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Effects of Sugars on Leucine and Lysine Uptake by Intestinal Cells from Rats Fed Sucrose and Stock Diets (38984)

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One of the most interesting aspects of the changing pattern of carbohydrate consumption in the United States (1) is the relationship between the increased intake of sucrose and the etiology of various diseases. Several controversial hypotheses have implicated the increased ingestion of sucrose, as compared to ingestion of the more complex carbohydrates, as an important factor in the etiology of coronary heart disease (2–4) and diabetes (4, 5). If dietary sucrose is an etiological factor in any disease, an understanding of the specific metabolic characteristics of fructose may provide information as to the mechanisms that may be involved.

Recent studies have shown that the small intestine contains a specific pathway that mediates the active transport of fructose (6–8). As part of a previous study, it was observed that fructose activated leucine uptake by intestinal epithelial cells (9). Since only a minor component of fructose has been shown to be metabolized in the small intestine of the rat during transport (10), the activation by fructose is difficult to attribute to an increase in a rate limiting energy supply. The effects of fructose, glucose and sucrose on the uptake of both a neutral and a basic amino acid by isolated rat intestinal cells were investigated in order to further define the scope of the possible interactions between sugar and amino acid transport. In addition, the effect of diet on these processes was also investigated since it has been shown that rats fed a high sucrose diet exhibited a greater intestinal transport of fructose, glucose, and sucrose than did rats fed a stock diet (11).

Materials and methods. Fourteen male weanling Wistar strain rats were randomly divided into two equal groups. One group of rats was fed a commercial ground stock diet. The other group was fed a diet containing 65% sucrose, 20% casein:lactalbumin (1:1), 5% corn oil, 5% Bernhart–Tomarelli salt mix, 4% cellulose and 1% vitamin diet fortification mixture. The animals were housed individually in stainless steel wire mesh cages in a temperature-humidity controlled room having equal 12-hr periods of light and dark. Food and water were available at all times. Body weight and food intake were determined weekly. The transport studies were conducted after the rats had been maintained on their respective diets for 9–10 weeks.

The methodology used to prepare the isolated intestinal cells has been described in detail (9). Briefly, the rats were killed by decapitation and the excised intestinal segments were filled with an oxygenated incubation medium containing 50 m*M* sodium citrate and 1.5 mg/ml hyaluronidase to dissociate the epithelial cells from the mucosa. After a 15-min incubation at 33°, the segments were drained, flushed with saline, and partially filled with an oxygenated collection medium. The epithelial cells were released from the underlying tissue by patting the serosal side with the fingers. The cells were collected by centrifuging the luminal contents for 5 min at 275*g* in a refrigerated centrifuge. The cell pellet was dispersed in 10 times its volume of ice cold Krebs–Ringer phosphate buffer, pH 7.1, filtered through a double layer of gauze, and then collected by centrifugation as indicated above.

To measure leucine and lysine uptake, 0.3 ml of the cells representing an average of 3.51 mg protein were added to 5 ml of a modified oxygenated Krebs–Ringer phosphate buffer (pH 7.1) in which two-thirds of the $CaCl_2$ was isotonically replaced by NaCl (12). The medium contained 120.8 m*M* NaCl, 4.7 m*M* KCl, 0.8 m*M* $CaCl_2$, 1.2 m*M* $MgSO_4$, 15.4 m*M* Na_2HPO_4, and radioactive (10,000–15,000 cpm/ml) and nonradioactive leucine or lysine to a final concentration of

1 m*M*. In addition, nonradioactive sugars were added to the medium to attain the desired final concentration indicated in the text. The reaction mixture was incubated at 37° by shaking for 5 min after which the reaction was halted by pouring the contents of the reaction mixture into a graduated centrifuge tube in an ice bath; the cells were centrifuged in the cold at 275*g* for 2 min. The cells were then washed and centrifuged three additional times with 5 ml of cold Krebs–Ringer phosphate. The uptake of the amino acids by the cells was determined as described previously (9, 13) and was expressed as the concentration of the amino acid in the cell water. This parameter was calculated with the use of the following relationship: m*M* amino acid (cellular water) = total amino acid taken up by the cells/(cell wet weight) (0.8). The cell wet weight was calculated as follows: (total mg protein in cells) (mg dry weight/mg protein) (5 mg wet weight/mg dry weight).

