Alterations in the Activity of Placental Amino Acid Transporters in Pregnancies Complicated by Diabetes

Thomas Jansson,[1] Ylva Ekstrand,[1] Caroline Björn,[1] Margareta Wennergren,[2] and Theresa L. Powell[1]

Alterations in placental transport may contribute to accelerated fetal growth in pregnancies complicated by diabetes. We studied the activity of the syncytiotrophoblast amino acid transporter system A and the transport of the essential amino acids leucine, lysine, and taurine. Syncytiotrophoblast microvillous plasma membranes (MVMs) and basal plasma membranes (BMs) were isolated from placentas obtained from normal pregnancies and pregnancies complicated by gestational diabetes mellitus (GDM) and type 1 diabetes, with and without large-for-gestational-age (LGA) fetuses. Amino acid transport was assessed using radio-labeled substrates and rapid filtration techniques. System A activity in MVM was increased (65–80%, $P < 0.05$) in all groups with diabetes independent of fetal overgrowth. However, MVM system A activity was unaffected in placentas of normal pregnancies with LGA fetuses. MVM leucine transport was increased in the GDM/LGA group. In BMs, amino acid transport was unaffected by diabetes. In conclusion, diabetes in pregnancy is associated with an increased system A activity in MVM, and MVM leucine transport is increased in the GDM/LGA group. We suggest that these changes result in an increased uptake of neutral amino acids across MVM, which may be used in placental metabolism or be delivered to the fetus. The increased MVM leucine uptake in the GDM/LGA group may contribute to accelerated fetal growth in these patients. *Diabetes* 51:2214–2219, 2002

Diabetes complicates 2–3% of all pregnancies, and despite major advances in clinical management, perinatal morbidity remains significant in this pregnancy complication (1). In gestational diabetes mellitus (GDM), which represents 90% of all cases, and in type 1 diabetes, problems include accelerated fetal growth and neonatal hypoglycemia (2,3). Fetal overgrowth, resulting in the delivery of a large-for-gestational-age (LGA) infant, represents a risk factor for operative delivery and traumatic birth injury (4). In addition, type 1 diabetes during pregnancy is associated with an increased risk for congenital malformations, intrauterine growth restriction, and infant respiratory distress syndrome (3). The metabolic disturbances in diabetes during pregnancy have consequences beyond life in utero, and the perinatal period since hyperglycemia during pregnancy is associated with obesity and diabetes (5) as well as GDM in the offspring (6).

Hyperglycemia has been proposed to be a major contributing factor to the development of many of the complications of diabetes in pregnancy, in particular accelerated fetal growth. Because of the facilitative nature of transplacental glucose transport, maternal hyperglycemia results in elevated fetal glucose levels, in turn stimulating fetal insulin release (7). However, birth weight is not clearly correlated to indexes of maternal blood glucose control in either GDM (8–10) or type 1 diabetes (3,11), suggesting a complex relationship between metabolic disturbance and fetal growth in diabetes during pregnancy. In this context, it has been emphasized that alterations in the metabolism of all energy substrates have to be considered to more fully understand the impact of maternal diabetes on the fetus (12,13).

Alterations in the activity and expression of placental nutrient transporters could represent an additional level of complexity to the relationship between maternal substrate levels and fetal growth in diabetes in pregnancy. The transporters of primary interest are localized in the plasma membranes of the syncytiotrophoblast, the transporting epithelium of the human placenta. The microvillous plasma membrane (MVM) faces the maternal circulation, whereas the basal plasma membrane (BM) represents the fetal-facing aspect of the cell. Indeed, the protein expression of the primary placental glucose transporter isoform GLUT1 as well as glucose transport activity has been shown to be increased in BM in type 1 diabetes (14,15). We have suggested that these alterations contribute to accelerated fetal growth in type 1 diabetes and may explain the occurrence of macrosomia in certain pregnancies despite rigorous maternal glycemic control (14). In contrast, GDM appears not to be associated with changes in placental glucose transporters (16).

Insulin is the primary growth-promoting hormone in fetal life. Amino acids are more potent stimulators of fetal insulin release than glucose (17–19), and changes in amino acid delivery to the fetus may have profound effects on fetal growth rate. The transport of neutral amino acids across isolated syncytiotrophoblast membranes has been assessed in two previous studies using the amino acid analogs aminoisobutyric acid, which is transported by several transporters for neutral amino acids, or methyl-

From the [1]Department of Physiology and Pharmacology, the Perinatal Center, Göteborg University, Göteborg, Sweden; and the [2]Department of Obstetrics and Gynecology, the Perinatal Center, Göteborg University, Göteborg, Sweden.

