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# THE ONTOGENY OF THE RABBIT HEPATIC GLUCOSE TRANSPORTER

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We delineated and characterized the fetal hepatic glucose transporter in the rabbit. Employing the 2-deoxy-D-glucose displaceable $^{3}$H-cytochalasin B binding assay we estimated the number and Kd of the GT per mg of liver protein. A gradual increase in the number was observed during development, the fetus (23.8±2.04 pmoles/mg) expressing a lesser amount when compared to the neonate (59.5±17 pmoles/mg; $p < 0.05$) and adult (142±11 pmoles/mg; $p < 0.05$). On the other hand the affinity of the glucose transporter was higher in the fetus (Kd 287±81 nM) when compared to either the neonate (988±222 nM, $p < 0.05$) or the adult (706±101 nM, $p < 0.05$). We conclude that the fetal hepatic GT is more efficient secondary to a higher affinity for glucose.
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The glucose transporter proteins are glycoproteins that transport glucose by facilitated diffusion intracellularly (1). These proteins have been extensively studied and characterized in various adult tissues (2-6). Depending on the tissue studied, the synthesis and translocation of the transporter from an intracellular pool to the cell surface have been observed to be either insulin-sensitive (7) or -insensitive (8).

Fetal glucose homeostasis is largely dependent on placental supply of glucose (9,10,11). Several investigators, have quantitated the fetal glucose turnover and utilization rates in vivo employing the sheep model (9,11), and demonstrated differences when compared to that of the adult. The fetal glucose utilization rates are higher (~4 to 5 fold) per unit mass and time (9,11). Studies in vitro employing isolated fetal rat

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Abbreviations: Glucose transporter = GT

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hepatocytes additionally demonstrated the glucose uptake by these cells to be different from that of the adult rat hepatocytes (12). Glucose to glycogen conversion in the adult was regulated by insulin, whereas in the fetus, insulin was incapable of this regulation (12). In the latter, glucose incorporation into glycogen was mainly dependant on the available substrate concentrations, namely glucose (12). Therefore particularly in the fetus, glucose and the glucose transporters are important and major determinants of the hepatic glycogen metabolism. In this study, we delineated the ontogeny and characterized the rabbit hepatic glucose transporter in an attempt to determine differences between the fetus, neonate and adult.

## Materials and Methods

Liver tissue preparation: Crude homogenates were obtained from New Zealand White rabbit fetal livers at 22 day and 30 day (n=5) gestation (term~31 d), 10 day old neonatal (n=5) and non-pregnant adult livers (n=5). Red blood cell contamination of hepatic tissue homogenate was determined by estimating hemoglobin content by the cyanomethemoglobin method (12) and found to represent ~2% of total tissue protein. Tissue protein content was determined by the Biorad assay using bovine serum albumin as a standard (13).

Cytochalasin B binding assay: Cytochalasin B is a fungal metabolite that is a specific and competitive inhibitor of the glucose transporter (15). We determined the glucose-displaceable binding of this compound to the hepatic tissues (in duplicate) as described previously (4) with minor modifications. Briefly, the assay mixture consisted of 0.25 mg of washed and dispersed liver homogenates along with 5 $\mu$M of cytochalasin E (dissolved in ethanol), 0.2 $\mu$Ci of $^{3}$H-cytochalasin B (specific activity = 18.5 Ci/mole) and varying concentrations of unlabeled cytochalasin B (ranging from 0 to 4 $\mu$M) in the presence and absence of 500 mM 2-deoxy-D-glucose in a final volume of 0.4 cc of phosphate buffered saline (PBS), pH 7.4. The mixture was incubated for 15 minutes at 4°C, the reaction terminated with ice cold PBS, the bound fraction separated from the free by rapid filtration and the radioactivity determined in a scintillation counter. Non-specific binding was defined as the radioactivity in the presence of a 1000-fold excess of unlabeled cytochalasin B and was subtracted from all the other experimental data points. Additionally, $^{3}$H-cytochalasin B binding in the presence of saturating concentrations of glucose (500 mM) was subtracted from the binding in the absence of glucose to derive the specific glucose-displaceable $^{3}$H cytochalasin B binding. This specific glucose-displaceable $^{3}$H cytochalasin B binding data was subjected to Scatchard analysis (16) using the equilibrium binding data analysis (EBDA) program (17) and, from this analysis, the number and the dissociation constant (Kd) of the liver glucose transporters was determined.

