Carbohydrate digestive capability in jejunum of rats subjected to simulated weightlessness

Toshinao Goda, Sachiko Takase, Hidehiko Yokogoshi, and Takeshi Hoshi

School of Food and Nutritional Sciences, The University of Shizuoka, 395 Yada, Shizuoka-shi, Shizuoka 422, Japan

Adult rats underwent a simulated weightlessness for 10 days by being confined within a jacket attached to the chains hanging from the corners of a cage. When the suspended rats had free access to diet they consumed less and showed a decreased jejunal mucosal weight. However, the specific activities (per mg of protein) of jejunal disaccharidases in the suspended rats were similar to those of normal rats fed ad libitum. When the suspended rats were force fed the regular amounts of diet, they again showed relatively high jejunal disaccharidase activities, the levels of which were similar to those of pair-fed control animals. In contrast, the liver glycogen contents were remarkably decreased in the suspended rats, regardless of whether they consumed sufficient amounts of diet. These results suggest that the digestive capability of carbohydrates is unaffected by the stress induced by the simulated weightless condition adopted in the present study, whereas hepatic glycogen metabolism is greatly altered in the simulated weightless condition.

Keywords: simulated weightlessness; disaccharidase; liver glycogen; stress

Introduction

Following space flight experimental animals exhibit changes of hepatic concentrations of glycogen.[1-3] These hepatic changes might be related to the stress accompanied by spaceflight, but a change in the rate of glucose supply originated from carbohydrate digestion in the small intestine is also likely, because eating behavior is presumably changed (less food intake) in animals in the weightless environment. This may lead to the changes in the digestive-absorptive capability of various nutrients in the small intestine, which in turn modifies the carbohydrate metabolism in the liver. However, no information is available as to whether intestinal disgestive/absorptive functions are fully preserved during and following spaceflight. The purpose of this study is to elucidate whether a simulated weightless condition alters the digestive capability of carbohydrates in the small intestine.

To partially simulate the state to which orbiter crews might be exposed, we utilized a procedure of prolonged immobilization/suspension in rats. Carbohydrate digestive capability was assessed by determining the activities of jejunal disaccharidases, which are known to play an essential role in the final stage of digestion and absorption of carbohydrates.[4,5] To evaluate the role of the change in the food intake, the rats were either fed a standard diet ad libitum or they were force-fed a regular amount of a synthetic diet.

Materials and methods

Animal procedure

Wistar male rats, 6 weeks of age, were purchased from Japan SLC, Inc., Hamamatsu, Japan. They were housed individually and had free access to a standard laboratory diet (MF; Oriental Yeast Co., Japan) until the start of the experiment. At 7 weeks of age, one group of animals was subjected to a state of simulated weightlessness according to the procedure described by Yokogoshi et al.[6] Briefly, a rat was fitted with a jacket to which metal chains were attached and it was suspended by hanging the chains to the four corners of a metal cage. The suspended animals were able to use their forelimbs to freely consume food

This study was supported by the Science and Technology Agency, Institute of Aerospace Technology, Tokyo, Japan, and the Ministry of Education, Institute of Space Science, Sagamihara, Kanagawa, Japan (U-9).

Address reprint requests to Dr. Goda at the Department of Nutrition, School of Food and Nutritional Sciences, University of Shizuoka, 395 Yada, Shizuoka 422, Japan.

Received September 25, 1990; accepted July 18, 1991

and water. The other group of animals was housed without the suspension. Throughout the entire experimental period, the animals had free access to water. Although the suspension of animals cannot be considered as simulation of zero-gravity, this model simulates another aspect of spaceflight, ie, prolonged immobilization. Indeed, the suspended animals in a previous study[6] exhibited similar physiologic changes, including muscle atrophy and bone demineralization, to those observed following spaceflight.

