Fructose Utilization by the Human Intestinal Epithelial Cell Line, Caco-2 (43556)

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Abstract. **The potential use of Caco-2 cells as a model for the study of fructose metabolism and transport in the intestine was evaluated, since this human cell line exhibits many of the anatomical and biochemical characteristics of mature enterocytes. Pre- and postconfluent cultures converted [^{14}C]fructose to CO_2, lipid, and glycogen. Apparent utilization of [^{14}C]fructose was less than that of [^{14}C]glucose. This difference was due in part to the more rapid uptake of glucose from medium as compared with fructose. Addition of glucose, galactose, and mannose to medium markedly decreased the metabolism, while slightly inhibiting the uptake, of [^{14}C]fructose. These data demonstrate that fructose can serve as a carbon and energy source for Caco-2 cells, and that common dietary monosaccharides affect the efficiency of fructose metabolism.**

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Fructose consumption has steadily increased during the past two decades, primarily due to the introduction of high fructose corn sweetners in foods and beverages (1). However, the mechanism of fructose absorption and the possible utilization of this monosaccharide by intestinal mucosal cells remain unclear. Results from studies using freshly isolated intestinal cells, everted sacs, and brush border membranes have revealed that fructose is transported across the apical surface by carrier-mediated processes (2–4) distinct from the D-glucose transport system (4, 5). Limited studies with humans have revealed that a small amount of dietary fructose can be converted to glucose and lactic acid by unknown cell types in the intestine, with the majority of the monosaccharide being transferred to the blood and metabolized by the liver (6–9). Several investigators have also reported that consumption of a high fructose diet is associated with increased fructokinase activity in the intestine of animals and humans (10, 11).

The human colon tumor cell line Caco-2 is a suitable *in vitro* model for the study of the transport and metabolism of nutrients and drugs by the intestinal absorptive epithelium. This cell line was established from a colonic adenocarcinoma and spontaneously differentiates into columnar cells displaying many of the morphologic and biochemical properties of mature enterocytes after cultures become confluent. These characteristics include the expression of disaccharidases and peptidases on the apical cell surface of polarized cells that possess tight junctions, the synthesis and secretion of lipoproteins, and a Na^+-dependent glucose transport system (12–16). The objectives of these experiments were to examine the uptake and possible utilization of fructose by Caco-2 cells and the influences of age of the culture and other monosaccharides on these parameters. Glucose uptake and metabolism by Caco-2 cells also were monitored in parallel studies for comparison.

Materials and Methods

Materials. Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD) at Passage 18. Dulbecco's modified Eagle's medium (DMEM) with 4.5 g glucose/liter, Hanks' balanced salt solution (HBSS), amino acid supplements, antibiotics, and fetal bovine serum were purchased from Sigma Chemical Co. (St. Louis, MO). Uniformly labeled [^{14}C]fructose

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(311.5 mCi/mmol) and [^{14}C]glucose (340 mCi/mmol) were obtained from Dupont/New England Nuclear (Wilmington, DE). Tissue culture flasks (75 cm^2 and 25 cm^2) were purchased from Falcon/Becton-Dickinson (Lincoln Park, NJ), while multiwell dishes (35 mm in diameter) were obtained from Costar Corp. (Cambridge, MA).

Cell Culture. Caco-2 cells from Passages 22 through 34 were used for all experiments. Cells were grown in DMEM supplemented with 20% fetal bovine serum, 2 m*M* L-glutamine, 1% nonessential amino acids, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 10 μg/ml of gentamicin, and 1 μg/ml of fungizone. Stock cultures were maintained in 75-cm^2 flasks in a humidified atmosphere of 95% air and 5% CO_2 at 37°C to 60–80% confluency. Cells were detached by exposure to 0.05% trypsin-0.5 m*M* EDTA in HBSS without calcium and magnesium. Fresh stock cultures were initiated with 5×10^5 cells/flask. For transport studies, isolated cells were added to 6-well culture dishes at 2×10^5 cells/well. Fructose and glucose metabolism were studied using cultures in 25-cm^2 flasks which had been initiated by the addition of 2×10^5 cells. Cultures generally reached confluency at 5–6 days after seeding. Spent medium was removed, the monolayer was washed once with HBSS, and fresh medium was added every second day. Unless otherwise indicated, monosaccharide metabolism and transport studies were done at 10–11 days after confluency, since cells exhibited maximal differentiation at this time as assessed by the activities of sucrase and alkaline phosphatase (data not shown).

