Monosaccharide Induction of 3-0-Methyl Glucose Transport Through the Rat Jejunum[1] (36258)

CLAUDE C. ROY AND REUBEN S. DUBOIS
(Introduced by Henry N. Claman)

Departments of Pediatrics, University of Colorado, Medical Center, Denver, Colorado and Ste. Justine Hospital, University of Montreal, Quebec, Canada

A number of recent studies on the small intestine have reported alterations in its absorptive capacity for monosaccharides in response not only to semistravation and starvation (1–4) but also to feeding patterns and specific diets (5–8). Although there have been a few reports on intestinal enzyme changes following feeding or the administration of certain diets (8–12), the relationship of the transport and enzyme changes to substrate specificity and intestinal cell metabolism remains unknown.

The following experiments were carried out in an effort to characterize the effect of substrate feeding on active transport using the previously described *in vitro* perfusion of isolated jejunal segments (13–15).

Material and Methods. The initial phase of the experiments consisted of a 48-hr period of constant *in vivo* duodenal feeding of either 3-0-methyl glucose (3-0-MG), glucose, or fructose. This was followed by the study of 3-0-MG absorption against a concentration gradient in extracorporeally perfused segments of upper jejunum removed from the animals with an intact superior mesenteric artery and portal vein.

1. In vivo *duodenal feeding of substrates.* Under pentobarbital sodium anesthesia, an intraduodenal cannula was positioned through a gastrostomy in six groups of male Sprague-Dawley rats weighing 200–250 g. The animals were then restrained, fasted, and intraduodenally perfused for 48 hr at a rate of 1 ml/hr with a physiological electrolyte solution or with a 50 m*M* concentration of either 3-0-methyl glucose (3-0-MG), fructose, or glucose by means of a Harvard constant flow pump (Model No. 975, Harvard Apparatus Co. Inc., Dover, MA.) The pH of these solutions and their osmolality were adjusted, respectively, to 6.9 and to 300 mOsm/kg. Two further groups of animals were similarly prepared and infused with a 1.39 *M* solution of either glucose or fructose. These solutions also had a pH of 6.9 but their osmolality was 1,600 mOsm/kg.

2. *In vitro study of 3-0-MG absorption.* After 48 hr of *in vivo* duodenal feeding of the various substrates, the active transport of 3-0-MG was studied. Briefly, the *in vitro* vascular perfusion of the intestine consists in a 20-cm segment of rat upper jejunum which is completely removed from the animal and perfused extracorporeally through the superior mesenteric artery. The blood perfusate consisted of 20% washed bovine red cells suspended in a physiological electrolyte solution with 2.5% dextran (mol wt 78,000), 2.5% bovine serum albumin, 100 mg% of D-glucose and 100 mg% of 3-0-MG. It was oxygenated by means of a rotating mesh screen using a humidified 95% O_2 and 5% CO_2 gas mixture. Circulation through the vascular system of the rat jejunal segment was maintained at a rate of 2.5 ml/min by a servo-controlled peristaltic roller-type pump with an asymmetrical head ("Ambec" extracorporeal perfusion unit model No. 100, MX International, Longmont, CO). All the venous effluent was collected through the cannulated portal vein in 1 min aliquots.

Both ends of the jejunal segment were can-

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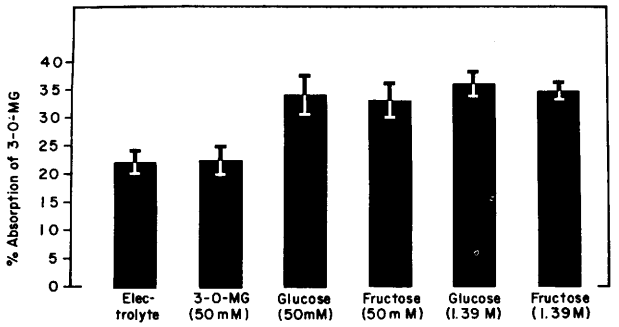

