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STRUCTURE, FUNCTION, AND REGULATION OF THE MAMMALIAN FACILITATIVE GLUCOSE TRANSPORTER GENE FAMILY

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ABSTRACT

The facilitative transport of glucose across the plasma membranes of mammalian cells is catalyzed by a family of glucose transport proteins (GLUT). Four glucose transport proteins and a fructose transport protein have been identified. These transport proteins have unique tissue distributions and biochemical properties under- lying specific physiologic functions. GLUT1, the first GLUT isoform identified, is expressed at highest levels in the endothelial of barrier tissues such as blood vessels and the blood-brain barrier. GLUT2, found predominantly in liver, intestine, kidney, and pancreatic β-cells, is a low-affinity glucose transport protein that is part of the glucose sensor in pancreatic β-cells and facilitates either glucose uptake or efflux from the liver depending on the nutritional state. GLUT3 is the glucose transporter responsible for maintaining an adequate glucose supply to neurons. GLUT4 is the insulin-regulated glucose transporter found in adipose tissues, heart muscles, and skeletal muscles that is responsible for insulin-regulated glucose disposal.

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INTRODUCTION

Glucose functions as precursor for the synthesis of glycoproteins, triglycerides, and glycogen and provides an important energy source by generating ATP through glycolysis. Because glucose is a polar molecule, it does not readily diffuse across the hydrophobic plasma membrane, and therefore, specific carrier molecules exist to mediate the specific uptake of this sugar. In polarized epithelial cells in the lumen of small intestine and in the proximal tubules of the kidney, there is an energy-dependent Na^+/glucose cotransporter (19, 55, 56, 64). This transport protein utilizes the movement of Na^+ down its electrochemical gradient to drive the complete uptake of glucose. In the intestines, this glucose transporter serves to efficiently absorb the dietary-presented glucose; in the kidney, it reabsorbs the filtered glucose back into the blood (reviewed in 127).

In contrast to the highly restricted tissue specificity of the Na^+-dependent glucose transporter, all mammalian cells contain one or more members of the facilitative glucose transporter gene family. These facilitative glucose transporters are characterized by a high degree of stereoselectivity, providing for the bidirectional transport of glucose, with passive diffusion solely down its concentration gradient. These facilitative glucose transporters function to regulate the movement of glucose between the extracellular and intracellular spaces within the body, thereby assuring that a relatively constant supply of circulating glucose will be available for metabolism.

The facilitative glucose transporters are a group of highly related integral membrane proteins that share significant sequence similarity. To date, seven members of this supergene family have been described, but only four have been documented as authentic glucose transporters. These proteins are named GLUT (for glucose transporters) 1–7, based on the chronologic order of their identification and isolation of cDNA clones (recently reviewed in 103). GLUT1, the first glucose transporter isoform to be described, was initially identified as the major glucose transport protein in human erythrocytes (77). Because of its high abundance in red cell membranes (3–5% of total membrane protein), GLUT1 could be isolated with a high degree of purity. The purification of the human erythrocyte glucose transporter allowed for the initial biochemical characterization of this protein and for generation of antibodies as tools for further biological study (1, 54, 104). The availability of GLUT1

antibodies provided the necessary reagents to clone the GLUT1 cDNA from human HepG2 cells (104) and rat brain (7). The identity of the GLUT1 cDNA was confirmed both by comparison with partial amino acid sequence of the human erythrocyte glucose transporter and by expression of a functional glucose transport activity (7, 104). In the decade since this work was accomplished, literally hundreds of research papers have been published on this topic, and our knowledge of glucose-transport biology, physiology, and pathophysiology has been enormously expanded. The purpose of this review is to discuss the current state of glucose-transporter biology, with an emphasis on the role of glucose transporters in mammalian nutrition and metabolism.

STRUCTURE AND FUNCTION OF THE GLUCOSE TRANSPORTERS

Tissue Distribution of the GLUT Isoforms

The facilitative glucose transporter gene family consists of six functionally distinct proteins of which four have been documented as functional facilitative glucose transporters. The cDNAs for GLUT1–4 have been isolated and characterized in terms of tissue specificity, expression, and functional activity. A related fifth member of this family (GLUT5) was originally thought to encode for an intestinal glucose transporter isoform; however, latter studies demonstrated this protein as a fructose transporter located in both apical and basolateral membranes (8, 18, 23). The sixth member of this family (GLUT6) is a pseudo gene containing multiple insertions and translation termination signals, and which does not encode for an expressed protein (80). Finally, an apparent glucose transport protein located in the endoplasmic reticulum termed GLUT7 has been identified as a component of the glucose-6-phosphatase complex in liver (141). However, the direct identification of this protein as a functional facilitative glucose transporter has not yet been established and has recently been questioned (124).

