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Effect of Glucose and Insulin on Small Intestinal Brush Border Enzymes in Fasted Rats[1] (41659)

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Abstract. Fasting reduced small intestinal length. It also decreased mucosal weight, DNA and protein content, and concentrations of enterokinase, maltase, and sucrase in both duodenal and jejunal segments. In contrast, the concentrations of lactase and leucine aminopeptidase were not affected. Concomitantly, serum insulin levels dropped to one-fifth of the control levels while serum glucose concentrations showed a lesser degree of reduction. Glucose supplementation alone raised the serum insulin level, prevented the decrease in DNA content, and showed a protective effect on mucosal protein, mucosal weight, mucosal thickness, and villus height. Glucose also protected the sucrase and maltase concentrations; more significantly for maltase in the jejunal segment. Insulin alone, although it increased the serum insulin level to that found with glucose supplementation alone, had no protective effect on the loss in protein, DNA, and most enzymes except for maltase concentration in the jejunal segment. Addition of insulin to glucose did not modify the glucose effect on the contents of DNA, protein, and concentrations of sucrase and maltase. These results suggest that the glucose effect on the mucosa is not mediated by insulin. In addition, the retention of both maltase and sucrase activities through only glucose supplementation suggests the loss of maltase and sucrase in fasting is due to nutrient rather than specific substrate restriction.

Fasting of experimental animals has been found to cause extensive changes in their small intestines. Rats fasted for 72 hr showed a loss of normal villous architecture (1), decrease in the number of cells/villus (2), and rapid loss of protein and DNA (3, 4). Concomitantly, the brush border enzymes are also affected. Sucrase and maltase concentrations exhibited drastic reductions following fasting in both the rat (4–6) and human (7). Alkaline phosphatase was reported to show a sharp decrease in an earlier study (8), but was found to be among the more resistant enzymes in the rat, in conjunction with lactase in a more recent report (4). Fasting may then have a selective effect on the brush border enzymes.

The factors that are involved in the adaptive changes of the small intestine following fasting have been studied to only a limited extent. Substrate and/or caloric availability are among some of the factors. Refeeding of fasted rats exclusively with sucrose led to a greater recovery of sucrase than maltase, while refeeding with maltose led to a better recovery of maltase than sucrase (6). The same study showed refeeding with lactose or glucose was ineffective. This suggests the operation of a "substrate induction" type of regulation. In contrast, in the human (7), refeeding with lactose or glucose had the effect of increasing protein and disaccharidase concentrations in the small intestinal mucosa from fasted individuals. Further delineation of the regulation by substrate and caloric availability is required.

Hormonal factors may also play a part. Previously, we have shown that fasting of rats drastically reduces their serum insulin levels (9). Insulin was recently demonstrated to be important in the maturation of disaccharidases *in vitro* (10, 11) and *in vivo* (12, 13). The reduction in insulin during fasting may retard the crypt to villus maturation process of intestinal epithelial cells, hence resulting in reduced levels of mucosal enzymes.

To evaluate whether caloric availability and/or insulin depletion are the causative factors, we examined their relative effectiveness

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in preventing small intestinal mucosal changes in fasting rats.

Materials and Methods. *Animals.* Male Sprague–Dawley rats (260–280 g) were housed in the animal unit on a 12-hr light–dark cycle and at an ambient temperature of 25°C. They were fed a standard laboratory chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, Ill.) and water *ad libitum* for 10 days before starting the experiment. Rats were divided into two groups. The control group was fed chow and water *ad libitum* throughout the entire study. The experimental group had their chow removed at the start of the experiment (Day 0) for 5 days. The experimental groups was further divided into four subgroups as follows: (I) fasting, (II) fasting with insulin supplementation, (III) fasting with glucose supplementation, (IV) fasting with insulin and glucose supplementation.

