Endocrine, *vol. 19, no. 1, 13–22, October 2002* 0969–711X/02/19:13–22/$20.00

Placental Glucose Transfer and Fetal Growth

Marc U. Baumann, Sylvie Deborde, and Nicholas P. Illsley

Department of Obstetrics, Gynecology and Women's Health, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ

One of the primary regulators of maternofetal glucose transfer is the density of glucose transporter proteins in the placenta. These transporters, members of the GLUT gene family of facilitated-diffusion transporters, are embedded in the microvillous (maternal-facing) and basal (fetal-facing) membranes of the syncytiotrophoblast, the main placental barrier layer. Eight members of this family have been described in human placental tissue, but only GLUT1 protein has been identified in the syncytium, where its distribution is asymmetric. The microvillous membrane contains markedly more transporter than the basal, and, as a result, the basal membrane acts as the rate-limiting step in transplacental glucose transport; thus, changes in the density of basal membrane GLUT1 will have a significant impact on transplacental glucose flux. What little is known about syncytial GLUT1 expression is restricted to factors associated with fetoplacental growth and metabolism; GLUT is inversely regulated by glucose concentration and basal membrane GLUT1 is positively regulated by insulin-like growth factor I, placental growth hormone, and hypoxia. In vivo, basal membrane GLUT1 is upregulated over gestation, increased in diabetic pregnancy, and decreased in chronic hypoxia, while microvillous membrane GLUT1 is unaffected. The contrast between in vitro and in vivo regulation and the specific changes in GLUT1 distribution suggest more complex regulatory interactions than those yet described.

Key Words: Glucose transfer; syncytiotrophoblast; placenta; hypoxia; rate-limiting step; GLUT.

Introduction

This review discusses three aspects of maternal-fetal glucose transfer, the transplacental mechanisms by which transfer takes place, the regulation of placental glucose transporters, and the role of placental glucose transporters in the pathophysiology of fetal growth disorders. We first describe what appears to be the consensus with respect to glucose transporter expression and distribution in the human placenta, concentrating on the trophoblast. The second element in the review concerns what little we know of the factors regulating glucose transporter expression and activity in the placenta. Although the list of factors modulating glucose transporter expression and activity is extremely long and varied, most of these factors have not been examined in the placenta. We have therefore focused on those agents that appear to regulate transporter expression and activity in circumstances relevant to the regulation of fetal growth. The final section examines glucose transporter expression and activity in pathophysiologies related to fetal growth and the potential consequences for glucose transfer.

This review is restricted to human pregnancy and cells and tissues from the human placenta. Although there are data concerning glucose transporters in the placenta or trophoblast of other mammalian species, these data are fragmentary, and as yet it is not possible to assemble a unified schema containing information on cell-specific transporter expression, distribution, and regulation. Furthermore, the marked differences in placental structure and function between human and other mammalian species confound extrapolation of this limited animal data to humans, and precludes significant conclusions regarding transporter function.

Transplacental Transfer of Glucose

Glucose is a primary energy source for the fetus. The absence of significant gluconeogenesis in the fetus means that the fetus must obtain this vital nutrient from maternal plasma. The high fetal demand for glucose, especially during the third trimester, when fetal growth is maximal, necessitates the presence of a rapid, high-volume system for maternal-fetal transfer of glucose. The low permeability of syncytiotrophoblast plasma membranes and the minimal cross-sectional area of syncytial paracellular channels do not provide sufficient transfer capacity to satisfy fetal demand *(1,2)*; thus, a carrier-mediated transport system is necessary to fulfill fetal needs.

Maternal-fetal glucose transfer is regulated by several factors: glucose supply, placental glucose metabolism, and placental glucose transporter density. Glucose supply is determined by both blood glucose concentration and blood flow. Glucose transfer across the placental barrier is a rela-

Received September 5, 2002; Revised September 16, 2002; Accepted September 16, 2002.

Author to whom all correspondence and reprint requests should be addressed: Dr. Nicholas P. Illsley, Department of Obstetrics, Gynecology and Women's Health, Medical Sciences Building, E506, UMD–New Jersey Medical School, 185 South Orange Avenue, Newark, NJ 07103-2714. E-mail: nick.illsley@umdnj.edu

Table 1
GLUT Glucose Transporter Isoform Distribution in the Human Placenta[a]

GLUT isoform	Protein	mRNA
GLUT1	Syncytiotrophoblast, cytotrophoblast, endothelium, vascular smooth muscle, stromal cells	Syncytiotrophoblast, cytotrophoblast, endothelium, vascular smooth muscle, stromal cells
GLUT3	First trimester: extravillous trophoblast, cytotrophoblast; third trimester: endothelium	First trimester: ND; third trimester: syncytiotrophoblast, cytotrophoblast, endothelium
GLUT4	Stromal cells	Stromal cells
GLUT8	ND	Term homogenate/distribution unknown
GLUT9	ND	Term homogenate/distribution unknown
GLU10	ND	Term homogenate/distribution unknown
GLUT11	ND	Term homogenate/distribution unknown
GLUT12	First trimester: extravillous trophoblast cytotrophoblast, syncytiotrophoblast; third trimester: vascular smooth muscle, stromal cells	First trimester: extravillous trophoblast, cytotrophoblast, syncytiotrophoblast; third trimester: vascular smooth muscle, stromal cells

[a]ND, not done.

tively rapid process compared to either the supply of (maternal) glucose or removal of (fetal) glucose from the site of transfer. Thus, glucose transfer can be defined as a flow-limited phenomenon, i.e., limited by movement to and from the transfer site *(3)*. Alterations in the maternal or fetal plasma glucose concentration will change the maternal-fetal glucose concentration gradient, resulting in an altered rate of glucose transfer (in the absence of changes in placental metabolism). Thus, changes in blood glucose, such as those seen in maternal circulation as a result of diabetic hyperglycemia, will serve to alter delivery of glucose to the fetus. Alterations in blood flow, such as the reduction in uteroplacental blood flow observed frequently in intrauterine growth retardation (IUGR), will also alter supply, leading to a change in glucose transfer to the fetus *(3)*. Glucose transfer is also affected by the metabolic status of the placenta; alterations in the supply of other energy-generating substrates, such as the reduction in oxygen delivery under hypoxic conditions, will produce changes in the placental metabolic demand for glucose, leading to alterations in maternal-fetal transfer *(4)*. Finally, glucose transfer is also modulated by the number of functional transporters. The transport rate will depend on the transporter density on the membranes delimiting the two compartments (i.e., the syncytiotrophoblast microvillous and basal membranes), and on the total surface area of these membranes. While these regulatory mechanisms are interdependent, the goal of this report is to review the localization, function, and regulation of glucose transporter proteins in the glucose transfer process and the extent to which this process is affected by conditions that produce abnormal fetal growth.