In general, the facilitative glucose transporters are distributed in a manner that underlies their specific physiologic function in maintaining whole body glucose homeostasis. Although there is a considerable degree of overlap in the tissue expression of the various glucose transporter isoforms, several overall themes have emerged. For example, GLUT1 is expressed at high levels in all fetal tissues (25, 118, 125, 139, 142). In adult tissues, GLUT1 is widely expressed, but it is most abundant in fibroblasts, erythrocytes, and endothelial cells with low levels of expression in muscle, liver, and adipose tissue (7, 35, 115). The expression of GLUT1 in endothelial cells is thought to provide a pathway by which glucose can be transported across the blood brain barrier to the central nervous system, which is dependent upon glucose as its primary

energy source (115). The GLUT2 protein is found on the basolateral membrane surfaces of liver cells, pancreatic β-cells, small intestine, and kidney, where its relatively high K_m results in transport activity in direct proportion to the physiologic range of glucose concentrations (137). GLUT3 is found at highest levels in neuronal tissue and is considered the major GLUT responsible for transporting glucose into the brain and peripheral nerves (81, 106). Interestingly, GLUT3 was originally cloned from a fetal skeletal muscle library, suggesting a role for GLUT3 in muscle development (81). In contrast, GLUT4 is predominantly expressed in adult skeletal and cardiac muscle, as well as in brown and white adipose tissue. This tissue distribution directly correlates with the cell types that display insulin-sensitive glucose transport (6, 17, 33, 68, 69).

Physiologic Functions of the GLUT Isoforms

The physiologic functions of the GLUT isoforms are also imparted by their distinct kinetic and substrate specificities. Several studies have examined the kinetic transport properties of these different isoforms. It is important to recognize that the use of glucose as a substrate will not reflect the true kinetics of transport because it is readily metabolized and transport may not necessarily be rate limiting under all conditions. Therefore, various nonmetabolizable glucose analogues have been developed. For instance, 2-deoxyglucose, which can be phosphorylated but not metabolized, is useful under conditions where phosphorylation is not rate limiting. 3-*O*-Methylglucose, although technically more difficult to use, is neither phosphorylated nor metabolized, making it the preferred substrate for kinetic analysis.

Glucose transport kinetics by individual transporter isoforms has also been difficult to determine because of a lack of mammalian cells, which express only a single isoform. Early kinetic studies utilized human erythrocytes, which contain only GLUT1, as a model system for studying glucose transport kinetics. The apparent K_m for glucose and 3-*O*-methylglucose transport in erythrocytes from humans and other species ranges from 1–38 mM, depending on the methods used (reviewed in 15). Similarly, the reported V_{max} for glucose transport in erythrocytes has varied from 15–366 mmol • liter of H_2O^{-1} • min^{-1} (15).

To circumvent the problem that multiple GLUT isoforms are present in most mammalian cells, *Xenopus laevis* oocytes have been widely used for measuring glucose transport kinetics catalyzed by a single GLUT isoform. Oocytes have low levels of endogenous glucose transport and are amenable to expression of specific transport proteins. An advantage of this system, although artificial, is that transport kinetics for various GLUT isoforms can be compared within an identical cell context. Results of glucose transport assays under equilibrium exchange conditions show an apparent K_m for 3-*O*-methylglucose transport by

both human and rat GLUT1 that ranges from 16.9 to 26.2 mM (45, 46, 82, 111). Under the same transport conditions, GLUT4 has an apparent K_m of 1.8–4.8 mM (82, 111), whereas GLUT3 has a K_m of 10.6 (46). Although K_m measurements for 3-*O*-methylglucose in oocytes are quantitatively different from those determined for the endogenous glucose transporters, these data demonstrate that both GLUT3 and GLUT4 have a higher affinity for glucose than does GLUT1. These higher affinities insure that glucose transport will be maximal in tissues containing these GLUT isoforms even when substrate concentrations are relatively low. For the brain, this is particularly important because of the reliance of neuronal tissue on glucose as its sole energy source.

In contrast, GLUT2 has the lowest affinity for glucose with a K_m of 40 mM for 3-*O*-methylglucose (46). Because normal circulating concentrations of glucose range between 3.9 and 5.6 mM, the rate of transport through this carrier will be directly proportional to the change in glucose concentration. Thus, when circulating levels of glucose are high (postprandial state), there is a net flux of glucose into the hepatocytes of the liver and β-cells of the pancreatic islet of Langerhans. In contrast, during periods of low circulating glucose (fasting), intracellular concentrations of free glucose increase in hepatocytes as a result of increased glycogenolysis and gluconeogenesis. When the intracellular glucose levels exceed the plasma glucose concentration, GLUT2 can then function to transport glucose out of the liver into the circulation. In addition to its ability to transport glucose, GLUT2 has also been shown to be a low-affinity fructose transporter (20, 46). This is consistent with the liver being the primary site for fructose metabolism.