Plasma glucose and insulin concentrations: were measured by the glucose oxidase method (18) and the double antibody technique (18) described previously.

Statistics: Inter-group comparisons were validated by one-way analysis of variance followed by Neuman Kuel's t-test. Correlations between groups of values were performed by regression analysis.

## Results

The optimal conditions for $^{3}$H-cytochalasin B binding assay were predetermined with regards to time (figure 1A), protein concentration (figure 1B) and unlabeled cytochalasin B concentrations that covalently inhibit the 2-deoxy-D-glucose uptake (figure 1C). The optimal time was observed to be between 15 and 20 minutes, while the glucose displaceable $^{3}$H-cytochalasin B binding was noted to be linear between liver homogenate protein concentrations of 50 to 1000 $\mu$g. 100 to 3000 nM of cytochalasin B inhibited $^{3}$H 2-deoxy-D-glucose uptake conversely 100 to 600 mM

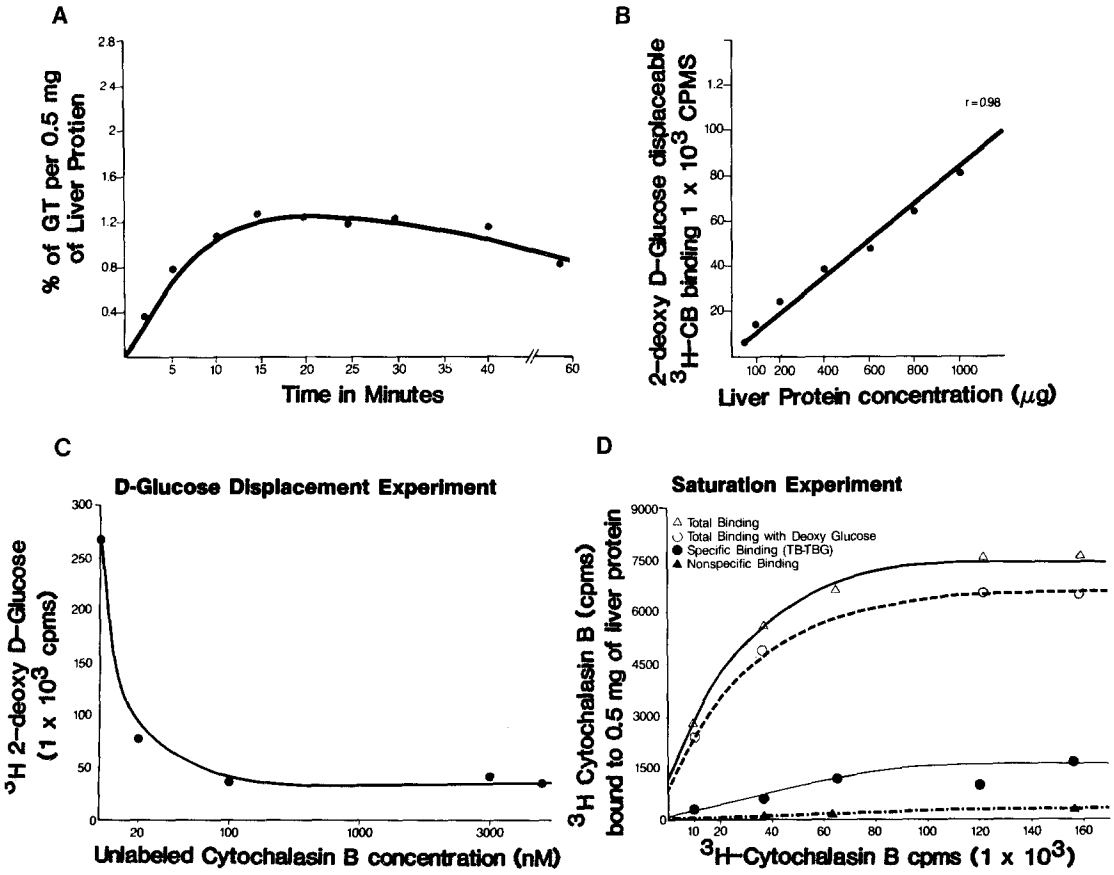


Figure 1: Optimal conditions for 2-deoxy-D-glucose displaceable $^{3}$H cytochalasin B binding: A) time B) liver homogenate protein concentration C) unlabeled cytochalasin B concentrations that inhibit glucose uptake and D) A representative set of $^{3}$H cytochalasin B saturation curves.

