Adaptive Changes of Sugar Absorption in Rat Small Intestine during Fasting and Refeeding

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Summary The effects of fasting and refeeding on the adaptive changes in intestinal sugar absorption was observed by measuring the transmural potential difference (ΔPD) evoked by glucose by infusion of sugars and compared with those in disaccharidase activities, glucose uptake and phlorizin binding activity to the brush border membranes, and morphological parameters.

Rats were fasted for 1, 3, or 5 days. The rats fasted for 5 days were refed for 1 or 3 days further. The ΔPD evoked by glucose, maltose, or sucrose in everted jejunal segments decreased within 1 day of fasting, remained at a same level during fasting, and then increased to the control level after refeeding. Maltase and sucrase activities did not change during the first day of fasting, but did so significantly after 3 days of fasting, in terms of both specific and total activities. On refeeding, disaccharidase activities increased rapidly. However, in spite of significant changes in ΔPD s and disaccharidase activities, glucose uptake by and phlorizin-binding to brush border membrane vesicles from jejunal mucosa did not change in either 5 day-fasted or refed animals compared with the control group. On the other hand, jejunal villus height in rats fasted for 5 days decreased significantly compared with that in control rats and was accompanied by a reduction in epithelial cell height and surface area of villi.

These results suggest that the absorption of sugar in small intestine could adapt to fasting and refeeding with changes in disaccharidase activity and surface area, without any change in glucose carrier.

Key Words: disaccharidase, small intestine, sugar absorption, phlorizin binding, brush border membrane

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Because of recent trends in popular use of intravenous alimentation in clinical nutrition, it is important to clarify the effect of luminal factors on the regulation of adaptive changes in intestinal absorption.

Many studies have shown that rapid alterations in mucosal functions are observed in response to the composition of diet [1], fasting [2], total parenteral nutrition [3], intestinal resection [4], or diabetes mellitus [5,6]. However, the mechanisms regulating the adaptation of intestinal absorption are incompletely understood. Especially, the observations on the effects of fasting are contradictory, i.e., fasting has been reported to increase [7,8] or decrease [9,10] the absorption of sugars and amino acids.

Glucose is generally accepted to be actively absorbed *via* carrier-mediated steps by the intestinal brush border membrane. A net mucosal-serosal flux of glucose is accompanied with that of Na^+ , which induces a transmural potential difference (ΔPD) [11]. Recently, it was reported that phlorizin binds specifically to the Na^+ -glucose cotransporter in the intestinal brush border membrane [12].

Therefore, we investigated the effects of fasting and refeeding on the adaptive changes in intestinal sugar absorption by measuring the ΔPD evoked by infusion of various sugars and comparing it with disaccharidase activities, glucose uptake, and phlorizin-binding activity to the brush border membrane vesicles, as well as with morphological parameters such as length of microvilli, absorptive surface area, etc.

MATERIALS AND METHODS

Animals. Male rats of the Wistar strain, weighing about 250 g, were kept at constant temperature ($23 \pm 1^\circ\text{C}$) and given a standard laboratory chow (Oriental Yeast Co.) and water *ad libitum* for a week before the initiation of the experiment. Then, the rats were fasted for 1, 3, or 5 days. The rats fasted for 5 days were refed a laboratory chow for 1 or 3 days further. Control rats were fed *ad libitum*. During the experiments, all rats had unrestricted access to water. The rats were killed by decapitation between 9:00 and 10:30. Blood glucose concentrations were tested by the glucose oxidase method of Dahlqvist [13].

Preparation of isolated intestinal segments. The small intestine from the ligament of Treitz to the ileocecal junction was quickly removed. The jejunal segment (3 cm in length) was taken out from the intestine beginning approx. 10 to 13 cm from the ligament of Treitz, rinsed with a standard buffer solution (composition described below), everted, and fixed over a small fenestrated polyethylene tube (5 mm outer diameter). Thereafter, each everted segment was tied at both ends with cotton threads over the tube [14]. The inside of the tube was filled with the standard solution and the mucosal side of the intestine was exposed to 9 ml of bathing solution (standard solution). The bathing solution was continuously aerated during experiments and its temperature was kept constant at 37°C . The standard solution

had the following composition (in mM): Na_2SO_4 , 50; mannitol, 160; CaSO_4 , 1.5; MgSO_4 , 1.0; KH_2PO_4 , 0.5; K_2HPO_4 , 2.0 (pH 7.4).

