“WILL THE ORIGINAL GLUCOSE TRANSPORTER ISOFORM PLEASE STAND UP!”

Anthony Carruthers¹, Julie DeZutter¹, Amit Ganguly² and Sherin U. Devaskar²*

¹ Department of Biochemistry & Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA.

² Division of Neonatology and Developmental Biology, Neonatal Research Center, Department of Pediatrics, David Geffen School of Medicine UCLA, Los Angeles, CA, USA

Running Title: Facilitative Glucose Transporter Isoform 1

*corresponding author

10833, Le Conte Avenue, MDCC-B2-375

Los Angeles, CA 90095-1752

Phone No. 310-825-9436

FAX No. 310-267-0154

Email: sdevaskar@mednet.ucla.edu

Copyright © 2009 by the American Physiological Society.
**ABSTRACT**

Monosaccharides enter cells by slow, trans-lipid bilayer diffusion by rapid, protein-mediated, cation-dependent co-transport and by rapid, protein-mediated equilibrative transport. This review addresses protein-mediated, equilibrative glucose transport catalyzed by GLUT1 - the first equilibrative glucose transporter to be identified, purified and cloned. GLUT1 is a polytopic, membrane-spanning protein that is one of 13 members of the human, equilibrative glucose transport protein family. We review GLUT1 catalytic and ligand binding properties and interpret these behaviors in the context of several putative mechanisms for protein-mediated transport. We conclude that no single model satisfactorily explains GLUT1 behavior. We then review GLUT1 topology, subunit architecture and oligomeric structure and examine a new model for sugar transport that combines structural and kinetic analyses to satisfactorily reproduce GLUT1 behavior in human erythrocytes. We next review GLUT1 cell biology and the transcriptional and post-transcriptional regulation of GLUT1 expression in the context of development and in response to glucose perturbations and hypoxia in blood-tissue barriers. Emphasis is placed on transgenic GLUT1 over expression and null mutant model systems, the latter serving as surrogates for the human GLUT1 deficiency syndrome. Finally we review the role of GLUT1 in the absence or deficiency of a related isoform, GLUT3, towards establishing the physiological significance of coordination between these two isoforms.

**KEYWORDS**

Glucose transport, facilitated diffusion, Major Facilitator Superfamily protein, glucose, blood-brain barrier, placenta, diabetes, GLUT1 deficiency syndrome, Development.
Most cells transport sugars rapidly down the prevailing concentration gradient into or out of the cell. This equilibrative transport process is mediated by a family of sugar transporters called GLUTs. GLUT1 was the first glucose transporter isoform to be identified, purified (66,132) and cloned (93) and is one of 13 proteins that comprise the human equilibrative glucose transporter family (63). GLUT1 is a membrane spanning glycoprotein containing twelve transmembrane domains with a single N-glycosylation site and its gene is located on chromosome 1 (1p35-31.3) (93). GLUT1 is expressed at highest levels in the plasma membranes of proliferating cells forming the early developing embryo, cells forming the blood tissue barriers, in human erythrocytes, astrocytes and in cardiac muscle (86). Having a catalytic turnover of about 1,200 per sec (115), GLUT1 provides an efficient pathway for cellular import and export of glucose. In addition, GLUT1 transports galactose and ascorbic acid (81, 107). This review examines the catalytic properties, structure, molecular regulation and physiology of GLUT1.

GLUT1 CATALYTIC PROPERTIES

Facilitated Diffusion

The cytoplasm of most cells equilibrates rapidly with nonmetabolizable, extracellular sugars. This process is mediated by sugar transport proteins that catalyze unidirectional sugar uptake and exit resulting in a net sugar movement down a concentration gradient from high to low sugar concentration. These sugar transport proteins are members of a family of integral membrane proteins called GLUTs that display strong specificity for D-stereoisomers of pentose and hexose monosaccharides adopting the chair configuration of the pyranose ring (e.g. D-glucose, D-galactose (7)), although some members of the protein family prefer fructose - a sugar
that adopts the furanose ring form (87). This equilibrative sugar transport is often called “facilitated diffusion” because it is several orders of magnitude faster than glucose diffusion across artificial lipid bilayers and the equilibrium transmembrane distribution of sugar is identical to that produced by transbilayer diffusion. Equilibrative sugar transport contrasts with that mediated by “active sugar transporters” or SGLTs which exploit the free energy available in transmembrane cation gradients to transport sugars against a concentration gradient (128,129).

**GLUT1- mediated sugar transport in Red Blood Cells**

GLUT1 comprises 10 - 20% of the integral membrane protein content of human red cells where it is quantitatively the only significant isoform of expressed GLUT (38). The availability of human red cells, their high GLUT1 content and the relative uniformity of red cell size and surface area have resulted in more than 60 years of sophisticated kinetic analysis of erythrocyte sugar transport.

**Steady-state kinetics of transport**

Accurate analysis of the concentration-dependence of sugar transport requires the measurement of “steady state” transport rates, which are obtained only when the concentrations of GLUT1-sugar intermediates involved in transport are unchanged during the transport assay. In practical terms, this means that transport measurements are made at very early time points where the amount of sugar in the cell increases (or decreases) linearly with time (the observed rate of transport is independent of time). This exposes the Achilles heel of sugar transport measurements in human red blood cells. GLUT1 is so abundantly expressed in human erythrocytes that it becomes necessary either to limit transport measurements to less than 1 sec at 37ºC or to lower temperature to 4ºC in order to satisfy this requirement. In spite of this complexity, glucose
transport in human red blood cells has been extensively characterized and has resulted in the
development of a number of revealing experimental conditions that permit full characterization
of any passive transport system (79).