Metabolism of Fructose and Glucose. Cultures (25-cm^2 flasks) were washed twice with HBSS at 37°C. Glucose-free DMEM (4 ml) containing 10 m*M* Hepes (pH 7.3) and supplemented with either 1 m*M* fructose plus 1 μCi of [^{14}C]fructose or 1 m*M* glucose plus 1 μCi of [^{14}C]glucose was introduced. Flasks were sealed immediately with serum tube stoppers with a suspended center well insert containing a folded strip of filter paper. Cultures were incubated at 37°C for 1, 2, or 4 hr before terminating metabolism by injection of 0.3 ml of 4*N* perchloric acid. $^{14}CO_2$ was trapped in filter paper by injecting 4*N* KOH (0.10 ml) into the center well and subsequently incubating flasks for 1 hr at 37°C. Filter paper and aqueous rinses of the center well were transferred into glass vials containing 10 ml of Opti-fluor scintillation cocktail (Packard Instrument Co., Downers Grove, IL) and radioactivity was measured by liquid scintillation spectrophotometry (Beckman model LS6800; Beckman, Downers Grove, IL). Acid-precipitated material was released from the surface with a plastic scraper before addition of 0.7 ml of 4*N* KOH. Flasks were incubated at 37°C overnight with gentle rocking to solubilize cellular material. To measure [^{14}C] glycogen, an aliquot of solubilized cellular material was added to a tube containing 50 mg of rat liver glycogen as carrier. Glycogen was precipitated with 95% ethanol, collected by centrifugation (1500*g* for 10 min), solubilized in 0.2% Triton X-100, and re-precipitated with 95% ethanol for collection by centrifugation. The final pellet was solubilized in 1*N* KOH and assayed as described by Van Handel (17). Lipids were extracted from an aliquot of solubilized cellular material using the chloroform:methanol (2:1) procedure (18). Solvent was removed under a stream of nitrogen and the residue was resuspended in hexane prior to determination of ^{14}C.

To study the influence of other sugars and culture age on the metabolism of fructose, cultures were incubated with glucose-free DMEM containing 10 m*M* Hepes (pH 7.3) and 1 m*M* fructose plus 1 μCi of [^{14}C] fructose with or without indicated monosaccharides for 2 hr at 37°C. Conversion of fructose to CO_2, glycogen, and lipid was determined as above.

Fructose and Glucose Uptake. Prior to initiation of an experiment, cultures were washed three times with phosphate-buffered saline (140 m*M* NaCl, 3 m*M* KCl, 1.5 m*M* KH_2PO_4, 4 m*M* Na_2HPO_4) containing 10 m*M* Hepes (pH 7.3) at 25°C. The medium used for uptake studies consisted of a glucose-free DMEM with 10 m*M* Hepes (pH 7.3) that was supplemented with either 5 m*M* fructose plus 1 μCi of [^{14}C]fructose or 5 m*M* glucose plus 1 μCi of [^{14}C]glucose. After addition of 2 ml of one of these solutions to each well, cultures were incubated with gentle rocking for 1 to 20 min at 25°C. In a second experiment, test monosaccharides (20 m*M*) were added individually to medium containing [^{14}C]fructose (5 m*M*) to evaluate their possible effects on fructose uptake during a 2-min incubation period at 25°C.

Uptake was terminated by washing three times with ice-cold phosphate-buffered saline-Hepes solution containing 0.5 m*M* phloretin and 0.5 m*M* phloridzin. Cellular material was solubilized in 1 ml of 1*N* NaOH. Aliquots were transferred into scintillation cocktail to determine ^{14}C. Separate aliquots were assayed for protein (19) using bovine serum albumin as standard.

Statistical Analysis. Presented data (mean ± SE) represent results from at least two separate experiments in which each parameter was tested in triplicate. Effect of sampling time or culture age on metabolism and transport were analyzed by repeated-measures analysis of variance. The effects of other carbohydrates on fructose transport and metabolism were evaluated by *t* test. Differences with *P*-values less than 0.05 were considered statistically significant.

Results

Metabolism of Fructose and Glucose. Ten-day postconfluent cultures of Caco-2 cells utilized medium [^{14}C]fructose as demonstrated by the presence of ^{14}C in

CO_2, lipid, and glycogen after 1–4 hr of incubation (Fig. 1). The amount of ^{14}C incorporated into CO_2 and lipid increased linearly with time in cultures containing [^{14}C] fructose, whereas [^{14}C]glycogen content remained constant from 1 to 4 hr. Apparent utilization of fructose by Caco-2 was less efficient than that of glucose, since the levels of ^{14}C in CO_2, lipid, and glycogen were significantly greater in cultures containing [^{14}C]glucose as compared with that with an equimolar amount of [^{14}C]fructose. The level of ^{14}C present in glycogen did not change between 1 and 4 hr when cells were incubated in medium containing [^{14}C]glucose.

