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Full Length Research Paper

Expression of glucose transporters in placenta from macrosomic infants

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Maternal glucose status and placental transport are related to macrosomia in pregnancies complicated with diabetes mellitus. However, there is little information on the placental expression of glucose transporters in non-complicated pregnancies. The objective of this study was to compare the expression glucose transporters GLUT1 and GLUT3 in placenta from macrosomic and normal weight newborns. Information on maternal factors was obtained from clinical records. Protein expression of GLUT1 and GLUT3 glucose transporters was determined by immunohistochemistry in archived term placenta samples of singleton, non-diabetic pregnancies, from macrosomic (≥4000g) and normal weight newborns, matched for gestational age (±1week) and gender (n=25 pairs) for a case-control study. Macrosomia was more frequent in newborns from women with pregestational overweight (body mass index ≥25 kg/m2, p=0.046) or with glucose concentration ≥85 mg/dL (p=0.01). Placentas in the highest tertile of GLUT3 expression had 5 times the chance of being from macrosomic newborns compared to controls (p<0.05). This association remained significant after adjusting for other maternal factors. Protein expression of GLUT1 was not different between groups. Term placentas from macrosomic newborns from uncomplicated pregnancies show increased protein expression of GLUT3 but not GLUT1.

Keywords: Macrosomic, placenta, glucose, GLUT, birth weight

ABBREVIATIONS

BMI; Body mass index, DM; Diabetes mellitus, GDM; Gestational diabetes mellitus, IUGR; Intrauterine growth retardation

INTRODUCTION

Fetal macrosomia has been defined as a birth weight ≥ 4000 g, independently of gestational age (Boulet, Alexander et al., 2003), occurs in about 7% of pregnancies (Forsbach, Contreras-Soto et al., 1988; Gyurkovits, Kallo et al., 2011) and it is related to a great number of adverse pregnancy outcomes, such as obstetric complications and perinatal morbidity and mortality (Boulet, Alexander et al., 2003).

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Fetal growth depends upon numerous maternal, fetal and placental factors. Their interaction across gestation may alter fetal growth patterns (Sacks, 2004; Mayer and Joseph, 2013). Maternal pre-gestational body weight, body composition, pathologies such as diabetes mellitus (DM) and preeclampsia, as well as weight gain and glucose levels during pregnancy, have been strongly associated with birth weight (Leguizamon and von Stecher, 2003; Sanin Aguirre, Reza-Lopez et al., 2004; Retnakaran, Qi et al., 2009; Vambergue and Fajardy, 2011; Dubova, 2013); chromosomal anomalies and gender also influence fetal development (Mayer and Joseph, 2013). Placental size, morphology, hormones, uteroplacental flow, and particularly, placental nutrient transport, are closely related to fetal growth (Jones, Powell et al., 2007; Jansson, Myatt et al., 2009).

Glucose is the main energy source for the fetus and it is essential for normal growth (Sacks, 2004; Hay, 2006). Glucose is transported by facilitated diffusion and there are at least three glucose transporters, named GLUT1, GLUT3 and GLUT4, expressed in placental tissue (Desoye, Gauster et al., 2011). In humans, GLUT3 is highly expressed in early pregnancy and decreases as gestation progresses (Brown, Heller et al., 2011), GLUT1 expression is relatively constant across gestation in normal pregnancies (Hahn, Hartmann et al., 1995), whereas GLUT4 is believed to serve only for transporting glucose to be used by the placenta itself (Desoye, Gauster et al., 2011). Recently, gene expression of other glucose transporters in placental tissue has been reported, but little is known about their specific function across gestation (Novakovic, Gordon et al., 2013).

Maternal pathologies, such as preeclampsia and diabetes mellitus have shown to alter the expression of glucose transporters in human (Gaither, Quraishi et al., 1999; Dubova, 2013) and animal placentas (Boileau, Mrejen et al., 1995). However, it is still unclear whether or not the expression of glucose transporters is altered in placentas from non-complicated pregnancies giving birth to macrosomic infants. Therefore, the objective of the study was to compare the expression of GLUT1 and GLUT3 in placentas from macrosomic and normal weight newborns.