**GLUT1 Substrate Specificity**

Competitive inhibition studies by Barnett et al (7) suggest that the OH groups at C1 and
C3 of D-glucose serve as hydrogen bond acceptors when D-glucose is seated in the GLUT1
sugar uptake site. C4 may form a hydrogen bond with GLUT1 because the C4 epimer of D-
glucose, D-galactose, has 10-fold lower affinity for GLUT1 than D-glucose. However, an
alternative explanation for this is that the non-gluco configuration of the sugar hydroxyl group
sterically hinders transport. The OH group at C6 seems not to hydrogen bond with GLUT1 and
bulky substitutions at this position are tolerated. However, bulky substitutions at C1 are not
tolerated. The reverse appears to be true for the sugar exit site. Bulky substitutions at C1 are
tolerated while substitutions at C6 are not. A similar pattern is observed for sugar interaction
with GLUT4 although the impact of non-gluco configurations of hydroxyls is less marked (106).
A remaining challenge is to understand the binding requirements at the C1 position of D-glucose.
Studies with fluoro-analogs of D-glucose suggest that the beta configuration of the C1 hydroxyl
(the OH-group is above the ring or positioned cis to the C5 -CH₂OH group) is preferred (8) while
studies with alpha- and beta-D-glucose indicate mixed results (for review see (74)). More recent
studies demonstrate that alpha and beta-D-glucose are transported with equal avidity by GLUT1
(74). Answers to these questions must await crystallization of the GLUT1-D-glucose complex.
Transport Kinetic Asymmetry

Sugar transport is termed “asymmetric” when $V_{\text{max}}$ and $K_m$ for sugar exit into sugar-free medium (zero-trans exit) are not identical to $K_m$ and $V_{\text{max}}$ for sugar entry into sugar-free cells (zero-trans entry). Although more complex than anticipated, this behavior does not violate the passive nature of transport. When [sugar] is much lower than $K_m$, the Michaelis-Menten expression for transport may be simplified to uptake, $v_{\text{out to in}} = \frac{V_{\text{max( entry)}} [S]_{\text{out}}}{K_{\text{m( entry)}}}$ and, because sugar uptake and exit must be identical when extracellular $[S] = $ intracellular $[S]$ uptake, $v_{\text{out to in}} = \frac{V_{\text{max( entry)}} [S]_{\text{out}}}{K_{\text{m( entry)}}} = v_{\text{in to out}} = \frac{V_{\text{max( exit)}} [S]_{\text{in}}}{K_{\text{m( exit)}}}$. Thus $V_{\text{max( entry)}} / K_{\text{m( entry)}}$ must equal $V_{\text{max( exit)}} / K_{\text{m( exit)}}$ and within experimental error this is satisfied.

Characteristics of Asymmetry: GLUT1-mediated sugar transport in human red cells is very asymmetric at low temperatures ($V_{\text{max}}$ for exit is 10-fold greater than $V_{\text{max}}$ for entry at $4^\circ\text{C}$) but, because $V_{\text{max}}$ and $K_m$ for entry increase more rapidly with temperature than do the exit parameters, asymmetry falls with increasing temperature (82). When studying red cell “ghosts” (cytoplasm is replaced with saline by reversible hypotonic hemolysis), transport asymmetry is greatly diminished ($V_{\text{max}}$ and $K_m$ for uptake increase and approach $V_{\text{max}}$ and $K_m$ for exit; (18)). This is caused by the loss of cytoplasmic ATP which allosterically modifies the catalytic properties of GLUT1 by binding reversibly to a GLUT1 ATP binding site (10, 16).

Physiologic significance of Asymmetry: Simulations of transport reveal important insights into transport function. Using standard transport equations that are independent of presumed transport mechanism (15), we can examine the consequences of asymmetry on net sugar import by red cells initially lacking intracellular sugar. Setting $V_{\text{max}}$ for zero-trans sugar entry as a constant, we ask what happens as we vary $V_{\text{max}}$ for zero-trans sugar exit from 10-fold less than $V_{\text{max}}$ for entry through 10-fold greater than $V_{\text{max}}$ for entry. The results show that the rate constant for
equilibration of cytoplasmic water with extracellular sugar increases in a saturable manner with
increasing asymmetry ($V_{\text{max \ exit}}/V_{\text{max \ entry}}$). Asymmetry of the kind observed in red cells allows
cells to equilibrate much more rapidly with extracellular sugar. Thus, glucose-depleted red cells
emerging from glucose-consuming organs such as the brain or placenta, are more readily refilled
upon re-entering glucose-rich circulation.

**GLUT1 displays Accelerated exchange**

Accelerated exchange transport describes the stimulating effect that the presence of sugar
at the opposite or “trans” side of the membrane exerts on the rate of unidirectional sugar flux
from the cis- to the trans-side. It is for this reason that accelerated exchange is also called “trans-
acceleration.” In an accelerated exchange uptake experiment, cells are first loaded with various
concentrations of unlabeled sugar and the rate of unidirectional, radiolabeled sugar uptake is
measured at a fixed extracellular sugar concentration. At 20ºC and lower where transport can be
accurately measured, unidirectional sugar uptake is accelerated several-fold by loading cells with
unlabeled sugar and exit is accelerated by extracellular sugar (73).

**Characteristics of Accelerated Exchange:** Two types of accelerated exchange or trans-
acceleration experiments have been described (78,96). In “equilibrium exchange” studies,
intracellular [sugar] = extracellular [sugar] and unidirectional sugar uptake or exit are measured
by using radiotracer sugars. In infinite trans experiments the concentration of unlabeled sugar at
the opposite, trans-side of the membrane is saturating and the concentration of sugar (plus
radiotracer sugar) at the cis side is varied. Unidirectional radiotracer sugar flux is then measured
in the direction cis to trans. Data obtained from these experiments have revealed much about the
GLUT1 transport mechanism.
V_max and K_m for equilibrium exchange are 50-fold greater than V_max and K_m for zero-
trans sugar uptake and 5- to 10-fold greater than V_max and K_m for zero-trans sugar exit at 4 °C (24,82), for review see (80). As temperature increases, the difference between exchange and net transport parameters decreases (82). GLUT1-mediated sugar transport in dolphin erythrocytes displays remarkably similar exchange transport properties (26). The availability of cytoplasmic ATP exaggerates trans-acceleration in human erythrocytes by suppressing the maximum rate of zero-trans sugar uptake and by decreasing K_m for equilibrium exchange (23).

**Physiologic significance of exchange transport:** How does accelerated exchange impact net sugar export from red cells initially containing 5 mM D-glucose into serum containing 2 mM sugar? Assume that transport is asymmetric (V_max exit = 10 V_max entry) and varying V_max for exchange from 50-fold greater than V_max for zero-trans entry to V_max for exchange ≈ V_max for zero-trans entry, our simulations show that the half-time for loss of intracellular sugar to serum increases as V_max for exchange transport falls. Thus red cells deliver their intracellular glucose content to serum more rapidly when V_max for exchange transport is greater than V_max for zero-trans entry. This lends support to the hypothesis that human and dolphin red cell sugar transport have evolved to deliver intracellular glucose to glucose-dependent tissues such as the brain and placenta (26).