Recording of transport-related increments in the transmural potential. ΔPD was led out by connecting calomel half cells to the mucosal and serosal media by means of 2% agar containing 3 M KCl and was recorded by a high-sensitivity DC pen-recorder (National VP 6541A). The polarity of the electrode in contact with the mucosal medium was negative with respect to that in contact with the serosal medium. After the spontaneous ΔPD had attained a steady level, a certain small amount of sugar, dissolved in 1 ml of standard solution, was rapidly injected into the mucosal medium (9 ml). The final concentration of sugar was 20 mM for D-glucose, 10 mM for maltose, or 40 mM for sucrose.

Preparation of brush border membrane vesicles. Jejunal mucosa was scraped by a glass slide from the proximal half of the small intestinal segment. Brush border membrane vesicles were prepared from the jejunal mucosa according to the method of Toggenburger *et al.* [12]. Mucosal scrapings were suspended in 25 vol of ice-cold 50 mM mannitol in 2 mM Tris-HCl buffer (pH 7.1) and homogenized using a Polytron (Brinkman Instruments) for 1 min. Calcium chloride was added to the homogenate to give a final concentration of 10 mM. After being kept in the cold for 20 min, the suspension was centrifuged at $3,000 \times g$ for 15 min, and the resulting supernatant was centrifuged at $27,000 \times g$ for 30 min. The pellet was then resuspended in 2 vol of 100 mM mannitol in 10 mM Tris-HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (pH 7.5) and centrifuged once more at $27,000 \times g$ for 30 min. The pellets, which contained almost pure brush border membrane vesicles, were resuspended in 100 mM mannitol in 10 mM Tris-HEPES buffer (pH 7.5). The suspended vesicles contained about 6–8 mg protein per ml.

Measurement of glucose uptake and phlorizin-binding activity to brush border membrane vesicles. Uptake of glucose and phlorizin binding to brush border membrane vesicles were determined by the nitrocellulose membrane filtration technique described by Hopfer *et al.* [15] and by Klip *et al.* [16]. They were both measured at room temperature (ca. 22°C). Twenty microliters of vesicles were carefully placed in the bottom of a clear polystyrene test tube. The same volume of 2.0 mM [^3H]glucose or 10 μM [^3H]phlorizin containing 100 mM mannitol, 10 mM Tris-HEPES, and 100 mM NaSCN or KSCN was placed as a separate drop at the bottom of the tube. The uptake or binding was started by rapid mixing using a vortex and terminated after 15 s by dilution with 2.5 ml of ice-cold 1 mM Tris-HCl buffer (pH 7.0) containing 250 mM NaCl. The diluted sample was immediately transferred onto a nitrocellulose filter (0.45 μm pore size, Schleicher Schuell, Inc.) and rapidly rinsed further with 2.5 ml of the ice-cold 250 mM NaCl/1 mM Tris-HCl buffer. Na^+ -dependent glucose uptake or phlorizin-binding activity was calculated by subtracting the amount of substrate in the presence of K^+ from that in the presence of Na^+ . These assays were done within 9 h after the rats had been sacrificed.

Scanning electron microscopy. A small area of jejunal tissue next to the everted jejunal segment used for Δ PD measurements was quickly flushed with 0.1 M sodium phosphate buffer (pH 7.2), slit open lengthwisely on filter paper, and fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 2 h. After fixation, the tissues were rinsed with the phosphate buffer and placed in the same buffer containing 1% osmium tetroxide for 2 h. The tissues were dehydrated through a graded series of ethanol and isoamylacetate, carefully fractured in liquid nitrogen, and dried in a critical point dryer (HCP-1, Hitachi) using CO₂ as the transitional fluid. All samples were coated in a gold vapor coater (Hummer II, Technics) and examined by scanning electron microscopy (S-800, Hitachi). From the scanning electron micrographs, thickness of the jejunal wall, height of villi and crypts, height and surface area of epithelial cells, number of microvilli per square micrometer, and length and diameter of microvilli were measured. For these estimations from the scanning electron micrographs, the jejunal villous surfaces examined were limited to the area on the middle part of the villi.

Disaccharidase assay and protein determination. After the measurement of Δ PD evoked by sugars, jejunal mucosa from each everted segment was homogenized with 9 vol of the standard solution (pH 7.4) and stored at -20°C until used. An aliquot of the homogenate was used for the assay of maltase and sucrase activities. An aliquot of brush border membrane vesicles was used for the assay of sucrase activity. Disaccharidase activities were determined by the method of Dahlqvist [13]. Protein concentration was determined by the method of Lowry *et al.* [17] using bovine serum albumin as a standard.

