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Biology of Intestinal Sugar Transport

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Textbooks of physiology and gastroenterology describe the small intestine as having an enormous capacity to absorb sugars. For example, Crane (1) calculated that

> the total daily capacity [of human intestine] is 10,211 g of a mixture of glucose and fructose, an amount equivalent to over 22 pounds of sugar and more than 50,000 calories. . . . Such a capacity for sugar absorption is ten times more than would be needed to provide for even the most unreasonable individual caloric requirements, since foods in addition to sugars are generally also eaten, and its great size indicates that control of sugar absorption is not exerted at the level of the processes of digestion and absorption at the brush border membrane.

This conclusion has always been puzzling from the perspective of evolutionary biology and common sense. One does not observe animals supporting large expensive structures that serve no function, for the reason that biosynthetic energy is at a premium. Animals that waste energy will be outcompeted by animals that use available energy productively. Considerations of economy in the broadest sense are the reason why natural selection ultimately causes cave animals living in dark environments to lose functional eyes, and bacteria or animals with free access to nutrients to lose the metabolic machinery for synthesizing those nutrients (2,3).

From this point of view, it was unexpected that the intestine should possess a capacity for absorbing sugar far beyond what it might be called upon to use. Sugar transporters, like any proteins, cost biosynthetic energy and occupy space. Even more puzzling were discoveries of the past decade, showing that intestinal transporters for sugars as well as for virtually every other nutrient examined are regulated closely according to physiological demand (4,5). The number of intestinal sugar transporters varies adaptively with dietary carbohydrate content, metabolic rate, stage of ontogenetic development, and evolutionary constraints imposed by the natural diet (6–16). If the intestine really did possess absorptive capacity in enormous excess, there would seem to be no reason to regulate absorptive capacity.

I shall describe here the recent resolution of these paradoxes. It turns out that intestinal sugar transport exemplifies nature's universal principle, "Enough but not too much." I begin with some brief background on intestinal sugar transport. Next, I summarize the evidence for adaptive regulation of intestinal sugar transport. Fi-

nally, I describe the resolution of these paradoxes through recent remeasurements of sugar concentrations in the intestinal lumen during absorption.

BACKGROUND ON INTESTINAL SUGAR TRANSPORTERS

Many dietary complex carbohydrates are hydrolyzed in the lumen of the small intestine to disaccharides, which are then hydrolyzed to monosaccharides by brush-border-bound disaccharidases. The monosaccharides are then transported into the bloodstream across the mucosa of the intestinal epithelium, which poses two membrane barriers in series to the transepithelial movement of solutes. The first barrier is the so-called apical or brush-border membrane, which separates the intestinal cell cytoplasm from the intestinal lumen. The second membrane is the so-called basolateral membrane, which separates the cell cytoplasm from the interstitial fluid and bloodstream.

The brush-border membrane contains several transport proteins responsible for sugar uptake. Best known is the cotransporter that conveys D-glucose and sodium, and there is a separate sodium-independent transporter for fructose. There appear to be several glucose transporters that differ in their capacities, Michaelis-Menten constants, relative affinities for glucose and galactose, and distribution along the intestinal axis from duodenum to ileum. The basolateral membrane contains a different set of transporters responsible for sugar egress from the cell.

Our work has focused on the brush-border transporters responsible for sugar uptake. We measure uptake by the everted sleeve technique, an *in vitro* method in which a cylindrical sleeve of intestine is excised, turned inside out so that the epithelial cells face outward, and mounted on a solid glass rod over a stirring bar (17). The tissue sleeve is incubated for 30 seconds to 8 minutes in a solution containing radiolabeled sugar plus a marker for adherent extracellular fluid. At the end of the incubation, we count the tissue to determine the uptake of sugar across the brush-border membrane. In the steady state, this must equal the egress of sugar across the basolateral membrane plus metabolic consumption of sugar by the intestinal cells themselves.

REGULATION OF SUGAR ABSORPTION

Intestinal sugar absorption is regulated at at least four levels: by dietary sugar levels in the adult, by overall metabolic rate, by natural diet on an evolutionary time scale, and by the sequence of ontogenetic demands faced by the growing animal.

