Comparison of glucose and fructose transport into adipocytes of the rat

Mitchell L. HALPERIN and Surinder CHEEMA-DHADLI

Department of Medicine, St. Michael's Hospital, University of Toronto, Toronto, Ont. M5B 1A6, Canada

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The purpose of these studies was to define the properties of the systems that transport hexoses into adipocytes. Glucose appears to enter adipocytes on a single transport system whose maximum velocity is stimulated by insulin and which is competitively inhibited by cytochalasin B, 5-thioglucofucose, fructose, mannose and 3-*O*-methylglucose. In contrast, fructose enters adipocytes by at least two separate mechanisms, one an insulin-sensitive transporter (probably the glucose transporter) and the other a mechanism that is insensitive to insulin. The fructose concentration required for half-maximal rates of transport is at least an order of magnitude higher than that for glucose and the maximum velocity of fructose transport is more than double that for glucose.

When the effect of insulin on glucose transport was investigated in adipocytes of the rat, we observed that the maximum velocity of transport was increased and the glucose concentration required for half-maximal rates of transport (K_T) was not altered by insulin (Halperin *et al.*, 1978). These results were similar to those reported by other investigators using different techniques to monitor glucose transport (Vinten *et al.*, 1976; Wardzala *et al.*, 1978; Olefsky, 1978; Livingston *et al.*, 1978; Czech, 1980).

Fructose metabolism in adipocytes can also be stimulated by insulin, but the magnitude of this stimulation is less than that observed when glucose was the substrate (Froesch & Ginsberg, 1962; Gutman *et al.*, 1966; Froesch *et al.*, 1967; Coore *et al.*, 1971; Sooranna & Saggerson, 1975). However, insulin did not stimulate fructose metabolism in all studies. For example, Lawrence & Lerner (1978) found that glycogen synthase was not activated by insulin in rat adipocytes when 5 mM-fructose was the substrate, whereas it was when similar concentrations of glucose, mannose or 2-deoxyglucose were present.

Another characteristic of fructose metabolism in adipocytes is that fructose uptake and metabolism increase to a large extent with a rising hexose concentration, whereas this is not true with glucose (Froesch & Ginsberg, 1962). These results suggest that fructose has a lower affinity than glucose for its transport system as well as a higher maximum velocity of transport. When Froesch & Ginsberg (1962) investigated the influence of glucose on fructose metabolism, they found no significant

inhibition of one hexose on the other in the absence of insulin, whereas in the presence of insulin glucose did compete with the stimulated, but not the basal, rate of fructose uptake. These observations led those authors to propose that fructose entered adipocytes in two separate ways, one that was independent of glucose and probably represented a separate specific transport system for fructose, and a second that was sensitive to glucose and could be stimulated by insulin. This latter method of entry may correspond to the glucose-transport system. Support for this concept was initially derived when Fain (1964) demonstrated that 2-deoxyglucose markedly inhibited glucose metabolism in adipocytes, whereas it had only a small effect on fructose metabolism; also, 2-deoxyglucose abolished the effect of insulin on fructose metabolism.

As pointed out above, much information existed on fructose metabolism in adipocytes, but there were few investigations designed to study fructose transport more directly. Schoenle *et al.* (1979) described a technique to monitor the counter-transport of intracellular [^{14}C]fructose with extracellular fructose. These experiments were performed in the presence of 30 mM-2-deoxyglucose, which is required to block fructose metabolism, but the concentration of this metabolite probably also inhibits the glucose-transport system. However, the effect of such a high 2-deoxyglucose concentration on the other fructose-transport system is unknown. Nevertheless, these authors observed that insulin had no effect on fructose transport under these conditions, and that the K_T for fructose transport was very high (25 mM).

The purpose of the present investigation was to study the pathways of fructose transport into adipocytes to help to define the hexose-transport systems. Results reported below confirm that two transport systems appear to be involved with fructose transport and only one of these is sensitive to insulin and cytochalasin B.