Insulin and glucose administration. Starting at Day 0, rats from the experimental group designated to receive insulin were given insulin either intramuscularly (long-acting protamine zinc iletin, Eli Lilly, Indianapolis, 1 unit/kg/injection) twice daily at 9:00 AM and 9:00 PM, or by continuous infusion (regular insulin, Eli Lilly, 2 units/day) with an ALZET mini osmotic pump (Alza Corporation, Palo Alto, Calif.) implanted subcutaneously on the dorsal side posterior to the scapular area. To prevent precipitation of insulin inside the pump, glutamate and aspartate were each added at equal concentrations of 3.5 mg/ml and the final pH of the insulin solution was adjusted to 3.5 according to Bringer *et al.* (14). Since both modes of insulin delivery showed essentially similar results, the data were combined and presented as one group. An equal volume of 0.9% saline was given to all other rats that were not receiving insulin by intramuscular injection. Injections were continued daily until 9:00 PM on Day 4; no injection was given in the morning of Day 5 before sacrifice. Glucose was supplied orally as a 20% aqueous solution *ad libitum.*

Preparation of mucosal homogenate. At Day 5, rats from all groups were sacrificed by decapitation. The whole intestine (from the pyloric end to the ileal–cecal region) was removed, trimmed of fat and mesentery. The length of the intestine was measured by attaching a 5-g weight to one end. A 15-cm length was removed from the proximal end representing the duodenal segment. Another 15-cm length was removed from the 30- to 45-cm section which was taken as the jejunal segment. Each segment was split and the intestinal content was removed by gently wiping with tissue paper. The mucosa was scraped and weighed separately. The mucosal preparation from each segment was separately homogenized with a Potter–Elvehjem homogenizer using a Teflon pestle with the vessel immersed in crushed ice. Homogenates obtained were used for the determination of DNA, protein, lactase, maltase, sucrase, enterokinase, and leucine aminopeptidase.

Biochemical determinations. Protein was determined by the technique of Lowry (15) using bovine serum albumin fraction V as the standard. DNA was first precipitated with cadmium chloride and then measured by the calorimetric reaction with diphenylamine reagent according to Burton (16) using highly polymerized calf thymus DNA as the standard.

Maltase, lactase, and sucrase were assayed by the method of Dahlqvist (17) using maltose, lactose, and sucrose as the corresponding substrate. Disaccharidase activities were expressed as micromoles of disaccharides hydrolyzed per minute per gram of protein.

Enterokinase was assayed in two steps. The mucosal homogenate was preincubated with trypsinogen and aliquots of the incubation mixture were then determined for trypsin formed. Trypsin activity was assayed according to the method of Erlanger (18) using benzoyl-DL-arginine-*p*-nitroaniline as the substrate. Activity was expressed as micromoles of substrate hydrolyzed per gram protein.

Leucine aminopeptidase was assayed by the method of Szasz (19) using L-leucine-*p*-nitroanilide as the substrate. The activity was expressed as micromoles of substrate hydrolyzed per minute per gram of protein.

The serum concentration of glucose was assayed using the Worthington Diagnostic Flozyme reagent. Serum insulin level was determined by radioimmunoassay using human insulin as the standard.

Morphometric measurements. Small sections of duodenum from control, fasted, and fasted-plus-glucose rats were excised and fixed immediately in 10% buffered Formalin. Fixed tissues were then carefully oriented and

embedded in paraffin. For each specimen, six serial sections of 5 μm thickness were made, fixed on slides, and stained with hematoxylin and eosin. The sections were then examined under light microscope at 400× magnification. Measurements of the sections were done with the help of a micrometer disc, scale 10 mm/100 units mounted in the eye piece. Vertical measurements were performed according to Fluge and Aksnes (20) to obtain the villus height, crypt depth, and the mucosal thickness. Ten separate villi were measured from each of two to three sample sections. Values were combined to obtain a mean with statistical variations.

Statistics. All results are presented as mean ± SEM. Differences in mean values between any two groups were evaluated by unpaired Student's t test with $P < 0.05$ considered as significant.

