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Glucose transporters (GLUT 1, GLUT 3) mRNA in human placenta of diabetic and non-diabetic pregnancies

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Summary. - Glucose transporters (GLUT) catalyse the transport of glucose in many human tissues, including the placenta. On the other hand glucose concentrations can affect both glucose transport activity and level of GLUT mRNA and protein. Up to now very few studies, concerning GLUT in the placenta appeared and studies *in vivo* in human diabetic pregnancy are lacking. Therefore we investigated placental GLUT 1 and GLUT 3 mRNA in 10 diabetic (5 IDDM, 2 NIDDM, 3 GDM) and 9 non-diabetic women. GLUT 1 mRNA was found significantly correlated with maternal age (> 30 *vs* < 30 years: $p < 0.025$), with placental weight (> 575 *vs* < 575 g: $p < 0.05$), while GLUT 3 mRNA decreased significantly in late gestation of diabetic women (38-40 *vs* < 38 weeks: $p < 0.025$). In addition GLUT 3 was significantly lower in the diabetic than in non-diabetic women in late gestation. These preliminary results deserve to better elucidate feto-maternal carbohydrate metabolism at the placental level in normal as well as diabetic pregnancy.

Key words: glucose transporters (GLUT), GLUT 1 mRNA, GLUT 3 mRNA, placenta, diabetes, pregnancy.

Riassunto *(Trasportatori del glucosio (GLUT 1, GLUT 3) mRNA nella placenta delle donne diabetiche e di controllo).* - I trasportatori del glucosio (GLUT) servono per trasportare il glucosio nelle cellule di numerosi tessuti, tra cui la placenta. D'altro canto le concentrazioni del glucosio nel mezzo possono influenzare questa attività di trasporto modificando i GLUT mRNA e la proteina prodotta. Finora pochissimi studi sono apparsi sul GLUT placentare e studi *in vivo* nelle gravidanze complicate da diabete non sono ancora stati descritti. Sono stati studiati il GLUT 1 e GLUT 3 mRNA in 10 diabetiche (5 IDDM, 2 NIDDM, 3 GDM) e 9 donne di controllo. Il GLUT 1 mRNA è stato trovato correlato significativamente ($p < 0,025$) con l'età materna e con il peso della placenta ($p > 0,05$), mentre il GLUT 3 mRNA si riduceva significativamente ($p < 0,025$) nelle diabetiche nelle ultime settimane di gravidanza. Il GLUT 3 mRNA, inoltre, al termine della gravidanza era significativamente inferiore nelle diabetiche che nei controlli. Questi risultati preliminari sono utili per una migliore conoscenza del ricambio glicidico materno fetale a livello placentare nella gravida normale e in quella diabetica.

Parole chiave: trasportatori di glucosio (GLUT), GLUT 1 mRNA, GLUT 3 mRNA, placenta, diabete, gravidanza.

Introduction

Metabolic homeostasis during pregnancy undergoes significant changes to favour an advantageous environment for fetal growth and survival.

The placenta plays a central role in maintaining an adequate transport of maternal fuels to the foetus. Glucose is a primary substrate for fetal energy metabolism, and the placental transport constitutes the only supply for the fetus until late in the development [1].

In mammalian cells glucose transport is mediated by a class of proteins, the glucose transporters (GLUT), which exhibit specific tissue distribution and kinetic properties. Two glucose transporters isoform (GLUT 1 and GLUT 3) have been identified in homogenates of placental tissue [2, 3] and they might play a role in the altered glucose of pregnancies complicated by diabetes mellitus.

GLUT 1 protein and its mRNA are present in many tissues [4].

The wide distribution and kinetic properties of this facilitative glucose transporter suggest that it may mediate basal glucose uptake. This transporter functions as a unidirectional transporter under conditions where extracellular glucose is low and/or intracellular demand for glucose is hight [5].

Glucose exposure leads to a decrease in glucose transport, glucose transporter number and glucose transporter mRNA in cultured cells [6, 7] suggesting a down regulation of glucose transport across the plasma membrane by high glucose concentration in the medium.

GLUT 3 mRNA was initially reported to be present in many human tissues [8], subsequent studies detected GLUT 3 protein in several human tissues including placenta [9].

The high GLUT 3 protein expression in tissues such as brain, in which glucose is the main metabolic fuel,

and its kinetic properties suggest that this transporte could work in tandem with GLUT 1 to ensure an adeguate glucose support also under unfavourable conditions like high glucose demand or hypoglycaemia.