MATERIAL AND METHODS

We conducted a case-control study, including archived samples from previous studies (Rodriguez-Moran, Levario-Carrillo et al., 2007; Bravo-Cano, 2009). Placental samples from singleton macrosomic newborns (cases, n=25), with a birth weight ≥4000 g were compared with newborns with birth weight adequate for gestational age (controls, n=25), matching for gestational age (±1 week) and gender. Both, case and controls were obtained from the “Hospital of Ginecologia #15” of the “Instituto Mexicano del Seguro Social”, in Chihuahua, Mexico. Placentas from newborns with birth defects or born to women with diagnosis of hypo/hyperthyroidism, preeclampsia/eclampsia, or diabetes mellitus (DM) pregestational or gestational, were excluded. Information on maternal clinical characteristics, biochemical measures, and newborn anthropometry was obtained from research and clinical records.

Placenta sample collection and analysis

Placenta samples were obtained and analyzed as previously described (Acosta-Maldonado, Sanchez-Ramirez et al., 2009). Briefly, placentas were collected following the delivery and the maternal blood clot was removed. Placenta weight and diameters were measured and samples from the central area of placentas were dissected from macroscopically lesion-free site and were fixed in a 10% formol solution, embedded in paraffin, and stained with hematoxylin and eosin. Placenta samples were analyzed by immunohistochemistry according to the protocol previously described (González-García, Sánchez-Ramírez et al., 2008) with the following modifications: after blocking (PBS pH 7.4, containing 10% non-fat milk), slides were incubated for 1 hour at 37° C with the corresponding polyclonal goat anti-human GLUT1 (Santacruz Biotechnology, CA, catalog # sc-1605) or GLUT3 (Santacruz Biotechnology, CA, catalog # sc-7582) (1:750 dilution in PBS pH 7.4 containing 1 % non-fat milk). Slides were washed and exposed to the secondary (affinity-purified biotinylated rabbit anti-goat IgG) antibody for 1 hour at room temperature. The signal was showed using avidin-peroxidase and freshly prepared diaminobenzidine substrate. Stained slides were dehydrated for permanent coverslipping with entellan resin. All samples were analyzed on the same day. For immunolocalization, slides for each GLUT transporter were contrasted with hematoxilin (Zymed, San Francisco, CA) for 10 min before dehydration.

The level of GLUT1 and GLUT3 expression was obtained by optical density measured in a BX41 Olympus microscope equipped with a Pixera-CCD camera and analyzed with the IMAGE pro-plus 4.1 software (Media Cibernetics, Silver Spring, MA). Five representative microphotographs were taken from each placenta at 60X and six measures of optical density (in a perimeter of 5 µm each) were performed for each sample (González-García, Sánchez-Ramírez et al., 2008). Measures were made after calibrating the microscope, with an individual pixel resolution of 175 grey levels. All determinations were made the same day to diminish calibration or lighting errors. Samples used for image analysis were not contrasted to avoid background signal for hematoxilin.

Table 1. Maternal and clinical characteristics of macrosomic and control newborns

	Control (n=25)		Macrosomic (n=25)		
Variable	**Mean**	**SD**	**Mean**	**SD**	**p**
Age (years)	23.6	4.6	27.2	4.8	0.01
Pregestational body weight (kg) [a]	59.5	11.0	73.3	13.1	<0.01
Height (cm)	160.0	6.5	162.2	7.0	0.27
Pregestational body mass index (kg/m^2) [a]	23.2	3.8	27.8	4.3	<0.01
Gestational weight gain (kg)	12.5	4.7	9.7	3.4	0.05
Number of pregnancies	1.8	0.9	2.5	0.9	0.01
Education (years)	9.8	4.0	10.7	3.1	0.39
Hemoglobin (g/dl)[b]	12.2	1.3	12.2	1.2	0.88
Glucose (mg/dl)[c]	76.1	10.6	82.6	7.1	0.01

[a] Case n=18, control n=24
[b] Case n=25, control n=18
[c] Fasting glucose concentrations were obtained during the third trimester of pregnancy
SD: Standard deviation

