Aquaporins and Fetal Membranes From Diabetic Parturient Women: Expression Abnormalities and Regulation by Insulin

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Context: During pregnancy, aquaporins (AQPs) expressed in fetal membranes are essential for controlling the homeostasis of the amniotic volume, but their regulation by insulin was never explored in diabetic women.

Objective: The aim of our study was to investigate the involvement of AQPs 1, 3, 8, and 9 expressed in fetal membranes in diabetic parturient women and the control of their expression by insulin.

Design and Participants: From 129 fetal membranes in four populations (controls, type 1, type 2 [T2D], and gestational diabetes [GD]), we established an expression AQP profile. In a second step, the amnion was used to study the control of the expression and functions of AQPs 3 and 9 by insulin.

Main Outcomes and Measures: The expression of transcripts and proteins of AQPs was studied by quantitative RT-PCR and ELISA. We analyzed the regulation by insulin of the expression of AQPs 3 and 9 in the amnion. A tritiated glycerol test enabled us to measure the impact of insulin on the functional characteristics. Using an inhibitor of phosphatidylinositol 3-kinase, we analyzed the insulin intracellular signaling pathway.

Results: The expression of AQP3 protein was significantly weaker in groups T2D and GD. In non-diabetic fetal membranes, we showed for the amnion (but not for the chorion) a significant repression by insulin of the transcriptional expression of AQPs 3 and 9, which was blocked by a phosphatidylinositol 3-kinase inhibitor.

Conclusion: In fetal membranes, the repression of AQP3 protein expression and functions observed in vivo is allowed by the hyperinsulinism described in pregnant women with T2D or GD. (J Clin Endocrinol Metab 100: E1270–E1279, 2015)

Diabetes mellitus is one of the world’s most widespread chronic illnesses. The World Health Organization estimated that the total number of cases of adult diabetes is expected to continue rising and reach 439 million adults in 2030 (ie, 7.7% of the world’s population) (1). Pregnant women are concerned by two types of diabetes: gestational...
diabetes, which occurs during pregnancy, and pregestational diabetes (type 1 or 2 diabetes) present before pregnancy (2). Type 1 diabetes (T1D) arises from the specific destruction of the beta cells of the pancreatic islets of Lang erhans and is responsible for an absolute deficiency in insulin secretion and a strict insulin dependency (3). Type 2 diabetes (T2D) and gestational diabetes (GD) both arise from insulin resistance associated with abnormal insulin secretion (4). Polyhydramnios, defined by an excess of amniotic fluid, is a complication in diabetic parturient women. Diabetes is the cause of 25% of hydramnios cases. This complication occurs more frequently when blood glucose is badly controlled, and several physiopathological mechanisms have been proposed. Among these, fetal hyperglycemia responsible for polyuria through an osmotic effect is the one most commonly hypothesized. Diabetic children with macrosomia (defined by a weight > 4000 g at term gestation or > the 95th percentile for gestational age body mass index [BMI]) also have increased heart rate and plasma volume, which increases glomerular filtration rate and thereby urine production (5, 6).

The fetal membranes, composed of the amnion lining the amniotic cavity and the chorion facing the maternal decidua, are also involved in the physiology of the amniotic fluid (7). At the cellular and molecular levels, water flows through the membranes via different passive permeation transport systems such as diffusion facilitated by aquaporins (AQPs) (8). The AQPs are small membrane proteins (24–30 kDa) whose name comes from their ability to increase the water permeability of the membrane lipid bilayers. At present, the AQP family is known to have 13 members (AQP 0–12) (9). In fetal membranes, AQPs 1, 3, 8, 9, and 11 are expressed throughout pregnancy in both amnion and chorion and play a role in the intramembranal aqueous regulation of the amniotic fluid (10). AQPs 1 and 8 are pure AQPs (ie, water-specific). AQPs 3 and 9 are aquaglyceroporins, being permeable to both water and glycerol (9). AQP 11 belongs to a third group, the superaquaporins, for which no real transmembrane role is established (11). Most studies that have focused on the role of AQPs in polyhydramnios have done so in an idiopathic context. They have reported an increase in the expression of the AQPs usually found on these membranes at full term. The expression of AQPs 1 (12), 3, and 9 (13) increases in both amnion and chorion; that of AQP8 increases only in the amnion (14). For Mann and coworkers, the increased expression of AQP1 is seen as a mechanism designed to compensate for the increase in volume of the amniotic fluid, rather than causing it (12). Thus, mice knocked out for the AQP1 gene present polyhydramnios (15). A recent study including a small number of diabetic pregnant women (n = 13) launched work on the involvement of AQPs in fetal membranes (16), but nothing is known about regulation by insulin in these tissues. We know, however, that in other tissues, in particular adipose and hepatic tissues, insulin regulates the expression of aquaglyceroporins that this is deregulated under conditions of insulin resistance (17).

