Hormonal regulation of glucose and system A amino acid transport in first trimester placental villous fragments

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ALTERATIONS IN PLACENTAL GLUCOSE transfer have been implicated in fetal growth abnormalities. In pregnancies complicated by diabetes, accelerated fetal growth has been suggested to be a direct consequence of maternal hyperglycemia. High maternal glucose levels increase placental glucose transfer, causing fetal hyperglycemia and increased fetal insulin secretion, which subsequently stimulates fetal growth (31). However, excessive fetal growth occurs in diabetic pregnancies despite rigorous glycemic control of the mother (3, 11). Glucose transporter 1 (GLUT1) is likely to be the primary glucose transporter protein isoform mediating glucose transport across the syncytiotrophoblast, the transporting epithelium of the human placenta (18, 20). The expression of GLUT1 and mediated glucose uptake have been shown to be increased in the basal plasma membrane of the syncytiotrophoblast of term placenta in insulin-dependent diabetes mellitus (IDDM) (10, 21). It is likely that this upregulation of the GLUT1 transporter contributes to accelerated fetal growth in diabetic pregnancies; however, the mechanisms of regulation are not well established. Furthermore, first-trimester glycosylated hemoglobin A1C (HbA1C) values in diabetic pregnancies have been identified as a strong predictor of accelerated fetal growth (32). This may suggest that fetal growth rates are determined already in early pregnancy and that important regulation of placental glucose transporters expression and activity takes place in the first trimester.

The expression of GLUT1 protein has previously been shown to be regulated by several different factors, including growth factors and insulin and glucose concentrations, in a number of tissues (4). Regulation of the placental glucose transporters have mainly been studied in cell cultures or in tissues isolated from term placentas. In a study of glucose transport in cultured cells of trophoblast origin isolated from first trimester placenta, insulin and insulin-like growth factors (IGF) I and II were found to stimulate glucose transport after 1 h of incubation (24). We (6) recently reported that 1-h incubation with insulin increased mediated glucose transport into primary villous fragments from first trimester placentas at 6–8 wk of gestation. These data suggest that placental glucose transport is hormonally regulated in the first trimester of pregnancy. Furthermore, glucose transport in cultured first-trimester trophoblast cells has been shown to be regulated by glucose concentrations after a few hours of incubation (12, 14).

Amino acids are potent stimulators of fetal insulin release (27), and system A is a key sodium-dependent transporter of neutral amino acids such as alanine, glutamine, and glycine (26). System A is more abundant in the microvillous membrane (MVM) than in the basal plasma membrane of the syncytiotrophoblast cell of human placenta (15, 23). The activity of system A has been shown to be reduced in the syncytiotrophoblast MVM in intrauterine growth restriction (5, 25). Moreover, in pregnancies complicated by IDDM or gestational diabetes mellitus, system A activity has been shown to be increased in the MVM (19). In a recent study (17), placental system A activity was shown to be upregulated by insulin and leptin at term. The placenta produces a number of hormones, and in some cases the actual hormone receptors are also

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expressed on the syncytiotrophoblast, suggesting an autocrine/paracrine function. For example, leptin is produced by the syncytiotrophoblast cell (9), and the receptor is expressed on its surface. These findings suggest a role of the placenta in hormonal regulation of nutrient transport, thereby affecting fetal growth.

We have previously studied short-term hormonal regulation of the system A transporter in villous fragments at term (17). In the present study, we used similar approaches to assess effects of hormones on system A activity in the first trimester. In addition, we investigated short-term hormonal regulation of placental glucose transport in term and first trimester villous fragments and tested the hypothesis that glucose transport is sensitive to regulation primarily in early pregnancy.

MATERIALS AND METHODS

**Tissue collection.** First trimester trophoblast tissue was collected at terminations performed at 6–13 wk postmenstruation. Full-term placentas were obtained immediately after vaginal delivery or caesarean section from women with uncomplicated pregnancies. Placentas were dissected, and the amniotic sac, chorionic plate, and decidua were removed. Villous tissue was collected from different parts of the placenta. Tissue was rinsed in physiological saline at room temperature (RT) and transported in DMEM (Sigma) diluted 1:3 in Tyrode buffer (in mM: 135 NaCl, 5 KCl, 1.8 CaCl$_2$, 1 MgCl$_2$, 10 HEPES, and 5.6 glucose; pH 7.4) to the laboratory (transport time of 30 min). Placental tissue was collected under conditions of informed consent, and all protocols were approved by the Committee for Research Ethics at Gothenburg University.