Chemicals. Glucose and sucrose were obtained from Wako Pure Chemical Industries Ltd. Maltose was provided by Hayashibara Co., Ltd. Phlorizin was purchased from Sigma Chemical Co. Glucose oxidase and peroxidase were from Worthington Biochem. Co., glutaraldehyde was from TAAB Laboratories, and osmium tetroxide, from E. Merck. D-[³H]Glucose and [³H]phlorizin were purchased from New England Nuclear Corp. Other reagents were all analytical grade chemicals.

RESULTS

Effect of fasting and refeeding on body weight, intestinal weight, and blood glucose. As shown in Table 1, the most marked weight loss occurred during the first day of fasting, the rats losing about 30 g in 24 h. Similarly the weight gain was the most marked after the first day of refeeding. Adult male rats lost 22% of their initial body weight during the 5 days of fasting. On refeeding, animals recovered weight losses up to the initial level within 3 days. Their food intake was almost the same amount, about 30 g/day/rat, during the experiment. The weight of mucosal scrapings from the jejunum did not change during the first 24 h fasting, but did so after 3 days (Table 1).

Blood glucose concentration decreased from 108 mg/dl to 71 mg/dl after 1 day

Table 1. Effects of fasting and refeeding on body weight, food intake, intestinal weight, and blood glucose.

	Control (15)	Number of days fasted			Number of days refed		
		one (8)	three (7)	five (10)	one (7)	three (7)	
Body weight (g)	301 ± 4	273 ± 9*	244 ± 8*	234 ± 5*	263 ± 9*	292 ± 8	
Food intake (g/day)	27 ± 2	—	—	—	29 ± 2	28 ± 3	
Jejunum weight (g)	3.79 ± 0.23	3.03 ± 0.08*	2.04 ± 0.08*	1.82 ± 0.04*	2.73 ± 0.07*	3.43 ± 0.16	
Jejunum mucosal weight (g)	1.74 ± 0.13	1.61 ± 0.06	0.95 ± 0.05*	0.83 ± 0.05*	1.48 ± 0.09*	2.06 ± 0.17	
Blood glucose (mg/dl)	108 ± 3	71 ± 2*	64 ± 3*	65 ± 2*	109 ± 2	108 ± 4	

Results are expressed as means ± SE. (), Number of animals. *, Significantly different from control rats at $p < 0.05$.

of fasting, maintained the same level during fasting, and then increased to the control level after refeeding.

Effect of fasting and refeeding of sugar-evoked transmural potential differences and disaccharidase activities in rat jejunum. The changes in sugar-evoked potential

Fig. 1

Fig. 1. Transmural potential difference evoked by glucose, maltose or sucrose in the rat jejunum during fasting and refeeding. Results are expressed as means $\pm$ SE of 7–15 animals. Δ PD was measured at the concentration of 20 mM glucose (A), 10 mM maltose (B), or 40 mM sucrose (C) in the standard solution containing 50 mM Na_2SO_4 (pH 7.4). *, Significantly different from control at $p < 0.05$.

Fig. 2. Sucrase and maltase activities in the rat jejunum during fasting and refeeding. Results are expressed as means $\pm$ SE of 7–15 animals. Enzyme activities were measured in the homogenate of jejunal mucosa after the measurement of Δ PD. Substrate concentration were 40 mM sucrose (A) or 10 mM maltose (B) in the standard solution (pH 7.4). *, Significantly different from control at $p < 0.05$. Specific activity, μmol substrate/ mg protein/h. Total activity, μmol substrate/ cm-gut/h .

Fig. 2

difference (Δ PD), when sugars were added to the mucosal side, are presented as increments in mucosal negativity in everted jejunum of fasted and refed rats (Fig. 1). On the first day of fasting, the jejunal Δ PD evoked by glucose was significantly decreased from that of the control group. During fasting, the Δ PD evoked by glucose showed a progressive small decrease, but it was not significant. After refeeding was started, the Δ PD evoked by glucose increased to the level of control rats within 24 h. The Δ PD evoked by maltose was almost the same as that by glucose, while the Δ PD evoked by sucrose was lower than that by glucose. All three sugars elicited similar Δ PD patterns during fasting and refeeding (Fig. 1).

After the measurement of potential difference, maltase and sucrase activities were assayed in the mucosal homogenate of jejunal everted intestine. The time course of the changes in specific (per unit of protein) activities of maltase and sucrase during 5 days of fasting and a successive refeeding for 3 days is shown in Fig. 2. A significant decrease in sucrase specific activity appeared on the third day of fasting. It decreased to three-fourths of the control level and did not change further during the period of starvation. Refeeding of the laboratory chow increased sucrase activity very rapidly to that seen in the control group within 24 h and even above that level by 3 days of refeeding. Similar trends were observed in maltase activity, but the effect of fasting on maltase activity was not so big as that on sucrase activity. These changes in disaccharidase activities were magnified when expressed as total activity per 1 cm of jejunal segment (Fig. 2).