**GLUT1 Cooperativity**

Two fundamentally different models have been suggested for protein-mediated sugar transport (the simple carrier and fixed-site transporter models, see Equilibrium Ligand Binding below). Multiple, independent analyses of human red cell sugar transport steady-state kinetics have demonstrated persistent deviations of sugar transport behavior from the behavior expected of these models (5,24, 36,40,44). Some studies have suggested that the behavior of the red cell
transport system is compatible with these models (82, 126). In combination, these studies suggest
either that transport is more complicated than anticipated or that previous transport
measurements are technically flawed. Recent published reports confirm the former interpretation
(9,74).

Characteristics of cooperativity: Measurements of $V_{\text{max}}$ and $K_m$ for zero-trans and equilibrium
exchange sugar transport permit computation of a predicted $K_m$ for infinite-trans exit
(unidirectional sugar exit from red cells into solutions containing saturating sugar concentrations
(79)). The experimental $K_m$ for infinite-trans exit is consistently 5- to 10-fold lower than that
predicted by standard models for transport (5,24,36,40,44). It appears, therefore, that saturation
of the external sugar binding site increases the affinity of the internal sugar binding site(s) for
sugar.

Other interesting trans-effects are also observed. Low concentrations of cytochalasin B
and forskolin (inhibitors of glucose transport that bind at or close to the sugar export site)
increase the affinity of the external site for transported sugars (25). Extracellular maltose, which
binds to the sugar uptake site but is too large to translocate through the transporter, stimulates
sugar uptake at extremely low maltose concentrations and inhibits transport as its concentration
is raised (41). Thus endofacial and exofacial inhibitors accelerate sugar import at subsaturating
inhibitor concentrations. These observations suggest that multiple ligand binding sites exist on
the glucose transporter that modulates the affinity of adjacent cis- or trans-sites for transported
substrate.

Physiologic significance of Cooperativity: The net impact of the high affinity exit site is best
illustrated when we compare simulated net sugar uptake with measurements of sugar uptake.
Simulated net uptake at 20 mM extracellular sugar proceeds relatively linearly with time because
the internal sugar exit site is predicted to have low affinity for sugar. Measured net sugar uptake slows from the predicted rate at relatively low intracellular sugar levels due to the high affinity sugar exit site. Net uptake is, therefore, inhibited significantly at low intracellular sugar levels due to the high affinity sugar exit site. This may serve to inhibit further glucose uptake when sufficient intracellular glucose is available to saturate hexokinase.

**Equilibrium ligand binding**

GLUT1 ligand binding studies have significantly extended our understanding of the sugar transport mechanism by permitting the direct quantitation of transporter sugar binding sites and by allowing for experimental approaches to questions such as “Can sugars bind simultaneously at sugar import and export sites or are binding sites only alternately accessible?”

**GLUT1 ligand binding sites:** Two experimental approaches can assess whether GLUT1 exposes sugar import and sugar export sites simultaneously or alternately. The first makes direct measurements of ligand binding to GLUT1 and examines the effects of exo- and endofacial site substrates on ligand binding. The second examines the effects of the simultaneous presence of endo- and exofacial inhibitors on glucose transport.

The textbook model for glucose transport (variously termed the simple carrier, the alternating conformer carrier, the mobile carrier or the iso-uni-uni model for glucose transport (60,79,127)) proposes that the transporter exposes one sugar binding site (an import site or an exit site) at any instant and that conversion of the import to the export site through a substrate binding induced conformational change is the central catalytic step mediating substrate translocation. While the various models listed above may represent different physical mechanisms, their King-Altman representations describing the key transport intermediates and
their inter-conversions are identical. Alternatively, the fixed-site or two-site transport mechanism describes a transporter that presents sugar import and sugar export sites simultaneously. Sugars bind at these sites then dissociate into a central cavity that is sufficiently large as to allow sugars to bypass each other en route to the trans-binding site. If the fixed-site model is correct, then a ligand such as cytochalasin B or forskolin that binds close to the endofacial sugar binding site should not eliminate the exofacial sugar binding site unless occupancy of the endofacial site by cytochalasin B greatly reduces the affinity of the exofacial site for sugar. If the simple carrier mechanism is correct, a ligand such as cytochalasin B or forskolin that binds close to the endofacial sugar binding site should eliminate the exofacial sugar binding site because both sites cannot exist simultaneously. In this case, the binding of cytochalasin B and exofacial ligands should be competitive. The available data suggest that GLUT1 ligand binding is compatible with the fixed-site transport mechanism although simple carrier behavior is observed under special circumstances.

**Characteristics of ligand binding:** The early purifications of human erythrocyte GLUT1 also demonstrated that cytochalasin B and exofacial inhibitor binding to GLUT1 are mutually exclusive (6,37,118). This behavior is compatible with the simple carrier mechanism for sugar transport. This approach was quickly followed up by ingenious studies (71) of sugar transport in human red cells which asked the question: Is the inhibition of sugar transport produced by pairs of inhibitors (one acting at the import site and one acting at the export site) consistent with a model in which inhibitor binding sites are mutually exclusive (the simple carrier) or co-exist (the fixed-site transporter)? The answer appeared to be that the simple carrier model is upheld with the proviso that should fixed site transporter import and export sites show negative cooperativity
(i.e. binding of inhibitor to one site reduces the affinity of the remaining trans-site for ligand), this conclusion would be negated. The outcome was therefore ambiguous.

Later studies using purified GLUT1, red cell membranes, and intact red cells (13,17,52) supported a fixed-site transport mechanism with interacting (negatively cooperative) import and export sites. The type of cooperativity between exo- and endofacial binding sites is strongly dependent on the exofacial ligand. For example, the exofacial ligand phloretin exerts a very strongly negative cooperative effect on $K_d$ for cytochalasin B binding to the endofacial site by increasing $K_d$ for cytochalasin B binding by 40-fold (52). At low concentrations (< 1 mM), maltose and ethylidene glucose enhance cytochalasin B binding to GLUT1 (positive cooperativity). At higher concentrations these ligands have a negative cooperative effect on binding (25,52). Exofacial D-glucose and endofacial cytochalasin B binding are independent (17).