Metabolism of monosaccharides was compared at various times after seeding flasks to determine whether the degree of cellular differentiation affected the absolute or relative utilization of fructose and glucose. In general, confluent cultures of Caco-2 cells converted greater amounts of [^{14}C]glucose to CO_2 and cellular lipid than that produced from [^{14}C]fructose (Fig. 2). The quantities of the monosaccharides oxidized to CO_2 progressively increased between 4 and 16 days in culture, with 2- to 4-fold more $^{14}CO_2$ produced from medium glucose than from fructose throughout the culture period. Confluent cultures incubated in medium containing either [^{14}C]fructose or [^{14}C]glucose accumulated significantly more ^{14}C in cellular lipids than preconfluent cultures. The maximum level of ^{14}C in lipid in cultures incubated with [^{14}C]glucose was observed at 4 days after confluency. Pre- and newly confluent cultures of Caco-2 cells incubated with fructose contained about twice as much ^{14}C in glycogen as cultures at 4–10 days after confluency. Four- to 10-day postconfluent cultures incubated with glucose contained significantly more ^{14}C in glycogen than replicate cultures exposed to fructose.

In general, [^{14}C]fructose utilization was decreased by the addition of equimolar amounts of other metabolizable monosaccharides to the medium (Table I). Glucose, galactose, and mannose significantly decreased cellular conversion of fructose to CO_2 and lipid without affecting the level of incorporation into glycogen. In contrast, metabolism of medium fructose was generally increased when 3-*O*-methylglucose and mannitol were added to cultures. Addition of sorbose, a stereoisomer of fructose, to medium increased cellular oxidation of fructose, but did not alter accumulation of ^{14}C in either cellular lipid or glycogen.

Uptake of Fructose and Glucose. We examined the possibility that the lower degree of fructose utilization as compared with glucose metabolism by Caco-2 cells was due in part to differences in the uptake of these monosaccharides from the medium. Uptake of [^{14}C]fructose by Caco-2 cells was characterized by an apparent initial rate of about 4 nmol mg^{-1} min^{-1} from 1 to 5 min and a slower rate of 1.4 nmol mg^{-1} min^{-1} between 5 and 20 min (Fig. 3). [^{14}C]Glucose uptake