Glucose Transporter Structure

Hydropathy analysis of the predicted amino acid sequence from the GLUT1 cDNA predicted a protein containing 12 membrane-spanning domains with both the amino and carboxyl termini oriented intracellularly (104). In addition, a single extracellular N-linked glycosylation site was predicted to be located between membrane-spanning helices 1 and 2, with a large intracellular loop located between membrane-spanning helices 6 and 7. Protease digestion studies have confirmed the cytoplasmic orientation of the carboxyl terminus and the presence of a hydrophilic loop spanning membrane domains 6 and 7 (13, 24). More recently, the presence of 12 membrane-spanning domains has been confirmed by glycosylation site scanning mutagenesis (61). In these studies, an N-linked glycosylation consensus site was independently inserted into each putative hydrophilic region of an aglyco-GLUT1 mutant cDNA construct. Expression of these constructs in *Xenopus* oocytes confirmed the exofacial and cytoplasmic orientation of each hydrophilic region.

A comparison of deduced amino acid sequences of the other GLUT isoforms

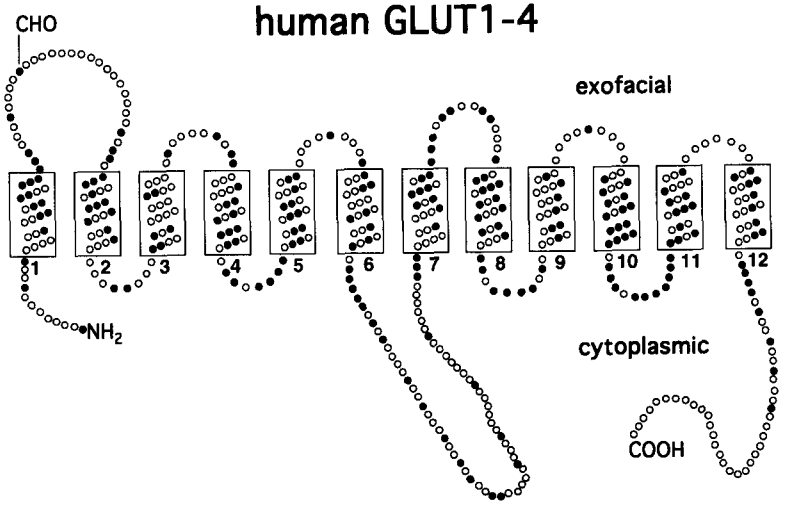

Figure 1 The predicted secondary structure of human GLUT1. The 12 predicted membrane-spanning domains are shown in boxes numbered 1 through 12. The closed circles depict the amino acids that are identical between GLUT1 and GLUT4. The N-linked glycosylation site (CHO) is also conserved in each of the four GLUT isoforms.

has revealed a similar size between these proteins (~500 amino acids), with the same overall topology (reviewed in 4). These proteins are all characterized by a topology identical to that proposed for GLUT1, including the conservation of the N-linked glycosylation site. Despite this overall similar structural organization, there is a considerable divergence in the specific amino acid sequences. Only 38% of all the amino acids are conserved between the GLUT1–4 isoforms, and in general, the greatest degree of amino acid sequence identity lies within the transmembrane domains, with the most divergence in the large hydrophilic domain and in the amino and carboxyl termini (Figure 1).

Early kinetic analysis of glucose transport suggested that GLUT1 functions through alternating conformational changes that sequentially expose a hexose-binding site to the external and internal surfaces of the transporter (2, 60). Thus, GLUT1 has both exofacial and endofacial ligand-binding sites, which can be distinguished by using site specific and impermeable hexose analogues (60). For example, cytochalasin B is a high-affinity inhibitor of glucose transport and binds to an endofacial domain of GLUT1 (3). The alternating conformation model is consistent with the ability of cytochalasin B to be a competitive inhibitor of glucose efflux but a noncompetitive inhibitor of glucose entry (26, 44, 87). In addition, deletion of the carboxyl terminal domain of GLUT1 locks the transporter into the outward-facing conformation, which cannot switch to the inward-facing state (112). Furthermore, mutational analy-

sis of some conserved amino acids have caused impaired glucose transport by inhibition of either the exofacial or the endofacial substrate–binding sites. Mutation of conserved residues Gln 161, Gln 282, Pro 385, and Trp 412 each abolish external ligand binding (53, 78, 105, 135). These mutations affect changes in transmembrane domains 5, 7, 10, and 11, respectively. Substitution of Tyr 293 in transmembrane domain 7 results in a total loss of the endofacial-binding site (102). Together, these data provide compelling evidence for distinct extracellular and intracellular binding sites. In addition, they support a proposed model in which the amphipathic residues found within the transmembrane domains cluster together to form a structure through which glucose can pass (104).