of 2-deoxy-D-glucose uptake was inhibited by these same cytocholasin B concentrations. Figure 1D demonstrates the saturation curve of $^{3}H$-cytochalasin B binding both in the presence and absence of 2-deoxy-D-glucose. As noted the total $^{3}H$ cytochalasin B binding is saturable at ~75,000 cpms (10 nM), however the glucose-displaceable cytochalasin B binding which represents the glucose transporter alone, was saturable at ~20% of that attained with total $^{3}H$ cytochalasin B binding.

Figure 2 demonstrates the glucose displaceable $^{3}H$-cytochalasin B binding in the presence of varying concentrations of unlabeled cytochalasin B. The Scatchard plots derived from this data are depicted in the inset. There was no difference in the glucose-displaceable $^{3}H$ cytochalasin B binding in both the 22 and 30 day old fetal livers, however there was a progressive increase observed in the newborn (three-fold) and adult (four-fold) livers. Analyzing (by Scatchard analysis) the high affinity sites alone, it is seen that there is a progressive increase in the number of glucose transporters/mg protein with development, i.e. from the fetus to newborn to adult (table 1).

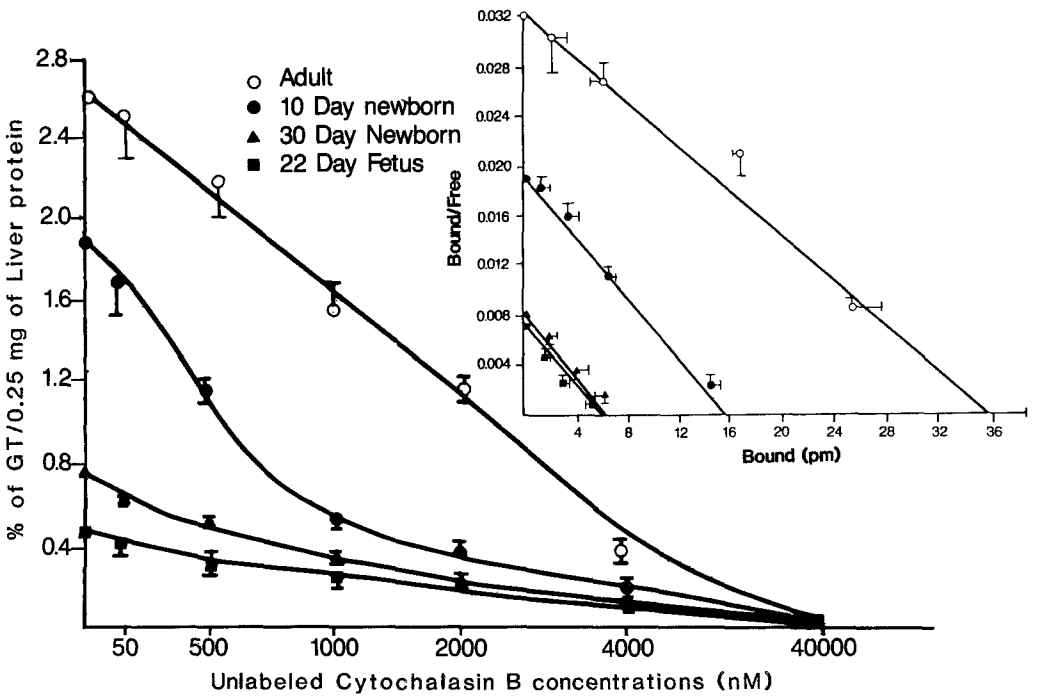


Figure 2: Glucose-displaceable $^{3}H$ cytochalasin B binding curves obtained at different ages. The data is represented as mean ± SEM. The inset depicts the corresponding Scatchard plots.

Table 1: The number and Kd of hepatic glucose transporters at different ages. Mean ± SEM

| Groups (n) | GT picomoles/mg | Kd nM |
|---|---|---|
| Fetus 22d (5) | 23.8±2.04 | 287±81 |
| Fetus 30d (5) | 24.4±1 | 463±74 |
| Newborn (4) | 59.5±17* | 988±222* |
| Adult (5) | 142±11* | 706±101* |

*Newman-Keuls: when compared to the fetus (22 and 30 d), significantly different at $p < 0.05$.