Glucose Transporters in Placenta

Term Trophoblast

The main barrier layer in the human placenta is the syncytiotrophoblast, composed of nonmitotic, multinuclear cells that possess an epithelial structure comprising the microvillous (maternal-facing) and basal (fetal-facing) membranes. Most investigations of syncytial glucose transporters have been performed on membranes derived from term placental tissue. The initial conclusions drawn from these investigations have been that the transporters present in both microvillous and basal membranes are sodium-independent moieties operating by facilitated diffusion *(5–7)*. Further investigations have shown the presence of sodium-independent transporters in the human placenta belonging to the GLUT family of glucose transporter proteins, a group of integral, transmembrane proteins that contains (at least) 12 isoforms (GLUT1–GLUT12) *(8)*. While many of these isoforms have been identified in human placental tissue, it is important to note that the only transporter present in the syncytium as a functional protein near term is the GLUT1 isoform *(9,10)*. Current information on the distribution of GLUT glucose transporter isoforms is summarized in Table 1. GLUT1 is found in both membranes, with an approximately threefold higher quantity in the microvillous membrane compared with the basal *(10)*. This asymmetric expression of GLUT1 is paralleled by the measurements of transport activity that show a similar disparity between microvillous and basal membranes. Note here, however, that both the measurements of expression and activity were made per unit of membrane protein; when membrane folding on the microvillous or brush

border surface is taken into account *(11)*, the difference in GLUT1 expression or transport activity between the two epithelial faces is magnified substantially, potentially reaching a level of 15- to 20-fold. This asymmetry has significant implications for changes in microvillous or basal membrane transporter content, as discussed in the last section of this review. GLUT1 is also found in the mononuclear, undifferentiated cytotrophoblasts, the cells that are the precursor to the syncytiotrophoblast *(12,13)*.

The other isoform that has been mentioned frequently in connection with the syncytiotrophoblast is GLUT3. Although initial reports identified GLUT3 as being present in the term syncytium, most subsequent analyses have not found GLUT3 protein in either microvillous or basal membranes, nor has it been detected in term cytotrophoblast cells *(9, 10,12)*, although it has been identified in placental endothelium *(14)*. Despite the absence of GLUT3 protein in the syncytiotrophoblast, GLUT3 mRNA has been identified in term cytotrophoblast and in the syncytium, with a distribution similar to that of GLUT1 *(14,15)*. A recent report also noted GLUT4 in plasma and intracellular membrane fractions of JAr choriocarcinoma cells; however, the levels of expression were extremely low compared with those of GLUT1 and GLUT3 in the same cells *(16)*, suggesting that GLUT4 does not contribute significantly to cellular glucose uptake in these cells. The majority of the GLUT4 was localized to low-density microsomes, a distribution that was not altered by insulin treatment, suggesting that GLUT4 is not subject to the regulation by insulin. GLUT4 has not been identified in membranes from primary syncytio- or cytotrophoblast cells; thus, it is unlikely that GLUT4 makes any appreciable contribution to trophoblast glucose uptake in vivo, especially at term.

Preterm Trophoblast

Unlike term trophoblast, GLUT3 protein has been observed in trophoblast cells from first-trimester tissue *(17)* as well as in choriocarcinoma cell lines obtained from early placental tissue *(12,13,16)*. The presence of GLUT3 in first-trimester tissue and in these choriocarcinoma lines suggests that GLUT3 may be an important transport component early in gestation. It is well known that GLUT3 has a lower binding constant for glucose *(18,19)*, and it is possible that early in gestation, when the maternal circulation is not yet fully established, the presence of GLUT3 may be important for fetoplacental nutrition. Where trophoblast is in contact with maternal blood lacunae, the lower circulatory turnover may result in a reduction in blood glucose concentrations below those normally observed in the maternal circulation. A higher-binding-affinity transporter such as GLUT3 would permit continued uptake of glucose under conditions of decreased substrate concentration. Later in gestation, once the maternal circulation is established and intervillous space glucose concentrations approximate those in the maternal peripheral circulation, this type of transporter would no longer be necessary.

Clarson et al. *(12)* have suggested that GLUT3 may be a feature of dividing trophoblast cells; thus the protein would not be observed in syncytiotrophoblast; and its expression in cytotrophoblast might be expected to decrease over gestation as increasing numbers of cytotrophoblast exit the cell cycle, preparatory to incorporation into the syncytium. The expression of GLUT3 protein in the preterm period and the continuing expression of GLUT3 mRNA at term suggest some form of active regulation that suppresses expression of the protein. Indeed, there is evidence that the level of GLUT3 mRNA in term trophoblast responds actively to stimuli such as hypoxia *(20)*, supporting the idea of a translational block. We have preliminary evidence suggesting that increased expression of GLUT1 may be one means whereby GLUT3 expression is decreased; overexpression of GLUT1 appears to correlate with a reduction in GLUT3 expression in BeWo choriocarcinoma cells (unpublished data). Interestingly, following induction of cellular differentiation by 8-bromo-cyclic adenosine monophosphate (cAMP), GLUT1 but not GLUT3 expression was stimulated in BeWo choriocarcinoma cells *(17)*. The same group reported opposite effects of 8-bromo-cAMP on GLUT1 mRNA transcription in a murine model *(21)*, emphasizing again the difficulty of extrapolating data obtained in animal models to the human.

The only other isoform to be examined in detail in the trophoblast is GLUT12. Gude et al. *(22)* have measured GLUT12 expression in first-trimester and term tissue. They concluded that, whereas this isoform is expressed in first-trimester syncytial and villous cytotrophoblast, it is absent at term in these cells. It is possible that GLUT12, like GLUT3, performs tasks that are vital to trophoblast function in early gestation but that are superfluous to placental function later in gestation.