These different behaviors are determined by the redox state of the transporter. GLUT1 purified in the presence of reductant appears to behave as a simple carrier while transporter obtained in the absence of reductant or studied in situ appears to function as a fixed-site transporter (48,49,133). We shall return to this observation when we consider glucose transporter quaternary structure.

**Transient Kinetics**

Steady state kinetic studies allow the observer to build models that describe the intermediates (e.g. GLUT1-sugar complexes) involved in sugar transport. Transient kinetics studies provide a more critical test of the proposed models by monitoring the rate of transition of one conformational state to another.
Four important studies of GLUT1 transient kinetics have been reported. Appleman and Lienhard (3) studied substrate-induced changes in GLUT1 conformational states by monitoring the intrinsic tryptophan fluorescence of the purified transporter. Their studies employed reduced GLUT1 and support the hypothesis that exofacial ligands can trap GLUT1 in one conformational state that subsequently relaxes to a second state upon dilution of exofacial ligand. These findings are compatible with both simple carrier and fixed-site transporter models for transport. Similar studies with non-reduced GLUT1 (120) demonstrate that μM levels of exofacial ligands promote one conformational state while higher, mM levels promote a second, inhibited state. These findings are compatible with only the fixed-site transporter model for transport. Rapid quench transport measurements in red cells (83) showing transient acceleration of glucose uptake upon dilution of extracellular maltose are inconclusive being consistent with the findings of Appleman and Lienhard (51), with fixed-site transporter predictions (14,97,98) and may be explained by the stimulatory effect of maltose on transport (41).

More recent studies demonstrate 3 phases of sugar uptake by red cells (9). The first, rapid (but quantitatively smallest) phase describes sugar association with GLUT1 (1 mol sugar : 1 mol GLUT1). The second, fast phase describes sugar import into cytosol and accounts for two-thirds of total D-glucose uptake by red cells. The third and slowest phase describes a slowing of transport as endofacial sugar binding sites become saturated (74). The potential significance of these results will be discussed below.

**GLUT1 STRUCTURE**

Human GLUT1 is a strongly hydrophobic protein comprising 492 amino acids. Hydropathy analysis suggests 12 hydrophobic, membrane-spanning alpha-helices (TMs, (93)). This conclusion is supported by scanning-glycosylation mutagenesis (55), mass spectrometry
GLUT1 presents cytoplasmic N- and C-termini, a long cytoplasmic loop connecting TMs 6 and 7, a long C-terminus and a glycosylated extracellular loop between TMs 1 and 2. In spite of its hydrophobicity, GLUT1 is readily accessible to solvent water (2, 64). Indeed 8 of the 12 GLUT1 putative TMs are amphipathic (93) suggesting that GLUT1 presents a water-filled channel for sugar translocation. Two TMs (1 and 8) are poised at the limits of bilayer solubility (TM1 is released by trypsin treatment of GLUT1 and TM 8 is released by addition of endofacial ligand to trypsinized GLUT1 (11)). GLUT1 undergoes significant conformational change upon binding substrates (22), inhibitors (2) and ATP (10). Interactions between the GLUT1 C-terminus and middle loop may control transport activity and are regulated by ATP (10).

**GLUT1 Topology and Architecture**

Our understanding of GLUT1 architecture has been shaped by three types of study: 1) Biochemical analyses; 2) Molecular Biology / mutagenesis approaches and, 3) Homology-modeling GLUT1 structure using the crystal structures of distantly related proteins from the Major Facilitator Superfamily (MFS). The results of these studies have transformed our understanding of GLUT1 architecture and have revealed new insights into determinants of stereospecificity. However, much remains to be achieved before a detailed understanding of the transport mechanism emerges.

GlpT and LacY were the first MFS proteins to yield high resolution crystal structures upon X-ray diffraction analysis (1, 56). Both structures comprise N-and C-terminal halves each containing 6 trans-membrane alpha-helices (TMs). The N- and C-terminal domains are connected by a long cytoplasmic loop and, when reviewed in isolation, are almost structurally
super-imposable (Fig 1A). In the crystal structure, the C-terminal domain is rotated 180 degrees about the central axis normal to the plane of the bilayer (Fig 1B). Each domain contains two strongly hydrophobic TMs (3 and 6 in the N-terminal domain and 9 and 12 in the C-terminal domain) that anchor 4 more amphipathic TMs (1, 2, 4 and 5 in the N-terminal domain and 7, 8, 10 and 11 in the C-terminal half). The subunit structure approaches that of a "Mayan Temple with a flat rectangular top and bottom" (56). The homology-modeled GLUT1 structure (109) closely resembles GlpT and LacY structures.

The MFS structure presents an open cavity to cytoplasm and appears to be sealed to the interstitium. This structure is visually consistent with theoretical models for carrier mediated sugar transport in which GLUT1 isomerizes between import and export states and may represent the export (also called the el) state of GLUT1. No MFS protein structures representing the import (or e2) state have been described to date, hence it is possible that the Mayan temple structure represents a more fixed or rigid architecture and that the flexible domains associated with transport conformations are more deeply buried within the temple's core.

Blodgett et al have compared the proposed topology of GLUT1 (109) with the trypsin and NHS-ester accessibility of GLUT1 (11). Making the assumption that trypsin cleavage, lysine-modification and cysteine-modification do not radically alter the architecture of GLUT1, only minor adjustments in the threaded topology of GLUT1 are necessary. These include adjustments to TM5, loop 5-6, and TM6 and TM12. Figure 1C summarizes the proposed modified GLUT1 topology and highlights 7 different categories of amino acids: 1) residues that are accessible to trypsin, NHS-LC-biotin and iodoacetamide (11); 2) residues which when mutagenized to cysteine allow sugar transport inhibition by extracellular PCMBs (94); 3) amino acids which when mutagenized to cysteine afford sugar-dependent protection against transport
inhibition by pCMBS (94); 4) residues whose substitution by cysteine results in transport inhibition (94); 5) amino acids whose mutagenesis results in GLUT1 deficiency syndrome (69, 124); 6) amino acids whose positions are inferred glucose binding sites in docking studies with Glpt-threaded GLUT1 structures (27, 109) and, 7) residues thought to discriminate between glucose and fructose (87).