Results. Fasting for 5 days led to a significant loss in body weight in all groups (Table I). The reduction in body weight was minimized by supplementing fasting rats with glucose. Insulin alone had no effect. Adding insulin to glucose did not improve the condition achieved by glucose alone. The serum level of insulin was severely depleted in fasting rats. Glucose supplementation alone increased the serum insulin to a level comparable to that found in the insulin supplemented fasting rats but the levels were still only about one-half that of the control animals. Glucose and insulin, when given together, further increased the serum insulin level, i.e., higher than that of either insulin or glucose alone but still did not attain the level found in controls. The serum glucose level showed a decrease in fasted animal. This was corrected by glucose feeding alone or in combination with insulin. Insulin given to fasted rats did not lead to a further decrease in serum level of glucose.

The length of the small intestine was affected by fasting such that a significant shortening was observed (Table II). Glucose supplementation did not prevent this shortening, but insulin, either alone or in combination with glucose, lessened the degree of shortening but did not prevent it entirely.

Other measurements showed a significant decrease in mucosal weight, protein, and DNA content in both duodenal and jejunal segments of the fasted rats. Supplementation with glucose alone or in combination with insulin partially prevented the loss in mucosal weight and protein content but completely eliminated the effect of fasting on the DNA content. Supplementation with insulin alone did not affect any of the changes due to fasting.

The mucosal concentrations of various brush border enzymes in both the duodenal and jejunal segments of the small intestine are presented in Table III. Fasting resulted in a sharp drop in the concentration of enterokinase, sucrase, and maltase but did not change the concentrations of leucine aminopeptidase and lactase. Fasting had no effect on the proximal–distal gradient of all the five enzymes studied. Glucose feeding during fasting selectively prevented the decline of sucrase and maltase concentrations in the duodenal segment. In the jejunal segment, glucose supple-

TABLE I. EFFECT OF FASTING ON BODY WEIGHTS AND SERUM LEVELS OF INSULIN AND GLUCOSE

Treatment	No. of rats	Body weight			Serum concentration	
		Initial (g)	Final (g)	Δ (g)	Insulin (μU/ml)	Glucose (mg/dl)
Control	20	321 ± 13.7	356 ± 20.8	18.8 ± 3.3	36.2 ± 2.8	120.6 ± 8.8
Fasted	18	314 ± 10.1	249 ± 9.2[a]	(−61.0 ± 3.5)[a]	6.0 ± 0.6[a]	96.3 ± 4.6[a]
+ Glucose	14	313 ± 10.9	284 ± 9.8[a,b]	(−29.4 ± 3.9)[a,b]	13.6 ± 1.8[a,b]	110.4 ± 4.6[b]
+ Insulin	18	308 ± 5.7	235 ± 4.0[a]	(−67.4 ± 3.8)[a]	16.3 ± 2.4[a,b]	88.1 ± 8.3[a]
+ Glucose and insulin	11	310 ± 5.6	271 ± 7.8[a,b]	(−26.4 ± 3.8)[a,b]	23.0 ± 3.7[a,b,c]	125.2 ± 8.6[b]

Note. Values for body weight and serum concentrations are given as the mean ± SEM.

[a] Significantly different from control rats ($P < 0.05$).

[b] Significantly different from fasted rats ($P < 0.05$).

[c] Significantly different from fasted rats supplemented with glucose alone ($P < 0.05$).

TABLE II. EFFECT OF FASTING ON INTESTINAL LENGTH, MUCOSAL WEIGHT, MUCOSAL PROTEIN, AND DNA CONTENTS OF DUODENAL AND JEJUNAL SEGMENTS OF SMALL INTESTINE