Nontrophoblastic Transporter Expression and Other Placental Glucose Transporters

The GLUT3 isoform is expressed in placental endothelial cells, along with GLUT1 *(14)*. There is preliminary evidence suggesting that its localization within the vasculature may be more specific. Head et al. *(23)* found that GLUT3 was expressed in endothelial cells only in the arterial sections of the placental vasculature. It is tempting to speculate that its presence there may relate to its higher binding affinity; blood returning from the fetus contains reduced levels of glucose, and the presence of GLUT3 may aid in extraction under conditions of reduced blood glucose concentration. This, in turn, may decrease the glucose concentration of blood flowing to placental exchange sites, increasing the transplacental gradient and maximizing transplacental transfer.

A variety of other GLUT transporter isoforms have been identified in the placenta. GLUT4 and GLUT8–12 have all

been identified elsewhere in the placenta through either measurements of protein or mRNA. GLUT4 has been localized to stromal cells in the villous tissue *(24)*. GLUT12 has been localized to the vascular smooth muscle and villous stromal cells at term *(22)*. The distribution of GLUT8–11 in the human placenta is unknown at this time; experiments describing expression of these isoforms were performed on RNA extracted from tissue homogenates, precluding cell-specific localization *(25–29)*.

Placental Glucose Transporter Regulation

The ubiquitous nature of GLUT1 has encouraged studies investigating the regulation of its expression in a variety of cell types. These investigations have defined multiple regulatory mechanisms and a broad range of regulatory factors. GLUT1 protein levels can be altered by changes in transcription rate *(30,31)*, by stabilization of GLUT1 mRNA *(32,33)* or protein *(34)*, or by recruitment of presynthesized GLUT1 protein from intracellular stores to the cell surface *(35,36)*. GLUT1 expression is influenced by a wide range of extracellular agents, including growth factors, cytokines, and steroids; however, few investigations have been carried out examining GLUT1 regulation in trophoblast. We next summarize and discuss GLUT1 regulatory mechanisms and pathways in the trophoblast.

Regulation by Glucose

Several studies have investigated the effect of extracellular glucose concentration on GLUT expression in trophoblast. The conclusion drawn from in vitro studies is that an inverse relationship exists between extracellular glucose concentration and the expression and activity of the GLUT1 transporter system. Under hypoglycemic conditions (0–1 m*M* glucose), GLUT1 mRNA and protein are upregulated in primary trophoblast *(13,37)*. Conversely, hyperglycemia (20–25 m*M* glucose) decreased GLUT1 mRNA and protein *(13,37,38)*. In the JAr choriocarcinoma cell line, both GLUT1 mRNA and glucose transporter activity were augmented under hyperglycemic conditions, whereas the level of GLUT3 transcripts was decreased *(39)*. Under similar hyperglycemic conditions, no changes in protein expression or glucose uptake were observed in the JEG-3 cell line, a more differentiated trophoblast-derived, choriocarcinoma cell line *(13,38)*. These findings raise the possibility that GLUT1 glucose transporter expression is regulated not only in a cell-specific manner, but also depends on the degree of trophoblast cell differentiation, as observed in other cell types *(40)*. This point is supported by the changes observed in the distribution of GLUT3 in trophoblast cells over gestation, changes that appear to parallel the alterations in degree of trophoblast differentiation.

It is possible that changes in glucose transporter activity reflect altered cell metabolism, because hyperglycemia has been shown to inhibit proliferation rates and mitochondrial activity in BeWo, JAr, and JEG-3 choriocarcinoma cell lines *(41)*. In fact, there is evidence suggesting that there are short-term regulatory influences that can act through nontranscriptional mechanisms to alter transporter activity, either through substrate effects or through the action of growth factors *(35,42)*. We demonstrated a suppression of GLUT1 expression in primary syncytial cells following exposure to 20 m*M* glucose and an elevation of GLUT1 expression following exposure to 0 m*M* glucose, but no differences were observed at glucose concentrations between these extremes. Nevertheless, transport activity was inversely related to glucose concentration over the entire concentration range *(13)*. These findings, together with the observations made by Hahn et al. *(38)* of substantial decreases in transport rates associated with only a relatively modest decrease in GLUT1 protein levels, are suggestive of additional, posttranscriptional mechanisms modulating the activity of the GLUT1 transport system. Recently, an electron microscopic study indicated translocation of GLUT1 from the cell surface to the intracellular compartment of primary trophoblast cells in response to hyperglycemia *(43)*, one example of a potential posttranscriptional mechanism. Another study, reported in preliminary form, suggests that human growth hormone (GH) also regulates transporter activity in a short-term manner, by nontranscriptional mechanisms *(44)*, similar to previous reports in other tissues *(40,45)*. Glucose has the potential to regulate the transcription of genes encoding for glycolytic and lipogenic enzymes *(46–48)*. One question that is still unclear and applies to both transcriptional and post-transcriptional regulation is, What is the actual messenger involved in producing these changes in expression? It is possible that the regulatory agent may be glucose itself; however, it is also possible that downstream metabolites may also be involved in the regulatory mechanisms *(35)*.

Insulin

Insulin has been shown to upregulate GLUT1 in a variety of different cell types, via mechanisms that include the Akt/PKB and mitogen-activated protein kinase (MAPK) pathways *(30,31,36,42)*. However, there is little evidence of insulin-stimulated effects on trophoblast glucose transporters. Although insulin receptors are plentiful on the basal membrane in the first trimester, at term these receptors are sparsely distributed on the microvillous membrane and absent from the basal membrane *(49)*. Brunette et al. *(50)* reported that pretreatment of placental tissue with insulin and Mn^{2+} enhanced glucose uptake in microvillous vesicles subsequently isolated from the tissue. Insulin enhanced trophoblast protein kinase B and MAPK phosphorylation in JAr cells in vitro, but no changes were observed in glucose uptake rates *(16)*. Increased GLUT1 expression and glucose uptake following insulin treatment were reported previously in the first-trimester trophoblast-derived ED27

cell line *(51,52)*. These data must be treated with caution, however, because this cell line appears, while having a distinct phenotype, to be genetically identical to the HeLa cell line *(53)*. In the placental dual-perfusion model, insulin had no effect on glucose uptake, despite concentrations an order of magnitude higher than maternal physiologic insulin levels (~10 μU/mL) during pregnancy *(54)*. When insulin was increased by three orders of magnitude over physiologic (12.5 mU/mL), glucose transport was stimulated in superfused microcarrier cultures of choriocarcinoma cells *(55)*. At such high concentrations, however, the possibility of insulin effects mediated through the type 1 insulin-like growth factor (IGF) receptor cannot be ruled out because insulin binds to the type 1 IGF receptor, although with a lower binding affinity than IGF-1 *(56,57)*. Finally, although the presence of GLUT4 has been reported in JAr cells, primarily in the intracellular, low-density microsomal fraction, neither GLUT4 translocation nor glucose uptake was affected by insulin treatment *(16)*, despite its stimulatory action on mitogenesis.