Previous studies investigated the localization of GLUT 1 and GLUT 3 proteins in human and animal placenta. Immunoistochemical data demonstrated that GLUT 1 is aboundant in apical and basal membranes of syncytotrophoblast [10-12] and in cytotrophoblast [10]. This arrangement provides the basis for the maintenance of glucose concentration in the fetal circulation at level that is only 1 mmol/l below maternal concentration, despite a very high rate of placental glucose consumption.

Because of its low K_m (1.8 mmol/l) GLUT 3 would be capable of scavenging maternal glucose for use by the placenta and fetus suggesting a key role in protecting fetus from maternal hypoglycemia [11].

GLUT 1 and GLUT 3 mRNA are aboundantly expressed in human and animal placenta [13]. Previous studies of human placenta cutured cells revealed that GLUT 1 mRNA and protein are downregulated by glucose concentrations [14], as well as in cultured rat conceptuses [13].

GLUT 1 mRNA displayed also an age-specific pattern of expression in human pregnancy [15] and in the rat uteroplacenta from the time of implantation through term [16]. These findings are in agreement with the idea that GLUT 1 functions as a constitutive, growth associated glucose transport which ensures that the growing placenta will get enough glucose from the maternal circulation to grow large enough to support the gestation.

The data about GLUT 3 mRNA age-specific pattern of expression are lacking in human placenta, but studies performed on rats demonstrated that the expression of GLUT 3 gene does not change during pregnancy [16].

Maternal diabetes during pregnancy is associated with placental morphological and functional abnormalities which are suspected to be related to poor fetal outcome [17].

As glucose is the main fuel in fetal metabolism several studies have investigated the effect of maternal diabetes on placental glucose uptake and metabolism. Moreover in human diabetes and in rat streptozotocin induced diabetes two- to tenfold increases in placental glycogen level were found during pregnancy [18].

The hyperglycemia of maternal diabetes alters materno-fetal glucose transfer kinetics [19] and this observation suggested that maternal diabetes can affect GLUT 1 and GLUT 3 gene expression in the placenta [13].

Subjects

Ten diabetic pregnant women and 9 non diabetic pregnant women were included in the study. They were recruited consecutively from the obstetrician clinic of the Policlinico Umberto I, in Rome.

None of the patients had kidney or liver diseases as evaluated by clinical and standard laboratory examinations.

Patients were treated either with diet or insulin and diet.

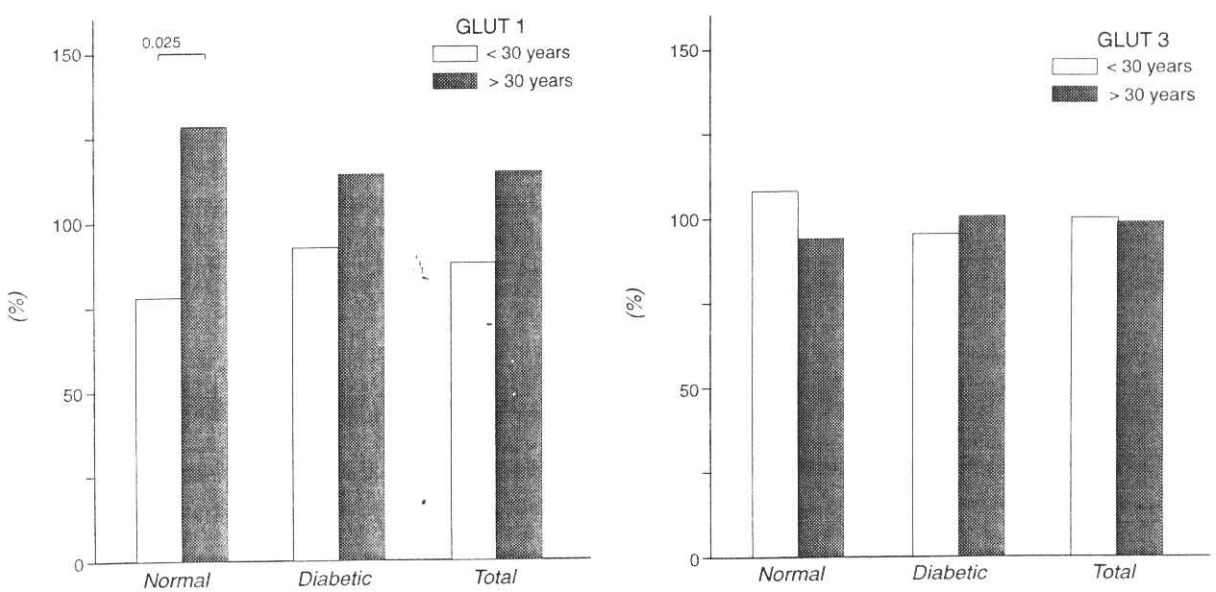

Fig. 1. - Placental glucose transporters mRNA and maternal age.