Treatment	Intestinal length (cm)	Duodenal segment			Jejunal segment		
		Mucosal wt (mg)	Protein (mg)	DNA (mg)	Mucosal wt (mg)	Protein (mg)	DNA (mg)
Control	126.9 ± 1.2	359.0 ± 23.4	46.2 ± 3.5	2.39 ± 0.23	322.1 ± 29.7	41.9 ± 4.1	1.87 ± 0.37
Fasted	111.5 ± 1.7[a]	173.5 ± 12.7[a]	19.7 ± 1.5[a]	1.11 ± 0.08[a]	138.3 ± 13.0[a]	17.1 ± 1.0[a]	0.73 ± 0.10[a]
+ Glucose	114.0 ± 1.7[a]	254.4 ± 37.2[a,b]	32.6 ± 3.6[a,b]	2.40 ± 0.15[b]	256.0 ± 23.6[b]	30.0 ± 3.7[a,b]	1.99 ± 0.12[b]
+ Insulin	118.8 ± 1.5[a,b]	193.5 ± 15.3[a]	19.8 ± 1.9[a]	1.00 ± 0.11[a]	161.2 ± 10.8[a]	19.0 ± 1.6[a]	0.70 ± 0.10[a]
+ Glucose and insulin	119.0 ± 1.6[a,b]	245.0 ± 4.9[a,b]	29.2 ± 4.6[a,b]	2.20 ± 0.43[b]	253.6 ± 36.7[b]	26.1 ± 5.1[a,b]	1.92 ± 0.15[b]

Note. Values are given as mean ± SEM.
[a] Significantly different from control rats ($P < 0.05$).
[b] Significantly different from fasted rats ($P < 0.05$).

mentation, either alone or with insulin, not only prevented the decline but increased the concentrations of both sucrase and maltase to a level higher than that found in the controls. In contrast, glucose feeding, with or without insulin, led to further decline in the concentration of enterokinase and to a reduction in leucine aminopeptidase and lactase. Insulin alone did not prevent the decline of enterokinase, sucrase, and maltase in the duodenal segment but seemed to partially relieve the adverse effect of fasting on the maltase concentrations. There was also a significant increase in the concentration of lactase in the jejunal segment with insulin supplementation.

Changes in the intestinal contents of these enzymes were also observed (Table IV). Fasting led to a general reduction of the contents of all enzymes studied. Glucose supplementation served to maintain sucrase and maltase contents in both duodenal and jejunal segments. Enterokinase, leucine aminopeptidase, and lactase were also preserved at levels significantly higher than the fasting level but only in the jejunal segment. Insulin alone also tended to preserve these enzymes such that a majority of the enzymes had segmental contents significantly higher than those in fasted rats. However, the contents of maltase and sucrase retained were substantially lower than those found in the glucose-supplemented rats. Insulin and glucose when given together resulted in enzyme contents similar to those found in rats receiving glucose alone.

Since glucose treatment has been shown to cause significant increases in villus heights, similar mechanisms may be operating in the suppression of the atrophic effect in fasting by glucose. To investigate this possibility, morphometric parameters of the duodenal mucosa were measured and the data are presented in Table V. Fasting not only led to significant reductions in mucosal thickness and villus height, but also to an increase in crypt depth and hence a lower villus/crypt ratio. Glucose supplementation seemed to partially preserve the mucosal thickness, villus height, and the crypt depth and resulted in an improved villus/crypt ratio over that of fasting alone.

Discussion. Fasting is known to lead to atrophy of the small intestine. Present studies show that total inanition reduces not only the mucosal weight, its protein, DNA, and enzyme