IGF-1/Placental GH

IGF-1 has been implicated in the regulation of glucose transporters in a number of cells and tissues *(32,58–61)*. The signaling pathways by which IGF-1 exerts its actions are similar in part to those employed by insulin, including the PI-3 kinase/Akt1 and the MAPK pathways *(62,63)*, although there is also some divergence *(61,63)*. IGF-1 and insulin appear to act through similar transcription factors, including activator protein (AP-1) and hypoxia-inducible factor-1/2 (HIF-1/2) *(64–66)*. Placental growth hormone (PGH) is similar in structure to pituitary GH but lacks a 13 amino acid sequence such that it is unable to bind to prolactin receptors. It is secreted only into the maternal circulation and in a tonic fashion, unlike the cyclic secretion of GH. Production of PGH is detectable by 20 wk of pregnancy, and its concentration increases linearly in the maternal circulation to term, completely replacing maternal pituitary GH *(67,68)*. Maternal IGF-1 levels appear to be regulated positively by PGH *(69)*. The factors (or factor) regulating PGH secretion are not yet clear; Patel et al. *(70)* demonstrated in vitro that PGH secretion was downregulated by glucose, but McIntyre et al. *(71)* have shown in vivo that PGH secretion correlates with maternal glycemic status. Both IGF-1 and PGH are also associated with fetal growth and placental nutrient transport *(71–74)*. We have investigated the effects of IGF-1 and GH on glucose transporters in BeWo cells and demonstrated a substantial upregulation of GLUT1 mRNA and protein expression, increases that are paralleled by increases in cellular glucose uptake and epithelial transfer *(75)*. IGF-1 and GH showed similar effects on basal membrane GLUT1 in a placental explant model *(75)*. While PGH presumably acts via receptors on the microvillous membrane, it is not yet clear whether the trophoblast response to IGF-1 is mediated via the receptors on both microvillous and basal membranes (i.e., response to maternal and fetal circulating IGF-1).

Hypoxia

Regulation of GLUT1 by hypoxia has been extensively studied in a variety of tissues. In hypoxic conditions, GLUT1 gene transcription is increased through the HIF pathway as a result of decreased degradation of the HIF-1 transcription factor *(76)*. Furthermore, hypoxia and inhibitors of oxidative phosphorylation decrease GLUT1 transcript degradation *(77)*. A number of studies have investigated the trophoblast response to low oxygen tension. Most of these studies were concerned with the role of hypoxia on the differentiation of the trophoblast and its potential for invasion of the maternal spiral arteries. Only a few reports on the regulation of placental or trophoblast glucose transport by hypoxia have been published *(20,78,79)*, in part because of the problems associated with assessing the oxygen concentrations to which trophoblast cells are exposed during normal pregnancy. There are only a few studies reporting oxygen measurements at the maternal-fetal interface *(80–82)*. The consensus is that the partial pressure of oxygen (Po_2) rises from below 20 mmHg at <10 wk of gestation to between 40 and 60 mmHg by midgestation. It is important to realize, however, that because of mixing and shunting, the intervillous space contains a mixture of arterial and venous blood, and thus trophoblast cells may be exposed to a range of oxygen pressures. For practical use and as a well-established reference point, normoxia has most commonly been defined for cells exposed to 21% partial oxygen tension (120–130 mmHg), although an O_2 concentration of 10% may be a more realistic value. Hypoxia has usually been defined as a concentration between 1 and 5% O_2. Following exposure to 14 mmHg of Po_2 (~1% O_2), GLUT1 and GLUT3 transcription were stimulated in trophoblast isolated from term placenta *(20)*. It was also reported that both glucose consumption and lactate production were increased under hypoxic conditions, while glucose uptake was reduced. Our results in BeWo cells show that GLUT1 and GLUT3 protein are upregulated following exposure to cobalt or desferroxamine (agents that simulate hypoxia) or to 1% O_2, as early as 12 h after treatment, and remain elevated for at least 72 h *(83)*. These data confirm that isolated trophoblast cells respond to hypoxia in a manner similar to a variety of other cells types, increasing GLUT levels in both the acute and chronic phases.

Placental Glucose Transporters and Fetal Growth

This section discusses the increasing volume of information relating to changes in placental glucose transporter expression and activity in common perinatal pathologies and the potential consequences of such changes. However, it is important to ask first, Do changes in glucose transporter expression and activity have any effects on transplacental glucose transfer, and, if so, to what extent? In the

absence of such information, observed alterations in expression and activity, while noteworthy, have unknown physiologic significance.

Rate-Limiting Step in Glucose Transport

The localization of GLUT1 glucose transporters in the human placental syncytium described earlier shows a level of expression on the microvillous or maternal-facing surface that is severalfold above that observed on the syncytial basal membrane *(9,10)*. This asymmetric localization led to the hypothesis that the basal membrane is the rate-limiting step in transsyncytial transport of glucose. Although this may be a fairly obvious conclusion, confirmation of this role for the basal membrane has been hindered by a lack of understanding of the intrasyncytial glucose transport and metabolic processes. Could steps such as glucose phosphorylation or dephosphorylation become rate limiting in transfer of glucose across the syncytial epithelium? Is it possible that glucose being transported across syncytial cells might bypass these steps, following a channel directly from apical (microvillous) to basolateral (basal) surfaces, another potential rate-limiting transport step?

To address these questions, the effects of variation in microvillous or basal membrane glucose transporter activity on transepithelial glucose transport have been determined *(4)*. The BeWo choriocarcinoma cell line was used as a model because it also shows an asymmetry in glucose transporter expression, transport across the apical membrane being approximately fourfold higher than that across the basal membrane. These experiments demonstrated that changes in carrier-mediated transepithelial transport were proportional to basal membrane glucose transport activity. Conversely, carrier-mediated transepithelial glucose transport was relatively insensitive to changes in microvillous membrane glucose transport activity. These data strongly support the hypothesis that in such an asymmetric transport system, the basal membrane is the rate-limiting step in glucose transport. The probability that the basal membrane is the rate-limiting step in glucose transport is even greater in the primary placental syncytium since the asymmetric distribution of transporters and transport activity is more pronounced than that observed in BeWo cells *(10)*.