FIG. 1. Absorption of 3-O-methyl glucose from isolated perfused jejunal segments after various *in vivo* perfusion regimens. Results are expressed as % absorption/min/g of dry mesentery-free jejunal segment. Each column represents the mean ± the SE of 8 experiments except for the glucose 1.39 *M* group which is made up of 7 experiments.

nulated. The intestinal lumen was perfused at a rate of 1 ml/min with 100 mg% of 3-O-MG (Calbiochem, Los Angeles) and 2 μCi of ^{14}C-labeled 3-O-MG (New England Nuclear Corp., 5.0 mCi/m*M*) in the same physiological electrolyte solution used as a vehicle for the duodenal substrate feeding and for the artificial plasma. Once placed in the tissue chamber at 37°, the intestinal preparation was perfused for 5 min before the constant luminal perfusion of 3-O-MG was started. As shown previously (13–15), after an initial 8–10-min period of rapid substrate accumulation, a relatively steady rate of 3-O-MG influx into the portal venous effluent was noted. The experimental period during which plasma ^{14}C activity was assayed in 1-min aliquots in a Packard 300 Tri-Carb liquid scintillation spectrometer was begun 12 min after starting the luminal perfusion and extended for 24 min. The mean percentage minute absorption of 3-O-MG was calculated by dividing the concentration of substrate in the portal venous effluent times the minute volume with the mean concentration of luminal substrate. The absorption of 3-O-MG/min was then corrected per gram of lyophilized, mesentery-free jejunal segment.

3. *Biochemical studies.* Jejunal biopsies were taken 20 cm from the ligament of Treitz at the time of preparing the isolated extracorporeally perfused segment of upper jejenum. Protein (16), glucose, and hexokinase activity were measured. The full thickness biopsy was plunged into boiling water after rinsing and blotting its mucosal surface. It was homogenized, then deproteinized with 5% $ZnSO_4$ and 0.3 *N* NaOH before analyzing the supernates by the D-glucose oxidase method (17). Jejunal mucosal scrapings were weighed and frozen at —40°. The particle-free supernatant fractions were assayed for hexokinase activity (18).

Results. A graphic representation of the percentage minute absorption of 3-O-MG is shown in Fig. 1. In those animals prepared for the *in vitro* study by a 48-hr duodenal infusion of electrolyte or of 50 m*M* 3-O-MG, their mean absorption was significantly different from the values obtained in the other four groups. A one-way analysis of variance gave an *F* ratio of 4.96 which corresponds to a $p < .01$. However, comparison between the electrolyte or the 3-O-MG group with either of the others using the Newman–Keuls method for multiple comparison of the means reduces the level of significance to a $p < .05$.

The caloric value of the substrates given in the duodenum varied between 0 and 25 cal/24 hr (Table I). Although some animals infused with 1.39 *M* glucose or fructose were noted to have loose stools, their percent weight loss during the 48-hr period of substrate feeding did not differ from that incurred by the other groups (Table I). The average weight loss was higher in the electrolyte and 3-O-MG groups but a one way analysis of variance shows no significance to these differences.

Table II summarizes the effect of substrate

TABLE I. Weight Loss in Relation to Calories Fed and Substrate Infused.

Substrate infused	No.	Calories fed	% Weight loss[a] ($\bar{x} \pm$ SE)
Electrolyte solution	8	0	14.8 ± 1.65
3-O-MG (50 m*M*)	8	0	14.1 ± 1.35
Glucose (50 m*M*)	8	0.9	10.7 ± 1.68
Fructose (50 m*M*)	8	0.9	11.5 ± 1.38
Glucose (1.39 *M*)	7	25	11.7 ± 1.25
Fructose (1.39 *M*)	8	25	11.2 ± 0.90

[a] The percent weight loss represents the difference between the weight recorded at the time of duodenal intubation through a gastrostomy and the weight at the termination of the 48-hr period of steady duodenal substrate infusion. Calories were calculated on the basis of 4 cal/g of metabolizable carbohydrate.

feeding on the endogenous D-glucose content of whole jejunal wall homogenates. There was no difference in protein or in glucose content expressed as a glucose/protein ratio among the four groups of animals where this was measured. Hexokinase activity of the particle-free supernatant fraction of mucosal scrapings was significantly higher in the animals fed 3-O-MG and glucose than in the ones receiving an identical concentration of fructose.