TABLE III. EFFECT OF FASTING ON MUCOSAL ENZYME CONCENTRATIONS IN DUODENAL AND JEJUNAL SEGMENTS OF THE SMALL INTESTINE

	Enzyme concentration (units/g protein)				
	Duodenal segment				
Treatment	Enterokinase	Leucine aminopeptidase	Lactase	Sucrase	Maltase
Control	4.5 ± 0.4	23.7 ± 1.6	11.8 ± 2.4	84.8 ± 7.2	236.3 ± 12.8
Fasted	2.3 ± 0.2[a]	20.8 ± 1.7	10.1 ± 2.0	39.9 ± 4.4[a]	141.0 ± 9.8[a]
+ Glucose	1.5 ± 0.1[a]	13.5 ± 2.8[a,b]	4.8 ± 1.5[a,b]	81.4 ± 9.0[b]	246.7 ± 19.8[b]
+ Insulin	2.9 ± 0.2[a]	17.9 ± 1.8[a]	10.8 ± 2.4	51.7 ± 6.4[a]	146.8 ± 1.6[a]
+ Glucose and insulin	1.2 ± 0.2[a]	12.6 ± 2.5[a,b]	2.1 ± 1.6[a,b]	80.1 ± 4.6[b]	250.4 ± 18.5[b]
	Jejunal segment				
Control	1.1 ± 0.1	46.2 ± 3.8[a,b]	21.8 ± 2.6	116.5 ± 8.8	352.2 ± 4.3
Fasted	0.5 ± 0.1[a]	48.7 ± 3.3	22.6 ± 3.7	64.9 ± 6.8[a]	223.6 ± 11.8[a]
+ Glucose	0.5 ± 0.16[a]	37.3 ± 4.1	17.7 ± 1.8	197.6 ± 31.4[a,b]	515.5 ± 31.1[a,b]
+ Insulin	0.6 ± 0.1[a]	51.8 ± 3.0	38.4 ± 5.3[a,b]	74.2 ± 5.0[a]	314.3 ± 20.8[b]
+ Glucose and insulin	0.7 ± 0.1[a]	29.3 ± 2.2[a,b]	19.4 ± 4.0	223.1 ± 25.3[a,b]	529.1 ± 15.5[a,b]

Note. Values are given as mean ± SEM.
[a] Significantly different from control rats ($P < 0.05$).
[b] Significantly different from fasted rats ($P < 0.05$).

contents, but also leads to a shortening of the small intestine. More importantly, fasting affects the specific activities of different brush border enzymes differently. Of the five enzymes studied, the specific activities of sucrase, maltase, and enterokinase were reduced by

TABLE IV. EFFECT OF FASTING ON MUCOSAL ENZYME CONTENTS IN DUODENAL AND JEJUNAL SEGMENTS OF THE SMALL INTESTINE

	Enzyme content (Units/15 cm)				
	Duodenal segment				
Treatment	Enterokinase	Leucine aminopeptidase	Lactase	Sucrase	Maltase
Control	0.2 ± 0.03	1.08 ± 0.007	0.57 ± 0.13	3.7 ± 0.36	10.5 ± 0.83
Fasted	0.04 ± 0.005[a]	0.43 ± 0.06[a]	0.20 ± 0.04[a]	0.79 ± 0.10[a]	2.43 ± 0.28[a]
+ Glucose	0.05 ± 0.007[a]	0.39 ± 0.05[a]	0.13 ± 0.04[a]	2.52 ± 0.28[a,b]	7.33 ± 0.51[a,b]
+ Insulin	0.063 ± 0.006[a,b]	0.38 ± 0.04[a]	0.21 ± 0.05[a]	1.10 ± 0.17[a,b]	3.24 ± 0.40[a,b]
+ Glucose and insulin	0.029 ± 0.005[a]	0.30 ± 0.05[a,b]	0.04 ± 0.03[a,b]	2.25 ± 0.31[a,b]	6.84 ± 0.74[a,b]
	Jejunal segment				
Control	0.05 ± 0.002	1.96 ± 0.14	0.97 ± 0.15	4.81 ± 0.32	14.5 ± 1.05
Fasted	0.009 ± 0.003[a]	0.68 ± 0.09[a]	0.38 ± 0.07[a]	1.09 ± 0.01[a]	3.82 ± 0.04[a]
+ Glucose	0.022 ± 0.004[a,b]	1.12 ± 0.11[a,b]	0.57 ± 0.08[a,b]	7.18 ± 0.06[a,b]	15.6 ± 0.85[b]
+ Insulin	0.010 ± 0.007[a]	0.92 ± 0.25[a,b]	0.64 ± 0.08[a,b]	1.35 ± 0.12[a,b]	5.3 ± 0.46[a,b]
+ Glucose and insulin	0.021 ± 0.004[a,b]	0.77 ± 0.13[a]	0.54 ± 0.14[a]	6.0 ± 1.1[b]	13.0 ± 2.0[b]

Note. Values are given as mean ± SEM.
[a] Significantly different from control rats ($P < 0.05$).
[b] Significantly different from fasted rats ($P < 0.05$).