There are significant consequences for the identification of the basal membrane as the rate-limiting step. Changes in the expression of glucose transporters at this surface will produce proportionate changes in the transsyncytial flux of glucose. Given the high degree of permeability of the capillary endothelium *(84–86)*, the transsyncytial flux of glucose is therefore likely to equate to the transplacental flux of glucose. Thus, changes in syncytial basal membrane glucose transport activity will have a profound impact on transplacental glucose transfer. Alterations in microvillous surface activity will be of little consequence unless transporter activity is reduced to a level similar to that on the basal membrane.

Gestational Development

Longitudinal studies have demonstrated increases in GLUT1 expression over gestation *(10,37,87)*. Although the asymmetry in the distribution of glucose transporters and glucose transport activity was first observed in term placental tissue, a similar asymmetry in transporter distribution was observed in preterm placental tissue *(10)*; expression of GLUT1 on the microvillous membrane was unchanged over the late second and third trimesters, whereas basal membrane expression increased by approx 50% over the same period *(10)*. It has long been recognized that the supply of glucose to the fetus increases substantially over the second half of pregnancy, paralleling the growth of the fetus. Part of the increase in glucose supply is attributable to the increase in uteroplacental and umbilical blood supply but by itself is insufficient to account for the increase in glucose supply necessary to meet fetal demand. It is therefore probable that the increase in the basal rate-limiting glucose transport step accounts for a significant portion of the increased supply of glucose to the fetus in the latter part of gestation.

Glucose Transport in Diabetic Pregnancy

The primary manifestation of diabetes is the increase in plasma glucose concentration due to the alterations in glucose homeostasis. Although diabetes is a maternal condition, during pregnancy the changes in maternal glycemic status are transmitted to the fetus as a result of the passive, facilitated-diffusion placental transport system by which maternal-fetal glucose transfer takes place. Analysis of placental glucose transporter expression and activity in diabetic pregnancy has been described in two recent studies *(88,89)*. Microvillous and basal membrane GLUT1 expression and glucose transporter activity were determined in placental tissue from women with pregestational (White Class B) and gestational diabetes (White Class A1, diet controlled; and A2, insulin controlled). The expression of GLUT1 glucose transporters and glucose transport activity in the microvillous membrane was unchanged between control subjects and all groups with diabetes. Basal membrane transporter expression and transport activity were increased by 97 *(88)* and 59% *(89)*, respectively, in women with pregestational diabetes. In the former study, basal membrane transporter expression and activity were also increased to a similar extent in both classes of women with gestational diabetes, whereas no such changes were observed in the latter study. The reasons for the difference between the studies with respect to women with gestational diabetes is not clear, but it may be related to gestational age at diagnosis or the duration and degree of maternal glycemic control exerted following diagnosis. What is clear, however, is that in at least one group of women with diabetes, maternal hyperglycemia provoked an increase in basal membrane glucose transporter expression and activity. Interestingly, although there was a demonstrated increase in transporter expression and activity at

(term) delivery, maternal glycemic status was not abnormal at term or in the weeks immediately prior to delivery. In both studies, mothers were euglycemic, as determined by plasma glucose and HbA_{1c} measurements. Thus, the response of the placental glucose transporter system appears to be remote from the maternal diabetic stimulus. The mechanism whereby this response is transmitted and sustained is not yet apparent; nevertheless, the consequence of this change, according to the basal rate-limiting step model, will be to increase maternal-fetal glucose flux in women with diabetes. As already noted, a variety of studies have also been performed to investigate directly the effects of hyperglycemia on trophoblast GLUT1 expression and glucose transporter activity. The conclusion from these studies is that under hyperglycemic conditions, transporter expression and activity are suppressed compared with that in euglycemic control subjects. This outcome is contrary to the in vivo observations in women with diabetes, suggesting that the in vivo regulation of glucose transporters may be considerably more complex than a simple effect of hyperglycemia.

The prediction of an increased flux in women with diabetes is at odds with recent in vitro perfusion studies. In the first of these studies, measurements of glucose transport in tissues from women with gestational diabetes treated by dietary intervention showed a decreased maternal-fetal transfer compared with control, nondiabetic tissue *(90)*. In the second study, women with diabetes who were insulin treated showed a greater rate of maternal-fetal transfer compared with those treated by dietary intervention, although, in this report, neither group was different from the control group *(91)*. Microvillous and basal membrane transporter expression and activity measurements were not performed in these studies, and, thus, it is difficult to know whether these women with diabetes showed an increase in basal membrane expression and activity similar to those reported previously *(88, 89)*. The apparent contradiction between the measurements of glucose transporter expression and activity, on the one hand, and the in vitro perfusion data, on the other hand, does raise a significant question. To what degree are the results observed in humans with diabetes the result of treatment to normalize glycemic status as opposed to the effects of the original maternal diabetic stimulus? In the absence of untreated diabetes, this may be a difficult question to resolve.

Glucose Transport in Hypoxia

For almost all studies concerning fetoplacental hypoxia in vivo, the hypoxic insult is associated with another pathology, such as preeclampsia or diabetes, precluding analysis of the consequences of a hypoxic insult alone. There is, however, one model that permits such an approach—the high-altitude pregnancy model, in which studies are performed on placental tissue obtained from women with normal pregnancies, residing at high altitude, in a condition of chronic hypoxia (i.e., reduced Po_2). We have performed measurements of GLUT1 glucose transporter expression in microvillous and basal membranes of placental tissue obtained from pregnant women residing at 3100 m and compared them with expression in membranes from pregnancies at a moderate altitude (1600 m). In these studies, we found no changes between expression of microvillous GLUT1 between the two groups. However, mean basal membrane GLUT1 expression was reduced by 40% *(92)*. Moreover, basal membrane GLUT1 expression was significantly correlated with birth weight in the high-altitude group, whereas no correlation was found for the moderate-altitude group. These data suggest a response to chronic hypoxia opposite to that observed in vitro, i.e., a reduction in GLUT1 compared to the increase in expression in the in vitro experiments. It is possible, however, that the reduced blood flow that is also observed in pregnancy at high altitude *(93)* may lead to reductions in circulating growth factors with consequent effects on placental glucose transporter expression.