Figure 1 illustrates how the intestinal brush border's capacity to absorb glucose varies with dietary carbohydrate levels. When mice are switched from a no-carbohydrate diet to a high-carbohydrate diet, their brush-border capacity to take up glucose doubles within about a day and declines again reversibly within about 3 days after the mice are returned to a no-carbohydrate diet. Activity of the separate transporter for fructose similarly increases reversibly with dietary carbohydrate levels.

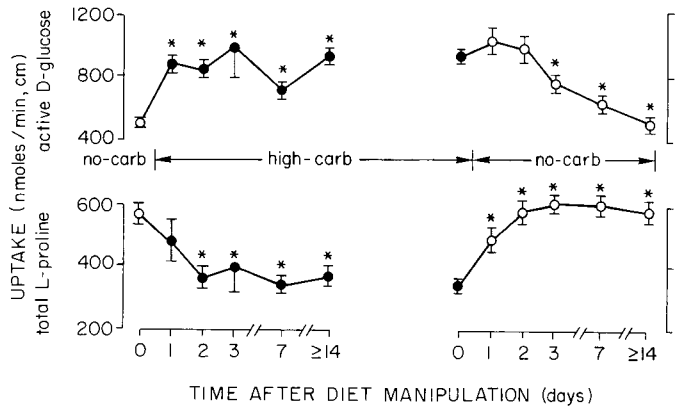

FIG. 1. This figure illustrates the reversible regulation of intestinal sugar and amino acid transport by dietary carbohydrate and protein levels. **Left:** Mice were switched from a no-carbohydrate (no-carb) high-protein diet (○) to a high-carbohydrate (high-carb) low-protein diet (●) at time $t = 0$. Subsequent points give intestinal brush border uptake rates for active D-glucose transport **(top)** and total transport of L-proline **(bottom)** after the indicated number of days on the high-carbohydrate low-protein diet. **Right:** Mice were switched back from a high-carbohydrate low-protein diet to a no-carbohydrate high-protein diet at $t = 0$. Subsequent points give uptake rates after the indicated number of days on the no-carbohydrate high-protein diet. Uptakes were measured *in vitro* in proximal jejunal sleeves incubated in solutions containing D-glucose or L-proline at 50 mM. Asterisks indicate uptake values that differ significantly at the $p < 0.05$ level from the corresponding $t = 0$ value. Note that D-glucose uptake quickly doubles in mice consuming the high-carbohydrate diet and declines more slowly in mice consuming the no-carbohydrate diet, whereas L-proline uptake similarly quickly increases in mice consuming the high-protein (no-carbohydrate) diet and declines more slowly in mice consuming the low-protein (high-carbohydrate) diet. From Karasov WH, et al. (7).

Measurements of the number of glucose transporters by means of phlorizin binding (phlorizin being an inhibitor of the glucose transporter) demonstrate that these diet-induced changes in brush-border transporter activity arise from changes in the number of transporters in the brush border (11).

Recall that enterocytes are being produced continually in intestinal crypts, mature as they migrate along the villi, and eventually slough off the villus tips after several days. At what level along this crypt-villus axis does dietary regulation of sugar transport activity take place? Does dietary programming of transport activity occur irreversibly once-and-for-all in developing cells in the crypts, or does it operate reversibly in mature enterocytes? We studied this problem by measuring numbers of glucose transporters (detected as phlorizin binding sites) as a function of enterocyte position from crypt to villus and also as a function of time after a change in dietary carbohydrate levels. It turned out that dietary regulation operates irreversibly at the crypt cell stage. Sugar transporter activity is fixed in crypt cells by dietary sugar levels prevailing at that time and is not reset in mature villus cells even if dietary carbohydrate levels change during cell migration from crypt to villus.

Intestinal transporters for all classes of amino acids, all trace minerals studied, and several water-soluble vitamins also are regulated by dietary levels of their sub-

strates (4). If the intestine really did possess absorptive capacity in enormous excess, the regulation of this capacity that I have described would pose a second paradox. We would have to ask not only why excess capacity is present at all but also why it should be regulated.