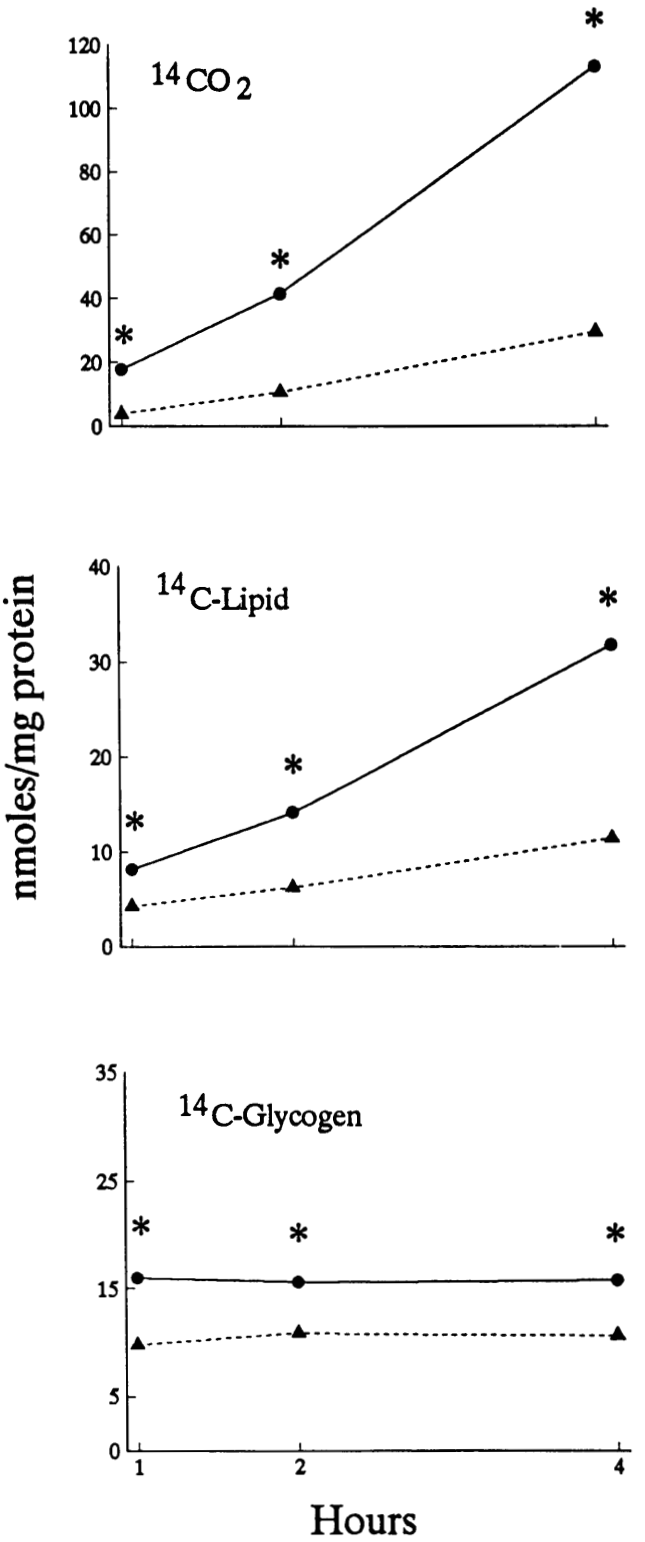

Figure 1. Metabolism of [^{14}C]fructose and [^{14}C]glucose by Caco-2 cells. Serum-free, modified DMEM with 10 m*M* Hepes (pH 7.3) plus either 1 m*M* fructose and 1 μCi of [^{14}C]fructose (▲) or 1 m*M* glucose and 1 μCi of [^{14}C]glucose (●) was added to washed 10-day postconfluent cultures. Flasks were incubated at 37°C for 1 to 4 hr and the levels of ^{14}C in CO_2, lipid, and glycogen were determined as described in Materials and Methods. Each point represents mean (SE negligible) for triplicate cultures from at least two separate experiments. An asterisk indicates that the values for cultures exposed to [^{14}C]glucose and fructose are significantly different ($P < 0.05$) at that time.