PHYSIOLOGY OF GLUCOSE TRANSPORTERS (GLUT1–3)

Glucose Transporters in the Brain

In contrast to most tissues in the body that utilize both glucose and fatty acids, the adult central nervous system predominantly relies on glucose as its main energy source. However, for glucose to reach neurons within the brain, it must first be transported across the endothelium of blood brain barrier into the interstitial space. Once in this compartment, glucose must then cross the plasma membrane of the individual brain cells. To accomplish this task, the central nervous system takes advantage of the functional properties of the GLUT1 and GLUT3 isoforms.

Western blot analysis has revealed the presence of brain GLUT1 as multiple-molecular-weight species between 45 and 55 kDa (reviewed in 95). The larger-molecular-weight species are predominantly present in microvessels (88, 95), whereas vessel-free preparations of brain membranes appear to contain the smaller-molecular-weight GLUT1 proteins (94, 115). An intermediate 46- to 48-kDa form of GLUT1 has been localized to the choroid plexus (88). These differences in molecular mass of GLUT1 are all accounted for by specific differences in N-linked glycosylation (88). The consequences of variable glycosylation states on GLUT1 function have yet to be established. In addition to the microvasculature, immunohistochemical and cell biological studies have colocalized GLUT1 mRNA with the glial-specific marker protein, glial fibrillary acidic protein (9, 25, 89).

Although GLUT3 is found in other tissues, it is expressed at highest levels in the brain of all species studied (50, 81, 96, 106). In contrast to GLUT1, GLUT3 expression has not been detected in the microvasculature of human or rat brains (94) and is primarily localized to neurons (39, 94, 96, 108). This 496–amino acid protein has a relatively low K_m, which suggests that glucose transport via GLUT3 is near maximal at normal plasma glucose concentrations (46).

The expression of GLUT1 and GLUT3 in the brain is regulated both in development and by changes in metabolic state. In rodents, fetal and neonatal brains rely primarily on the expression of GLUT1 in all cell types, but neurons apparently convert to the expression of GLUT3 approximately 10 days after birth (109). GLUT3 mRNA levels are also up-regulated by hypoglycemia in mouse brain and primary cultured neurons, which may be a protective mechanism against energy depletion when circulating glucose levels are reduced (107).

Various conditions that result in altered neuronal activity also regulate the expression of GLUT3. Chronic depolarization of neurons is associated with increased oxidative metabolism and induction of GLUT3 expression (92). GLUT3 in the neurohypophysis has been shown to increase in streptozotocin-induced diabetes and water deprivation, conditions that increase activity in the neurohypophyseal-hypothalamic axis (93, 140). A reduction of GLUT3 has been observed in necropsy samples obtained from patients with Alzheimer's disease (52, 128). The areas of the brain in which GLUT3 is reduced correspond to regions exhibiting deficits in glucose utilization measured by positron emission tomography scans. A loss of GLUT3 mRNA is also observed in experimentally induced ischemia, which may reduce availability of glucose to injured regions and reduce the viability of ischemically damaged neurons (100).

Glucose Transporters in Pancreatic β-Cells and Liver

GLUT2 is a 524–amino acid protein that is predominantly expressed in hepatocytes and pancreatic β-cells with lower levels in the kidney and intestines. Kinetically, GLUT2 is distinguished from the other GLUT isoforms by being a low-affinity glucose transporter with a high turnover rate (46). These marked kinetic differences and the fact that GLUT1 mRNA and protein could not be detected in liver directly led Thorens and coworkers to screen a liver cDNA library for the presence of a GLUT1-related facilitative glucose transporter (137).

These kinetic properties of GLUT2 underlie its specific function in the liver, where glucose transport must not be rate limiting for either influx or efflux. In the postprandial state, when circulating levels of glucose are high, there needs to be net hepatocyte uptake of glucose as the intracellular free glucose is metabolized or converted into glycogen. Conversely, in the postabsorptive or fasting state, the liver must be able to freely export glucose into the plasma despite relatively high steady-state circulating glucose concentrations. Hepatocytes accomplish this by utilizing a low-affinity, high-capacity transporter that is coupled with a regulated glucose phosphorylating activity provided by glucokinase (or hexokinase type IV). Thus, during states of glycogen synthesis, glucokinase is up-regulated and can increase the formation of intracellular

glucose-6-phosphate (91). This not only provides the precursor for glycolysis and glycogen synthesis, it also maintains a low intracellular concentration of free glucose, allowing for the continuous influx of glucose. In contrast, during states of glycogenolysis and gluconeogenesis, the reduction in glucokinase and increase in phosphoenolpyruvate carboxykinase levels allow hepatocytes to increase their intracellular concentration of free glucose greater than that present in the plasma. Under these conditions, there is a net efflux of glucose from the hepatocytes into the circulation.