Table 2. Characteristics of newborn and placentas

Variable	Control (n=25)		Macrosomic (n=25)		
	Mean	**SD**	**Mean**	**SD**	**P**
Gestational age (weeks)	39.4	1.0	39.7	0.7	0.31
Weight (g)	3293	360	4216	283	<0.01
Length (cm)	53.5	2.3	55.0	3.4	0.06
Placenta weight (g)	498	90.9	647.2	94.5	<0.01
Birth weight/placenta weight ratio	6.8	1.2	6.6	1.0	0.64
Placental expression of GLUT1 [a, b]	2.2	1.3	2.1	1.4	0.66
Placental expression of GLUT3 [b]	1.7	0.4	1.9	0.4	0.07

[a] Log-transformed for analysis, geometric mean presented
[b] Samples from the central area of placentas were dissected from macroscopically lesion-free site were analyzed by immunohistochemistry. The level of GLUT1 and GLUT3 expression was obtained by optical density. Five microphotographs were taken from each placenta at 60X and six measures of optical density (in a perimeter of 5 µm each) were performed for each sample. Data is expressed in arbitrary units
SD: Standard deviation

Statistical analyses

Data is presented as mean ± standard deviation or frequency and percentage, according to the measuring scale of the variables. Comparison between groups was performed by t-test or Wilcoxon rank sum test for quantitative variables and by χ^2 or Fisher exact tests for variables measured in nominal scale. Tertiles of protein expression of GLUT1, GLUT3, and concentrations of maternal glucose distribution were calculated and included as categories in bivariate and multivariate models. Age was also included as <25 and ≥25 years (above/below the median). Body mass index (BMI) was included as <25 and ≥25 kg/m^2, according with the cut off suggestive of overweight (WHO, 1997). Conditional logistic regression models were used to evaluate the relationship between macrosomia and selected variables. The Spearman correlation coefficient was calculated to establish the relationship between maternal glucose concentrations and glucose transporters protein expression. Statistical significance was declared when $p<0.05$. Stata v. 11.0 (Stata Corp.,College Station, TX) was used for statistical analyses.

RESULTS

Mothers from macrosomic newborns were older, with higher pregestational weight and BMI, as well as higher weight gain and blood glucose concentrations during pregnancy than those from the control group (Table 1). Macrosomic newborns were heavier and larger than controls and their placenta weight was also greater (Table 2).

Table 3. Relationship between macrosomia, maternal factors and protein expression of glucose transporters

Variable	Control (n=25)	Macrosomic (n=25)	OR	$CI_{95\%}$	P
Pregestational BMI (kg/m^2)[a]					
<25	12	4	1.0	1.0-13.1	0.046
≥25	5	13	3.7		
Age (years)					
<25	16	9	1.0	1.0-10.1	0.054
≥25	9	16	4.5		
Parity [b]					
Primiparous	10	4	1.0	0.8-18.8	0.080
Multiparous	14	20	4.0		
Glucose (mg/dL)					
<77	12	3	1.0		
77.0-84.9	8	9	6.8	0.8-56.7	0.075
85.0-99.9	5	13	20.3	1.8-223.6	0.014
GLUT1 [c]					
<1.90	10	8	1.0		
1.90-2.49	7	9	1.7	0.4-7.1	0.476
>2.5	8	8	1.2	0.4-3.7	0.733
GLUT3 [c]					
<1.58	11	5	1.0		
1.58-1.97	9	8	2.0	0.5-8.4	0.345
≥1.98	5	12	5.0	1.0-24.5	0.047

[a] n=17 pairs
[b] n=24 pairs
[c] Samples from the central area of placentas were dissected from macroscopically lesion-free site were analyzed by immunohistochemistry. The level of GLUT1 and GLUT3 expression was obtained by optical density. Five representative microphotographs were taken from each placenta at 60X and six measures of optical density (in a perimeter of 5 µm each) were performed for each sample. Data is expressed in arbitrary units. Categories correspond to tertiles of the distribution
OR: Odds ratio, obtained by conditional logistic regression (bivariate analysis)
BMI: Body mass index, calculated dividing the pregestational body weight (kg) by the squared of height (m^2), analysis based on 17 pairs.

Pregestational overweight, blood glucose, and GLUT3 protein expression in the upper tertile were associated with macrosomia (Table 3). The association between GLUT3 expression and macrosomia remained significant after adjusting for age, parity, and glucose concentrations (Table 4).

Glucose concentrations did not correlate to the abundance of glucose transporters in any of the study groups (GLUT1: in normal weight, rho=0.11, p=0.59; macrosomic, rho=-0.05, p=0.82; GLUT3: in normal weight rho=-0.04, p=0.86, macrosomic rho=0.16 p=0.44).