Reflecting the glucose-displaceable $^3H$ cytochalasin B binding data, no difference in the number of GTs was observed between the 22d (early gestation) and 30d (late gestation) fetal livers. On the other hand, Kd is lower in the fetus when compared to both the newborn and adult.

Plasma glucose and insulin concentrations are shown in figure 3 along with the number of glucose transporters. The fetal glucose values are lower than the neonatal and adult values, following a similar trend as the number of glucose transporters ($r = 0.94$, $p < 0.0005$), while they inversely correlate with the affinity of the GT ($r = 0.76$, $p < 0.025$).

## Discussion

We have described the hepatic glucose transporter and determined its development with maturation in the rabbit. When compared to the glucose transporters in adult cells such as erythrocytes (19) and adipocytes (20), the hepatic glucose transporter has a 10-fold lower binding affinity (4). In addition, antibodies raised against the erythrocyte and adipocyte glucose transporters fail to recognize the hepatic protein, suggesting that the hepatic transporter may be a different protein or, at least one, with distinctly different antigenic properties.

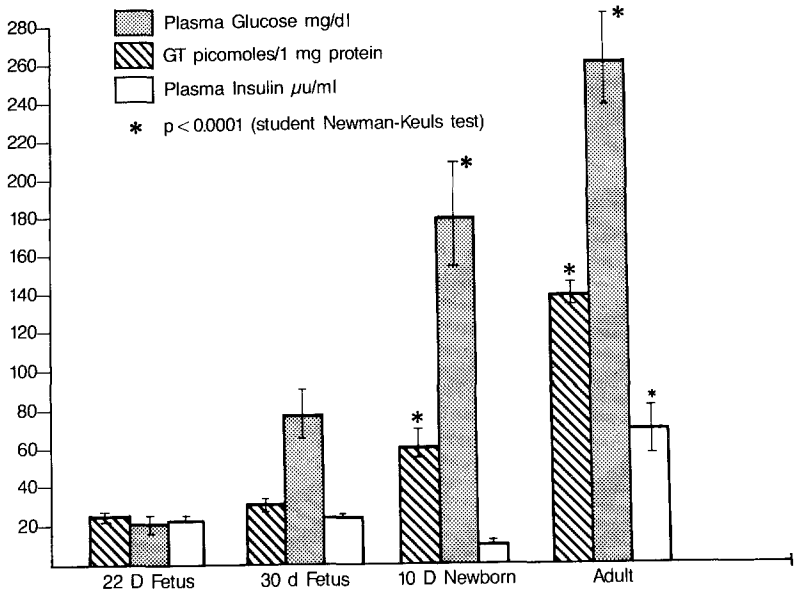


Figure 3: Plasma glucose and insulin concentrations shown along with the number of glucose transporters at different ages. Mean ± SEM. Newman-Keuls test failed to reveal any significant differences between the insulin concentrations at different ages. Student's t-test when applied to the neonatal and adult groups alone, revealed a difference that was significant at $p < 0.05$.

Our present report demonstrates fewer GTs/mg of protein in the fetal liver when compared to the neonate and adult. In separate studies by others, employing a GT cDNA generated from the HepG2 cell line, a lower abundance of GT mRNA (per unit RNA) was detected in the fetal rat liver when compared to the adult (21), confirming our protein data here. Additionally, in our present report, the fetal GTs demonstrate a higher affinity for glucose suggesting a more avid binding between the substrate and the transporter resulting in a rather efficient fetal glucose transport system. This appears to be borne out in studies of glucose utilization in vivo. Here glucose utilization rate in the fetus has been found to be in the range of 5 to 8 mg/kg/min, whereas in the adult it is 2 to 3 mg/kg/min (9,10). Since the liver to total body weight ratio is significantly higher in the fetus than the adult, the fetal hepatic GT with its higher affinity, contributes towards the building of hepatic glycogen stores, thereby meeting the fetal requirements for glucose adequately. In addition, as discussed earlier, most of the fetal glucose requirements are met by the placental supply of glucose, endogenous glycogen stores being utilized only when there is a

shortage in the maternal glucose supply (11). This unique fetal situation may explain the need for distinctly different GTs, i.e. one with a higher affinity. Due to a significantly higher contribution by hematopoeitic elements in the fetal liver one could argue that the fetal hepatic GT has a higher affinity due to the contamination of the liver tissue with erythrocytic elements (22). However, it has previously been demonstrated that the rabbit erythrocytes lack a GT (23) and in this study we have determined the hemoglobin concentration in the final liver tissue preparation to be similar in the fetus and adult.