A similar situation also holds for pancreatic β-cells, which must be highly sensitive to changes in plasma glucose concentrations to appropriately regulate the amount of insulin secretion. Thus, the presence of a high K_m glucose transporter will insure that the transporter is not saturated at physiologic levels, and that glucose flux will be directly proportional to the plasma glucose concentration. As in the liver, glucokinase activity regulates the entry of glucose into the glycolytic pathway and is therefore thought to function as the β-cell glucose sensor (40, 57, 99). The functional importance of glucokinase has been demonstrated by the identification of various functional glucokinase defects in several genetic lineages having MODY (maturity onset diabetes of the young), a condition of insulin resistance, hyperglycemia, and relative insulinopenia (29, 32, 43, 132, 134). Recently, transgenic mice in which glucokinase is knocked out either in liver or in both liver and pancreatic β-cells clearly document that glucokinase expression in the pancreatic β-cells is absolutely essential for maintaining normal plasma glucose levels and rates of insulin secretion (47).

Although substantial data support a critical role of glucokinase in insulin secretion, several lines of evidence also suggest that GLUT2 functionally coordinates with glucokinase. For example, the expression of GLUT2 in insulinoma cells is able to reconstitute glucose-stimulated insulin secretion (30). Furthermore, the pituitary cell line (ATT-20), when transfected with the insulin cDNA, is capable of secreting insulin in response to various agonists but not in response to glucose. Transfection of these cells with GLUT2 imparts glucose-sensitive insulin secretion, although at glucose concentrations significantly lower than normal (62). Nevertheless, the requirement for GLUT2 is specific because expression of GLUT1 was unable to impart glucose-dependent insulin secretion (63). Together, these data support a model whereby glucose transport mediated by GLUT2 is coupled to glucokinase function, and this coordinated regulation is required for appropriate glucose sensing by the β-cells.

Intestinal and Kidney Glucose Transporters

The small intestines and the kidneys express multiple glucose transporter isoforms, including the Na^+-dependent glucose transporter. These tissues ex-

press the facilitative glucose transporters GLUT1, GLUT2, and GLUT3, as well as the fructose transporter GLUT5 (34, 81, 119, 137). The relative abundance of these GLUT isoforms is markedly different, and it is now established that GLUT2 is the primary GLUT isoform responsible for glucose transport across the basolateral membrane of intestinal epithelial cells (136). In contrast, the Na^+-dependent glucose transporter is localized to the brush-border membrane, whereas GLUT5 is equally abundant in both the apical and the basolateral membrane of the intestine (8). Fructose uptake from the intestinal lumen and exodus from the intestinal epithelia is largely conducted by GLUT5 (8). Although GLUT2 can transport fructose as well as glucose, it does so with a sixfold lower affinity for fructose than GLUT5 (20).

Regulation of intestinal GLUT2 and GLUT5 is controlled by the levels of dietary sugars. GLUT2 mRNA is increased by D-glucose, D-galactose, and D-fructose, but not by 3-*O*-methylglucose, D-mannose, or D-xylose (101). GLUT5 mRNA is up-regulated only by D-fructose (11, 101). Interestingly, a high-sucrose (a disaccharide consisting of glucose and fructose) diet does not increase GLUT5 mRNA, which suggests that an alternative transport pathway for monosaccharides generated from sucrose, or that sucrose, may be transported prior to hydrolysis. In addition to effects of dietary sugars, GLUT5 expression shows a circadian rhythm in rat intestine (16). A 12-fold increase in GLUT5 mRNA was observed at the end of the 12-h light cycle and at the beginning of the dark cycle, a time that immediately preceded the animal feeding period. The expression of GLUT5 in intestine is also developmentally regulated in that GLUT5 mRNA and protein are found primarily in adult enterocytes (16, 23). GLUT2 expression is also developmentally regulated; however, unlike GLUT5, it begins to appear late in gestation (23).