Glucose uptake and phlorizin-binding activity to the jejunal brush border membrane vesicles of fasted and refed rats. In order to determine whether the changes in Δ PD evoked by glucose were accompanied by changes in the glucose transporter, Na^+ -dependent glucose uptake and Na^+ -dependent phlorizin-binding activity to the brush border membrane vesicles of fasted and refed rats were measured. The specific activity of sucrase in brush border membrane vesicles was increased about 15-fold compared with that of the mucosal homogenate, and recovery of the activity

Table 2. Effect of fasting and refeeding on sucrase activity, glucose uptake, and phlorizin-binding activity in brush border membrane vesicles from rat jejunum.

	Control	Number of days		
		fasted		refed
		one	five	three
	(7)	(5)	(7)	(5)
Sucrase activity (μmol substrate/mg protein/h)	69.8 $\pm$ 2.4	77.7 $\pm$ 5.6	39.4 $\pm$ 4.5*	94.7 $\pm$ 9.6*
Glucose uptake (nmol/mg protein/15 s)	1.05 $\pm$ 0.15	1.02 $\pm$ 0.14	1.20 $\pm$ 0.20	1.31 $\pm$ 0.17
Phlorizin binding (pmol/mg protein/15 s)	57.3 $\pm$ 4.0	60.1 $\pm$ 6.0	56.6 $\pm$ 5.3	59.8 $\pm$ 7.2

Results are expressed as means $\pm$ SE. (), Number of animals. *, Significantly different from control rats at $p < 0.01$.

Table 3. Morphological parameters of jejunal villi and microvilli in control and 5 day-fasted rats.

		Control	5 days-fasted
Thickness of intestinal wall (μm)	(10)	554 ± 20	$418 \pm 21^*$
Height of villi (μm)	(10)	374 ± 12	$244 \pm 12^*$
Height of crypt (μm)	(10)	97 ± 3	102 ± 5
Height of epithelial cells (μm)	(50)	34.6 ± 0.9	$23.5 \pm 0.8^*$
Surface area of epithelial cell ($\mu\text{m}^2/\text{epithelial cell}$)	(50)	17.5 ± 0.01	$9.17 \pm 0.02^*$
Number of microvilli/ μm^2 of epithelial cell surface	(21)	57 ± 4	$83 \pm 6^*$
Length of microvilli (μm)	(63)	0.83 ± 0.01	$1.12 \pm 0.02^*$
Diameter of microvilli (μm)	(63)	0.132 ± 0.001	$0.098 \pm 0.001^*$

Results are expressed as means $\pm$ SE. (), Number of measured sites. *, Significantly different from control rats at $p < 0.01$.

was about 40% in all groups. As shown in Table 2, sucrase specific activity in the jejunal brush border membrane vesicles did not decrease compared with that in the control group after 1 day of fasting, but dropped significantly after 5 days of fasting. Then it increased considerably above the control level after 3 successive days of refeeding, in good agreement with the results for the jejunal homogenate.

Na^+ -dependent glucose uptake by brush border membrane vesicles did not change in any of the groups. Similarly phlorizin-binding activities remained the same in control, fasted, and refed rats (Table 2).

Changes in morphological parameters of villi and microvilli in the fasted rat jejunum. Five day-fasted jejunum which showed the most significant decreases in ΔPDs evoked by sugars and disaccharidase activities, was further examined by scanning electron microscopy, and the results were compared with those of the control jejunum.

The thickness of the jejunum was decreased in five day-fasted rats compared with that in control rats, in good agreement with the reduction in mucosal weight. The decrease in jejunal wall thickness was mainly dependent on the decrease in villous height rather than in crypt height (Fig. 3-1, 2, Table 3). Enterocyte height in fasted rats was a little shorter than that in control rats. Moreover, the surface area per epithelial cell was significantly decreased by fasting (Fig. 3-3, 4) with a change of microvillous shape (Fig. 4, Table 3). The length of microvilli was increased by fasting, whereas their diameter was rather decreased (Fig. 4, Table 3). On the assumption that microvilli can be geometrically represented as dome-capped cylinders, the surface areas of microvilli were determined from these measurements, as suggested by Anderson and Taylor [18]. As a result, the surface area of one microvillus was estimated as $0.346 \mu\text{m}^2$ for control animals and $0.345 \mu\text{m}^2$ for fasted animals. On the other hand, the number of microvilli per square micrometer of villous surface was greater in fasted animals than in control ones, though the number of microvilli possessed by one epithelial cell was much less in fasted than in control animals due to a significant reduction in epithelial cell surface area by

Fig. 3. Scanning electron micrographs of jejunal villi (1, 2) and surface of jejunal epithelial cells (3, 4) in control and 5 day-fasted rats. 1 and 3 are from control animals, and 2 and 4, from fasted ones. Magnification $\times 130$ (1 and 2), $\times 3,000$ (3 and 4).