**Oligomeric structure**

The crystal structure of the MFS protein LacY, which contains a bound substrate, suggests that the catalytic unit of the transporter (and that of its structurally similar, relative GlpT) is the protein monomer. Studies with purified GLUT1 indicate that reduced GLUT1 binds 1 mol cytochalasin B per mol GLUT1 while nonreduced GLUT1 binds 0.5 mol cytochalasin B per mol GLUT1 (49, 117, 132). This suggests that each reduced GLUT1 molecule provides 1 fully functional sugar export site and, since exofacial ligands competitively displace cytochalasin B, each subunit also presents a sugar import site. It is probable, therefore, that each GLUT1 molecule represents a fully functional catalytic unit. Freeze fracture electron microscopy suggests that reduced GLUT1 is a dimer (40, 117) and that nonreduced GLUT1 is a tetramer. Hydrodynamic size analysis of detergent solubilized GLUT1 and chemical cross–linking studies support this conclusion (40, 49, 132) although some detergents disrupt GLUT1 oligomeric structure (40, 43).

Co-immunoprecipitation studies using GLUT1-GLUT4 chimeras also confirm that GLUT1 forms oligomeric complexes (104) but that GLUT hetero-complexes are not found. Unpublished findings using GLUT1-GLUT3 chimeras demonstrate that GLUT1 TM9 is essential for GLUT1 dimerization (Levine, DeZutter and Carruthers, unpublished). The reductant
sensitivity of tetrameric GLUT1 has been ascribed to a specific transporter fold deriving from an intramolecular disulfide bridge or from 2 mixed disulfides (involving Cys 347-and Cys 421) present in each subunit (49,133).

Functional co-expression studies in Xenopus oocytes suggest that mutant forms of GLUT2 and GLUT3 do not suppress the activity of wild-type GLUT (12). Heterologous expression of mutant GLUT1 in mammalian cells demonstrates either unaltered parental GLUT1 activity (59,101) or modified parental GLUT1 activity (76,77). We have proposed that dimeric GLUT1 (observed with reduced GLUT1 or GLUT1 expressed in Xenopus oocytes) comprises two structurally associated but functionally independent GLUT1 subunits (133). Tetrameric (non reduced) GLUT1 comprises a dimer of GLUT1 dimers in which subunits interact cooperatively. The net result is a complex simultaneously presenting at least two exofacial and two endofacial ligand binding sites (25,41,133).

**UNIFYING MODEL FOR STRUCTURE AND TRANSPORT**

Recent docking studies have suggested that GLUT1 presents two exofacial and endofacial glucose binding sites connected by a cavity sufficiently large to contain a glucose molecule (27,109). It should be noted, however, that homology modeling is not immune to over-interpretation. Homology-modeled LacY structure using GlpT as a template approximates transporter topology and architecture but less successfully reproduces the spatial arrangement of amino residues involved in substrate binding to LacY (75). Naftalin has suggested that the simple carrier hypothesis is flawed on thermodynamic grounds (99). This dogma-challenging hypothesis reasons that the high affinity exofacial form of GLUT1 (e2) must reorient to a high affinity endofacial form before relaxing to the low affinity endofacial form (el). Similarly, the
low affinity endofacial form \( e_1 \) must isomerize to a low affinity exofacial form before relaxing to the high affinity exofacial state \( e_2 \).

Previous studies have demonstrated that the complexities of erythrocyte sugar transport are not explained by classical simple carrier or fixed-site transporter models (see above). Naftalin proposes a variant of the fixed-site transporter in which sugar can bind to co-existent endo- and exofacial sugar binding sites and exchanges between these sites via a connecting, central cavity. Exchange between binding sites and cavity is accelerated when binding sites are occupied by sugar via process termed “geminate exchange”. This model not only explains the stereospecificity and complexity of steady-state sugar transport (99) but also accounts for multiphasic sugar transport kinetics (74), the ability of GLUT1 to trap or occlude a sugar molecule within a central cavity (9) and cooperative, multi-site ligand binding (41, 71). The enhanced cooperativity of tetrameric GLUT1 may result from additional subunit interface interactions that are present in the tetramer. Future structural, mutagenesis and ligand binding studies will resolve this question by demonstrating co-existent or mutually exclusive import and export sites.

CELL BIOLOGY OF GLUT1

Approximately 20 to 40% of total cellular GLUT1 is expressed at the cell surface of cardiomyocytes (84), blood brain barrier endothelial cells (116), adipocytes (54) and astrocytes (85). Intracellular GLUT1 is located within the endoplasmic reticulum, the Golgi and endosomes (84). Endosomal GLUT1 cycles between the plasma membrane and endosomal compartments in cardiomyocytes in an AMP kinase and PI-3 kinase dependent manner (84) but this recycling compartment is distinct from that of the insulin-responsive glucose transporter isoform GLUT4 in adipose, heart and muscle (89). It has been suggested that acute regulation of cell surface
GLUT1 levels could provide an effective mechanism for controlling sugar transport across the blood brain barrier and in astrocytes (116) but supporting evidence is unavailable.

**EARLY IMPLANTATION AND EMBRYO DEVELOPMENT**

GLUT1 plays an important role in mediating implantation of the embryo. This is a highly synchronized process that occurs between an activated blastocyst and the receiving endometrium. Investigators have employed endometrial stromal cells in both mouse and human and observed that a fine balance has to be struck between the two sex-steroids, namely progesterone and estrogen. While estrogen primes the endometrium, it is progesterone that transforms the endometrium to provide a receptive environment for the embryo. Progesterone increases GLUT1 and GLUT1-dependent glucose uptake in endometrial stromal cells. Estrogen demonstrates no effect on its own, however when introduced in the presence of progesterone, estrogen brings the progesterone induced increased GLUT1 expression and glucose uptake back to normal untreated concentrations. Further, in the presence of a progesterone receptor antagonist (RU486), progesterone effect on GLUT1 and glucose uptake is abolished. Thus it appears that the progesterone induced receptivity by the endometrium is reliant on GLUT1 mediated glucose uptake by the endometrial stromal cells (33).

