containing 1.5 % lactosucrose group). On the other hand, the increase

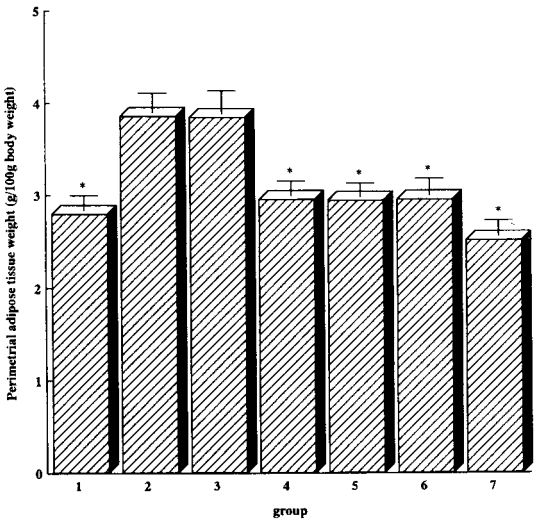

Fig. 3 Effect of lactosucrose on perimetrial adipose tissue weight of mice made obese with a high-fat diet.
1, Control group ; 2, High fat diet-treated group ; 3, high-fat diet plus 1.5 % LC ; 4, high-fat diet plus 3 % LC ; 5, high-fat diet plus 6.25 % LC ; 6, high-fat diet plus 12.5 % LC ; 7, high-fat diet plus 17.5 % LC ; Each point represents the mean±s.e.m. of 1.5 mice. * : Significantly different from high-fat diet treated group, $p<0.05$. LC: Lactosucrose.

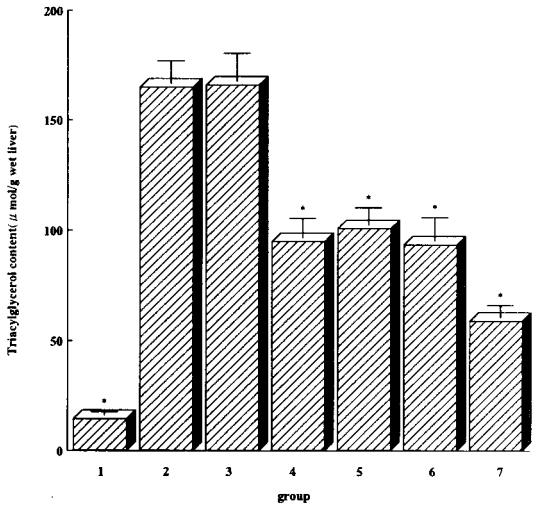

Fig. 4 Effect of lactosucrose on hepatic triacylglycerol in mice made obese with a high-fat diet.
The procedure was as described in Materials and Methods. 1, Control group; 2, High fat diet-treated group; 3, high-fat diet plus 1.5 % LC; 4, high-fat diet plus 3 % LC; 5, high-fat diet plus 6.25 % LC; 6, high-fat diet plus 12.5 % LC; 7, high-fat diet plus 17.5 % LC; Each point represents the mean±s.e.m. of 15 mice. * : Significantly different from high-fat diet treated group, $p<0.05$. LC: Lactosucrose.

of liver weight induced by a high-fat diet was reduced by the feeding lactosucrose (data not shown). Furthermore, the liver weight by the feeding lactosucrose plus high-fat diet was increased more than that of normal mice (data not shown).

There are a number of studies describing high-fat diet-induced obesity.[11-14] We therefore designed experiments to clarify whether high-fat diet-induced obesity in female mice might be prevented by lactosucrose, possibly due to inhibition of the intestinal absorption of dietary fat. Although a significant reduction in body weight was found in mice fed a high-fat diet containing 40 % beef tallow with 3 % or 17.5 % lactosucrose, lactosucrose decreased the perimetrial adipose tissue weight of the mice fed the high-fat diet at doses of 3 %, 6.25 %, 12.5 % and 17.5 % (Table I and Fig. 3), whereas no differences in the energy consumed were found among the experimental groups (except the laboratory pellet chow group). In a preliminary experiment, it was found that the weights of body and perimetrial adipose tissue were not affected by the reduction of casein contents in a high-fat diet.[7] Based on these experimental results, it may be safety concluded that lactosucrose prevents the obesity induced by a high-fat diet. In addition to its anti-obesity activity, lactosucrose was found to reduce the triacylglycerol content in fatty liver induced by the high-fat diet.

It seems likely that lactosucrose may prevent the increase of body weight and fatty liver by fat storage through inhibiting the intestinal absorption of dietary fat in a high-fat diet fed mice. Experiments are now in progress to prove this hypothesis.

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和文抄録

ラクトスクロースは2-モノグリセロールのラット小腸刷子縁小胞への取り込みを試験管内で阻害した。また，コーンオイル脂質エマルジョンをラットに経口投与した後の血中トリグリセライドの含量をラクトスクロースが低下することが明らかになった。これらの成績はラクトスクロースは食事中の脂質の腸管吸収を阻害する可能性を推測させるものである。この実験結果を踏まえてラクトスクロースの抗肥満作用を検討したところ，40％牛脂を含む高脂肪食投与による雌性マウスの子宮傍脂肪組織重量と肝臓中のトリグリセライド含量を低下させることが明らかになった。

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