Materials and methods

RNA isolation

Placental tissue was homogenized in a denaturating solution (solution D). Sequentially a phenol-chloroformio extraction followed according to the Chomczynski-Sacchi method [20]. Quantity and purity of RNA was assessed by UV absorbance at 260 and 280 nm.

Northern blot

20 µl RNA per sample was denaturated and then separated by gel electrophoresis on 1% formaldeide agarose gels and finally transferred to nylon membranes (Hybond N.; Amersham). The fixation of the samples to the filter was performed by UV exposition.

Slot blot

20 µl RNA per sample were denaturated and sequentially slot blot under vacuum was performed followed by UV fixation.

The Northern blot and the slot blot filters were hybridized with radioactive probe GLUT 1 cDNA (vector pGEM 4Z) or probe GLUT 3 cDNA (vector pBluescript SK+) (kindly provided by Dr. G.I. Bell, Howard Hughes Medical Institute Research Laboratories, University of Chicago, Chicago, Illinois).

Subequentially, a new hybridization with beta actin probe was performed. Probe beta actin was kindly furnished by Dr. G. Pugliese (Policlinico Umberto I, II Clinica Medica, Roma).

All probes were random primed with the megaprime DNA labeling system, Amersham.

mRNA abundance was then quantitated by densitometry. The beta actin mRNA levels, evaluated by densitometry served as internal controls to standardize the amount of RNA loaded for each sample. The quantitative results of specific mRNA/beta actin ratios were transformed and expressed as a percent of control.

Results

Mean expression of GLUT 1 and GLUT 3 mRNA in diabetic and non-diabetic control women were similar. Among the diabetic patients GLUT 1 and GLUT 3 mRNA were lower in the GDM + type 2 than in type 1-IDDM women, although without significant difference.

No correlation between GLUT 1 and GLUT 3 transcript levels and both fasting blood glucose and glycosylated haemoglobin was found, showing that there is no influence of maternal metabolic control in diabetic women on the expression of these transporters.

GLUT 1 mRNA expression was found correlated to maternal age (> 30 *vs* < 30 years) although it was significantly ($p < 0.025$) only in control pregnant women (Fig. 1). No age related differences in GLUT 3 mRNA expression were observed in both normal and diabetic women (Fig. 1).

According to the time of delivery GLUT 1 and GLUT 3 mRNA levels were higher in diabetic women which delivered before 38 weeks (Fig. 2), however significant differences ($p < 0.01$) were observed only for GLUT 3. In addition, diabetic patients showed significantly lower GLUT 3 mRNA levels as compared to controls in late pregnancy (> 38 weeks) (Fig. 3). No significant differences were found in GLUT 1 transcript between diabetic and control women.

In the whole group of pregnant women (controls + diabetics) the placental weight (mean: 575 g) was inversely correlated to GLUT 1, but not to GLUT 3 mRNA (> 575 *vs* < 575 g) (Fig. 4).

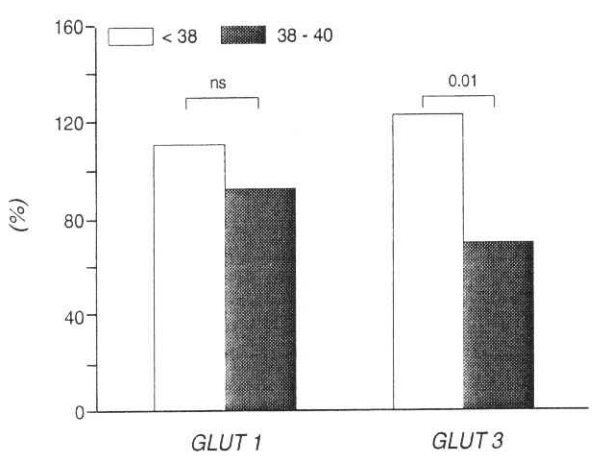

Fig. 2. - GLUT 1 and GLUT 3 mRNA before and after 38 weeks of delivery in diabetic patients.