Address correspondence and reprint requests to Dr. Thomas Jansson, Perinatal Center, Department of Physiology and Pharmacology, Göteborg University, Box 432, S-405 30 Göteborg, Sweden. E-mail: thomas.jansson@fysiologi.gu.se.

Received for publication 21 December 2001 and accepted in revised form 12 April 2002.

AGA, appropriate-for-gestational-age; BM, basal plasma membrane; GDM, gestational diabetes mellitus; LGA, large-for-gestational-age; MeAIB, methylaminoisobutyric acid; MVM, microvillous plasma membrane; OGTT, oral glucose tolerance test.

aminoisobutyric acid (MeAIB), which is exclusively transported by system A. The uptake of aminoisobutyric acid was not affected in MVMs isolated from placentas obtained from pregnancies complicated by diabetes but associated with normal fetal growth (20). Subsequently, Kuruvilla et al. (21) demonstrated a decrease in the uptake of MeAIB in MVMs prepared from placentas of women with diabetes. Therefore, the impact of diabetes in pregnancy on placental transporters for neutral amino acids is not well established. In addition, other unresolved questions remain. For example, to assess transplacental transport, both polarized plasma membranes of the syncytiotrophoblast have to be studied. Previous reports have mainly addressed the transport of certain neutral amino acids, whereas transport of essential amino acids have not been studied in detail.

The system A amino acid transporter is ubiquitously expressed in human tissues, transports a broad range of neutral amino acids, and is characterized by being highly regulated. The primary objective of the present investigation was to study the activity of the placental system A transporter in type 1 diabetes and GDM, with and without accelerated fetal growth, as well as in placentas obtained from pregnancies with LGA infants but without signs of altered glucose metabolism. In addition, we studied the activity of three transport systems for essential amino acids. We isolated MVMs and BMs simultaneously from term placentas and assessed the activity of amino acid transporters using radioactively labeled substrates employing rapid filtration techniques.

RESEARCH DESIGN AND METHODS

Patients. The collection of placental tissue was approved by the Committee for Research Ethics at Göteborg University. Placentas were obtained from six groups of patients: control subjects, patients with GDM delivering appropriate-for-gestational-age (AGA) infants (GDM) or LGA infants (GDM/LGA), patients with type 1 diabetes delivering AGA infants (type 1 diabetes) or LGA infants (type 1 diabetes/LGA), and patients without indications of diabetes giving birth to LGA infants (LGA). Control placentas were obtained from uneventful term pregnancies with birth weights classified as AGA. These women had either spontaneous vaginal delivery or elective cesarean sections because of maternal indication. Screening for GDM was carried out routinely in all pregnancies by measuring capillary blood glucose in the nonfasting state on at least four occasions starting in weeks 8–12 of gestation. If nonfasting blood glucose was ≥7.0 mmol/l, patients were referred to a 75-g oral glucose tolerance test (OGTT) after overnight fasting. In the LGA group, blood glucose measurements were carried out frequently in the third trimester because signs indicated accelerated fetal growth. Nonfasting blood glucose levels were found to be <7.0 mmol/l in all instances. In two patients in the LGA group, an OGTT was carried out and found to be normal. GDM was defined as a fasting blood glucose level ≥6.1 mmol/l or blood glucose level ≥9.0 mmol/l 2 h after an OGTT in a pregnant woman without prior known diabetes. These pregnancies were not associated with any major complications in addition to GDM. Assessment of blood glucose control was performed by self-monitoring fasting and postprandial blood glucose levels at least twice a week. Patients with GDM were treated with diet adjustments only, except in seven patients (1/10 in GDM group and 6/10 in GDM/LGA group), in which insulin was administered in a five-dose regimen. The criterion for initiating insulin therapy was elevated blood glucose levels (fasting glucose ≥6.1 and/or postprandial blood glucose ≥8.0) despite consistent dietary adjustments.

Type 1 diabetic patients were classified as Diabetes White D, defined as onset of disease before 10 years of age or a history of diabetes longer than 20 years, or Diabetes White C, defined as onset of disease at 10–19 years of age or a duration of 10–19 years (22). Type 1 diabetic patients had no other major complications. Patients carried out self-monitoring of capillary blood glucose concentrations at least four times daily, and HbA_{1c} was measured every 2–6 weeks. All patients in the type 1 diabetes group received insulin in a five-dose regimen.

All women with diabetes attended the obstetric antenatal clinic at Sahlgren's University Hospital, Göteborg. Estimated date of delivery was determined from the last menstrual period and confirmed by ultrasound at 16–18 weeks of gestation. LGA neonates were defined as having a birth weight greater than the mean birth weight plus 2 SDs using intrauterine growth curves based on ultrasonically estimated fetal weight (23).