Glucose Transport in IUGR

Measurements of fetal plasma glucose in IUGR have consistently shown a decreased concentration of glucose in IUGR fetuses *(94,95)*. A decreased expression of glucose transporters has been suggested as one possible mechanism by which fetal plasma glucose concentration is reduced. However, studies investigating placental transporter expression and activity in human IUGR are limited. An initial study carried out in our laboratory showed that glucose transporter expression and activity in both preterm and term IUGR was unchanged when compared with that of age-matched controls, on both microvillous and basal membranes *(10)*. This study examined transporters in idiopathic IUGR, identified solely by low birth weight (<3rd centile), raising the possibility that the subjects were a heterogeneous group, encompassing different etiologies that might or might not demonstrate a glucose transporter response. A recent, more detailed study, however, has reported very similar results *(96)*, showing that in well-defined IUGR, no changes in microvillous or basal membrane glucose transporter expression or activity were present compared with age-matched, normally grown controls, at any level of severity of IUGR.

As described earlier, multiple in vitro studies examining the effects of hypoglycemia on transporter expression and activity have shown that reduced levels of glucose lead to an upregulation of transporter expression or activity, although the thresholds at which these changes occur are not clearly defined. Extrapolation of these in vitro results to the in vivo situation are complicated by the fact that while fetal plasma glucose levels appear to be decreased in IUGR, the glucose concentrations existing in the syncytiotrophoblast in vivo are not known. With a relatively normal maternal plasma glucose concentration, it is possible that intrasyncytial glucose concentrations in IUGR are close to those in normal pregnancies.

Contrasts Between In Vivo and In Vitro Studies

One of the themes that emerges from consideration of the data that we have described is the contrast between those results using tissue obtained in vivo, from diabetic pregnancies, in hypoxia and IUGR, and the results obtained by in vitro experimentation. The data obtained from diabetic pregnancies (increased syncytial basal membrane GLUT1 expression) are the obverse of results obtained in vitro under hyperglycemic conditions (reduced trophoblast GLUT1). The results obtained for chronic hypoxia in vivo (decreased basal, unchanged microvillous GLUT1 expression) differ significantly from those obtained for hypoxia using isolated, in vitro methods (increased cellular GLUT1 expression). The absence of alterations in trophoblast GLUT1 in IUGR contrasts with the increased expression of GLUT1 observed in vitro under hypoglycemic conditions. It is clear that although the results from in vitro research provide valuable insights into the role of individual effectors of glucose transporter expression and activity, the results in vivo, obtained under conditions in which these effectors might be predicted to play a role, are not consistent with the in vitro results. It is probable that in vivo, as might be expected, there are multiple factors influencing transporter expression and activity. As we become better able to define the variety of agents that affect glucose transporters, it may be possible to sum the effects of these agents and reach an understanding of the complex factors that modulate not only glucose transporters but also other nutrient transporters such as the amino acid transporters. For example, the role of hypoxia in IUGR has not been examined with any rigor. Although the definitions of IUGR are much improved over those relying simply on birth weight, the other elements, relating to blood flow alterations and fetal distress/fetal metabolic condition, are primarily clinical in nature and do not address the potential extent of chronic hypoxia that may underlie some of the changes we observe in placental tissue collected from IUGR pregnancies. To do so will require parallel assessment of clinical and biochemical markers of hypoxia.

Another contrast between in vitro and in vivo research observations is the difference in structural and spatial organization of the syncytiotrophoblast. Most in vitro research has been concerned with overall effects on trophoblast cells, combining measurements of microvillous and basal membrane transporters. Demonstration of the different roles played by microvillous and basal glucose transporters in transepithelial transport suggests that the simple reporting of global changes in cellular expression and activity are insufficient to permit analysis of the physiologic significance of such changes. Another domain in which spatial effects may be important is the site of action of effectors or messengers. It is clear that receptor expression can differ between syncytial microvillous and basal membranes for agents such as insulin, IGF-1, and PGH *(49,57,97)*, and, thus, the syncytial responses mediated by these receptors may differ depending on the source of the stimulus, maternal or fetal. Moreover, it is unknown whether the binding of a growth factor such as IGF-1 has the same effect when binding to the basal membrane as it does when binding to the microvillous membrane. Among the factors affecting the response to IGF-1 are the presence of insulin receptors that can hybridize with the type 1 IGF receptors *(98)* and the presence of differing quantities of IGF-binding proteins on the opposing plasma membranes *(57)*. Studies on integrated models will become much more important as we are forced to examine not only the effects of multiple factors but also at the effects of spatial differences in the pathways that regulate transporter expression.

Acknowledgment

This work was supported in part by National Institutes of Health grant R01 DK55369.

References

1. Illsley, N. P., Hall, S., Penfold, P., and Stacey, T. E. (1985). *Contrib. Gynecol. Obstet.* **13,** 92–97.
2. Jansson, T., Powell, T. L., and Illsley, N. P. (1993). *J. Physiol.* **468,** 261–274.
3. Illsley, N., Hall, S., and Stacey, T. (1987). *Troph. Res.* **2,** 535–544.
4. Vardhana, P. and Illsley, N. P. (2002). *Placenta* **23,** 653–660.
5. Johnson, L. W. and Smith, C. H. (1980). *Am. J. Physiol.* **238,** C160–C168.
6. Bissonnette, J., Black, J., Wickham, W., and Acott, K. (1981). *J. Membr. Biol.* **58,** 75–80.
7. Johnson, L. W. and Smith, C. H. (1985). *Biochim. Biophys. Acta* **815,** 44–50.
8. Joost, H.-G. and Thorens, B. (2001). *Mol. Membr. Biol.* **18,** 247–256.
9. Barros, L. F., Yudilevich, D. L., Jarvis, S. M., Beaumont, N., and Baldwin, S. A. (1995). *Placenta* **16,** 623–633.
10. Jansson, T., Wennergren, M., and Illsley, N. P. (1993). *J. Clin. Endocrinol. Metab.* **77,** 1554–1562.
11. Teasdale, F. and Jean-Jacques, G. (1985). *Placenta* **6,** 25–31.
12. Clarson, L. H., Glazier, J. D., Sides, M. K., and Sibley, C. P. (1997). *Placenta* **18,** 333–340.
13. Illsley, N. P., Sellers, M. C., and Wright, R. L. (1998). *Placenta* **19,** 517–524.
14. Hauguel-De Mouzon, S., Challier, J., Kacemi, A., Caüzac, M., Malek, A., and Girard, J. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2689–2694.
15. Jansson, T., Cowley, E. A., and Illsley, N. P. (1995). *Reprod. Fertil. Dev.* **7,** 1425–1430.
16. Boileau, P., Causaz, M., Pereira, M., Girard, J., and Hauguel-De Mouzon, S. (2001). *Endocrine* **142,** 3974–3979.
17. Ogura, K., Sakata, M., Okamoto, Y., et al. (2000). *J. Endocrinol.* **164,** 171–178.
18. Maher, F., Davies-Hill, T., and Simpson, I. (1996). *Biochem. J.* **315,** 827–831.
19. Arbuckle, M., Kane, S., Porter, L., Seatter, M., and Gould, G. (1996). *Biochemistry* **35,** 16519–16527.
20. Esterman, A., Greco, M. A., Mitani, Y., et al. (1997). *Placenta* **18,** 129–136.
21. Yamaguchi, M., Sakata, M., Ogura, K., and Miyake, A. (1996). *J. Endocrinol. Invest.* **19,** 567–569.
22. Gude, N., Stevenson, J., Rogers, S., et al. (2002). *Placenta,* in press.