Alternatively, the possibility exists that the fetal glucose transporter may be innately different from that of the adult form. In other cell systems, such as the adipocytes, an immature form of the protein has been detected within the intracellular pool that differs by way of its migration to a separate isoelectric point from the mature membrane-associated form (3). In this case both the immature and the mature forms of the GT were observed within the same cell. Whether a similar situation exists in the fetal liver (immature form) when compared to the adult (mature) remains to be elucidated.

Further, in spite of the fact that there was some relationship observed between the plasma glucose concentrations and both the number and affinity of the hepatic GTs in this report, the effect of varying circulating glucose concentrations on these glucose transporters needs to be investigated.

In summary, we have delineated the ontogeny of the hepatic glucose transporter and observed that although there are less number of transporters per mg of liver tissue, the fetal transport system may be a more efficient system when compared to the adult counterpart.

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References

1. Wheeler, T.J. and Hinkle, P.C. (1985) Ann. Rev. Physiol. 47:503-517.
2. Leinhard, G.E., Baldwin, J.M., Baldwin, S.A. and Gorga, F.R. (1983) Structure and function of membrane proteins. pg. 325-333 Elsevier Biomedical Press, Amsterdam.

3. Jones, M.N. and Nickson, J.K. (1981) Biochemica et Biophysica Acta 650:1-20.
4. Wheeler, T.J. and Hinkle, P.C. (1982) Membrane and transport.pg. 161-167 Plenum Publishing Corp., New York,
5. Mueckler, M.M., Caruso, C., Baldwin, S.A., Panico, M., Blench, I., Morris, H.R., Allard, J.W., Leinhard, G.E. and Lodish, H.F. (1985) Science (Wash DC) 229:941-945.
6. Wang, C. (1985) Proc. Natl. Acad. Sci. 82:3621-3625.
7. Kahn, B.B. and Cushman, S.W. (1985) Diabetes/Metabolism Reviews 1(3):203-227.
8. Chin, J.J., Jung, E.K.Y. and Jung, C.Y. (1986) J. Biol. Chem. 261:7101-7104.
9. Block, C.A., Banach, W., Landt, K., Devaskar, S. and Sperling, M.A. (1986) Am. J. Physiol. E448-E456.
10. Hay, W.W. Jr., Sparks, J.W., Quissell, B.J., Battaglia, F.C., Meschia, G. (1981) Am. J. Physiol. 240:E662-E668.
11. Hay, W.W. Jr., Sparks, J.W., Wilkening, R.B., Battaglia, F.C., Meschia, G. (1984) Am. J. Physiol. 246:E237-E242.
12. Sinha, M.K., Miller, J.D., Sperling, M.A., Suchy, F.J. and Ganguli, S. (1984) Diabetes 33:864-871.
13. Matsubara, T. (1978) Clinica Chemica Acta 93:113-114.
14. Bradford, D.M., (1976) Anal. Biochem. 72:248-250.
15. Tauerna, R.D. and Langdon, R.G. (1984) Biochem. Biophys. Acta 323:207-217.
16. Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51:660-672.
17. McPherson, G.A. (1985) J. Pharmacol. Methods 14:213-228.
18. Devaskar, S., Grim, P. and Devaskar, U. (1985) Pediatric Research 19:192-198.
19. Jung, C.J. and Rampal, A.L. (1977) J. Biol. Chem. 252:5456-5463.
20. Wardsala, L.J., Cushman, S.W. and Salans, L.B. (1978) J. Biol. Chem. 253:8002-8005.
21. Flier, J.S., Muechler, M., McCall, A.L. and Lodish, H.F. (1987) J. Clin. Invest. 79:657-661.
22. Allard, W.J. and Leinhard, G.E. (1985) J. Biol. Chem. 260:8668-8675.
23. Lains, V.R., Lee, D.P. and Shenolikar, S. (1987) J. Biol. Chem. 262:14571-14575.