Preparation of syncytiotrophoblast plasma membranes. Placentas were placed on ice immediately after delivery, and the membrane isolation procedure was started within 30 min. MVMs and BMs were prepared simultaneously from the same placenta according to a well-characterized protocol (24) with modifications (25). Briefly, after initial homogenization and centrifugation steps (at 4°C in 250 mmol/l sucrose, 10 mmol/l HEPES-Tris, pH 7.4), BMs were separated from MVMs by Mg^{2+} precipitation and further purified on a sucrose step gradient. Samples were snap-frozen in liquid nitrogen and stored at −80°C. MVM and BM enrichments were assessed using standard activity assays for adenylate cyclase (26) and alkaline phosphatase (27). The production of cAMP by adenylate cyclase was measured by radioimmunoassay (New England Nuclear, Boston, MA).

Activity of amino acid transporters. The activity of the system A transporter was assessed using a modification of the protocol of Mahendran et al. (28). The uptake into plasma membrane vesicles of the three essential amino acids L-lysine, L-leucine, and taurine was studied as described (29,30) with minor modifications. Vesicles were preloaded by incubation in 300 mmol/l mannitol and 10 mmol/l HEPES-Tris, pH 7.4, overnight at 4°C. Subsequently, vesicles were pelleted and resuspended in a small volume of the same buffer (protein concentration ~5–10 mg/ml). Membrane vesicles were kept on ice until immediately before transport measurements, when samples were warmed to 37°C. At time 0, 30 μl of vesicles was rapidly mixed (1:2), with the appropriate incubation buffer including ^{14}C-methyl-aminoisobutyric acid (MeAIB, 150 μmol/l), ^{3}H-L-lysine (0.15 μmol/l), ^{3}H-L-leucine (0.375 μmol/l), or ^{3}H-taurine (0.75 μmol/l). Uptake of radio-label was terminated by addition of 2 ml ice-cold PBS after 30 s (MeAIB, MVM), 150 s (MeAIB, BM) (31), or 8 s (all three essential amino acids) (29,30). Subsequently, vesicles were rapidly separated from the substrate medium by filtration on mixed ester filters (0.45 μm pore size; Millipore Corporation, Bedford, MA) and washed with 3 × 2 ml PBS. In studies of MeAIB and taurine transport, 150 mmol/l NaCl and 150 mmol/l KCl was used in incubation buffers to assess total and sodium-independent uptake, respectively. In lysine uptake experiments, incubation buffers included 78 mmol/l NaSCN, 144 mmol/l mannitol, and 10 mmol/l HEPES-Tris, pH 7.4. In experiments assessing the contribution of the y^+ system, 1.5 mmol/l mannitol was exchanged with 1.5 mmol/l L-glutamine (32). Possible contribution of the $b^{0,+}$ transporter to lysine uptake was investigated in the presence of 100 μmol/l leucine and in the absence of sodium. Nonmediated lysine flux was measured in the presence of 10 mmol/l unlabelled substrate. In leucine transport experiments, NaSCN was replaced by NaCl in the incubation buffer, and nonmediated flux was studied in the presence of 20 mmol/l unlabeled L-leucine.

In all uptake experiments, each condition was studied in triplicate for each membrane preparation. Filters were dissolved in 2 ml liquid scintillation fluid and counted. Appropriate blanks were subtracted from counts, and uptakes were expressed as picomoles per milligram protein. Na^+-dependent uptake of MeAIB (corresponding to system A activity) and taurine was calculated by subtracting Na^+-independent uptake from total uptakes. For lysine and leucine, mediated uptake was calculated by subtracting nonmediated transport from total uptake. Protein content of the vesicles was determined by the method of Bradford (33).

Statistics. Results are given as means ± SE. ANOVA followed by Dunnett's test or Student Newman-Keuls test was used as appropriate to evaluate data statistically. $P < 0.05$ was considered significant.

RESULTS

Selected clinical characteristics for the patients are given in Table 1.

The six groups were comparable with regard to maternal age and gestational age. Maternal weight early in pregnancy in the two GDM groups was significantly higher than that in control subjects. As expected, placental weight and birth weight were significantly higher in the three LGA groups. In the two type 1 diabetes groups, HbA_{1c} was measured regularly throughout pregnancy, and mean values in the three trimesters are presented in Table 2.