INSULIN-REGULATED TISSUES (ADIPOSE TISSUE, SKELETAL MUSCLE, AND HEART)

Studies on glucose transporter biology have focused predominantly on the insulin-regulated glucose transporter system. It is this process that provides the regulation of whole body glucose homeostasis and that, when dysregulated, is central in the pathogenesis of diabetes mellitus. Insulin-stimulated glucose transport is almost entirely accounted for by the GLUT4 isoform, which is also the most abundant glucose transporter present in tissues that display insulin-sensitive glucose transport (6, 17, 59, 67–69, 83, 97). Although these tissues also express the GLUT1 isoform, its abundance is substantially less than that of GLUT4 (59, 83, 97). Of these tissues (adipose, skeletal muscle, and cardiac muscle) skeletal muscle accounts for the majority of postprandial glucose disposal because of its significantly larger mass in lean individuals.

Acute Regulation of GLUT4 by Insulin

Early studies, performed prior to the purification and cDNA cloning of the GLUTs, demonstrated that adipocytes displayed a highly sensitive insulin-stimulated increase in glucose transport activity that occurred in a protein synthesis-independent but energy-dependent manner (85). In addition, the ability of insulin to activate glucose transport was readily reversible upon removal of insulin. Subsequent studies demonstrated that adipocytes contained a large intracellular pool of glucose transporter proteins, which were rapidly translocated or recruited to the plasma membrane in response to insulin, giving rise to the "Recruitment Hypothesis" (21, 133). This distribution of glucose transporters was quite different from that observed in other tissues, in which all the glucose transporter proteins were found exclusively at the plasma membrane.

In contrast to adipocytes, insulin-stimulated regulation of GLUT4 has not been as extensively studied in cardiac and skeletal muscle because of technical difficulties in isolation and subcellular fractionation. Nevertheless, recent studies using a photolabelling technique have demonstrated a direct correlation between the appearance of GLUT4 at the cell surface with glucose transport activity (143). As in adipocytes, the insulin-stimulated increase in glucose transport activity was fully accounted for by an increase in surface-accessible GLUT4 protein. This study strongly supports both immunocytochemical evidence and subcellular fractionation studies, which have demonstrated GLUT4 translocation to the plasma membrane of skeletal muscle (31, 49, 58, 97, 123). Although most of the characterization of GLUT4 vesicles has been carried out in adipose tissue, a biochemical comparison of GLUT4 vesicles in rat adipose and muscle have indicated that these structures are biochemically indistinguishable in terms of buoyant densities, sedimentation coefficients, and protein composition (75). For a detailed review of GLUT4 vesicle trafficking and molecular actions of insulin on glucose transport regulation, please refer to two recent reviews (22, 131).

Regulation of GLUT4 in Insulin-Resistant States

Insulin resistance occurs in various pathophysiologic states, resulting in a decrease in either insulin sensitivity and/or responsiveness. The streptozotocin (STZ)-induced diabetic rat is an insulinopenic animal model similar to patients with insulin-dependent diabetes mellitus, which also results in an insulin-resistant phenotype. Early studies using cytochalasin B binding as a measure of glucose transporter protein demonstrated a reduction in glucose transporters in adipocytes isolated from STZ-diabetic rats (76). After cloning and identifying GLUT4 as the predominant GLUT isoform in adipocytes, several groups demonstrated that both GLUT4 protein and mRNA, but not GLUT1, were

markedly reduced in adipose tissue from these animals (5, 36, 70, 130). Based on these data, a model was proposed in which the down-regulation of intracellular GLUT4 protein levels resulted in a reduction in the number of transporters that could be recruited to the plasma membrane (12). Since insulin stimulation resulted in a reduced number of glucose transporters at the cell surface, there would be a decrease in glucose transport and, hence, a state of insulin resistance.

This reduction in GLUT4 protein is primarily due to a decrease in GLUT4 mRNA levels. However, GLUT4 is apparently a very stable protein, whereas the GLUT4 message has a relatively rapid turnover rate. Thus, the depletion of GLUT4 mRNA substantially precedes any decrease in GLUT4 protein levels. The decrease in GLUT4 mRNA in STZ-diabetic rats results from, at least in part, decreased transcription of the GLUT4 gene (41, 113). Furthermore, there appears to be a compensatory mechanism that reduces the rate of GLUT4 protein degradation in STZ-diabetic rats, which spares GLUT4 at a time when there is a pretranslational synthetic defect (84).

Not surprisingly, the regulation of GLUT4 expression in cardiac and skeletal muscle is not identical to that observed in adipose tissue. In the heart, STZ-diabetes results in a depletion of GLUT4 mRNA and protein similar to that found in adipose tissue (14, 74, 113, 114). However, GLUT4 in skeletal muscle displays a modest reduction in GLUT4 protein and mRNA during STZ-induced diabetes (10, 14, 73, 74, 122). Interestingly, the effect of STZ-diabetes on GLUT4 in skeletal muscle is fiber type specific with differential responses, depending on composition of the muscle group and duration of STZ treatment (10, 74, 122). These changes in GLUT4 mRNA are also due, in part, to a decrease in transcription rate of the GLUT4 gene (110).