The second type of regulatory response of intestinal absorption involves adaptations to overall metabolic rate. The examples of the preceding paragraphs involve reversible changes in specific transporters in response to dietary levels of their particular substrates, but there are also circumstances under which an animal experiences a reversibly increased need for all nutrients. Such circumstances include pregnancy, lactation, chronic exercise, and low environmental temperatures. Under all such circumstances, one observes hyperplasia of the intestinal mucosa itself, whose mass may double (6). The result of more intestinal mucosa is increased absorptive capacity for all nutrients. Whereas reversible regulation of specific transporters by their dietary substrates operates on a time scale of ½ to 3 days, intestinal hyperplasia is a slower response that involves up to several weeks.

The third level of regulation of intestinal sugar transport is much slower on an evolutionary time scale. Animal species vary greatly in the carbohydrate/protein ratios normally encountered in their natural diets. Carnivores normally consume a high-protein/low-carbohydrate diet, herbivores consume a high-carbohydrate/low-protein diet, and omnivores face variable dietary carbohydrate and protein levels. Figure 2 illustrates that intestinal sugar uptakes are higher in herbivores than in omnivores and higher in turn in omnivores than in carnivores (13–15). In our experience, the record for intestinal sugar absorptive capacity is held by hummingbirds, whose natural diet consists of nectar with sugar concentrations often exceeding 1 M (10). Since I have already explained how intestinal sugar transporters are regulated reversibly (at least in mice) by dietary sugar levels, one might wonder whether these species differences are reversibly determined by differences in the diet that each species happened to be consuming at the time of study. However, when one studies intestines from carnivorous, omnivorous, and herbivorous species all eating identical diets, sugar transport rates are still highest in herbivores, lower in omnivores, and lowest in carnivores (13).

The evolutionary forces of natural selection have programmed not only the activity levels of sugar transporters but also their regulatory apparatus, according to each species' natural diet. That is, it is not only the case that herbivores have higher sugar transport than do carnivores consuming the same diet. It is also the case that strict carnivores, such as cats, adult bullfrogs, and trout, whose natural diets always contain very low carbohydrate levels, lack the regulatory apparatus for upregulating intestinal sugar transport when the animal is placed on an (unnaturally) high-carbohydrate diet. In contrast, omnivorous species, such as mice and rats, and carnivorous species belonging to omnivorous families, such as carnivorous mink in the omnivorous weasel family, do possess the ability to regulate intestinal sugar transport reversibly according to dietary carbohydrate levels. Thus, not only transporter activities but also their regulatory machinery are adapted to a species' natural diet.

The remaining level on which intestinal sugar transport is regulated occurs during