In contrast, HbA_{1c} was measured only sporadically in GDM groups (Table 2) and not at all in the control and

TABLE 1
Selected clinical data

	Control	Type 1 diabetes	Type 1 diabetes/LGA	GDM	GDM/LGA	LGA
n	35	8	13	10	10	8
Maternal age (years)	32.5 ± 1.0	30.2 ± 1.7	33.0 ± 1.0	31.6 ± 1.2	35.4 ± 1.9	30.5 ± 1.7
Maternal weight (kg)	65.4 ± 1.9	74.2 ± 8.1	70.8 ± 4.5	80.7 ± 4.3*	80.3 ± 5.0*	73.6 ± 3.5
Gestational age (weeks)	38.4 ± 0.2	38.6 ± 0.6	37.9 ± 0.3	39.4 ± 0.4	38.7 ± 0.3	38.9 ± 0.6
Placental weight (g)	637 ± 17	665 ± 56	999 ± 44*	687 ± 70	905 ± 46*	921 ± 63*
Birth weight (g)	3,230 ± 57	3,283 ± 191	4,379 ± 104*	3,477 ± 184	4,400 ± 110*	4,169 ± 171*

Data are means ± SE. Maternal weight was registered around gestational week 10. *$P < 0.05$ vs. control subjects.

LGA groups. In both the type 1 diabetes and type 1 diabetes/LGA groups, HbA_{1c} values were highest in the first trimester and decreased toward term. In addition, first trimester HbA_{1c} values were significantly elevated in both of these groups when compared with the normal range for this variable (3.9–5.3) (Table 2). However, HbA_{1c} values remained significantly elevated in the third trimester only in the type 1 diabetes group with accelerated fetal growth.

In the control group, enrichment of alkaline phosphatase activity in MVM was 16-fold, and enrichment of adenylate cyclase activity was 31-fold in BM. Marker enzyme activities in the other study groups were not significantly different from control vesicles (data not shown). We have previously shown that isolated syncytiotrophoblast plasma membranes show only little or no contamination by markers for intracellular organelles (34). These results confirm that this plasma membrane isolation procedure produces MVMs and BMs with high enrichment and low contamination (24).

System A activity in BMs was only 4% of that in MVMs (Fig. 1). The activity of system A in MVM was increased by 65–80% in all four groups associated with diabetes (Fig. 1*A*). Placentas obtained from pregnancies complicated with diabetes and LGA infants showed the same degree of changes as placentas from pregnancies not associated with accelerated fetal growth. In contrast, in pregnancies with LGA infants but without signs of impaired glucose metabolism (LGA group), system A activity in MVMs was unaltered. In BMs, system A activity was unaffected by diabetes and/or accelerated fetal growth (Fig. 1*B*).

The activity of syncytiotrophoblast transport systems for essential amino acids was studied in type 1 diabetes/LGA and GDM/LGA groups. The main transporter for cationic amino acids in MVMs obtained from control, type 1 diabetes/LGA, and GDM/LGA groups was the y^+ system, and the y^+L transporter represented the primary transporter in BMs, whereas no evidence for $b^{0,+}$ transporter activity was found (data not shown). These findings are consistent with previous extensive studies on cationic amino acid transport in syncytiotrophoblast plasma membranes of control placentas (35). The mediated transport of lysine and taurine in MVM and BM was not significantly different in these two groups compared with control subjects (Table 3). However, MVM uptake of leucine was increased in association with GDM and LGA infants (Fig. 2).

DISCUSSION

This is the first study to assess the transport of neutral amino acids in both polarized plasma membranes of the placental transporting epithelium isolated from pregnancies complicated by GDM or type 1 diabetes with and without accelerated fetal growth as well as from pregnancies associated with fetal overgrowth despite apparently normal glucose metabolism. The transport of amino acids across MVMs is a secondary active process and represents the rate-limiting step in transplacental transfer of amino acids (36). System A is a Na^+-dependent transporter mediating the uptake of neutral amino acids such as alanine, serine, and glutamine. In the current article, we demonstrate that amino acid transport mediated by system A is markedly increased in the syncytiotrophoblast MVM in association with diabetes during pregnancy. We suggest that these changes result in an increased uptake of neutral amino acids across MVM into the syncytiotrophoblast cytoplasm, which may increase the delivery of amino acids to the fetus. However, our findings that the increased MeAIB uptake in MVM vesicles was independent of the occurrence of LGA infants suggest that these changes are not the primary cause for accelerated fetal growth. It is possible that alterations in other syncytiotrophoblast nutrient transporters, such as the demonstrated increased expression and activity of glucose transporters in the type 1 diabetes/LGA group (14), are more important. System A mediates the transport of nonessential amino acids that are subjected to catabolism in the placenta, which is compatible with the possibility that a substantial portion

TABLE 2
HbA_{1c} in pregnancies complicated by diabetes

	Type 1 diabetes (*n*=6–7)		Type 1 diabetes/LGA (*n*=9–10)		GDM (*n*=2)		GDM/LGA (*n*=3)	
Trimester	HbA_{1c}	95% CI	HbA_{1c}	95% CI	HbA_{1c}	95% CI	HbA_{1c}	95% CI
First	6.33 ± 0.29*	5.56–7.10†	7.02 ± 0.38*	6.14–7.91†	—	—	—	—
Second	4.97 ± 0.16	4.52–5.42	5.46 ± 0.22	4.95–5.97	—	—	—	—
Third	5.07 ± 0.29	4.22–5.91	5.72 ± 0.15	5.38–6.05†	4.27 ± 0.09	3.89–4.65	4.57 ± 0.38	2.95–6.18

Data are means ± SE. *$P < 0.05$ vs. the second and third trimester; †HbA_{1c} value significantly different from normal range of HbA_{1c} in uncomplicated pregnancies (3.9–5.3).