Table 4. Relationship between GLUT3 protein expression and macrosomia, adjusted for selected factors

Model	OR	CI 95%	P	Pseudo R^2
Model 1				0.44
GLUT3 [a]				
1.58-1.97	7.2	0.7-74.9	0.100	
≥1.98	33.0	1.8-593.5	0.018	
Parity [b]	12.2	1.1-132.5	0.039	
Model 2				0.49
GLUT3 [a]				
1.58-1.97	4.5	0.6-34.7	0.149	
≥1.98	17.3	1.0-285.8	0.046	
Glucose (mg/dl) [c]				
77.0-84.9	10.3	0.9-119.7	0.062	
85.0-99.0	70.2	2.2-2206.5	0.016	
Model 3 [d]				
GLUT3 [a]				
1.58-1.97	3.5	0.4-28.5	0.238	0.35
≥1.98	6.7	0.7-66.4	0.106	
BMI≥25.0 (kg/m^2) [e]	5.0	1.0-24.6	0.047	
Model 4				
GLUT3 [a]				
1.58-1.97	2.8	0.5-15.4	0.237	0.35
≥1.98	12.4	1.2-123.3	0.032	
Maternal age ≥25 years [f]	12.4	1.1-143.1	0.044	

[a] Placental protein expression of GLUT3. Values correspond to tertiles, Reference: GLUT3 <1.58 (tertile 1)
[b] Reference: primiparous women
[c] Maternal fasting glucose concentrations during the third trimester of pregnancy. Values correspond to tertiles. Reference: glucose <77.0 (tertile 1)
[d] n=17 pairs
[e] Maternal pregestational BMI. Reference: BMI<25.0 kg/m^2
[f] Reference: age<25 years (below the median), obtained from clinical records
OR: Odds ratio, by conditional logistic regression models

DISCUSSION

Our results show an increased protein expression of glucose transporter GLUT3 in placentas from macrosomic newborns. Placentas with greater abundance of GLUT3 had 5 times the chance of being from macrosomic newborns compared to controls. Expression of GLUT1 was not different between groups.

The relationship between glucose transporters in the human placenta and birth weight has been studied primarily in pregnancies complicated with intrauterine growth retardation (IUGR) or DM. In human placentas from IUGR pregnancies, GLUT3 was either undetected or found increased in the maternal aspect of the placenta, whereas protein expression of GLUT1 was not different from controls (Jansson, Wennergren et al., 1993; Janzen, Lei et al., 2013). GLUT3 expression has been found decreased in placentas from DM complicated pregnancies (Sciullo, Cardellini et al., 1997), whereas GLUT1 has been found unaltered or increased (Sciullo, Cardellini et al., 1997; Colomiere, Permezel et al., 2009). However, the increased levels of GLUT1 were found only in placentas from non-obese women with insulin-controlled gestational diabetes mellitus (GDM) compared with those from diet-controlled GDM and normoglycemic women (Colomiere, Permezel et al., 2009). In these studies, glucose transporter expression was not associated with higher birth weight, in contrast to our results in uncomplicated pregnancies in which higher protein expression of GLUT3 was more frequently found in placentas from macrosomic newborns than in controls.

Results from animal studies have shown a relationship between fetal weight and the expression of glucose transporters in restricted or hyperglycemic conditions. In rats, a restricted diet during the last week of pregnancy resulted in decreased fetal body weight and placental

GLUT3 protein, but not GLUT1 (Lesage, Hahn et al., 2002). However, in moderate hyperglycemic conditions, the expression of glucose transporters was unrelated to fetal growth. In this animal model, hyperglycaemia resulted in decreased GLUT1 mRNA levels, but unchanged GLUT3, in placentas from both macrosomic and normosomic rat pups (Cisse, Fajardy et al., 2013). This suggests that GLUT1 and GLUT3 protein expression may respond to different stimulus.

GLUT1 is decreased in conditions of glucose abundance, such as maternal hyperglycemia. mRNA and protein expression of GLUT1 are decreased at a glucose concentration of 20-25 mmol/L in cultured trophoblast cells from human term placentas (Hahn, Barth et al., 1998), values that can be reached in *in vivo* conditions by uncontrolled diabetic women, but above than those recently reported in women with GDM (Goldberg, Ye et al., 2012). Because all women included in our study were considered normoglycemic (i.e. fasting glucose <5.55 mmol/l) it is unlikely that they could have reached glucose concentrations to induce a decrease in GLUT1 expression.