In contrast, the time-dependent decrease in GLUT4 expression occurred significantly more slowly than did the decrease in insulin-stimulated glucose transport activity (73, 122). These data have been interpreted to suggest a translocation defect in STZ-diabetic skeletal muscle apart from a latter depletion of GLUT4 protein pool size. Consistent with this interpretation, skeletal muscle GLUT4 protein levels from non-insulin-dependent diabetes (NIDDM) or insulin-dependent diabetes mellitus do not display significant differences compared with nondiabetic controls (27, 28, 37, 72, 116). This is in contrast to adipose tissues, which have a reduction in GLUT4 protein and mRNA in NIDDM patients (38, 129, 128). Nevertheless, these data indicate that insulin-resistant glucose transport in skeletal muscle is not directly correlated with a reduction in GLUT4 protein pool size, as is the case in adipose tissue.

In rodents, high-fat feeding also results in altered glucose homeostasis and is associated with decreased glucose utilization and insulin resistance (86). Chronic feeding of a high-fat diet to rats reduces GLUT4 gene expression in both adipose tissue and muscle (71, 84, 117). These animals also have markedly

reduced plasma insulin levels, which may be responsible for the decreased levels of GLUT4 (71, 117).

GLUCOSE TRANSPORT IN TRANSGENIC MICE

To examine the physiologic effect of increased glucose uptake into muscle, the GLUT1 cDNA was overexpressed in transgenic mice with the skeletal muscle–specific myosin light chain-2 promoter (98, 120). Because GLUT1 is localized to the cell surface membrane, these animals had an increased rate of basal skeletal muscle glucose uptake, which correlated with a decrease in plasma glucose levels. Following a glucose challenge, the high basal rate of glucose transport caused a marked reduction in insulin-stimulated glucose excursion consistent with enhanced whole body glucose disposal. Examination of muscle glucose transport activity demonstrated an increase in basal glucose transport with a relatively reduced extent of insulin-stimulated transport. Similarly, these GLUT1 transgenic mice also displayed a reduction in contraction-stimulated glucose uptake (48). Although the molecular basis for this phenomenon has not been established, this unexpected insulin- and contraction-stimulated resistance of glucose transport in skeletal muscle appears to directly result from the increased flux of glucose into this tissue.

As described previously, GLUT4 is the predominant GLUT isoform in muscle and adipose tissue. Although GLUT4 expression can be modulated in various insulin-deficient, as well as in some insulin-resistant, states, it is unlikely that a reduction in GLUT4 pool size can completely account for the insulin-resistant phenotype in NIDDM. Nevertheless, several studies in transgenic mice have demonstrated that increased expression of GLUT4 can enhance insulin sensitivity (65, 90, 126). To specifically express GLUT4 in adipose tissue, the human GLUT4 cDNA driven by the adipose-specific fatty acid–binding protein promoter (aP2) was used to generate transgenic mice (126). These animals displayed fat hyperplasia with enhanced glucose disposal (126). Independently, the human GLUT4 protein was also expressed in adipose tissue and in heart and skeletal muscle of transgenic mice with a GLUT4 mini-gene driven by its own promoter (90). These animals also displayed a marked reduction in circulating glucose levels and enhanced insulin sensitivity (90, 138). However, in these animals, increased tissue-specific expression of GLUT4 did not lead to an increase in adipose cell number but did have a small effect on adipose cell size (90). The increased adiposity of the animals expressing GLUT4 from the aP2 promoter most likely results from the effect of increased glucose flux on adipose cell fat determination. The aP2 promoter functions earlier in fetal development than does the endogenous GLUT4 promoter and thereby enhances glucose uptake during the commitment stage of adipose tissue differentiation (126).

The hallmark of the mice overexpressing GLUT4 in a tissue-specific manner is enhanced glucose disposal, low plasma insulin levels, increased plasma lipids, increased plasma free fatty acids, increased plasma β-hydroxybutyric acid, and increased insulin sensitivity (51, 90, 121, 138). The increase in GLUT4 levels also resulted in a higher basal glucose transport rate because of increased amounts of GLUT4 found at the plasma membrane, which probably results from saturation of the GLUT4 intracellular retention signal. Nevertheless, insulin is capable of inducing a large recruitment of the intracellular localized GLUT4 protein to the plasma membrane concomitant with a proportional increase in glucose transport activity.