Materials and methods

Chemicals

Enzymes, metabolic intermediates and cofactors were obtained from Boehringer-Mannheim Corp., Montreal, Quebec, Canada. Collagenase (EC 3.4.24.3) was obtained from Worthington Biochemical Corp., Freehold, NJ, U.S.A. Phenazine methosulphate, mannose, cytochalasin B, insulin, 5-thioglucoase, 3-*O*-methylglucose and bovine serum albumin were supplied by Sigma Chemical Co., St. Louis, MO, U.S.A. D-[1-¹⁴C]Glucose, D-[1-¹⁴C]- and D-[U-¹⁴C]-fructose and Hyamine hydroxide were purchased from Amersham-Searle Corp., Don Mills, Ont., Canada. However, D-[1-¹⁴C]fructose was in very short supply.

Rats

Male Wistar rats (125–175 g) were supplied by Canadian Breeding Labs, LaPrairie, Quebec, Canada. They were allowed free access to laboratory chow until the time of killing (approx. 10:00h). Adipocytes were prepared as described by Rodbell (1964), modified such that the glucose concentration was 1.67 mM. The cells were washed twice in glucose-free media and preincubated at 37°C for 15 min.

Assay of hexose transport

To monitor glucose transport, adipocytes were incubated for 45 min in a bicarbonate-buffered medium containing D-[1-¹⁴C]glucose, albumin (20 mg/ml) and 20 μ M-phenazine methosulphate as previously described (Taylor *et al.*, 1976), except that NaF was not added. To monitor fructose transport, a similar technique was used. The rate of fructose transport was linear with time over a 60 min period with 2.5 mM- and 10 mM-fructose, and the rate of ¹⁴CO₂ production from D-[U-¹⁴C]fructose was 3 times that with D-[1-¹⁴C]fructose in the presence and absence of insulin (results not shown). These observations were similar to those with glucose as substrate (Taylor & Halperin, 1979). Incubations were terminated by the addition of 0.8 ml of 0.5 M-HClO₄. The ¹⁴CO₂ was trapped on filter paper impregnated with Hyamine hydroxide contained in suspended centre wells. Radioactivity was assayed in a Beckman model LS230 liquid-scintillation counter as previously described (Snyder, 1971).

Results and discussion

The rate of glucose transport was directly proportional to glucose concentrations when the glucose concentration was less than 1 mM. However, when the glucose concentration was increased from 1 to 20 mM, there was little further rise in the rate of glucose transport. In contrast, when fructose transport was studied, there were several differences noted; the fructose concentration required for half-maximal rates of transport was much higher than that for glucose, and the maximum rate of transport was also higher (Fig. 1). The rate of fructose transport increased further when the fructose concentration was increased to 50 mM, suggesting that there was now an appreciable rate of hexose diffusion under these conditions.

Insulin caused the maximum velocity of glucose transport to increase almost 2-fold, but the glucose concentration required for half-maximal rates of transport remained unchanged under these conditions (Halperin *et al.*, 1978). Insulin also stimulated fructose transport when the fructose concentration

Fig. 1. Effect of glucose and fructose concentrations on the rate of hexose transport

Isolated adipocytes (20–50 mg of lipid) from normal fed rats were incubated for 45 min at 37°C in 3 ml of bicarbonate-buffered medium containing D-[1-¹⁴C]glucose or D-[1-¹⁴C]fructose at the concentrations noted as described in the Materials and methods section. Results are reported as means of seven experiments, each assayed in triplicate.

was 1 and 10mM (Table 1). Cytochalasin B, a known inhibitor of glucose transport in adipocytes (Mizel & Wilson, 1972; Czech *et al.*, 1973; Loten & Jeanrenaud, 1974; Jarrett & Smith, 1979), inhibited glucose transport in a competitive fashion in the presence and absence of insulin.

Cytochalasin B also inhibited fructose transport; it abolished the stimulation of fructose transport by insulin (Table 1). Furthermore, it appeared that maximum inhibition was achieved with a low concentration of inhibitor and that more than half the rate of fructose transport was insensitive to this inhibitor (Fig. 2). The concentrations of fructose used in these studies were less than those that saturated the fructose-transport system (see Fig. 1).

When other non-metabolizable hexose derivatives were employed, both 5-thioglucose and 3-*O*-methylglucose were competitive inhibitors for glucose transport, but they could only inhibit a portion of the rate of fructose transport at the concentrations employed (Fig. 2). Like cytochalasin B, they inhibited the effect of insulin on fructose transport (results not shown).