GLUT3 has been found altered in both restricted and in hyperglycaemic maternal conditions. Thus we could speculate that GLUT3 mediates a bidirectional feto-placental glucose transport, possibly to protect the fetus from extreme alteration in maternal glycemia. In line with this, experiments in rats show that fetal glucose levels are maintained, despite maternal hypoglycaemia (Lesage, Hahn et al., 2002). Although the mechanisms are currently unclear, the high-glucose affinity of GLUT3 and its increased expression found in human placentas from IUGR complicated pregnancies (Janzen, Lei et al., 2013), suggest a role for this transporter in preserving fetal glucose levels in restricted conditions. On the other hand, both acute and chronic hyperglycaemic conditions in rats also result in increased placental GLUT3, which appear to be sensitive to environmental glucose concentrations (Boileau, Mrejen et al., 1995). Furthermore, in human and rabbit placentas from normal pregnancies, GLUT3 is asymmetrically expressed showing great abundance in endothelium, and predominantly around fetal vessels (Hauguel-de Mouzon, Challier et al., 1997; Khan, Kusakabe et al., 2011). Thus, based on histological patters, it is likely that GLUT3 could also mediate glucose back-flux from the fetal circulation to the placenta in order to maintain normal fetal glucose levels (Hahn, Blaschitz et al., 2001). However, it is still unclear whether or not GLUT3 is regulated by maternal, placental or fetal glucose concentrations.

Several factors such as gestational age, maternal pathologies, and hormonal changes may modulate the expression of placental glucose transporters. We included only placentas at term from non-complicated pregnancies, thus observed changes in GLUT3 are not likely attributed to maternal pathologies. However, whether similar results are observed at earlier gestational age is currently unknown. We obtained biochemical measures from clinical records, and were unable to include information on maternal hormone status. Obtaining information on insulin and adipokines could have provided further information on maternal factors.

The finding that both the glucose transporter expression and higher glucose levels were related with macrosomia in our study, suggests that these two conditions may independently contribute to fetal growth. This is supported by two observations: first, the possibility of having a macrosomic newborn was 20-fold higher in the upper tertile of glucose concentrations, even though all glucose values were in the normal range and none of the women were diagnosed with diabetes; and second, maternal glucose concentrations were uncorrelated to the protein abundance of GLUT3. This observation is consistent with other studies that found no correlation between GLUT1 and GLUT3 mRNA levels and fasting glucose or glycosylated haemoglobin in women with or without diabetes (Sciullo, Cardellini et al., 1997). Similarly, experiments in rats giving glucose treatments in early pregnancy (glucose injections) led to fetal overgrowth, without change in placental glucose transporters (GLUT1 and GLUT3) (Ericsson, Saljo et al., 2007). Recently, it has been reported that the 1-hour postprandial glucose peak relates to fetal growth in non-diabetic pregnancies (Parretti, Mecacci et al., 2001). However, maternal fasting glucose has been reported to be a better predictor of large-for-gestational-age newborn than glucose concentrations at 1 or 2 hours in glucose tolerance tests (Retnakaran, Qi et al., 2009). Although maternal glucose concentrations are recognized to strongly influence fetal growth, the role of fetal, placental, or maternal glucose concentrations at different stages of pregnancy, on modulating the expression of glucose transporters requires further studies.

Maternal age, pregestational BMI and parity were also greater in women who delivered a macrosomic infant. Similar to our results, macrosomia has been found to be more frequent as maternal age increases (Boulet, Alexander et al., 2003), and probably related to parity. Pregestational BMI is also strongly associated to macrosomia (Castro and Avina, 2002), a relationship that we confirmed in this study.

CONCLUSION

In conclusion, term placentas from macrosomic newborns from uncomplicated pregnancies show increased protein expression of GLUT3 but not GLUT1. The results of the study contribute to characterize glucose transporters GLUT1 and GLUT3 protein expression in placentas from non-diabetic pregnancies. Further research is warranted to identify specific mechanisms regulating fetal growth in macrosomia.

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