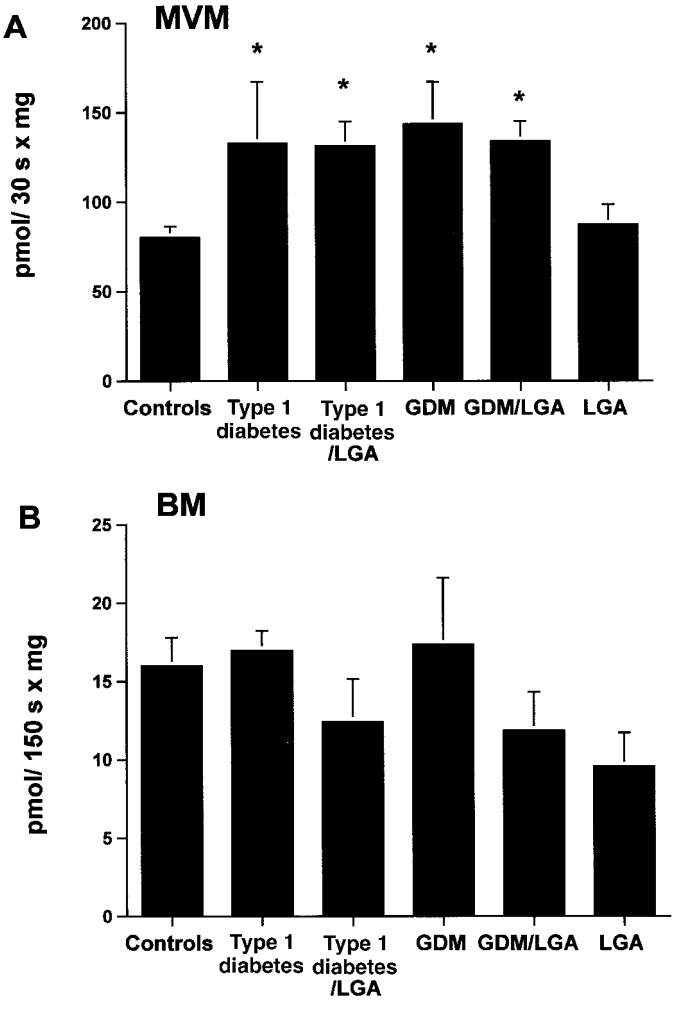

FIG. 1. Na^+-dependent uptake of MeAIB (system A activity) in MVM vesicles (*A*) and BM vesicles (*B*) isolated from placentas obtained from six different groups. The studied groups were control subjects (n = 35), type 1 diabetes with normal fetal growth (type 1 diabetes, n = 8), type 1 diabetes with LGA infants (type 1 diabetes/LGA, n = 13), GDM with normal fetal growth (GDM, n = 10), GDM with LGA infants (GDM/LGA, n = 10), and women giving birth to LGA infants but without signs of altered glucose metabolism (LGA, n = 8). *P < 0.05 vs. control subjects, determined by ANOVA.

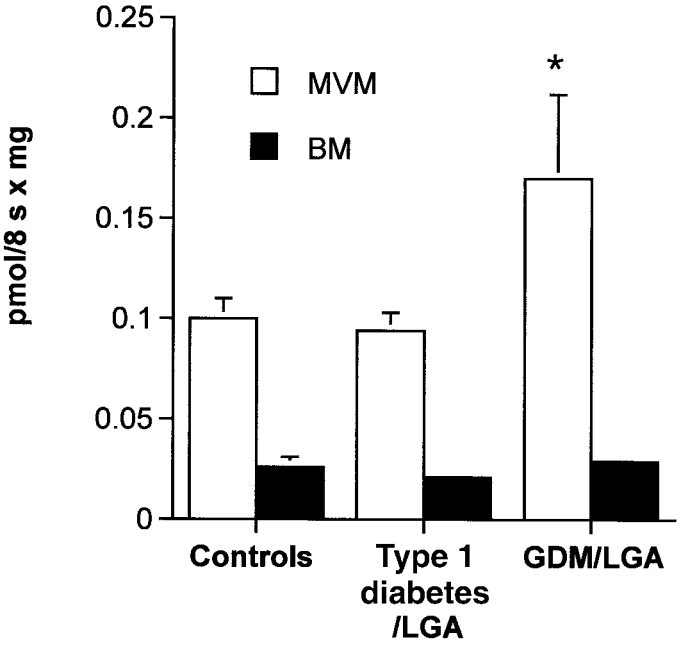

FIG. 2. The transport of leucine in MVM and BM vesicles in control (n = 13), type 1 diabetes/LGA (n = 8), and GDM/LGA (n = 4) groups. *P < 0.05 vs. control subjects, determined by ANOVA.

of the amino acids taken up by System A are used in placental metabolism. The increased MVM system A activity in pregnancies complicated by diabetes may therefore be related to an increased amino acid catabolism in the placenta.