Radioactive leucine and lysine were obtained from New England Nuclear, Boston, MA[1] and had specific activities of 279 mCi/mmole and 280 mCi/mmole, respectively.

A paired-difference *t* test was used to determine whether the presence of the sugars or differences in diet produced significant differences in the parameters measured. References to statistical significance pertain to the 5% probability level or below. Data are reported as the mean ± SEM.

Results. After 8 weeks the rats fed the stock diet weighed an average of 313 ± 11 g and the rats fed the high sucrose diet weighed an average of 345 ± 13 g. These weight differences were not statistically significant. The average daily food intake of the stock-fed and sucrose-fed rats at this time was 29.4 ± 1.3 g and 28.3 ± 2.3 g, respectively.

Table I shows the effect of 10 m*M* fructose, 10 m*M* glucose, and 20 m*M* sucrose on the uptake of 1 m*M* leucine and 1 m*M* lysine by intestinal cells isolated from stock-fed rats after a 5-min incubation. Leucine uptake was increased by 32% ($P < .005$) in the presence of fructose. Conversely, glucose and sucrose significantly decreased leucine uptake (by about 30%). In contrast, the uptake of lysine appeared to be much less sensitive to the addition of the sugars. Fructose and sucrose did not affect lysine uptake, and glucose produced a small (14%) but significant inhibition.

The transport of fructose, glucose, and sucrose had been shown to be greater in the intestine of rats fed the high sucrose diet as compared to that in the intestine of rats fed a stock diet (11). Therefore, it was of interest to determine whether sucrose feeding would influence the nature or the magnitude of the effects of the sugars on amino acid transport observed in the stock-fed rats. The patterns of leucine and lysine uptake in the presence of the sugars in the sucrose-fed rats (Table II) were essentially the same as the patterns noted in the stock-fed rats (Table I). The two major differences noted were a greater inhibition of leucine uptake by glucose ($P < .025$) and the absence of a significant decrease in lysine uptake by glucose.

In the controls, sucrose feeding slightly increased the uptake of leucine (13%) and lysine (8%) as compared to stock feeding; however, these increases were not statistically significant. In contrast the uptake of 1 m*M* glucose, run concurrently in these studies, was increased significantly (58.5%) by sucrose feeding (4.02 ± 0.45 m*M*/5 min in stock-fed rats and 6.37 ± 0.66 m*M*/5 min in sucrose-fed rats) thus confirming previous results (11).

Discussion. The complex interactions between the pathways that mediate the intestinal transport of amino acids and sugars are illustrated by these results. Leucine uptake was extremely sensitive to the presence of dietary sugars while lysine uptake was virtually unaffected by the sugars. The mechanism by which the inhibitory interaction between Na^+-dependent transport systems for sugars (e.g., glucose) and amino acids (e.g., leucine) occurs is still a subject of controversy. Possible explanations include competition for a common energy source

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

TABLE I. EFFECT OF SUGARS ON THE UPTAKE OF 1 m*M* LEUCINE AND 1 m*M* LYSINE BY INTESTINAL CELLS ISOLATED FROM RATS FED A STOCK DIET.[a]

Sugar	Leucine uptake (m*M*/5 min)	Control (%)	*P*	Lysine uptake (m*M*/5 min)	Control (%)	*P*
None (Control)	2.44 ± 0.27			2.76 ± 0.14		
10 m*M* Fructose	3.22 ± 0.35	132.0	<.005	2.63 ± 0.20	95.3	
10 m*M* Glucose	1.70 ± 0.23	69.7	<.025	2.38 ± 0.17	86.2	<.050
20 m*M* Sucrose	1.72 ± 0.17	70.5	<.005	2.62 ± 0.25	94.9	

[a] Each value represents the mean from seven rats ± SEM. A paired-difference *t* test was used to obtain the probability values. A *P* of 0.050 or less was considered significant and only these probability values are shown.

TABLE II. EFFECT OF SUGARS ON THE UPTAKE OF 1 m*M* LEUCINE AND 1 m*M* LYSINE BY INTESTINAL CELLS ISOLATED FROM RATS FED A SUCROSE DIET.[a]

Sugar	Leucine uptake (m*M*/5 min)	Control (%)	*P*	Lysine uptake (m*M*/5 min)	Control (%)
None (Control	2.76 ± 0.36			2.99 ± 0.25	
10 m*M* Fructose	3.71 ± 0.39	134.4	<.005	3.05 ± 0.29	102.0
10 m*M* Glucose	1.17 ± 6.22	42.4	<.005	2.84 ± 0.26	95.0
20 m*M* Sucrose	1.78 ± 0.34	64.5	<.050	2.90 ± 0.11	97.0

[a] Each value represents the mean from seven rats ± SEM. Probability values were obtained and expressed as in Table I.