Fig. 4. Scanning electron micrographs of jejunal microvilli in control and 5 day-fasted rats. 1 and 3 are from control animals, and 2 and 4, from fasted ones. 1 and 2 show the luminal surface of microvilli. 3 and 4 show longitudinal views of microvilli. Magnification $\times 30,000$ (1 and 2), $\times 40,000$ (3 and 4).

fasting. Therefore, the entire surface area of microvilli per epithelial cell seemed to be significantly decreased by fasting.

DISCUSSION

In the present study, the effects of fasting and refeeding on the adaptive changes in sugar absorption were observed in rat small intestine.

In good agreement with a previous report that fasting causes a decrease in intestinal weight [19], we found that the mucosal weight decreased after 3 days of fasting and increased very quickly after refeeding. The response of refeeding seemed to be more rapid than that of fasting. Fasting also reduced the height of the jejunal villi and the surface area per epithelial cell with a change in microvillous shape. It is reported that mucosal cell proliferation in crypts is greatly influenced by fasting in rats. Number of mucosal cells, DNA content, and mucosal uptake of [^3H]thymidine were shown to be reduced as was the migration rate of cells up to the villous tip [19–21]. Therefore, the entire surface area of the jejunal villus was much smaller in fasted than in control animals.

Total deprivation of diet significantly decreased maltase and sucrase activities in the jejunal mucosa. A highly significant decrease in both specific and total activities of the enzymes occurred after 24 h of fasting. Refeeding the diet increased maltase and sucrase activities very rapidly. These results confirmed previous studies [1,2]. On the other hand, the transmural potential difference (ΔPD), which is induced by a net mucosal-serosal flux of glucose accompanied with that of Na^+ , was significantly decreased in fasted animals within 24 h, despite no change in disaccharidase activity. ΔPD s evoked by maltose and sucrose, which are supposed to be induced by glucose released from the hydrolysis of these disaccharides, were also changed during fasting and refeeding in the same manner as the glucose-evoked ΔPD . Debnam and Levin [9] reported that the decrease in ΔPD evoked by glucose after 3 days of fasting correlated with a decrease in enterocyte number. However, it is interesting to note the difference between ΔPD s evoked by disaccharides and disaccharidase activity on the first day of fasting. There appears to be no direct correlation between digestion and absorption, when disaccharidase activity is relatively high. Murray and Wild [22] reported that $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity of rat jejunum decreased within 17 h after the beginning of fasting. Therefore, a decrease in the electrochemical gradient generated by Na^+ in jejunal tissue could contribute to a decrease in Na^+ -coupled transport.

Glucose absorption in the small intestine occurs by a carrier-mediated transport mechanism in the brush border membrane of epithelial cells [12,15]. In order to determine whether decreased or increased ΔPD s are due to intrinsic alterations in the plasma membrane properties of the epithelial cells, we measured glucose uptake by and phlorizin binding to the Na^+ -glucose cotransporter of brush border membrane vesicles. In spite of a significant change in sucrase activity, both the capacity of glucose uptake and the phlorizin-binding activity did not change during

fasting and refeeding. These results suggest that the number of glucose transporters in the brush border membrane is not affected by fasting when expressed as a function of the membrane unit. However, the total number of transporters could be decreased because of the reduction in the amount of brush border membrane which occurs during fasting. It is reported that a starved animal shows a decreased $V_{\max}$ without any change in K_m for glucose transport and luminal nutrients maintained the capacity of glucose absorption [23]. Consideration of adaptive changes in glucose absorption is needed to interpret the changes in the surface area of intestine or in the amount of brush border membrane, as well as changes in specific activity. Furthermore, it is also important to consider the metabolism of epithelial cells. For example, it is known that the effect of fasting on active uptake of monosaccharides seems to be largely due to the withdrawal of dietary carbohydrate [24] and that glucose transport is induced by an increase in blood glucose in diabetic rat intestine [25]. In our study the changes in blood glucose paralleled the Δ PD evoked by glucose during fasting and refeeding.

In conclusion, our results have shown that the absorption of sugar in the small intestine decreases or increases by fasting or refeeding very rapidly, although further studies will be required to unravel the mechanism of the adaptive changes in brush border membranes and the metabolism of enterocytes in response to luminal factors.

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