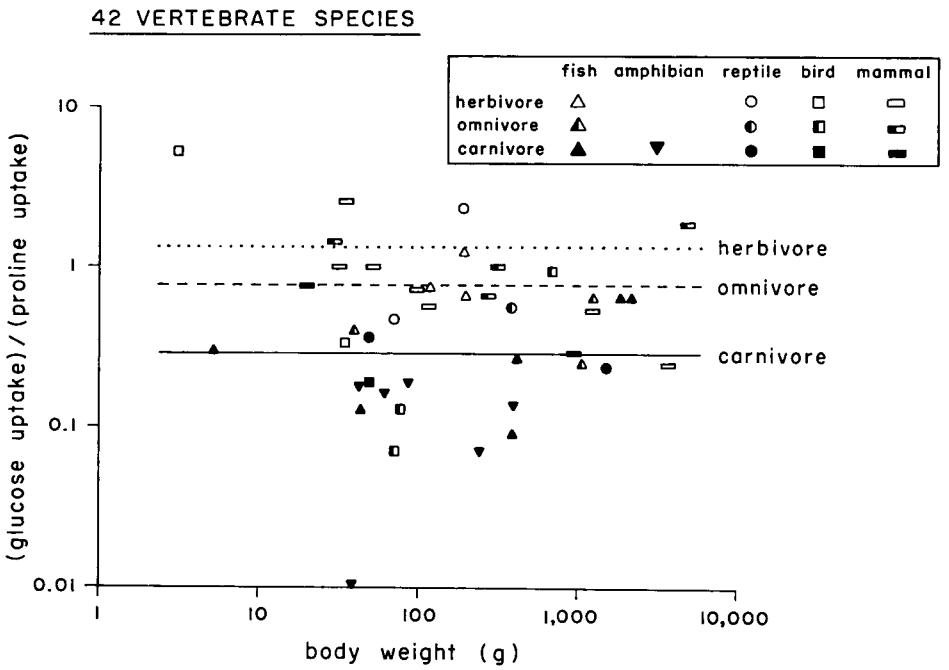

FIG. 2. Relative rates of sugar and amino acid transport in intestines of herbivorous, omnivorous, and carnivorous species of all five higher vertebrate classes, as measured *in vitro* by the everted sleeve technique. Each species was eating its natural diet or one of nutrient composition similar to the natural diet. The ordinate is the ratio of the intestine's total uptake capacity (i.e., the uptake capacity of the whole length of the intestine or small intestine) for the sugar D-glucose to its uptake capacity for the amino acid L-proline. Note that this ratio is highest for herbivores, intermediate for omnivores, and lowest for carnivores. Most of the variation is in glucose uptake (highest in herbivores, lowest in carnivores) rather than in proline uptake. There is no significant dependence of these ratios on body mass, so the horizontal lines depict average values of the ratio for each trophic group. Thus, intestinal rates of glucose absorption vary among species according to the carbohydrate contents of their natural diet (highest in herbivores, lowest in carnivores). Karasov WH, Diamond JM (15).

ontogenetic development. Whereas dietary carbohydrate levels may change repeatedly and unpredictably in adults of omnivorous species, most species traverse a predictable sequence of dietary carbohydrate levels as they proceed through ontogenetic development. For example, baby cats start off on high-carbohydrate milk and grow up to consume a low-carbohydrate meat diet as adults. Humans consume most of their carbohydrate as lactose while babies, but much of it as complex polysaccharides as adults. Herbivores, such as rabbits, consume much carbohydrate both in the suckling stage and as adults, but fructose does not normally enter the diet until after weaning. As Fig. 3 illustrates, intestinal fructose absorption is very low in suckling rabbits and increases 10-fold around the time of weaning, when rabbits normally begin to ingest solid food and encounter dietary fructose (18). On the other hand, cats normally encounter negligible fructose in their adult diet, and

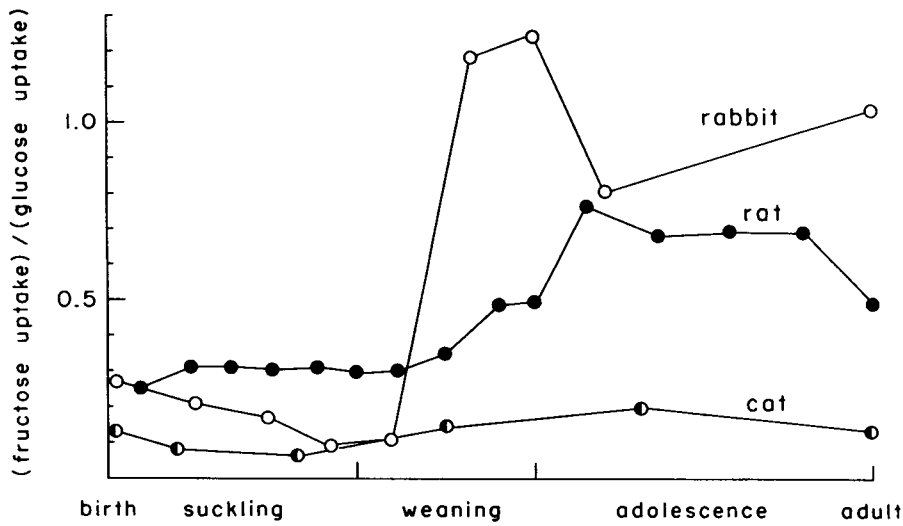

FIG. 3. Brush border uptake of the sugar fructose, divided by uptake of the sugar D-glucose, as a function of postpartum developmental stage in three mammal species. Uptakes were measured *in vitro* at 50 mM by the everted sleeve method. Note that fructose uptake rises steeply at the time of weaning in rabbits and does so less steeply in rats but that there is no significant change in fructose uptake at the time of weaning in cats. Thus, only in herbivorous and omnivorous species, in which fructose suddenly begins to appear in the natural diet at the time of weaning, does the intestinal fructose transporter turn on at that time. From Buddington RK, Diamond JM (16).

there is correspondingly no turn-on of intestinal fructose transport in cats around the time of weaning.