In the preliminary experiment the suspended rats consumed less diet when they had free access to the standard laboratory diet. To eliminate a possible effect of modified food intake in the first experiment, one group of control rats was pair-fed the same amount of diet that the suspended rats consumed on the previous day. In the second experiment, to ensure a regular food intake, the suspended rats were force-fed a synthetic diet that contained 20 energy% casein, 5 energy% corn starch, 45 energy% sucrose and 30 energy% corn oil, according to the procedure described previously.[7,8] One group of unsuspended rats (referred to as "normal") had free access to the synthetic diet; the food intake of this group was used as a reference for the amount of force-feeding. The other group (control) of unsuspended rats was force-fed the synthetic diet on the same schedule as the suspended rats. Force feeding was performed four times a day, at 14:00, 19:00, 23:00 and 6:00 hr in consideration of the circadian rhythmicity of the rats' food consumption.[9] The diet contained 71 kJ (6.8 mL) per feeding. The experimental procedures used in the present study met the guidelines for animal usage of the committee of the University of Shizuoka.

Preparation of intestinal homogenates

Ten days after the start of the experiment, rats were killed by decapitation at 10:00 hr. Blood samples were collected and the entire small intestine and the liver were immediately removed. The duodenum was discarded and the jejunoileum was divided into three equal parts along its length. The proximal third of jejunoileum (jejunum) was scraped using a microscope slide. Jejunal mucosa was homogenized with four volumes of 10 mmol/L potassium phosphate buffer (pH 7.0).

Assay procedures

Sucrase, isomaltase, and trehalase activities were assayed according to Dahlqvist[10] using 28 mmol/L sucrose, palatinose, and trehalose as substrate, respectively. Lactase activity was assayed according to Koldovský et al.[11] The liver was immediately frozen in liquid nitrogen following dissection and stored at −80° C. The liver glycogen was determined according to the method of Seifter et al.[12] Serum glucose was determined by UV method using hexokinase and glucose-6-phosphate dehydrogenase by a biochemical analyzer (Shimadzu CL 7000, Shimadzu Manufacturing Co., Japan). Protein was determined by the method of Lowry et al.[13]

Statistics

All data were subjected to one-way analysis of variance. Differences in mean values between groups were tested using Tukey's multiple range test. *P*-values of less than 0.05 were considered to indicate statistical significance.

Results and discussion

Effects of simulated weightlessness on disaccharidases in rats fed ad libitum

Ad libitum food intake of suspended rats was 10.6 ± 0.7 g/day (mean ± SEM), declining to approximately 60% of that in the unsuspended rats (17.1 ± 0.4 g/day). As shown in *Table 1*, suspended rats lost weight when they had free access to standard laboratory diet. The decrease in the weight gain should be partially ascribed to the decreased food intake in these animals. However, the extent of the weight loss of suspended rats was significantly ($P < 0.05$) greater than that of pair-fed rats. The adrenal weight of suspended rats was significantly greater than their pair-fed control (36.6 ± 1.0 mg versus 33.6 ± 0.8 mg, mean ± SEM, $P < 0.05$), suggesting that the suspension procedure used in the present study was a stressor to the rats. The involvement of adrenals in the suppression of weight gain was also suggested in the literature, which demonstrated that the weight gain of adrenalectomized rats was remarkably greater than shamoperated rats when they were force-fed the same amount of diet.[7]

Table 1 Effect of simulated weightlessness on jejunal disaccharidase activities and liver glycogen in rats

Group	Normal fed ad libitum	Control pair-fed*	Suspension fed ad libitum (reference)
Body weight (g)			
on day 0	207 ± 4	210 ± 4	210 ± 4
at killing (day 10)	242 ± 5[a]	195 ± 2[b]	174 ± 6[c]
Food intake (g/day)	17.1 ± 0.4[a]	10.9 ± 0.1[b]	10.6 ± 0.7[b]
Jejunal mucosa protein (mg)	146 ± 15[a]	69 ± 4[b]	72 ± 7[b]
Sucrase activity†	4.37 ± 0.36	4.94 ± 0.28	6.00 ± 0.36
Isomaltase activity†	0.97 ± 0.07	1.12 ± 0.05	1.22 ± 0.13
Lactase activity†	1.00 ± 0.11	1.15 ± 0.12	1.56 ± 0.25
Trehalase activity†	3.07 ± 0.29	3.02 ± 0.34	3.42 ± 0.66
Serum glucose (mg/100mL)	117 ± 2	110 ± 2	120 ± 8
Liver glycogen (mg/g)	42.6 ± 0.4[a]	39.1 ± 0.2[a]	19.2 ± 0.1[b]

* A group of control animals were pair-fed the same amount of a standard laboratory diet as the suspended animals consumed on the previous day.