In pre-implantation embryos, GLUT1 is expressed on the basolateral surface of the polarized trophectodermal cells and the inner cell mass. Maternal diabetes with hyperglycemia causes a suppression of GLUT1 expression in the pre-implantation embryos at 48 and 96 hours after conception (92). This suppression interferes with glucose uptake resulting in upregulation of Bax and DNA fragmentation consistent with apoptosis (91). Maternal diabetes in Bax-/- genotypic mice resulted in suppression of GLUT1 expression and glucose uptake without an increase in apoptosis, confirming a role for Bax in mediating apoptosis due to GLUT1 reduction.
More importantly during the late gestation stages of fetal development, GLUT1 is expressed by most tissues, specifically at e10, it is noted in the neural tube, gut, heart and optic vesicle (90). As the fetus matures into late gestation, most tissues demonstrate GLUT1 expression.

**REGULATION OF GLUT1 EXPRESSION**

Postnatally, through the suckling phase of life, various tissues such as brain (30,67,108), skeletal muscle (108,112) and myocardium (112) reveal high concentrations of GLUT1 expression. In the late gestation ovine fetus, insulin and glucocorticoids regulate skeletal muscle GLUT1 expression by increasing the concentrations (39,47). After weaning during the post-suckling phase and beyond into adult stages, there is a decline in GLUT1 expression in most tissues except the brain (30,67,108,112). This decline is replaced by the emergence of tissue-specific isoforms, such as the insulin responsive GLUT4 in skeletal muscle and myocardium (108,112). GLUT1 is expressed in white adipocytes even in the adult suggesting a mechanism of basal glucose uptake by these cells which provides a storage function for the body (65).

In addition to the developmental regulation noted with GLUT1 expression in various tissues, perturbations in circulating glucose concentrations demonstrate an ability to regulate GLUT1 expression (114). Further, hypoxic conditions as encountered in cancerous transformation or otherwise, result in enhanced GLUT1 expression (19). Regulation of GLUT1 expression occurs at two levels, the first is transcriptional and the second is post-transcriptional. Transcriptional regulation involves trans-activation by Sp1 and repression by Sp3. The ratio of these two nuclear factors determines the ultimate expression levels of GLUT1 mRNA and protein (57,110). Developmental regulation consisting of a post-suckling decline in myocardial
GLUT1 expression is mediated by myoD that inhibits Sp1 induced trans-activation of the GLUT1 promoter (123).

In addition, specific RNA binding proteins are known to bind a consensus sequence at the 3’-end of the GLUT1 gene mediating mRNA stabilization of the GLUT1 gene. Studies employing the bovine GLUT1 gene revealed nucleotides 2181 to 2190 to bind cytosolic proteins and provide mRNA stability (35). Glucose deprivation and hypoxia induced increase in GLUT1 expression is mediated by this cis-acting regulatory element in the 3’-untranslated region of the GLUT1 gene (35, 42). Further, RNA binding proteins hnRNP A2 and hnRNP L that complex with each other and independently bind the AU-rich response element in the GLUT1 3’-UTR regulate expression by translational repression and mRNA instability (42). Hypoxia and hypoglycemia both selectively decreased polysomal hnRNP A2 and L protein concentrations, thereby contributing to increased GLUT1 mRNA stability and translation efficiency with resultant increased GLUT1 expression (42). Other investigations have demonstrated binding of the RNA binding protein that belongs to the Hu/ELAV family (HuR) with the nucleo-cytoplasmic shuttling sequence that functions as an adaptor protein in the nuclear export of mRNAs that contain adenylate-uridylate rich elements in the 3’-UTR, thus contributing while in the cytoplasm towards mRNA stability and translational efficiency (35). In terminally differentiated adipocytes alone, HuR complexes with EBPβ and translocates to the cytosol where HuR binds the GLUT1 3’UTR thereby leading to increased expression of GLUT1. In contrast, in undifferentiated pre-adipocytes, HuR was observed within the nucleus alone (35).
Placental expression of GLUT1

GLUT1 is considered the major glucose transporter isoform of the syncytiotrophoblastic cells of the human placenta (58). Increased concentrations are observed on the microvillous surface of the trophoblastic layer which is the maternal surface as opposed to the basal layer facing the fetus (58). Investigations in the human diabetic placenta have revealed an increase in GLUT1 concentrations that correlate with a large infant (58). In contrast, the presence of intra-uterine growth restriction revealed a decrease in placental GLUT1 concentrations. While GLUT3, a related isoform also responsible for basal glucose transport is expressed in cytотrophoblasts during early gestation, its expression during late gestation is limited to the fetal vascular endothelium (70). Various investigations in mice/rats reveal that unlike the human placenta, GLUT1 co-exists with a related isoform, GLUT3, in syncytiotrophoblast layers of the placental labyrinthine region throughout gestation. GLUT1 is noted on the maternal and fetal-facing surfaces of syncytiotrophoblast layers I and II in rats (113). Maternal diabetes in the streptozotocin-treated pregnant rat and the non-obese diabetic (NOD) mouse failed to alter placental GLUT1 expression (28,29). In contrast, intra-uterine growth restriction in rats due to uterine artery ligation led to a 50% decrease in GLUT1 concentrations (28). In contrast, more recent experiments in high fat fed pregnant mice demonstrated increased trans-placental glucose transport mediated by increased placental GLUT1 concentrations during late gestation with resulting large fetuses (62). In chronic experiments of hyperglycemia in the sheep fetus an initial increase in placental GLUT1 over 48 hours was observed followed by a decline ultimately (28). In contrast, insulin induced chronic maternal hypoglycemia caused a decline in placental GLUT1 concentrations (29). Thus it appears that placental GLUT1 plays a vital role in mediating
materno-placental glucose transport, which in turn regulates the placento-fetal transport of glucose via the coordinate expression of GLUT3 (113).

**Blood-brain barrier GLUT1**

The endothelial cells of the brain microvasculature, the astrocytes and choroid plexus also express GLUT1. In the endothelial cells, GLUT1 plays a vital role in brain glucose uptake. Circulating glucose concentrations regulate the endothelial GLUT1 protein concentrations (30). While chronic hypoglycemia upregulates GLUT1 concentrations, hyperglycemia has no effect (114). The presence of GLUT1 on astrocytes suggests that glucose taken up by these cells is converted into lactate and released into the interstitium as an alternate substrate to be utilized by neurons, particularly in the absence of an adequate supply of glucose (103). Although controversial (115), this concept supports astrocytes as local energy storage sites necessary to fuel neurons, providing credence to the astrocyte-neuron shuttle theory (103). Further provision of an n-3 polyunsaturated fatty acid diet (docosahaeanoic and eicosapentaenoic acids) in rats led to a decrease in microvascular GLUT1 expression, while an n-3 PUFA rich diet increased this protein (105).