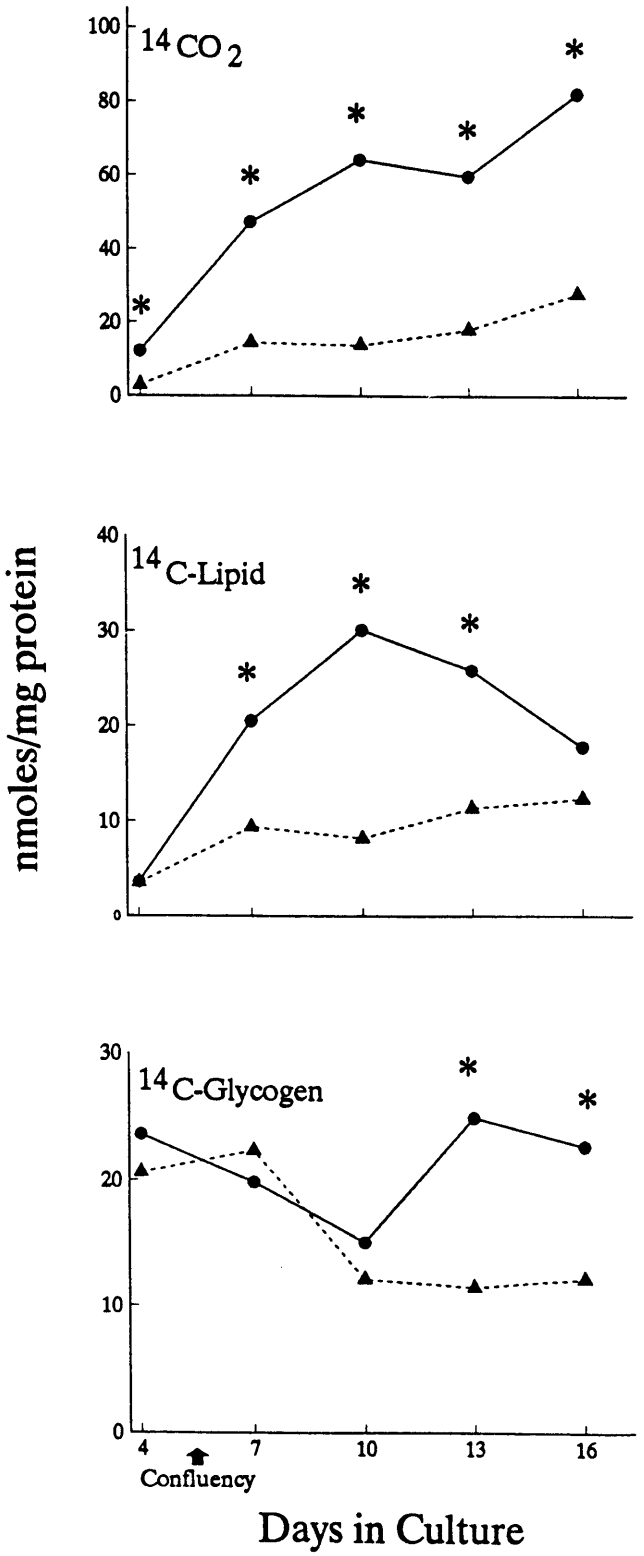

Figure 2. Influence of culture age on metabolism of [^{14}C]fructose and [^{14}C]glucose by Caco-2 cells. The arrow on the abscissa indicates that cultures reached confluency on the sixth day after seeding. Cultures were about 50% confluent after 4 days. At indicated times, the cultures were incubated in medium containing either 1 m*M* [^{14}C] fructose (▲) or 1 m*M* [^{14}C]glucose (●) for 2 hr. Data represent mean (negligible SE) for triplicate cultures from at least two separate experiments. Significant differences ($P < 0.05$) between cultures treated with [^{14}C]glucose and fructose are shown by an asterisk.

Table I. Effects of Various Monosaccharides on Fructose Metabolism by Caco-2 Cells[a]

Monosaccharide	[^{14}C]Fructose metabolism (nmol mg protein^{-1})		
	CO_2	Lipid	Glycogen
Fructose alone	10.1 ± 0.1	7.2 ± 0.3	6.9 ± 0.1
Fructose + glucose	2.1 ± 0.3[b]	5.2 ± 0.2[b]	8.3 ± 1.2
+ 3-*O*-methylglucose	15.8 ± 0.9[b]	9.3 ± 0.2[b]	10.3 ± 0.1[b]
+ galactose	5.3 ± 0.1[b]	5.9 ± 0.3[b]	7.7 ± 0.7
+ mannose	2.0 ± 0.1[b]	4.5 ± 0.3[b]	8.4 ± 0.4
+ mannitol	14.1 ± 0.1[b]	7.4 ± 0.2	10.2 ± 0.4[b]
+ sorbose	14.4 ± 0.3[b]	6.6 ± 0.2	6.6 ± 0.1

[a] Ten-day postconfluent cultures were washed with HBSS prior to the addition of serum-free, modified DMEM containing 10 m*M* Hepes (pH 7.3) plus 1 m*M* [^{14}C]fructose and equimolar levels of indicated monosaccharides. Flasks were incubated for 2 hr at 37°C before analysis of ^{14}C in indicated metabolites as described in Materials and Methods. Data are means ± SE for at least triplicate cultures from two separate experiments.
[b] Value is significantly ($P < 0.05$) different from that for cultures incubated in presence of fructose alone.

from medium during a 20-min incubation period was also characterized by a rapid apparent rate of accumulation during the initial 2 min of incubation (approximately 22 nmol mg^{-1} min^{-1}) and a slower rate of accumulation between 5 and 20 min (2.2 nmol mg^{-1} min^{-1}). The addition of glucose, 3-*O*-methylglucose, or galactose to medium slightly (20–30%), but significantly, decreased the initial apparent rate of [^{14}C]fructose uptake, whereas this parameter was not significantly altered by sorbose (Table II). The apparent rate of [^{14}C]fructose uptake increased 4.3-fold when the concentration of this monosaccharide was increased from 5 to 25 m*M*, which suggests that the transport process was not saturated at the higher level. [^{14}C] Glucose uptake was similar in the absence and presence of 20 m*M* fructose (18.3 vs 17.3 nmol mg^{-1} min^{-1}, respectively). However, increasing the concentration of glucose in medium from 5 to 25 m*M* only increased the apparent rate of uptake 2.1-fold (to 39.3 nmol mg^{-1} min^{-1}; $P < 0.05$), suggesting that the transport system was saturated at the higher concentration of this monosaccharide.