Both mannose, a hexose which is apparently a substrate for the glucose transporter (Wood *et al.*, 1961), and glucose competitively inhibited fructose transport with a similar affinity (Fig. 3). The above results suggest that, in the presence of insulin, all these hexoses share a common transport system. However, as with the non-metabolized transport inhibitors, it appears that fructose has an additional mechanism available for transport which is either insensitive or at least much less sensitive to inhibition with all these compounds.

To analyse the components of fructose transport separately, we re-evaluated the effect of insulin on fructose transport in the presence and absence of insulin and/or cytochalasin B. When the rate of fructose transport that was inhibited by cytochalasin B was plotted against the fructose concentration, insulin seemed to increase the maximum

velocity without influencing the apparent K_T (Fig. 4); these results are similar to the effects of insulin on glucose transport (Vinten *et al.*, 1976; Halperin *et al.*, 1978; Wardzala *et al.*, 1978; Olefsky, 1978;

Fig. 2. Effect of (a) cytochalasin B and (b) 3-*O*-methylglucose on fructose transport (Dixon plot) Results are reported as means of triplicate assays in a representative experiment; the number of separate experiments was four in each case. The fructose concentrations (shown on the lines) were 2.5 and 10mM, the cytochalasin B concentrations ranged from 0 to 20μM and the 3-*O*-methylglucose concentrations ranged from 0 to 9mM.

Table 1. Effect of cytochalasin B on the stimulation of fructose transport by insulin

For details, see legend to Fig. 1. Results are reported as the means ± s.e.m. for three separate experiments, each assayed in triplicate. ^a $P < 0.01$ for paired observations for effect of insulin; ^b $P < 0.01$ for paired observations for effect of cytochalasin B.

Additions to incubation medium			Rate of fructose transport (μmol/45 min per g of lipid)
Fructose	Insulin	Cytochalasin B	
1 mM	—	—	0.80 ± 0.07
1 mM	+	—	1.14 ± 0.03 ^a
1 mM	—	+	0.45 ± 0.08 ^b
1 mM	+	+	0.44 ± 0.04 ^b
10 mM	—	—	5.29 ± 0.37
10 mM	+	—	7.01 ± 0.27 ^a
10 mM	—	+	3.27 ± 0.50 ^b
10 mM	+	+	3.36 ± 0.25 ^b

Fig. 3. Effect of (b) mannose and (a) glucose on fructose transport (Dixon plot)

For details, see Fig. 2. Insulin was present at a concentration of 500 $\mu\text{units/ml}$. Results are reported as means of triplicate assays in a representative experiment. The number of experiments was three in each case.

Livingston *et al.*, 1978; Czech, 1980). This component of fructose transport is probably the one studied by the technique of Schoenle *et al.* (1979). By the technique described above, the apparent K_T for fructose on the insulin-sensitive transport system is close to 10 mM, whereas it was approx. 20 mM for the insulin-insensitive transport system. The maximum velocity of the insulin-insensitive transporter was approx. 7 $\mu\text{mol}/45 \text{ min per g of lipid}$, whereas it was 3 and 8 $\mu\text{mol}/45 \text{ min per g of lipid}$ in the absence and presence of insulin on the insulin-sensitive transport system.

Since the fructose concentration in the blood is always at least one order of magnitude lower than the apparent K_T for its transporter into adipocytes, this system will not transport appreciable quantities of this hexose *in vivo* unless there is a factor which increases its affinity for fructose. It is possible that this transport system transports another compound as its primary function, that it is an inactive system *in vivo* or that it is a modified form of the glucose transporter which is unable to transport glucose in one of its forms. With respect to this last speculation, the inactive form might represent the glucose transporter, which is inactivated by incubating adipocytes with ATP (Chang & Cuatrecasas, 1974; Halperin *et al.*, 1978).

In summary, fructose appears to enter adipocytes on two transport systems: (1) an insulin-sensitive system with lower apparent K_T , and (2) an insulin-insensitive system. Both systems have roughly equal maximum velocities in the presence of insulin. The physiological role of this latter transporter remains to be defined.

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Fig. 4. Effect of insulin on fructose transport

For details, see Fig. 2. Results are reported for a representative experiment; each point was assayed in triplicate. The number of experiments was three. Insulin was present at a concentration of 500 $\mu\text{units/ml}$ (○), or absent (●). For a direct comparison, the original data are shown in (a). In (b) the rate of fructose transport that could be inhibited by cytochalasin B is plotted against the fructose concentration.

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