In addition to these qualitative changes in intestinal sugar transport around the time of weaning, there are also quantitative changes. For example, even in herbivores, the intestine's sugar transport capacity normalized to body weight tends to decline with age, corresponding to the decrease in mass-specific metabolic rate with age (16).

LUMINAL SUGAR CONCENTRATIONS AND THEIR SIGNIFICANCE

Let us now return to the twin paradoxes with which I began this article. Why does the intestine waste biosynthetic energy on sugar transport capacity far in excess of what it will ever be called upon to use? Why waste energy and metabolic machinery on regulating sugar transport at so many levels if transport capacity is present in enormous excess?

The resolution of this paradox proves to hinge on correct values for luminal sugar concentrations in the intestine during absorption. In the early and middle part of this century, many attempts were made to measure concentrations of sugars and other nutrients in the intestine during absorption (19–21). These studies often re-

ported intestinal luminal sugar concentrations of 50 to 500 mM, several orders of magnitude greater than the Michaelis-Menten constants for the transporters. However, these older values were based on nonspecific methods for measuring glucose. Earlier authors also failed to realize the importance of centrifuging the intestinal contents and rapidly halting enzymatic activity liberating glucose from complex carbohydrates after drawing of luminal samples. When specific enzymatic methods were finally applied in 1977 to measuring intestinal sugar concentrations (22), it became apparent that most of the material recorded as glucose by older methods was not glucose at all.

Hence, my colleagues and I have been redetermining values for nutrient concentrations in the intestinal lumen in mammal species eating different natural diets, at different intestinal positions, and at different times of day, and while the animals were consuming different actual diets. We take care to separate the supernatant from intestinal contents and to halt hydrolytic activity quickly after drawing samples. With these methods, it turns out that luminal sugar concentrations are mostly in the range 1 to 30 mM. The validity of these modern measurements is confirmed by their yielding correct values for sugar concentrations added experimentally to intestinal lumen samples and by their accounting correctly for measured osmolarities of the samples.

These new values for intestinal lumen concentrations resolve four puzzles. First, we have recalculated intestinal capacity to absorb sugars, based on sugar concentrations prevailing physiologically rather than on enormously overestimated values. For all life stages of all species that we have analyzed, intestinal sugar absorptive capacity is only modestly in excess of normal sugar intake in that species at that life stage on that diet. Rarely is the excess capacity by as much as a factor of 2. Thus, sugar transporters conform to the motto, "Enough but not too much"—that is, the intestine synthesizes enough sugar transporters to cope with sugar intake and provide for a modest margin of safety but does not waste biosynthetic energy and space on unneeded transporters. If dietary carbohydrate levels increase, more sugar transporters are synthesized reversibly and then eliminated when dietary carbohydrate levels decline again. Throughout mammalian ontogenetic development, from birth through weaning to adulthood, there is a close match between normal sugar intake and intestinal absorptive capacity.

Second, the observation that virtually all intestinal nutrient transporters studied prove to be regulated by dietary substrate levels now makes sense. The function of regulation is to match absorptive capacity to momentary demand by synthesizing or eliminating needed or excess transporters as appropriate.

Third, effective Michaelis-Menten constants for intestinal sugar and amino acid transporters now also make functional sense. Under physiological conditions, these are typically of the order of a few millimolar. Much lower Michaelis-Menten constants are measured in vesicles where unstirred layers are much smaller, as a result of unstirred layer effects discussed by Barry and Diamond (23). In other systems, one observes Michaelis-Menten constants to be matched to prevalent substrate concentrations, with the effect of maximizing regulatory sensitivity (24). It would hardly make sense for the intestine to have transporters with Michaelis-Menten constants

on the order of a few millimolar if prevalent nutrient concentrations were actually 2 orders of magnitude greater. In reality, there now proves to be a good match between Michaelis-Menten constants and prevalent substrate concentrations for intestinal transporters, as for other enzymes.