Discussion

Previous studies with humans have shown that although the majority of absorbed dietary fructose enters the portal circulation unchanged, there is some conversion of the ketohexose to glucose and lactate (6–8). This finding is consistent with the presence of fructokinase (EC 2.7.1.3) and fructose-1-phosphate aldolase (EC 4.1.2.7) activities in jejunal biopsies (20). Since biopsy samples and mucosal scrapings contain various types of cells (e.g., enterocytes, goblet cells, enterochromaffin cells, and leukocytes), the origin of the enzymes involved in fructose metabolism is unknown. The re-

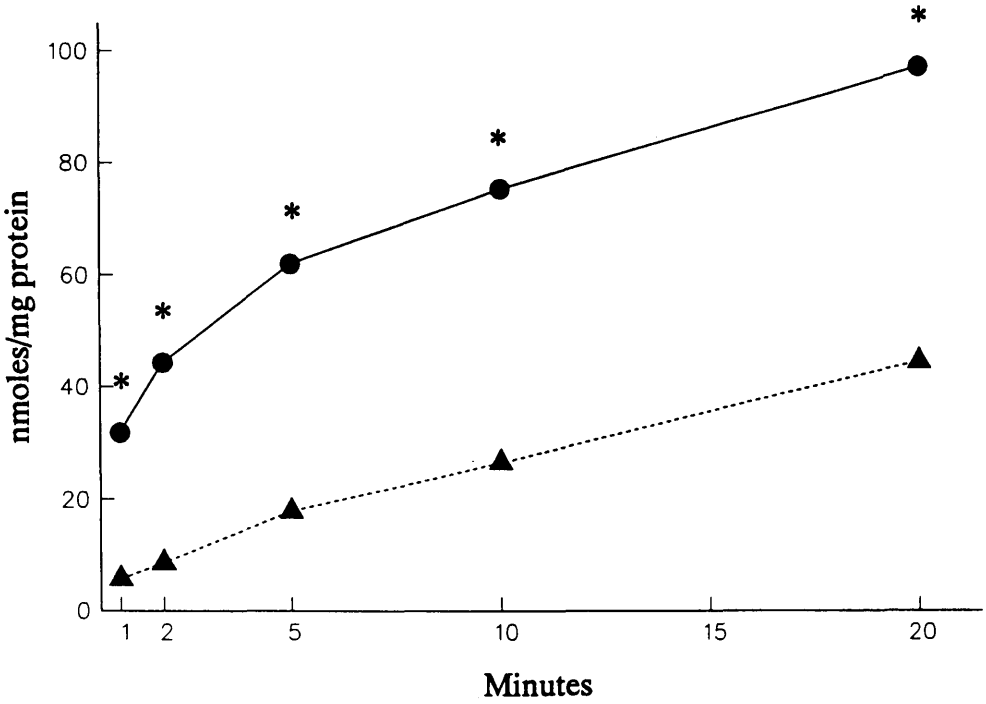

Figure 3. Uptake of [^{14}C]fructose and [^{14}C]glucose by Caco-2 cells. Ten- to 11-day postconfluent cultures were washed three times with phosphate-buffered saline containing 10 m*M* Hepes (pH 7.3) at 25°C. Serum-free, modified DMEM with either 5 m*M* fructose and 1 μCi of [^{14}C]fructose (▲) or 5 m*M* glucose and 1 μCi of [^{14}C]glucose (●) was added to monolayers and incubated at 25°C. Significant differences ($P < 0.05$) between cultures treated with [^{14}C]glucose and fructose are shown by asterisk.

Table II. Effects of Monosaccharides on [^{14}C]Fructose Uptake by Caco-2 Cells[a]

Monosaccharides	[^{14}C]Fructose uptake (nmol min^{-1} mg^{-1})
5 m*M* Fructose only	4.0 ± 0.1
5 m*M* Fructose + 20 m*M* glucose	2.9 ± 0.1[b]
+ 20 m*M* 3-*O*-methylglucose	2.8 ± 0.1[b]
+ 20 m*M* galatose	3.3 ± 0.1[b]
+ 20 m*M* mannose	3.5 ± 0.2
+ 20 m*M* mannitol	3.7 ± 0.1
+ 20 m*M* sorbose	3.9 ± 0.1
25 m*M* Fructose	17.1 ± 0.3[b]

[a] Ten- to 11-day postconfluent cultures were washed three times with phosphate-buffered saline containing 10 m*M* Hepes (pH 7.3), at 25°C. Serum-free, modified DMEM with indicated monosaccharides and 1 μCi [^{14}C]fructose was added and cultures were incubated for 2 min at 25°C. Transport was quenched and cultures were analyzed as described in Materials and Methods. Data are means ± SE for at least triplicate cultures from two separate experiments.