(14, 15), competition for a common polyfunctional carrier with resultant allosteric interactions (16), and an accelerated rate of efflux of solute from the tissue (17). The absence of a comparable inhibition of lysine transport by glucose may be attributed to the presence of a basic amino acid pathway that is largely mediated by a Na^+-independent exchange transport requiring minimal energy input (18).

Sucrose generally produced an inhibition of leucine uptake similar to that of glucose rather than an acceleration of leucine transport in the manner of fructose. Previous studies showed that after a 5-min incubation, sucrose hydrolysis by intestinal cells ranged from 3% in stock-fed rats to 5% in sucrose-fed rats (11). A similar rate of hydrolysis in the present study would produce 0.6–1.0 m*M* glucose and fructose during the incubation. The inhibitory action of sucrose can be explained if it is assumed that the inhibition of leucine uptake occurs at glucose concentrations as low as 0.6 m*M* while the activation by fructose requires concentrations greater than 1 m*M*. It is also possible that the inhibition by sucrose is the result of a specific property of a disaccharide transport system (12, 19).

The two diets were fed in this study to determine whether the increased sugar uptake observed in sucrose-fed rats (11) would result in increases in the magnitude of the inhibition or stimulation of amino acid uptake noted in the stock-fed rats. Only in the inhibition of leucine uptake by glucose did sucrose-feeding produce a significant increase. The concentration of the sugars used may have been high enough for optimum inhibition or activation to occur, even in the stock-fed rats. On the basis of these results, it appears that fructose concentrations between 1 and 5 m*M* and glucose concentrations less than 1 m*M* may present the best conditions for demonstrating differences in leucine transport due to diet. Although the major difference in the diets fed in this study was the nature of the utilizable carbohydrate, it is possible that differences other than sucrose versus starch contributed to the transport rates observed. Therefore, differences in the availability of the amino acids or glucose from these diets due to ingredients such as dietary fiber also

must be considered in evaluating these transport results.

These results have illustrated another specific metabolic effect attributable to fructose ingestion and have characterized an important dichotomy in the properties of glucose and fructose transport in rat intestine. The mechanism by which fructose activates leucine uptake has not been elucidated. Alvarado (20) has shown that hamster intestine preincubated with fructose will stimulate cycloleucine transport from a fructose-free medium. Preliminary results in our laboratory also indicate that intracellular as well as extracellular fructose stimulates leucine transport. Since fructose is poorly metabolized in rat intestine during transport (10), these results suggest an interaction between the leucine and fructose transport pathways such as an exchange transport between intracellular fructose and extracellular leucine. The presence of a more generalized stimulation of neutral amino acid transport by fructose and a concurrent inhibition of fructose transport by these amino acids would support an interaction mediated by exchange transport.

The physiological significance of the stimulatory effect of fructose on leucine transport is also a subject for conjecture. Most of the fructose in the diet originates from sucrose, yet sucrose was found to inhibit rather than stimulate leucine uptake. However, physiological sucrose digestion may produce varying local concentrations of luminal glucose and fructose (21, 22) which may provide the environment required for fructose stimulation of amino acid transport. One result of an increase in leucine uptake may be an elevated serum insulin since leucine is one of the most potent secretagogues of insulin (23). Increases in serum insulin in animals fed high sucrose diets have been reported (11, 24, 25).

Summary. The effect of various dietary sugars on the uptake of 1 m*M* leucine and 1 m*M* lysine by intestinal cells isolated from stock-fed and sucrose-fed rats was determined. Leucine uptake was activated by 10 m*M* fructose and inhibited by 10 m*M* glucose or 20 m*M* sucrose on both diets. The major dietary effect noted was a significant increase in the inhibition of leucine by glucose in the sucrose-fed rats. The uptake of lysine was minimally affected by the sugars irrespective of the diet fed. These results demonstrate an important dichotomy in the properties of glucose and fructose transport in the intestine and suggest that dietary fructose may increase the transport of certain amino acids.

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