Finally, remeasured luminal nutrient concentrations also make it possible to resolve a puzzle about the mode of intestinal sugar absorption. Although most studies of intestinal sugar absorption in recent decades have considered active transport to be responsible for most sugar absorption, Pappenheimer and Reiss (25) claimed that most sugar is absorbed passively by a mechanism that they termed solvent drag. However, their calculations of solvent drag were based on the luminal sugar concentrations of 50 to 500 mM reported in the older literature. Using correct values for luminal sugar concentrations makes it once again clear that the contribution of solvent drag to sugar absorption under physiological conditions is negligible and that most sugar absorption is by means of specific transporters.

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DISCUSSION

Dr. Cowett: It is important to point out that birth period is not a single time point but a heterogeneous period of development that may occur anywhere from 26 to 42 weeks and can result in a low-birthweight survivor. Are there any studies in the literature about the ontogeny of development of the transport systems you have discussed? There are attempts at very early nutritional support of the newborn per os to try to diminish the possibility of necrotizing enterocolitis. Studies of glucose transport would be important to establish the physiological basis for the success of early feeding of premature infants.

Dr. Diamond: An interesting question. When one studies experimental animals or the human fetus before term, at what stage during pregnancy do the transporters appear? Most of the transporters examined appear well before the normal time of birth, in the last trimester or even earlier. This means that the intestine has designed itself with protection against some uncertainty in timing of birth, such that the intestine will still be able to absorb if the animal is born early.

Dr. Kretchmer: Have you done the reverse? Have you brought up a cat on a sucrose diet maintaining the protein sufficiently high to prevent adverse reactions, and will the cat then develop fructose transporters?

Dr. Diamond: Another very interesting question. Let me answer in two ways. We tried to induce glucose transport in adult cats by putting them on a high-carbohydrate diet. Although we can induce glucose transport in herbivores and omnivores, we have not been able to do so in adults of any species of carnivore we have studied except for mink, which are a carnivorous species in an omnivorous family. We have not exposed cats to sucrose in infancy. The closest parallel that we have done was to ask whether there are any critical period effects of early diet in mice by exposing them through their mothers' gestation and then through the suckling period to either high-carbohydrate or high- protein diets and then seeing whether in adulthood those mice showed a permanent effect of the diet. There was no critical period effect on sugar or amino acid transport. We have not done the experiment you suggested.

Dr. Kretchmer: I am not sure my question is valid because the cat has very low sucrase levels, very low concentrations.

Dr. Holdsworth: As far as development of sugar absorption is concerned, active transport develops in the chick around the time of hatching or a day or two before. It has been done in man, and was found to be quite early—I believe in the first trimester.

Dr. Lentze: You showed the changes in glucose uptake according to the diet. I was very surprised by the kinetics of the change in respect to glucose and proline uptakes. Do you have any comment?

Dr. Diamond: As you point out, the switch from a no-carbohydrate to a high-carbohydrate diet or from a low-protein to a high-protein diet causes a rise in transporter activity that is more rapid than the subsequent decline of transporter activity. That is true for both glucose and proline (see Diamond, Fig. 1). I do not know whether the explanation relates to irreversible programming of transporter activity in the crypts and whether that might somehow account for this time assymetry.

Dr. Truswell: In Dr. Holdsworth's data, the absorption rate of galactose was slightly better than for glucose. In the newborn period in mammals, the load of galactose is much higher than at any other stage in life. Is it possible that there might be enhanced absorption of galactose over glucose at that period?

Dr. Diamond: In our experiments there is, but it is a second-order effect. We have compared glucose and galactose transport in developing rats and rabbits from birth to adulthood. There is a slight decline in the galactose/glucose transport activity ratio with developmental age, but it needed a refined statistical analysis to detect it. It is on the order of only 20%, so it is not likely to be of major physiological significance. Glucose and galactose, as you know, compete for the same transporter. There may be several glucose transporters whose proportions change developmentally.

Dr. Lifschitz: Do you have any information about transport sites in relation to intestinal hyperplasia following experimental bowel resection?

Dr. Diamond: There is substantial evidence that small bowel resection results in hypertrophy of the remaining bowel segments, which to some extent takes over the transport capacity of the segment that was removed.