TABLE V. EFFECT OF FASTING ON MORPHOMETRIC PARAMETERS OF DUODENAL MUCOSA

Treatment	Mucosal thickness (μm)	Villus height (μm)	Crypt depth (μm)	Villus/crypt ratio
Control	536.7 ± 42.0	203.5 ± 36.8	125.3 ± 10.7	2.6 ± 0.4
Fasted + glucose	416.6 ± 12.3*	231.2 ± 10.1*	150.1 ± 8.6	1.5 ± 0.1*
Fasted	379.5 ± 7.0*†	145.5 ± 7.9*†	216.3 ± 20.6*†	0.7 ± 0.03*†

Note. Values represent mean ± SEM of 12–20 measurements.
* Values significantly less than corresponding control values with $P < 0.05$.
† Values significantly less than corresponding fasted and glucose values with $P < 0.05$.

one-half in both the duodenal and jejunal segments, while those of leucine aminopeptidase, and lactase remained unchanged. The selective effect on the various brush-border enzymes confirms the study reported by Ecknauer *et al.* where a shorter term of fasting was used (4). These results suggest that there are at least two groups of enzymes in the brush border with each under a separate metabolic control. This hypothesis is further strengthened by the following observations. Only sucrase and maltase concentrations were protected from decline during fasting by glucose supplementation, either alone or in combination with insulin. The same supplement had no effect on enterokinase concentrations, and caused a reduction in the concentrations of leucine aminopeptidase, and lactase, both of which were not affected by fasting alone.

Analogous observations have been obtained following glucose infusion of a self-emptying blind loop of the rat jejunum in that glucose induces increases in disaccharidases but not dipeptidase activities (21). Further, the glucose treatment was shown to lead to a concomitant increase of villus height. Our results partially confirm this observation in that glucose feeding during fasting seems to preserve the mucosal thickness and villus height better than the fasted group.

The present study also investigated the possible regulating factors involved in the observed changes caused by fasting. The sharp decline in insulin, and the slight decrease in glucose concentration in the serum noted in fasted rats suggest a disturbance in glucose homeostasis which may cause the changes observed in the small intestine. In young suckling mice, insulin has been shown to elicit the development of brush-border enzymes, particularly disaccharidases, and promotes the maturation of villus (12, 13). In addition, insulin administration to young rats was found to increase both sucrase and lactase concentrations (22). In this study with adult rats, insulin had no effect on enzyme concentrations and only slight effect on the small intestinal enzyme contents. This suggests a relative insensitivity of the intestinal mucosa to this hormone in adults as compared to young rats.

Our results further showed that when glucose was given in addition to insulin, most of the atrophic effects were prevented. However, supplementation with glucose alone was as effective. In fact, glucose alone may be slightly better than a combination of glucose and insulin (Table I), although the latter regimen led to a better recovery of the serum level of insulin. Since the serum glucose in insulin-supplemented rats did not change significantly in spite of the increase in serum insulin, it suggests that the lack of responsiveness of the small intestinal mucosa to insulin in the fasting adult rats is not likely a result of limitation of glucose.

The observation that glucose supplementation alone can alleviate most of the atrophic changes during fasting is interesting. The glucose effect seems to be direct and not insulin mediated since insulin alone was not effective.

It has been shown that the recovery of maltase and sucrase in rats following fasting depended on the dietary carbohydrate source (6). A sucrose diet led to a greater increase in sucrase than maltase, and a maltose diet led to a higher increase in maltase than sucrase, while glucose or lactose was much less effective. In contrast, in fasted human volunteers, refeeding with glucose or lactose was found to be equally effective in increasing both sucrase and maltase concentrations in their mucosa (7). Our results are in agreement with

the human findings in that glucose supplementation prevents the decline in both sucrase and maltase equally well.

The increases in enzyme activities, mucosal protein, and DNA to glucose supplementation in fasting rats suggests that caloric repletion is adequate for the maintenance of protein, DNA, and enzymes especially that of maltase and sucrase activities.

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