Because these data demonstrated that increased tissue-specific expression of GLUT4 can enhance insulin sensitivity and glycemic control, genetically diabetic *db/db* mice were also engineered to overexpress GLUT4 in a tissue-specific manner (42). The diabetic state of these animals is characterized by obesity, hyperglycemia, and hyperinsulinemia similar to obese, insulin-resistant diabetic patients. Similar to the wild-type mice, increased expression of GLUT4 (three- to sixfold) in the *db/db* mice resulted in a reduction in circulating glucose levels, enhanced insulin sensitivity, and increased insulin-stimulated GLUT4 translocation. More recently, transgenic mice overexpressing mouse GLUT4 protein were observed to overcome insulin resistance associated with high-fat feeding (66). Taken together, these studies strongly support a role in increased GLUT4 gene expression as a potential therapy for insulin-resistant disease states. Even if the cause of insulin resistance is not due to a reduced GLUT4 pool size, it appears that increased tissue-specific GLUT4 expression may have substantial therapeutic benefit.

In contrast to GLUT4 transgenic mice, the GLUT4 gene has recently been disrupted by homologous recombination (79). Heterozygotic animals that had reduced GLUT4 levels were observed to develop a mild insulin resistance several months after birth but were not grossly abnormal. In contrast, homozygotic knockout mice resulted in the complete absence of GLUT4 protein. These mice had multiple abnormalities, including growth retardation, little or no adipose tissue, and cardiac hypertrophy, which directly resulted in decreased longevity. Surprisingly, however, these animals did not have elevated blood glucose levels. Several possible mechanisms could account for the lack of diabetes in these animals. Although the knockout mice did not have significant changes in muscle GLUT1 expression, increased GLUT1-intrinsic glucose activity might account for this observation. In addition, the major contributor to fasting hypergylcemia is glucose release from the liver due to glycogenolysis and gluconeogenesis. Because lactate levels are low in these animals, the release of glucose by the liver is probably very low. Furthermore, the cardiac hypertrophy will reduce blood flow and induce a relative tissue-deprived nutritional state. It is also important to

recognize that insulin-stimulated glucose transport into muscle was not determined, so insulin resistance at the cellular level was not established. Nevertheless, these results are highly provocative and will require further investigation into the molecular basis of the role of GLUT4 in insulin-sensitive whole body glucose disposal.

SUMMARY AND CONCLUSIONS

The regulation of glucose concentrations both intracellularly and extracellularly is critical to the normal function and survival of complex multicellular organisms. In particular, plasma glucose levels are tightly regulated by the endocrine system to insure adequate fuel supply for the central nervous system, which primarily relies on the metabolism of glucose for energy. The liver stores excess glucose in the form of glycogen, due to the increased flux of glucose into hepatocytes, and by activation of glucokinase activity. Insulin also functions to suppress liver glucose output and enhances peripheral tissue disposal by increasing the flux of glucose into muscle and adipose tissue. These critical physiologic regulatory mechanisms are carried out, in part, by the integrated function of insulin and glucose with the facilitative glucose transport proteins, particularly those in pancreatic β-cells, liver, adipose tissue, skeletal muscle, and cardiac muscle. Dysregulation of the mechanisms controlling any of these complex pathways mediating whole body glucose disposal can result in the pathophysiologic states associated with diabetes.

In particular, the GLUT2 and GLUT4 isoforms play central roles in these processes. In the intestines, GLUT2 serves to transport the dietary-absorbed glucose from the epithelium into the circulation, in the kidney it reabsorbs the filtered glucose, in the liver it mediates either uptake or release of glucose, and in the pancreas it is part of the glucose sensor apparatus mediating glucose-stimulated insulin release. On the other hand, GLUT4 functions to dispose of excess circulating glucose by increasing its uptake into muscle and adipose tissue in response to insulin. The acute effects of insulin result from a rapid recruitment of a preexisting intracellular pool of GLUT4 protein to the cell surface. In addition, chronic states of insulin deficiency down-regulate the expression levels of the GLUT4 gene, which may also serve to impair normal glucose homeostasis.

Over the past several years, we have developed a significantly greater understanding of these complex integrative functions of the facilitative glucose transporters. Obviously, there are numerous future challenges that still need to be resolved, including identification of the intracellular signaling pathways regulating GLUT4 translocation and the molecular basis for its impaired function in NIDDM. In addition, the relationship between glucokinase and GLUT2 in the control of liver glucose uptake and release, as well as in the regulation

of pancreatic β-cell insulin secretion, will require further study. Although GLUT1 and GLUT3 appear to have primarily a constitutive function, these isoforms are also regulated under a variety of conditions, including stress and cellular transformation. The complex integration of all these pathways in the maintenance of whole body glucose homeostasis and its dysregulation in various pathophysiologic states are important issues that will be receiving substantial attention in the near future.

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