The markedly increased system A–mediated transport in MVMs associated with diabetes observed in this study is unlikely to be caused by a general membrane effect in diabetes or technical difficulties inherent in the membrane isolation procedure. First, enrichments of membrane marker enzymes were similar between groups, and MVM transport systems for taurine and lysine were unaffected by diabetes. In addition, in studies of similar groups of placentas from patients with diabetes, we reported unaltered glucose transporter activity and expression in the MVM (14). Consequently, the effect of diabetes on MVM nutrient transporters may be specific for system A.

In experimental diabetes in rats, it has been demonstrated that system A activity is increased in skeletal muscle (37) and liver (38). The increase in system A–mediated amino acid transport in the human placenta may therefore represent a manifestation of a more general effect of diabetes on this amino acid transporter. Unlike most other amino acid transporters, system A is highly regulated, and system A activity in nonplacental tissues is influenced by a number of hormones (e.g., insulin, glucagon, and cortisol) and nutrition (39). In addition, insulin, dexamethasone, and glucagon have been shown to stimulate the uptake of MeAIB in cultured human trophoblast

TABLE 3
Mediated uptake of lysine and taurine

	Control	Type 1 diabetes/LGA	GDM/LGA
n	13	8	4
Mediated uptake of lysine			
MVM	0.35 ± 0.06	0.21 ± 0.04	0.41 ± 0.03
BM	0.065 ± 0.01	0.039 ± 0.01	0.032 ± 0.04
Mediated uptake of taurine			
MVM	1.03 ± 0.12	0.91 ± 0.08	1.20 ± 0.21
BM	0.048 ± 0.01	0.042 ± 0.01	0.051 ± 0.01

Data are means ± SE. Uptakes are given as picomoles per 8 seconds per milligram. Uptakes in type 1 diabetes/LGA and GDM/LGA groups were not significantly different from those in the control group.

cells (40). The factors causing the increase in MVM system A activity in diabetes in pregnancy remain to be identified.

In the current study, we measured the uptake of essential amino acids that are transported by three different transport systems. The uptake of leucine, but not lysine or taurine, was significantly increased in MVMs isolated from GDM/LGA pregnancies, whereas no change was observed in the type 1 diabetes/LGA group. This difference in the impact of GDM and type 1 diabetes on leucine transport probably reflects that these two types of diabetes are distinct diseases with differences in degree and timing of metabolic disturbance. Leucine is transported across the MVM, mediated almost exclusively by the L system (36), a broad scope Na^+-independent transporter that recently was characterized at the molecular level (41–43). Although the L transporter has been suggested to be relatively insensitive to regulation (39), the transporter has been shown to be stimulated by phorbol esters and calmodulin antagonists in a choricarcinoma cell line (44). Leucine has been shown to be an effective stimulus for fetal insulin in human fetal pancreas studied in vitro (18). In addition, in vivo studies applying stable isotope techniques have provided evidence to suggest that leucine taken up across the MVM is rapidly transferred to the fetus (45). Therefore, we suggest that the increased leucine transport across the MVM in the GDM/LGA group increases the leucine delivery to the fetus and contributes to the accelerated fetal growth in these patients.

Our data on MVM system A activity in diabetes are distinctly different from that of Kuruvilla et al. (21), who found significantly decreased MVM system A activity in diabetes with LGA infants and an unaltered activity in pregnancies complicated by diabetes associated with normal fetal growth. Furthermore, in the previous report, MVM leucine transport in diabetes was not significantly different compared with control MVMs (21). The reason for these different findings is not immediately apparent but may be related to methodological differences. We studied amino acid transport at 37°C, whereas room temperature (System A) or 4°C (leucine) was used in the previous study (21). In general, elevated temperature will increase measured transporter activity but may also alter the apparent behavior of a transporter. For example, in some studies, activity of the cationic amino acid transporter y^+L cannot be detected in syncytiotrophoblast plasma membranes at room temperature, whereas it is readily observed at 37°C (46). Differences in study populations represent another possible explanation for the discrepant results because the British and Swedish pregnant women who were studied may differ in some fundamental way with regard to ethnicity, nutrition, or clinical management. Finally, the discrepant results between the two studies concerning MVM leucine uptake in diabetes may be more apparent than real because, in the study of Kuruvilla et al., MVM leucine uptake in diabetic patients with macrosomia was 32% higher than that in control subjects. However, this difference was not significant, possibly because of the relatively small number of observations.