**Measurements of mediated glucose transport.** Individual villous fragments were used to assess glucose transporter activity according to techniques recently described and validated in detail for amino acid uptake (17). Villous tissues from first trimester and term placentas were further dissected into fragments ~2 mm in diameter and secured to one end of a silk suture, which in turn was attached to specially designed hooks constructed of a core of steel with a superficial layer of plastic in triplicate. Fragments were subsequently placed in fresh DMEM-Tyrode medium at RT. In experiments that assessed hormonal and substrate regulation, villous fragments were incubated in DMEM-Tyrode medium containing 300 ng/ml insulin, 500 ng/ml leptin, 340 ng/ml cortisol, 500 ng/ml GH, or 250 ng/ml IGF-I for 1 h at 37°C, and performing a 2-min wash in Tyrode with and without Na

**Uptake of [14C]-methylamino-isobutyric acid (MeAIB) into first-trimester villous fragments was carried out as previously described for term tissue (17), with a few modifications. An initial time course for first-trimester villous fragments was carried out by first attaching fragments to hooks, incubating fragments in DMEM-Tyrode medium for 1 h at 37°C, and performing a 2-min wash in Tyrode with and without Na

**System A activity.** Uptake of [14C]-methylamino-isobutyric acid (MeAIB) into first-trimester villous fragments was carried out by first attaching fragments to hooks, incubating fragments in DMEM-Tyrode medium for 1 h at 37°C, and performing a 2-min wash in Tyrode with and without Na

**Glucose transport.** Glucose transport was measured by uptake of 3-O-[methyl-14C]-D-glucose (20 Ci/ml) into DMEM-Tyrode buffer on ice and washed by 2 × 15-s agitation. The fragments were then lysed in distilled H$_2$O overnight and then transferred to 0.3 M NaOH overnight. Subsequently, scintillation fluid was added to the distilled H$_2$O, and released [14C]-glucose was counted in a 315-s-counter. Protein concentration was measured after denaturation in NaOH by using the Bradford assay (1). Subsequently, the uptake in Na

**System A activity.** System A activity was determined by subtracting the uptake in Na

**Electron microscopy.** Isolated primary villous fragments were processed identically to fragments used for measurements of mediated glucose transport and System A activity regarding temperature, incubation, and buffers. The villous fragments were fixed immediately after dissection (0 h) or after 1 or 2 h of incubation. Fixation and processing of the tissue for scanning electron microscopy and transmission electron microscopy were performed as previously described (17).

Data presentation and statistical analysis. Repeated measures ANOVA followed by Dunnett’s test was used to statistically evaluate differences between groups in the hormone studies. Linear regression and correlation were applied on time course data and in the assessment of changes in uptake between 6 and 13 wk of gestation. t-Test was used when comparing first trimester and term results on glucose uptake. Values are presented as means ± SE.

RESULTS

**Clinical data.** Gestational age and the number (n) of placentas from which villous tissue was used in the different groups are given in Table 1.

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**Table 1. Gestational age and number of placentas from which villous tissue was used in the different groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>GLUT</th>
<th>System A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean GA, wk</td>
</tr>
<tr>
<td>Time course, 1st trimester</td>
<td>20</td>
<td>38.84±0.19</td>
</tr>
<tr>
<td>Regulation study, term</td>
<td>20</td>
<td>9.69±0.36</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of placentas. GA, gestational age.

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Effects of hormones and glucose concentrations on glucose uptake. Villous fragments from term and first-trimester placentas were incubated with the hormones insulin, leptin, cortisol, GH, IGF-I, or prolactin (term fragments only) or under hypoglycemic or hyperglycemic conditions for 1 h. The response to hormonal stimulation was variable in first trimester, and no significant (ANOVA) short-term effects were observed on glucose uptake compared with control in first-trimester or term fragments (Fig. 1). We have previously demonstrated in a limited series of experiments that incubation of villous fragments from 6–8 wk of gestation in insulin for 1 h increased glucose uptake twofold (6). In the present study, the effect of hormones and glucose concentrations on glucose uptake in first trimester was therefore related to gestational age. Although this analysis did not result in any statistically significant correlations, there were clear trends toward a negative correlation between gestational age and percent change of glucose uptake after insulin, leptin, GH, and IGF-I incubation (data not shown).

Time dependence of MeAIB uptake in first trimester. Fragments isolated from first-trimester trophoblast tissue were incubated in [14C]-methylaminoisobutyric acid for 5, 10, 20, and 30 min (Fig. 2). Linear MeAIB uptake was observed up to 30 min. In subsequent experiments, we used the time point 20 min to measure Na⁺-dependent uptake of MeAIB, representing system A activity.