[b] Value differs significantly ($P < 0.05$) from the culture incubated with only 5 m*M* fructose.

sults of the present study demonstrate that Caco-2, the human epithelial cell line with properties similar to mature enterocytes, is capable of utilizing fructose as a carbon and energy source (Figs. 1 and 2; Table I). Moreover, our observations infer that fructokinase and fructose-1-phosphate aldolase are constitutively expressed in pre- and highly differentiated cultures of Caco-2, since cells had not been exposed to the monosaccharide before the addition of [^{14}C]fructose.

The incorporation of ^{14}C from fructose into CO_2 and lipid was markedly decreased by the addition of equimolar concentrations of glucose, galactose, and mannose to medium (Table I). These same monosaccharides had less, if any, impact on the uptake of [^{14}C]fructose by Caco-2 cells (Table II). Thus, it is likely that the apparent decrease in fructose utilization when the three metabolizable aldohexoses were present actually reflects a dilution of the pools of intermediary metabolites. This conclusion is supported by the observation that conversion of [^{14}C]fructose to CO_2 and lipid was not impaired by either 3-*O*-methylglucose or mannitol (Table I). 3-*O*-Methylglucose enters cells via the glucose transporter but is not metabolized, whereas mannitol is not taken up by intact cells.

The metabolism and uptake of [^{14}C]glucose by Caco-2 cells were monitored for comparison with fructose. In general, cultures appeared to utilize medium glucose more efficiently than fructose in 1 to 4 hr test periods (Figs. 1 and 2). However, this difference was largely due to the more rapid uptake of glucose from medium (Fig. 3). The rate of fructose absorption in the small intestine of humans and animals also is slower than that of glucose absorption (8, 21, 22). Fructose and glucose are transported across the intestinal brush border membrane of mammals by highly specific and

distinct carriers (5, 23). The presence of a sodium-dependent, phloridzin- and phloretin-sensitive sugar transport system similar to the glucose carrier has been demonstrated in the brush border membrane of Caco-2 (16). Moreover, the activity of this carrier is correlated with growth-related differentiation of brush border hydrolases. Our data provide preliminary evidence that Caco-2 cells possess separate carriers for glucose and fructose uptake. Glucose uptake was not affected by a 4-fold excess of fructose, and fructose uptake was minimally decreased by excess glucose and other monosaccharides known to use the glucose carrier system (Table II). It is also interesting that fructose, but not glucose, uptake was directly proportional to medium levels of fructose as high as 25 m*M*. This difference is consistent with reports that the rate of fructose uptake from intestinal lumen of monogastrics does not plateau until the concentration of the monosaccharide exceeds 200 m*M* (e.g., 5, 24). In contrast, the effective K_m for carrier-mediated glucose transport by small intestine is 2–5 m*M* after correction for effects of unstirred layers (25, 26).

Various investigators have recently used Caco-2 cells as a model for the study of nutrient metabolism and absorption, since confluent cultures exhibit many properties common to human intestinal absorptive cells. Several examples include the characteristics of phenylalanine (27) and folate (28) transport, secretogog-mediated regulation of chloride transport (29), and the influence of cellular iron status on transepithelial transport of iron (30). The results of the present study indicate that Caco-2 cells provide a useful *in vitro* system for a more detailed investigation of the characteristics of dietary fructose absorption, as well as possible influences of this monosaccharide on enterocyte function.