23. Head, J. R., Fujikawa, H., and Casey, M. L. (1999). *J. Soc. Gynecol. Invest.* **6,** 153A.
24. Xing, A. Y., Challier, J. C., Leperq, L., et al. (1998). *J. Clin. Endocrinol. Metab.* **83,** 4097–4101.
25. Carayannopoulos, M., Chi, M.-Y., Pingsterhaus, J., et al. (2000). *Proc. Natl. Acad. Sci. USA* **97,** 7313–7318.
26. Phay, J., Hussain, H., and Moley, J. (2000). *Genomics* **66,** 217–220.
27. McVie-Wylie, A., Lamson, D., and Chen, Y. (2001). *Genomics* **72,** 113–117.
28. Doege, H., Schürmann, A., Bahrenberg, G., Brauers, A., and Joost, H.-G. (2000). *J. Biol. Chem.* **275,** 16275–16280.
29. Joost, H.-G., Bell, G., Best, J., et al. (2002). *Am. J. Physiol.* **282,** E974–E976.
30. Barthel, A., Okino, S., Liao, J., et al. (1999). *J. Biol. Chem.* **274,** 20,281–20,286.
31. Taha, C., Liu, Z., Jin, J., Al-Hasani, H., Sonenberg, N., and Klip, A. (1999). *J. Biol. Chem.* **274,** 33085–33091.
32. Maher, F. and Harrison, L. C. (1990). *Biochem. Biophys. Res. Commun.* **171,** 210–215.
33. Boado, R. J. and Pardridge, W. M. (1993). *J. Neurochem.* **60,** 2290–2296.
34. Khayat, Z., McCall, A., and Klip, A. (1998). *Biochem. J.* **333,** 713–718.
35. Sasson, S., Kaiser, N., Dan-Goor, M., et al. (1997). *Diabetologia* **40,** 30–39.
36. Fujishiro, M., Gotoh, Y., Katagiri, H., et al. (2001). *J. Biol. Chem.* **276,** 19800–19806.
37. Hauguel-de Mouzon, S., Leturque, A., Alsat, E., Loizeau, M., Evain-Brion, D., and Girard, J. (1994). *Placenta* **15,** 35–46.
38. Hahn, T., Barth, S., Weiss, U., Mosgoeller, W., and Desoye, G. (1998). *FASEB J.* **12,** 1221–1231.
39. Hahn, T., Barth, S., Hofman, W., Reich, O., Lang, I., and Desoye, G. (1998). *Int. J. Cancer* **78,** 353–360.
40. Schwartz, J. and CarterSu, C. (1988). *Endocrine* **122,** 2247–2256.
41. Weiss, U., Cervar, M., Puerstner, P., et al. (2001). *Diabetologia* **44,** 209–219.
42. Sweeney, G., Somwar, S., Ramalal, T., Volchuk, A., Ueyama, A., and Klip, A. (1999). *J. Biol. Chem.* **274,** 10071–10078.
43. Hahn, T., Hahn, D., Blaschitz, A., Korgun, E., Desoye, G., and Dohr, G. (2000). *Diabetologia* **43,** 173–180.
44. Persson, A., Jansson, N., Powell, T., Hamark, B., and Jansson, T. (2002). *Placenta* **23,** 392–399.
45. CarterSu, C., Rozsa, F., Wang, X., and Stubbart, J. (1988). *Am. J. Physiol.* **255,** 723–729.
46. Vaulon, S., Vasseur-Cognet, M., and Kahn, A. (2000). *J. Biol. Chem.* **275,** 31,555–31,558.
47. Krones, A., Jungermann, K., and Kietzmann, T. (2001). *Endocrine* **142,** 2707–2718.
48. Koo, S.-H., Dutcher, A., and Towle, H. (2001). *J. Biol. Chem.* **276,** 9437–9445.
49. Desoye, G., Hartmann, M., Blaschitz, A., et al. (1994). *Histochemistry* **101,** 277–285.
50. Brunette, M. G., Lajeunesse, D., Leclerc, M., and Lafond, J. (1990). *Mol. Cell. Endocrinol.* **69,** 59–68.
51. Kniss, D. A., Shubert, P. J., Zimmerman, P. D., Landon, M. B., and Gabbe, S. G. (1994). *J. Reprod. Med.* **39,** 249–256.
52. Gordon, M. C., Zimmerman, P. D., Landon, M. B., Gabbe, S. G., and Kniss, D. A. (1995). *Am. J. Obstet. Gynecol.* **173,** 1089–1097.
53. Kniss, D., Xie, Y., Li, Y., et al. (2002). *Placenta* **23,** 32–43.
54. Challier, J. C., Hauguel, S., and Desmaizieres, V. (1986). *J. Clin. Endocrinol. Metab.* **62,** 803–807.
55. Eaton, B. and Sooranna, S. (1996). *Placenta* **17,** 209–215.
56. Cohran, V., Fang, J., Milio, L., Smith, C. H., and Fant, M. (1996). *Placenta* **17,** 313–320.
57. Fang, J., Furesz, T. C., Laurent, R. S., Smith, C. H., and Fant, M. E. (1997). *Pediatr. Res.* **41,** 258–265.
58. Wilson, C. M., Mitsumoto, Y., Maher, F., and Klip, A. (1995). *FEBS Lett.* **368,** 19–22.
59. Fladeby, C., Bjonness, B., and Sewrck-Hanssen, G. (1996). *J. Cell. Physiol.* **169,** 242–247.
60. Zhou, J., Bievre, M., and Bondy, C. (2000). *Growth Horm. IGF Res.* **10,** 111–117.
61. DeBosch, B., Baur, E., Deo, B., Hiraoka, M., and Kumagai, A. (2001). *J. Neurochem.* **77,** 1157–1167.