Effects of hormones on MeAIB acid uptake in first trimester. First-trimester fragments were incubated with 300 ng/ml insulin, 500 ng/ml leptin, 500 ng/ml cortisol, 500 ng/ml GH, or 250 ng/ml IGF-I for 1 h at 37°C. Furthermore, the fragments were incubated in [14C]-MeAIB for 20 min. GHs significantly down-regulated system A activity by 31% (P < 0.05, ANOVA; Fig. 3). Other hormones tested had no effect on Na⁺-dependent MeAIB uptake.

Gestational changes. The mediated uptake of 3-O-[methyl-14C]-D-glucose in nonstimulated (control) first-trimester (n = 14) and term fragments (n = 18) were compared (Fig. 4A). Term villous tissue had a 50% higher glucose uptake.
compared with first trimester ($P < 0.05$, t-test). The mediated glucose uptake in first-trimester nonstimulated control fragments ($n = 14$) was positively correlated to gestational age, whereas there was no significant relationship between system A activity and gestational age (data not shown). The Na$^+$-dependent transport of MeAIB in first trimester villous fragments (controls, $n = 15$) was 31% higher compared with previous measurements at term ($P < 0.05$).

**Fig. 2.** Time course of methylaminoisobutyric (MeAIB) acid uptake in villous fragments of first trimester placenta ($n = 5$). Uptake occurred in the presence (+Na$^+$) or absence (−Na$^+$) of sodium. System A activity corresponds to the Na$^+$-dependent uptake of MeAIB acid, which was linear between 0 and 30 min. $P < 0.05$.

**Fig. 3.** Isolated villous fragments from first-trimester placental tissue ($n = 11$) were incubated for 1 h at 37°C with the following effectors concentrations: 300 ng/ml insulin, 500 ng/ml leptin, 340 ng/ml cortisol, 500 ng/ml GH, or 250 ng/ml IGF-I. The uptake of Na$^+$-dependent MeAIB acid was measured after 20 min at 37°C. GH significantly reduced system A activity compared with control ($P < 0.05$).

**Fig. 4.** A: mediated uptake of 3-O-[methyl-14C]-d-glucose in villous fragments isolated from first-trimester ($n = 14$) and term placentas ($n = 18$, $P < 0.05$). B: Na$^+$-dependent uptake of MeAIB acid in villous fragments isolated from first-trimester ($n = 15$) and term placentas ($n = 14$; (17)).

**Electron microscopy.** In scanning electron microscopy, first-trimester placental villi had a slender appearance, with fewer branches compared with term. Proliferative zones and syncytial sprouts were abundant. The brush border of microvilli on the surface of the syncytium was rich and somewhat irregular in the pattern of cluster formation. The microvilli of larger stem villi appeared less slender and erect than those of intermediate villi. Syncytial sprouts exhibited an immature brush border that looked flatter. The specimens fixed at 0 h were well preserved, and ruptures of the MVMs were rare (Fig. 5A). An intact brush-border structure was also observed after 2 h of incubation, although the microvilli had a tendency to aggregate and seemed more bent (Fig. 5B). In transmission electron microscopy, sectioned villi revealed an intact MVM, a thick syncytium rich in intracellular vesicle content, and underlying cytotrophoblasts (Fig. 5C). The distribution and frequency of
cell organelles were similar after 2 h of incubation, although the cytoplasm of the syncytiotrophoblast appeared more condensed compared with 0 h (Fig. 5D).

DISCUSSION

We have studied the mediated uptake of glucose and MeAIB in primary villous fragments from human placenta, an experimental system that may have several potential advantages compared with cultures of transformed cells of trophoblast origin or isolated cytotrophoblasts. The cell characteristics are maintained, and polarization and cell-cell contact are likely to resemble the in vivo situation. Maintained ultrastructural integrity of term fragments after 1–3 h of incubation has been confirmed previously using electron microscopy and human placenta, and 17β-estradiol production has been shown to be stable (17). The electron microscopy data from first-trimester fragments in the present study indicate that the cellular morphology of fragments is well preserved for up to 2 h of incubation. Furthermore, other investigators have evaluated the use of similar models of human placental explants from first trimester and term villous fragments and demonstrated stable conditions up to 4 h, when assessing morphological, biochemical, and physiological parameters (34). The uptake of MeAIB acid by system A is Na⁺ dependent, which makes it unlikely that nonspecific MeAIB uptake contributes significantly to the measurements. The mediated uptake of methyl-D-glucose into intracellular space by facilitated GLUTs is effectively distinguished from nonspecific diffusion of glucose through GLUT inhibition by phloretin. Collectively, these data support the validity of first-trimester fragments for studies of nutrient uptake.