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1. Economic Research Service, USDA. Sugar and sweeteners. Situation and Outlook Report Yearbook. Washington, DC: U.S. Government Printing Office, p81, 1989.
2. Gracey M, Burke V, Oshin A. Active intestinal transport of D-fructose. Biochim Biophys Acta **266**:397–406, 1972.
3. Macrae AR, Neudoerffer TS. Support for the existence of an active transport mechanism of fructose in the rat. Biochim Biophys Acta **288**:137–144, 1972.
4. Hallfrisch J. Metabolic effects of dietary fructose. FASEB J **4**:2652–2660, 1990.
5. Sigrist-Nelson K, Hopfer V. A distinct D-fructose transport system in isolated brush border membrane. Biochim Biophys Acta **367**:247–254, 1974.
6. Cook GC. Absorption and metabolism of D(−) fructose in man. Am J Clin Nutr **24**:1302–1307, 1971.
7. Holdsworth CD, Dawson AM. Absorption of fructose in man. Proc Soc Exp Biol Med **118**:142–145, 1965.
8. Holdsworth CD, Dawson AM. The absorption of monosaccharides in man. Clin Sci **27**:371–379, 1964.
9. Kneepkens CMF. What happens to fructose in the gut? Scand J Gastroenterol **24**:1–8, 1989.
10. Marvis DA, Mayer RJ. Metabolism of fructose in the small intestine. I. The effect of fructose feeding on fructose transport and metabolism in rat small intestine. Biochim Biophys Acta **291**:531–537, 1973.
11. Shafrir E. Effect of sucrose and fructose on carbohydrate and lipid metabolism and the resulting carbohydrate metabolism. In: Beitner R, Ed. Regulation of Carbohydrate Metabolism. Boca Raton, FL: CRC Press, Vol **II**: Chap. 5, p95, 1985.
12. Pinto M, Robine-Leon S, Appay MD, Kedinger M, Triadoo N, Pussaulx E, Lacroix B, Simon-Assmann P, Haffen K, Fogh J, Zweibaum A. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. Bio Cell **47**:323–330, 1983.
13. Rousset M. The human colon carcinoma cell lines HT-29 and Caco-2: Two in vitro models for the study of intestinal differentiation. Biochimie **68**:1035–1040, 1986.
14. Ramond MJ, Martinot-Peignoux M, Erlinger S. Dome formation in the human colon carcinoma cell line Caco-2 in culture. Influence of ouabain and permeable supports. Biol Cell **54**:89–93, 1985.
15. Rousset M, Laburthe M, Pinto M, Chevalier G, Rouyer-Fessard C, Dussaulx E, Trugnan G, Boige N, Brun JL, Zweibaum A. Enterocytic differentiation and glucose utilization in the human colon tumor cell line Caco-2: Modulation by forskolin. J Cell Physiol **123**:377–385, 1985.
16. Blais A, Bissonnette P, Berteloot A. Common characteristics for Na^+-dependent sugar transport in Caco-2 cells and human fetal colon. J Membr Biol **99**:113–125, 1987.
17. Van Handel E. Estimation of glycogen in small amounts of tissue. Anal Biochem **11**:256–265, 1965.
18. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem **226**:497–509, 1957.
19. Nerurkar LS, Marino PA, Adams DO. Quantitation of selected intracellular and secreted hydrolases of macrophages. In: Chersowitz HB, Holden HT, Bellonti JA, Ghaffer A, Eds. Manual of Macrophage Methodology. New York: Marcel Dekker, pp229–258, 1981.
20. Rosenweig NS, Stifel FB, Herman FB, Zakim B. The dietary regulation of glycolytic enzymes. II. Adaptive changes in human jejunum. Biochim Biophys Acta **170**:228–234, 1968.
21. Vanden Berghe G. Fructose: Metabolism and short-term effects on carbohydrate and purine metabolic pathways. Prog Biochem Pharmacol **21**:1–32, 1986.
22. Truswell AS, Seach JM, Thorburn AW. Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. Am J Clin Nutr **48**:1424–1430, 1988.
23. Reiser S. Intestinal digestion of sucrose and absorption of fructose. In: Reiser S, Hallfrisch J, Eds. Metabolic Effects of Dietary Carbohydrates. Boca Raton, FL: CRC Press, pp11–23, 1987.
24. Schultz SG, Strecker CK. Fructose influx across the brush border of rabbit ileum. Biochim Biophys Acta **211**:586–588, 1970.
25. Medding JB, Westergaard H. Intestinal glucose transport using in vivo perfused rat jejunum: Model analysis and derivation of corrected kinetic constants. Clin Sci **76**:403–413, 1989.
26. Ferraris RP, Yasharpour S, Kent Lloyd KC, Mirzayan R, Diamond JM. Luminal glucose concentrations in the gut under normal conditions. Am J Physiol **259**:G822–G837, 1990.
27. Hidalgo IJ, Borchardt RT. Transport of a large neutral amino

acid (phenylalanine) in a human intestinal epithelial cell line: Caco-2. Biochim Biophys Acta **1028**:25–30, 1990.
28. Vincent ML, Russell RM, Vodek S. Folic acid uptake characteristics of a human colon carcinoma cell line, Caco-2. A newly described model for small intestinal epithelium. Hum Nutr Clin Nutr **39C**:355–360, 1985.
29. Burnham DB, Fondacaro JD. Secretagogue-induced protein phosphorylation and chloride transport in Caco-2 cells. Am J Physiol **256**:G808–G816, 1989.
30. Alvarez-Hernandez X, Nichols GM, Glass J. Caco-2 cell line: A system for studying intestinal iron transport across epithelial cell monolayers. Biochim Biophys Acta **1070**:205–208, 1991.