ACKNOWLEDGMENTS

This study was supported by grants from the Swedish Research Council (10838), the Swedish Diabetes Association, the Torsten and Ragnar Söderbergs Foundation, Frimurare-Barnhus-direktionen, the Åhlens Foundation, the Åke Wiberg Foundation, the Magnus Bergvall Foundation, the Hedberg Foundation for Medical Research, and the Willhelm and Martina Lundgrens Foundation.

REFERENCES

1. Landon M: Diabetes mellitus and other endocrine diseases. In *Obstetrics: Normal and Problem Pregnancies*. 3rd ed. Gabbe S, Niebyl J, Simpson J, Eds. New York, Churchill Livingstone, 1996, p. 1037–1081
2. Coustan DR: Gestational diabetes: a continuum of risk. *Eur J Endocrinol* 137:13–14, 1997
3. Garner P: Type I diabetes mellitus and pregnancy. *Lancet* 346:157–161, 1995
4. Casey BM, Lucas MJ, McIntire DD, Leveno KJ: Pregnancy outcomes in women with gestational diabetes compared with the general obstetric population. *Obstet Gynecol* 90:869–873, 1997
5. Pettitt DJ, Nelson RG, Saad MF, Bennett PH, Knowler WC: Diabetes and obesity in the offspring of Pima Indian women with diabetes during pregnancy. *Diabetes Care* 16 (Suppl. 1):310–314, 1993
6. Pettitt DJ, Bennett PH, Saad MF, Charles MA, Nelson RG, Knowler WC: Abnormal glucose tolerance during pregnancy in Pima Indian women: long-term effects on offspring. *Diabetes* 40 (Suppl.):126–130, 1991
7. Pedersen J: Weight and length at birth of infants of diabetic mothers. *Acta Endocrinol* 16:330–342, 1954
8. Miller J: A reappraisal of "tight control" in diabetic pregnancies. *Am J Obstet Gynecol* 147:158–162, 1983
9. Jacobson JD, Cousins L: A population-based study of maternal and perinatal outcome in patients with gestational diabetes. *Am J Obstet Gynecol* 161:981–986, 1989
10. Coustan DR, Imrah J: Prophylactic insulin treatment of gestational diabetes reduces the incidence of macrosomia, operative delivery and birth trauma. *Am J Obstet Gynecol* 150:836–842, 1984
11. Persson B, Hansson U: Fetal size at birth in relation to quality of blood glucose control in pregnancies complicated by pregestational diabetes. *Br J Obstet Gynaecol* 103:427–433, 1996
12. Freinkel N: Of pregnancy and progeny. *Diabetes* 29:1023–1035, 1980
13. Metzger BE, Phelps RL, Freinkel N, Navickas IA: Effects of gestational diabetes on diurnal profiles of glucose, lipids and individual amino acids. *Diabetes Care* 3:402–409, 1980
14. Jansson T, Wennergren M, Powell TL: Placental glucose transport and GLUT 1 expression in insulin dependent diabetes. *Am J Obstet Gynecol* 180:163–168, 1999
15. Gaither K, Quraishi AN, Illsley NP: Diabetes alters the expression and activity of the human placental GLUT1 glucose transporter. *J Clin Endocrinol Metab* 84:695–701, 1999
16. Jansson T, Ekstrand Y, Wennergren M, Powell TL: Placental glucose transport in gestational diabetes. *Am J Obstet Gynecol* 184:111–116, 2001
17. Hoffman L, Mandel TE, Carter WM, Koulmanda M, Martin FIR: Insulin secretion by human pancreas in organ culture. *Diabetologia* 23:426–430, 1982
18. Milner RDG, Ashworth MA, Barson AJ: Insulin release from human foetal pancreas in response to glucose, leucine and arginine. *J Endocrinol* 52:497–505, 1972
19. Maitland JE, Parry DG, Turtle JR: Perifusion and culture of human fetal endocrine pancreas. *Diabetes* 29:57–63, 1980
20. Dicke JM, Henderson GI: Placental amino acid uptake in normal and complicated pregnancies. *Am J Med Sci* 295:223–227, 1988
21. Kuruvilla AG, D'Souza SW, Glazier JD, Mahendran D, Maresh MJ, Sibley C: Altered activity of the system A amino acid transporter in microvillous membrane vesicles from placentas of macrosomic babies born to diabetic women. *J Clin Invest* 94:689–695, 1994
22. White P: Pregnancy complicating diabetes. *Am J Med* 7:609–616, 1949
23. Marsál K, Persson P-H, Larsen T, Lilja H, Selbing A, Sultan B: Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr* 85:843–848, 1996
24. Illsley NP, Wang ZQ, Gray A, Sellers MC, Jacobs MM: Simultaneous preparation of paired, syncytial microvillous and basal membranes from human placenta. *Biochim Biophys Acta* 1029:218–226, 1990
25. Johansson M, Jansson T, Powell TL: Na^+/K^+-ATPase is distributed to microvillous and basal membrane of the syncytiotrophoblast in the human placenta. *Am J Physiol* 279:R287–R294, 2000
26. Schultz G, Jakobs K: Adenylate cyclase. In *Methods of Enzymatic Analysis*. 3rd ed. Bergmeyer H, Ed. Weinham, Germany, Verlag Chemie, 1984, p. 369–378