**Blood-retinal and blood-neural barrier GLUT1**

The blood-retinal barrier consists of the retinal pigmented epithelial cells that are rich in GLUT1 (88). In addition to expression in the retinal pigmented epithelial cells, the choroidal, iridial and pars planus, retinal Mueller cells, the lens fiber cells, iridial microvascular endothelial cells and to a lesser extent the outer segments of the photoreceptor cells of the adult human eye expressed GLUT1. This distribution pattern was conserved throughout development and was evident as early as 8 weeks gestation in the human embryo (88). In addition, the endothelial cells
of vitreous hyaloid vessels expressed GLUT1 at 8 weeks gestation (88). Diabetes mellitus decreases GLUT1 expression in the vascular endothelium (4), while increasing GLUT1 in the blood-retinal barrier (72), thereby mediating increased glucose transport across the blood-retinal barrier. Further vascular endothelial growth factor mediates this increase in GLUT1 of the vascular endothelial cells (119). The presence of a polymorphism located in the GLUT1 gene was associated with diabetic nephropathy but not with diabetic retinopathy, GLUT1 being primarily expressed in the mesangial cells of the kidney (53).

Similarly the perineurial cells that form the blood-nerve barrier also demonstrate GLUT1 expression. Peripheral nerves are ensheathed by layers of flattened fibroblast-like cells that form the perineurium. The perineurium and the vascular endothelium together form the blood-nerve barrier and both are rich in GLUT1. This isoform mediates transport of glucose to the peripheral nerves (122). Thus under conditions of hyperglycemia related to diabetes, increased glucose transport across this barrier may mediate peripheral neuropathy. However in-vitro cultures of the sciatic nerve in the presence of 25 mM of glucose demonstrated a downregulation of GLUT1 expression (95). Whether similar changes occur in-vivo remains to be established.

**GLUT1 over expression investigations**

Transgenic overexpression of human GLUT1 in skeletal muscle leads to a large increase in basal glucose transport and metabolism but impaired stimulation of insulin induced glucose transport resulting in reduction in fasting and fed circulating glucose concentrations with enhanced glucose clearance. However, overexpression of GLUT1 in skeletal muscle (EDL) did not interfere with the insulin responsive translocation of GLUT4, but rather dissociated the intrinsic activity of GLUT4 from the plasma membrane concentrations of the protein (32,45). In
contrast, when transgenic overexpression of GLUT1 was accomplished in myocardium, a remodeling of fatty acid and ketone metabolic pathways was observed to accommodate the chronic increase in intracellular glucose concentrations targeted at maintaining cardiac energy and function. When exposed to a high fat diet however, the GLUT1 transgenic mice suffered from increased oxidative stress and contractile dysfunction (130).

**GLUT1 Null Mutations**

Various groups have created mutations in the GLUT1 gene to assess its functional role during development. Initially GLUT1 deficient transgenic mice were produced using antisense-GLUT1 constructs. In pre-implantation embryos obtained from these mice, glucose uptake was reduced when compared to wild type mice (51). In addition, homozygous GLUT1 mutant mouse embryos obtained from the antisense-GLUT1 transgenic mouse mating revealed severely impaired organogenesis consisting of caudal regression, agenesis of kidneys, stunted growth, absent head, microphthalmia and other malformations reminiscent of the human infant of a hyperglycemic diabetic mother (51). The rate of still births was higher than that seen in heterozygous mating of the antisense-GLUT1 and wild type mice. The offspring of this mating was stunted in growth but achieved adulthood with no differences in circulating blood glucose concentrations but demonstrated the antisense-GLUT1 transcript in the heart, spleen, lung, skeletal muscle, liver, kidney and adipose tissue (51). In these heterozygous mice, nitric oxide-dependent endothelial relaxation in aorta was reduced supporting an important role for GLUT1 dependent glucose metabolism in regulating endothelial function (102).

Using morpholinos and creating loss of GLUT1 function in the xenopus (frog) embryo resulted in microcephaly and an axis elongation error. Further, these morpholinos against
GLUT1 suppressed cell movement in dorsal marginal zones supporting an important role for GLUT1 during gastrulation cell movement (121). Using morpholinos targeted against GLUT1 in a Danio rerio (zebra fish) embryonic model, a decline in embryonic growth along with disruption of the developing brain structure was evident secondary to apoptosis (61). This GLUT1 deficient apoptosis was Bax dependent (61). In separate investigations, study of murine embryonic stem cells (GLUT1+/-) that demonstrate GLUT1 deficiency revealed a reduction of glucose uptake but enhanced apoptosis. Further, inhibition of oxidative phosphorylation led to impaired upregulation of GLUT1 protein in GLUT1+/− embryonic stem (ES) cells, which was evident in wild type cells (50).

Two different groups created GLUT1 null mutant mice disrupting exon 1 of the gene (100,125). The homozygous null mutation demonstrated embryonic lethality at e10 through e14. These embryos at e13 revealed morphological abnormalities consisting of a small size, lack of eyes, diminutive rostral embryonic pole and overall developmental delay versus the wild type littermates. No live birth occurred in any of them. In contrast, the heterozygous null mutation led to no decrease in body weight but a decrease in brain weight and hypoglycorrhachia in the absence of hypoglycemia. Further, abnormal motor performance was seen in the rotorod test that emerged early in adult life only to worsen with advancing age. Beam walking revealed abnormalities in balance and motor coordination and the footprint test showed no difference in stride length or hind paw base during gait (125). Further spontaneous seizures were observed along with a decline in brain glucose uptake as observed with positron emission tomography. These findings in mice paralleled that observed in human infants with the GLUT1 deficiency syndrome (G1DS) (31,68).