62. Butler, A., Yakar, S., Gewolb, I., Karas, M., Okubo, Y., and Le Roith, D. (1998). *Comp. Biochem. Physiol. B* **121,** 19–26.
63. Petley, T., Graff, K., Jiang, W., Yang, H., and Florini, J. (1999). *Horm. Metab. Res.* **31,** 70–76.
64. Teruel, T., Valverde, A., Navarro, P., Benito, M., and Lorenzo, M. (1998). *J. Cell. Physiol.* **176,** 99–109.
65. Zelzer, E., Levy, Y., Kahana, C., Shilo, B., Rubinstein, M., and Cohen, B. (1998). *EMBO J.* **17,** 5085–5094.
66. Akeno, N., Robins, J., Zhang, M., and Czyzk-Krzeska, M. (2002). *Endocrine* **143,** 420–425.
67. Alsat, E., Guibourdenche, J., Luton, D., Frankenne, F., and Evain-Brion, D. (1997). *Am. J. Obstet. Gynecol.* **177,** 1526–1534.
68. Alsat, E., Guibourdenche, J., Couturier, A., and Evain-Brion, D. (1998). *Mol. Cell. Endocrinol.* **140,** 121–127.
69. Caufriez, A., Frankenne, F., Hennen, G., and Copinschi, G. (1993). *Am. J. Physiol.* **265,** E572–E577.
70. Patel, N., Alsat, E., Igout, A., et al. (1995). *J. Clin. Endocrinol. Metab.* **80,** 1743–1746.
71. McIntyre, H. D., Serek, R., Crane, D. I., et al. (2000). *J. Clin. Endocrinol. Metab.* **85,** 1143–1150.
72. Currie, M. J., Bassett, N. S., Breier, B. H., et al. (1996). *Growth Reg.* **6,** 123–129.
73. Bauer, M. K., Harding, J. E., Bassett, N. S., et al. (1998). *Mol. Cell. Endocrinol.* **140,** 115–120.
74. Jenkinson, C. M., Min, S. H., Mackenzie, D. D., McCutcheon, S. N., Breier, B. H., and Gluckman, P. D. (1999). *Growth Horm. IGF Res.* **9,** 11–17.
75. Palta, V., Baumann, M., Deborde, S., Mascarina, M., and Illsley, N. (2002). *J. Clin. Endocrinol. Metab.,* submitted.
76. Semenza, G. (2000). *Biochem. Pharmacol.* **59,** 47–53.
77. Ebert, B., Firth, J., and Ratcliffe, P. (1995). *J. Biol. Chem.* **270,** 29083–29089.
78. Illsley, N. P., Aarnoudse, J. G., Penfold, P., et al. (1984). *Placenta* **5,** 213–225.
79. Das, U. G., Sadiq, H. F., Soares, M. J., Hay, W. W. Jr., and Devaskar, S. U. (1998). *Am. J. Physiol.* **274,** R339–R347.
80. Howard, W., Hunter, C., and Huber, C. (1961). *Surg. Gynecol. Obstet.* **112,** 435–438.
81. Rodesch, F., Simon, P., Donner, C., and Jauniaux, E. (1992). *Obstet. Gynecol.* **80,** 283–285.
82. Jauniaux, E., Watson, A., Hempstock, J., Bao, Y., Skepper, J., and Burton, G. (2000). *Am. J. Pathol.* **157,** 2111–2122.
83. Baumann, M., Deborde, S., Mascarina, M., and Illsley, N. (2002). *Am. J. Physiol.,* submitted.
84. Eaton, B. M., Leach, L., and Firth, J. A. (1993). *J. Physiol.* **463,** 141–155.
85. Leach, L. and Firth, J. A. (1995). *Reprod. Fert. Dev.* **7,** 1451–1456.
86. Leach, L. and Firth, J. A. (1997). *Microsc. Res. Tech.* **38,** 137–144.
87. Sakata, M., Kurachi, H., Imai, T., et al. (1995). *Eur. J. Endocrinol.* **132,** 206–212.
88. Gaither, K., Quraishi, A. N., and Illsley, N. P. (1999). *J. Clin. Endocrinol. Metab.* **84,** 695–701.
89. Jansson, T., Wennergren, M., and Powell, T. L. (1999). *Am. J. Obstet. Gynecol.* **180,** 163–168.
90. Osmond, D., Nolan, C., King, R., Brennecke, S., and Gude, N. (2000). *Diabetologia* **43,** 576–582.
91. Osmond, D., King, R., Brennecke, S., and Gude, N. (2001). *Diabetologia* **44,** 1133–1139.
92. Zamudio, S., Baumann, M., and Illsley, N. (2002). *Placenta,* submitted.

93. Zamudio, S., Palmer, S., Droma, T., Stamm, E., Coffin, C., and Moore, L. (1995). *J. Appl. Physiol.* **79,** 7–14.
94. Economides, D. and Nicolaides, K. (1989). *Am. J. Obstet. Gynecol.* **160,** 385–389.
95. Marconi, A. M., Paolini, C., Buscaglia, M., Zerbe, G., Battaglia, F. C., and Pardi, G. (1996). *Obstet. Gynecol.* **87,** 937–942.
96. Jansson, T., Ylven, K., Wennergren, M., and Powell, T. (2002). *Placenta* **22,** 392–399.
97. Hill, D. J., Riley, S. C., Bassett, N. S., and Waters, M. J. (1992). *J. Clin. Endocrinol. Metab.* **75,** 646–650.
98. Kasuya, J., Paz, B., Maddux, B., Goldfine, I., Hefta, S., and Fujita-Yamaguchi, Y. (1993). *Biochemistry* **32,** 13531–13536.