Discussion. In vitro studies with everted gut sacs have shown that semistarvation (19) enhances the normally occurring active transport of 3-O-MG (20), a nonmetabolizable glucose analog (21). These results were consistent with the idea that dietary restriction is an important stimulus to a nonspecific increase in intestinal absorptive capacity since these conclusions were also valid for amino acids and L-sugars. By contrast, a more recent report (12) shows a greater 3-O-MG transport in the fed vs. the fasted intestine. In the experiments described, it has been possible to measure absorptive changes of 3-O-MG in response to the administration of certain substrates at various concentrations. The data presented show an increased absorption in the animals intraduodenally perfused with the substrates, glucose and fructose (Fig. 1). These adaptive changes in 3-O-MG absorption were apparently independent of percent weight loss and calories fed. (Table I).

One of the important points is that substrate enhancement of 3-O-MG transport was not quantitatively related to the concentration of substrate infused. In fact, a 25-fold increase in the concentration of glucose or fructose was unsuccessful in potentiating the effect already observed with the lower concentration. It is surprising that fructose, a monosaccharide which is not accumulated in the cell against its own concentration gradient and is absorbed by facilitated diffusion (22), has the same effect as glucose on the transport of 3-O-MG, a glucose analog. There is, however, a recent report which suggests that fructose may be actively transported (23). Unlike glucose and fructose, 3-O-MG administered *in vivo* did not enhance the absorption of 3-O-MG studied *in vitro*. This observation suggests that the specific mechanism allowing the entry and transport of 3-O-MG across the lipid–protein membrane of the intestinal cell is adaptive only to a metabolizable monosaccharide, but in a nonspecific fashion, since both glucose and fructose were equally effective.

The sole function of the carrier in any transport system is to cause the solute to become temporarily soluble in the lipid layer of the membrane. The driving force for the actual translocation can be, either, a concentration gradient or a pump which is activated by a stepped-up metabolism. The active transport of L-glucose by everted intestinal sacs from semistarved animals has been hypothetically explained by an associated decrease in intestinal wall glucose content

TABLE II. Jejunal Glucose and Hexokinase Activity.[a]

Substrate infused	No.	Glucose ($\bar{x} \pm$ SE)	Hexokinase ($\bar{x} \pm$ SE)
Electrolyte	8	4.0 ± 0.6	—
3-O-MG (50 m*M*)	8	4.5 ± 0.6	22.3 ± 1.6
Glucose (50 m*M*)	8	3.8 ± 0.4	23.5 ± 1.5
Fructose (50 m*M*)	8	3.5 ± 0.5	18.2 ± 1.5

[a] Glucose determinations are expressed as μg/mg protein and were carried out on whole bowel wall homogenates. Hexokinase activity is in mμM/min/mg protein and was done on mucosal scrapings.

(24). The present experiments (Table II) showed no difference in bowel wall glucose content. There was, consequently, no correlation between endogenous D-glucose, the type or concentration of substrate fed, and the transport of 3-O-MG. Hexokinase activity was higher in the glucose than in the fructose-fed animals. However, this difference was not associated with either a lower bowel wall glucose content or a higher percentage minute absorption of 3-O-MG. Consequently, our data provide no support for adaptive changes in 3-O-MG absorption in terms of a favorable gradient or of a stepped-up metabolism of glucose accelerating the transport of 3-O-MG.

Summary. The effects of a 48-hr intraduodenal perfusion of electrolyte solution, 50 m*M* 3-O-methyl glucose (3-O-MG), glucose, or fructose, 1.39 *M* glucose or fructose on the subsequent absorption of 3-O-MG were studied in 20-cm segments of rat jejenum perfused extracorporeally through the superior mesenteric artery. The feeding of glucose or fructose enhanced the transport of 3-O-MG while 3-O-MG itself had no effect. This adaptive change was independent of substrate concentration, calories fed, weight loss, bowel wall glucose content, and hexokinase activity of mucosal scrapings.

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