27. Bowers G, McComb R: A continous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Clin Chem* 12:70–89, 1966
28. Mahendran D, Donnai P, Glazier JD, D'Souza SW, Boyd RDH, Sibley CP: Amino acid (System A) transporter activity in microvillous membrane vesicles from the placentas of appropriate and small for gestational age babies. *Pediatr Res* 34:661–665, 1993
29. Jansson T, Scholtbach V, Powell TL: Placental transport of leucine and lysine is reduced in intrauterine growth restriction. *Pediatr Res* 44:532–537, 1998
30. Norberg S, Powell TL, Jansson T: Intrauterine growth restriction is associated with a reduced activity of placental taurine transporters. *Pediatr Res* 44:233–238, 1998
31. Jansson T, Ylvén K, Wennergren M, Powell TL: Glucose transport and system A activity in syncytiotrophoblast microvillous and basal membranes in intrauterine growth restriction. *Placenta*. In press
32. Eleno N, Devés R, Boyd CAR: Membrane potential dependence of the kinetics of cationic transport systems in human placenta. *J Physiol* 479:291–300, 1994
33. Bradford MM: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254, 1976
34. Powell TL, Lundquist C, Doughty IM, Glazier JD, Jansson T: Mechanisms of chloride transport across the syncytiotrophoblast basal membrane in the human placenta. *Placenta* 19:315–321, 1998
35. Ayuk PT, Sibley CP, Donnai P, D'Souza S, Glazier JD: Development and polarization of cationic amino acid transporters and regulators in the human placenta. *Am J Physiol Cell Physiol* 278:C1162–C1171, 2000
36. Jansson T: Amino acid transporters in the human placenta. *Pediatr Res* 49:141–147, 2001
37. Gumà A, Mora C, Santalucia T, Vinals F, Testar X, Palacin M, Zorzano A: System A transport activity is stimulated in skeletal muscle in response to diabetes. *FEBS Lett* 310:51–54, 1992
38. Samson M, Fehjman M, Dolais-Kitabgi J, Freychet P: Amino acid transport in isolated hepatocytes from streptozotocin-diabetic rats. *Diabetes* 29:996–1000, 1980
39. McGivan JD, Pastor-Anglada M: Regulatory and molecular aspects of mammalian amino acid transport. *Biochem J* 299:321–334, 1994
40. Karl PI, Alpy KL, Fischer SE: Amino acid transport by the cultured human placental trophoblast: effect of insulin on AIB transport. *Am J Physiol* 262:C834–C839, 1992
41. Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E, Endou H: Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD 98). *J Biol Chem* 273:23629–23632, 1998
42. Pineda M, Fernández E, Torrents D, Estévez R, López C, Camps M, Lloberas J, Zorzano A, Palacin M: Identification of a membrane protein, LAT-2, that coexpresses with 4F2 heavy chain, a L-type amino acid transport activity with broad specificity for small and large zwitterionic amino acids. *J Biol Chem* 274:19738–19744, 1999
43. Segawa H, Fukasawa Y, Miyamoto K, Takeda E, Endou H, Kanai Y: Identification and functional characterization of a Na^+-independent neutral amino acid transporter with broad substrate selectivity. *J Biol Chem* 274:19745–19751, 1999
44. Ramamoorthy D, Leibach FH, Mahesh VB, Ganapathy V: Modulation of the activity of amino acid transport system L by phobol esters and calmodulin antagonists in a human placental choriocarcinoma cell line. *Biochim Biophys Acta* 1136:181–188, 1992
45. Cetin I, Marconi AM, Baggiani AM, Buscaglia M, Pardi G, Fennessey PV, Battaglia FC: In vivo placental transport of glycine and leucine in human pregnancies. *Pediatr Res* 37:571–575, 1995
46. Furesz TC, Smith CH: Identification of two leucine-sensitive lysine transport activities in human placental basal membrane. *Placenta* 18:649–655, 1997