**GLUT1 Deficiency Syndrome**

GIDS is an autosomal dominant disorder where differing mutations have been described resulting in haploinsufficiency of GLUT1. This interferes with red blood cell glucose transport and produces hypoglycorrhachia with unperturbed lactic acid in the cerebrospinal fluid, the presence of seizures, abnormal gait and developmental delays. Introduction of ketogenic diet early during infancy through teenage years ameliorates seizures, although resurgence can be encountered despite the ketogenic diet requiring additional anti-convulsant therapy. However, ketogenic diet does not reverse the neurobehavioral findings or developmental delays (31,68).

Further investigation of GLUT1 haplodeficient mice revealed that the peak decline in brain GLUT1 occurred at P14 with a compensatory increase in Mct1 (moncarboxylate transporter mediates ketone, lactate and pyruvate transport) seen at P0. However in the adult brain, a compensatory increase in GLUT1 expression occurred reaching levels seen in wild type mice (100). The functional implications of these developmentally sensitive observations remain to be determined. Fifty percent of adult patients with GLUT1 deficiency syndrome are able to function well within a social context but the rest are greatly disadvantaged due to neurodevelopmental impairments. In various experiments, phenobarbital has been observed to reduce GLUT1 protein concentrations. These observations have led to an avoidance of this anticonvulsant in infants suspected of being afflicted by the GLUT1 deficiency syndrome. Further other classes of drugs such as methyl xanthines, caffeine and tricyclic anti-depressants known to interfere with GLUT1 function are avoided (31, 68). Hence the mouse models of GLUT1 deficiency have provided valuable insights in understanding this syndrome that affects humans.
GLUT1 functions coordinately with GLUT3 in trophectoderm/placenta and brain

While it is clear that GLUT1 is essential for normal embryonic development, it is incapable of salvaging an embryo in the absence of a closely related isoform, namely GLUT3 (Km 0.8 mM). Thus, in the presence of a homozygous null mutation of GLUT3, despite the presence of intact GLUT1, there is embryonic loss at e8.5. Thus neither GLUT1 nor GLUT3 alone are sufficient for the well being of the early embryo (34). In contrast, another related isoform, namely GLUT8 when mutated, the homozygous null mutant mice are born normally and progress to adulthood (111). More importantly, in heterozygous null mutation of GLUT3 in the presence of intact GLUT1 in the placenta, a decrease in trans-placental glucose transport to the fetus is observed. Again this observation attests to the importance of coordinated functionality of GLUT1 with GLUT3 in mediating trans-placental glucose transport (34).

Further, recent studies in the heterozygous GLUT3 mutant mouse revealed that there was a compensatory increase in brain GLUT1 expression resulting in preserving glucose transport across the blood-brain barrier (131). However, since GLUT1 was only expressed in astrocytes and not neurons, despite this compensation by GLUT1 in brain, electroencephalographic seizures and cognitive deficits were observed (131). Therefore it appears that both GLUT1 and GLUT3 are necessary for normal brain development and function (125, 131). Thus there appears to be an interdependency of GLUT1 and GLUT3 that are coordinately expressed in certain tissues towards adequately mediating the glucose transport function necessary to meet the tissue requirements or that of other cells/tissues present beyond the barrier.

ACKNOWLEDGEMENTS

SD is supported by grants from the NIH HD 33997, 46979, 41230 and 25024. AC is supported by NIH grants DK 36081 and DK 44888.
REFERENCES


105. Pifferi F, Jouin M, Alessandri JM, Haedke U, Roux F, Perriere N, Denis I, Lavialle M, Guesnet P. N-3 fatty acids modulate brain glucose transport in endothelial cells of the


FIGURE LEGENDS

Figure 1 - GLUT1 structure.

A and B GLUT1 structure as modeled in (109) and drawn using VMD (v 1.8.5). The twelve transmembrane helices are shown in two forms - as a surface representation (left) or as a cartoon representation (right). Extracellular and cytoplasmic structures are omitted. The cartoon representations include the transmembrane helix number. A TMs are shown parallel to the membrane. In B the TMs are viewed along the membrane normal from the cytoplasmic side. C GLUT1 sequence and putative topology. Amino acids are shown using the 1 letter code. The 12 TMs are colored as in A and B. Some amino acids are numbered. Individual amino acids that are not colored white indicate: cyan, sites of trypsin cleavage or lysine modification by NHS esters (11); red, amino acids, which when mutagenized to cysteine are reactive with pCMBS in the external solvent (94); green, amino acids, which when mutagenized to cysteine are reactive with pCMBS in the external solvent in a substrate-protected manner (94); yellow, cysteine residues that are accessible to iodoacetamide (11); purple, amino acids, which when mutagenized to cysteine result in ≥ 90% inhibition of GLUT1 (94); orange, putative substrate binding sites predicted by docking studies (27,109); blue, amino acids implicated in substrate discrimination (87); black, sites at which mutations cause GLUT1 deficiency syndrome (69, 124). Some amino acids fall into multiple categories.

Figure 2 Model for GLUT1-mediated sugar transport.

A Schematic representation of the catalytic center of the transporter. Extracellular sugar (Go) and intracellular sugar (Gi) react with exo- and endofacial sites respectively to form G2 and G1 respectively. Sugar dissociates from these sites into the inter-site cavity to form Gc. When G2
and G1 are occupied, dissociation to Gc and reassociation are accelerated (red dissociation steps). Adapted from (99)). **B.** Simulation of biphasic exchange transport by this carrier mechanism (adapted from (74)). **C.** The GLUT1 model (adapted from Figure 1B with TMs 2 and 11 removed for clarity) showing intracellular sugar complexed to the G1 site, a putative G2 site, the small inter-site cavity (109), the TM9 oligomerization surface (Levine, DeZutter & Carruthers, unpublished), the 2 conformationally dynamic TMs (1 & 8) that are released into the aqueous solvent when the GLUT1 backbone is cleaved by trypsin (11), the three exofacial lysine residues exposed by D-glucose (11), the putative cytochalasin B binding hairpin (11,109).
A
outside inside

G0 ↔ G2 ↔ Gc ↔ G1 ↔ Gi

B

\[
\begin{align*}
G_1, G_2 & \text{ or } G_c \\
\text{(mM)} & \\
\end{align*}
\]

Gii/Go

Time (sec)

C

Inter-site cavity (Gc)

TM1

Glc-exposed lysines

TM9 oligomerization surface

TM10-11 hairpin

CB-sensitive TM8

CB