The human placental GH gene (hGH-V) is a variant of the pituitary GH gene (hGH-N), and placental GH is predominantly synthesized and secreted by the syncytiotrophoblast (22, 33). hGH-V has high somatogenic activity and low lactogenic activity. In a recently developed assay measuring hGH-V in maternal plasma, it was shown that hGH-V levels increased markedly between 20 and 30 wk of gestation and peaked around week 36 (37). Furthermore, hGH-V was present already at 7 wk of gestation, raising the possibility that GH may have a role in early placental development. Placental GH secretion has also been shown to be inhibited by glucose in villous explants and cultured trophoblast cells, which further suggests an important metabolic role for this hormone during pregnancy (30). In other tissues, GH has been demonstrated to regulate the transport of glucose and amino acids. In skeletal muscle and fibroblasts, GH was found to reduce basal glucose transport activity (2, 36). GHs have been shown to enhance amino acid uptake in the small intestine (16) and downregulate system A activity in the liver (29). In the present study, we found that GHs reduce system A activity in first trimester fragments. It may be speculated that GH is involved in a local homeostatic regulatory loop to adjust syncytiotrophoblast amino acid uptake in response to changes in glucose availability. For exam-
ple, hypoglycemia stimulates placental GH secretion (30), which in turn downregulates system A transporter activity. It has previously been shown that GH receptors are present in cell membranes of first-trimester and term placenta (7). The interaction between GH and its receptor is likely to occur on the microvillous side because placental GH is secreted only in the maternal circulation and is not detected in the fetal blood (8).

The effector concentrations used in our study were higher than physiological maternal and fetal plasma concentrations. However, high placental production and secretion of leptin and GH may result in substantially higher hormone concentrations locally in the placenta. Jansson et al. (17) recently showed that leptin and insulin increased system A-mediated amino acid uptake after 1-h incubation of term villous fragments. The present experiments extend the study of hormonal regulation of system A to early pregnancy. In marked contrast to term, leptin and insulin failed to stimulate system A activity in first-trimester and GH downregulated system A activity by 31%. Thus our findings suggest distinct gestational differences in hormonal regulation of amino acid transport by system A.

The Na\(^+\)-dependent MeAIB acid uptake in first trimester in this study was somewhat higher compared with uptake at term in the previous study. In contrast, villous fragment glucose uptake increased significantly from first trimester to term. Thus there appears to be a distinct difference in the gestational development of villous uptake between neutral amino acids and glucose. Glucose is the primary fuel for the rapidly growing baby in late pregnancy, and placental glucose transport therefore increases throughout gestation. The increase in glucose transport capacity is met in large part by increased uteroplacental blood flow, syncytiotrophoblast surface area, and glucose transporter density. The observed difference in glucose uptake capacity in our study between term and first-trimester villous fragments is likely due to the two latter factors. Moreover, gestational differences in expression of GLUT isoforms may be another possible factor to consider. Interestingly, GLUT3, GLUT4, and GLUT12 isoforms are highly expressed in first-trimester syncytiotrophoblast but not at term (6, 13, 28, 35).

In pregnancies complicated by IDDM, fetal overgrowth still constitutes a major clinical problem. Despite rigorous glycemic control of diabetic pregnant women, early pregnancy is often associated with increased maternal HbA1C values, indicating a control of diabetic pregnant women, early pregnancy is often constituted a major clinical problem. Despite rigorous glycemic at term (6, 13, 28, 35).

Interestingly, GLUT3, GLUT4, and GLUT12 isoforms are further expressed in human placental cells isolated from first trimester villous tissue, insulin and glucose have been shown to regulate glucose transport activity and the expression of GLUT1 mRNA (12). However, the effects demonstrated by Gordon et al. (12) were significant after several hours of incubation in insulin and glucose. We have previously shown an increase in glucose uptake due to insulin stimulation of villous fragments of 6–8 wk of gestation (6). The response to insulin in this extended study was variable and did not reach statistical significance. However, first-trimester trophoblast tissues used in this study were collected from placentas of 6–13 wk of gestation. We suggest that glucose transport is primarily sensitive to regulation by insulin after 1-h incubation before 8 wk of gestation. Compatible with this interpretation is the clear trend toward an inverse correlation between gestational age and percent change in glucose uptake after incubation of fragments in insulin. Furthermore, the presence of insulin-sensitive glucose transporter isoforms GLUT4 and GLUT12 may further support that insulin regulates glucose transport into the syncytiotrophoblast in early pregnancy. Long-term studies of the effects of hormones and varying glucose concentrations on glucose uptake in first-trimester placental tissue are needed.

GRANTS

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