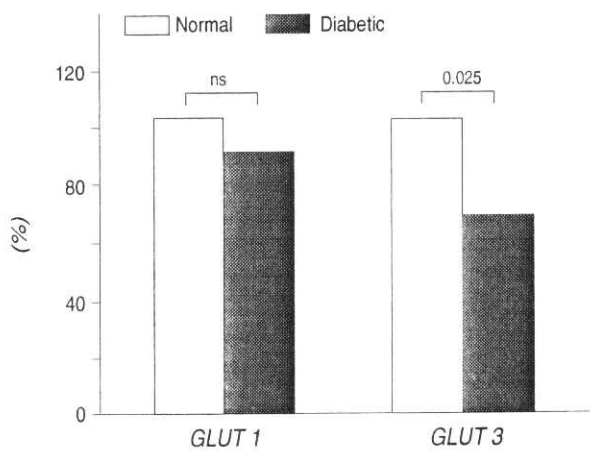

Fig. 3. - Placental GLUT 1 and GLUT 3 mRNA at 38-40 weeks of delivery.

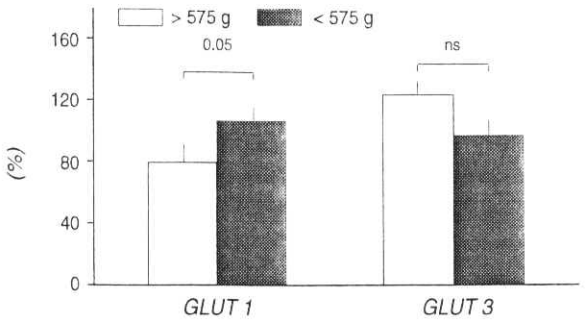

Fig. 4. - Correlation between glucose transporters mRNA and placental weight in controls + diabetics.

Finally neonatal macrosomia (LGA) was not correlated with both GLUT 1 and GLUT 3 expression in diabetic pregnancy, as well as no correlation between the birth weight and GLUT mRNA values was found in both diabetic and normal pregnancy.

Discussion

Freinkel and Metzger [21] indicated that the concentration of nutrient fuels in the maternal circulation is a key determinant of the composition of the fetal nutrient milieu throughout gestation. It was emphasized that, as in all tissue culture exercises, the developing cells may be greatly influenced by the nature of the incubation medium [22].

On the other hand it is well known that glucose is a primary substrate for fetal energy metabolism and in the absence of appreciable gluconeogenesis [13], placental transport constitutes the only supply for the fetus. Therefore the central role of both the placenta, and the glucose transport across the placenta by carrier-mediated facilitated diffusion clearly appear worthy of investigation. In fact the influence of maternal hyperglycemia, throughout pregnancy, on the fetus might be mediated by the placental structure and by presence and function of some specific glucose-transpoters (GLUT 1 and GLUT 3). In addition both insulin and glucose directly may modulate glucose transporter gene expression, as well as counter-regulator factors [3].

Our investigation, although limited for number of cases (diabetic and non-diabetic), is the first study performed *in vivo* in pregnant women with and without diabetes mellitus. The few cases, likely, do not allowed us to find significant differences between diabetic and non diabetic women in the GLUT 1 and GLUT 3 mRNA content, even if non insulin-dependent and gestational (NIDDM + GDM) diabetic women showed lower values of the two glucose-tramsporters. In addition no significant correlation was found between the metabolic control and the GLUT content, as well as no significant correlation has been found between the GLUT and feto-neonatal morbidity in diabetic pregnancy. On the other hand the GLUT 1 content was found correlated with the placental weight. A such observation is interesting in the light of the knowledge that the placental weight is generally increased in diabetic pregnancy. Moreover higher GLUT 1 values were found correlated with the age of the women. Would this observation be correlated with multiple pregnancies, gestational diabetes and glucose homeostasis? Interestingly GLUT 3 and GLUT 1 mRNA values in late pregnancy decreased in diabetic women. Up to now we cannot state if a same finding is observed in non-diabetic pregnant women, however at the end of gestation GLUT 3 values were lower in diabetic than in control women. A such condition in diabetic pregnancy could counterbalance the effect of hyperglycemia on the fetus.

The results are very preliminary, however they seem indicate for a further investigation to better elucidate fetomaternal correlation at the placental level in normal as well as in diabetic pregnancy, where a modified milieu may exert its effects, also by means of placental glucose transporters.

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