† Jejunal sucrase, isomaltase, lactase, and trehalase activities are expressed as μmol/mg protein/h. Mean ± SEM of five animals are shown.

[a-c] The values not sharing a common superscript are significantly different from each other by Tukey's multiple range test ($P < 0.05$).

Table 2 Effect of simulated weightlessness on jejunal disaccharidase activities and liver glycogen in rats force-fed a regular amount of diet

Group	Normal fed ad libitum	Control force-fed*	Suspension force-fed*
Body weight (g)			
on day 0	170 ± 3	171 ± 1	172 ± 3
at killing (day 10)	214 ± 4[a]	204 ± 1[a]	182 ± 1[b]
Food intake (kcal/day)	68.4 ± 1.1	68.4	68.4
Jejunal mucosa protein (mg)	128 ± 4[a]	147 ± 5[b]	152 ± 5[b]
Sucrase activity†	4.19 ± 0.03	4.38 ± 0.12	4.54 ± 0.12
Isomaltase activity†	0.80 ± 0.02	0.85 ± 0.03	0.81 ± 0.02
Lactase activity†	0.78 ± 0.03	0.80 ± 0.04	0.77 ± 0.03
Trehalase activity†	4.12 ± 0.14	4.15 ± 0.16	3.88 ± 0.11
Serum glucose (mg/100mL)	142 ± 3[a]	149 ± 3[ab]	152 ± 2[b]
Liver glycogen (mg/g)	54.3 ± 1.3[a]	58.6 ± 2.5[a]	27.6 ± 3.8[b]

* Suspended and a group of control animals were force fed a synthetic diet four times each day during the experimental period.
† Jejunal sucrase, isomaltase, lactase, and trehalase activities are expressed as μmL/mg protein/h. Mean ± SEM of five animals are shown.
[a-b] The values not sharing a common superscript are significantly different from each other by Tukey's multiple range test ($P < 0.05$).

Jejunal mucosa total protein was decreased in suspended rats as well as in the pair-fed rats as compared to the normal rats fed ad libitum. The specific activities (per mg protein) of sucrase, isomaltase, lactase, and trehalase in the suspended rats were similar to those of pair-fed control rats. In spite of decreased food intake, the suspended rats and the pair-fed control showed appreciable amounts of the specific activities of disaccharidases; the values were similar to those of normal rats (*Table 1*). These results suggested that the stress induced by the simulated weightlessness did not evoke any alteration of jejunal digestive capability of carbohydrates.

In contrast, liver glycogen content of suspended rats was remarkably lower than that of the pair-fed control, while the glycogen content of the pair-fed control was similar to that of normal rats. Serum glucose concentration at the time of killing was unaffected by the stress state. Because the suspended rats consumed insufficient amounts of the diet during the experimental period and lost weight, the interpretation of the first experiment was complicated due to possible nutritional deficiencies. Therefore, we considered it pertinent to ensure a regular food intake in the suspended rats. This was performed by force-feeding in the second experiment.

Effects of simulated weightlessness on disaccharidases in rats force fed a regular amount of diet

The results of the second experiment are summarized in *Table 2*. During the 10-day experiment period, the suspended rats received sufficient amounts of diet and they gained weight. However, the extent of the weight gain of suspended rats was again significantly ($P < 0.05$) less than the force-fed controls. Interestingly, the amount of jejunal mucosa total protein was greater in the force-fed rats than in rats fed ad libitum, regardless of whether they were suspended or not. Neither force feeding nor suspension of animals led to modifications of the specific activities of sucrase, isomaltase, lactase, and trehalase (*Table 2*). Serum glucose concentration of suspended rats was slightly but significantly higher than that of the control rats that had free access to the the diet, but it did not significantly differ from that of the force-fed and unsuspended rats. Force feeding of a synthetic diet did not affect liver glycogen content in control animals. However, the suspended rats again showed a remarkably decreased (by approximately 50%) liver glycogen content as compared with the control animals, in spite of the fact that a regular food intake was ensured in the suspended rats (*Table 2*).

The results of the present study suggested that even if chronically exposed to a state of simulated weightlessness, digestive capacity for carbohydrates in jejunum is well preserved when a regular food intake is maintained. This indicates that the metabolism of jejunal microvillar disaccharidases is possibly independent of stress-related hormone(s) in adults. From a development point of view, it is of note that in suckling rats stress was capable of inducing a precocious increase of sucrase activity.[14] Independent of the digestive capacity of carbohydrates in the small intestine, the machinery involved in expenditure and/or storage of liver glycogen is readily altered following exposure to simulated weightless condition.

References

1 Belitskaya, R.A. (1977). Carbohydrate and lipid content of rat liver tissue following a 22-day spaceflight. *Space Biol. Aerospace Med.* **4**, 97–99

2 Abraham, S., Lin, C.Y., Volkmann, C.M., and Klein, H.P. (1983). Biochemical changes in rat liver after 18.5 days of spaceflight. *Proc. Soc. Exp. Biol. Med.* **172**, 334–339

3 Merrill Jr, A.H., Wang, E., Jones, D.P., and Hargrove, J.L. (1987). Hepatic function in rats after spaceflight: effects on lipids, glycogen, and enzymes. *Am. J. Physiol.* **252**, R222–R226

4 Semenza, G. (1986). Anchoring and biosynthesis of stalked brush border membrane protein: glycosidases and peptidases of enterocytes and renal tubuli. *Annu. Rev. Cell Biol.* **2**, 255–313

5 Goda, T. and Koldovský, O. (1988). Dietary regulation of small intestinal disaccharidases. In *World Review of Nutrition and Dietetics*. (G.H. Bourne, ed.), Vol. 57, pp. 275–329, Karger, Basel, Switzerland
6 Yokogoshi, H., Takase, S., Goda, T., and Hoshi, T. (1990). Effects of suspension hypokinesia/hypodynamia on the body weight and nitrogen balance in rats fed with various protein concentrations. *Agric. Biol. Chem.* **54,** 799–789
7 Goda, T., Bustamante, S., Grimes, J., and Koldovský, O. (1984). Dietary induced increase of lactase activity in adult rats is independent of adrenals. *Experientia* **40,** 1287–1288
8 Goda, T., Bustamante, S., and Koldovský, O. (1985). Dietary regulation of intestinal lactase and sucrase in adult rats; quantitative comparison of effect of lactose and sucrose. *J. Pediatr. Gastroenterol. Nutr.* **4,** 998–1008
9 Siegel, P.S. (1961). Food intake in the rat in relation to the dark-light cycle. *J. Comp. Physiol. Psychol.* **54,** 294–301
10 Dahlqvist, A. (1964). Method for assay of intestinal disaccharidases. *Analyt. Biochem.* **7,** 18–25
11 Koldovský, O., Asp, N.G., and Dahlqvist, A. (1969). A method for the separate assay of "neutral" and "acid" -galactosidase in homogenates of rat small-intestinal mucosa. *Analyt. Biochem.* **27,** 409–418
12 Seifter, S., Dayton, S., Novic, B., and Muntwyler, E. (1950). Estimation of glycogen with the anthrone reagent. *Arch. Biochem.* **25,** 191–200
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275
14 Goda, T., Yamada, K., Bustamante, S., Edmond, J., Grimes, J., and Koldovský, O. (1985). Precocious increase of sucrase activity by carbohydrates in the small intestine of suckling rats. 1. Significance of the stress effect of sugar-induced diarrhea. *J. Pediatr. Gastroenterol. Nutr.* **4,** 468–475