In this context, our study set out to delineate the involvement of AQPs in fetal membranes in diabetic parturient women and to study the role of insulin in the expression profiles observed.

Materials and Methods

Collection of fetal membranes

We recruited 129 pregnant women in the maternity wards of the Lille and Clermont-Ferrand hospitals to collect their fetal membranes at full term. The study was approved by the appropriate institutional review boards of both hospitals (PHRC DIAMANT A00534–49). The women included agreed to the use of the collected membranes. The recruitment was organized in four preset groups: a control group consisting of nondiabetic women (n = 36), a T1D group (n = 35), a T2D group (n = 17), and a GD group (n = 41). For all 129 women, the clinical and biological data were prospectively collected by manual chart review on obligatory items. T1D and T2D patients were already identified before pregnancy by using the criteria proposed by the World Health Organization and the American Diabetes Association. All the GD patients were included during the first trimester following the guidelines of the French Obstetrics and Gynaecology Society based on the International Association of Diabetes Pregnancy Study Group. GD was first managed by a diet. Insulin therapy was required when preprandial and postprandial glycemias remained more than 0.95 g/L and/or 1.20 g/L respectively. Dose of insulin was adjusted to reflect these glycemic targeted levels.

Culture of amnion and chorion explants in the presence of insulin and an inhibitor of phosphatidylinositol 3-kinase

Delivery products from nonpathological postcesarean full-term births (37–39 weeks of amenorrhea) were collected at the Clermont-Ferrand Teaching Hospital. The amnion and chorion were separated by peeling, and the 1-cm² fragments (cut with a surgical and sterile scalpel) of both tissues were randomly taken to obtain homogeneity in terms of used tissues (only excluding the cervix side). They were immediately (<30 minutes after delivery) cultured in 6-well plates in Dulbecco’s modified Eagle medium (ThermoFisher Scientific). This culture was carried out in the presence and absence of insulin (Sigma-Aldrich) at a classically used concentration of 10 or 100 nM during 24 or 48 hours. For the assays with an inhibitor of phosphatidylinositol 3-kinase (PI3K), the culture was carried out in the presence and absence of insulin at 100 nM and an inhibitor of PI3K, LY294002 (Sigma-Aldrich), at 100 μmol/L during 48 hours. For each of the 10 membranes, all the induction experiments were conducted in triplicate.
Tritiated glycerol permeability test

Amnion explants from nondiabetic fetal membranes were cultured as previously described with and without insulin (100 nM) for 48 and 72 hours. The explants were exposed for 5 minutes with/without 0.3 mM HgCl2 purchased from Sigma-Aldrich (to block permeases). Explants were incubated with 1 /Ci of [2-3H]-glycerol (American Radiolabeled Chemicals) or 1.5 M glycerol for 5 minutes. Amnion explants were lyzed overnight in 0.3 N NaOH. Radioactivity was visualized by adding 5 mL of scintillation solution (Filtron-X). In parallel, proteins of control samples were quantified (Vista Siemens, according to the manufacturer’s protocol). Results were expressed as [2-3H]-glycerol (dpm)/g of proteins/min for the corresponding vehicle conditions.

Extraction of total RNA and qualitative and quantitative RT-PCR

Total RNA was extracted from 129 membranes using an RNeasyMini Kit (QIAGEN). The primer sequences used (Table 1) were previously described (10).

From these 129 membranes, the gene expression profile of the 13 AQPs, the insulin receptor, and the two main substrates IRS1 and IRS2 was determined by classical PCR. For each PCR, the expression of a housekeeping gene (RPLP0) was found. The products of amplification were sequenced to verify the amplification specific to each primer pair.

Quantitative RT-PCR reactions for AQPs 1, 3, 8, and 9 transcripts were quantified independently three times in two independent experiments using the geometric mean of three housekeeping genes (RPLP0, 18S rRNA, and β-actin). All of these steps followed the Minimum Information for Publication of Quantitative Real-Time PCR Experiment guidelines (18).

Extraction of protein, assay of total proteins, and ELISA assay of AQPs 3 and 9

Total proteins in fetal membranes and in amnion and chorion explants were extracted using a Membrane Protein Extraction Kit from BioVision. The assay of total proteins in our tissue extracts was carried out using a Vista analyzer (Siemens). The level of protein expression of the AQPs 3 and 9 was measured by the ELISA method on extracts of total proteins from the various tissue explants using USCNK kits following the manufacturer’s recommendations. The concentrations of the transcripts of the AQPs 3 and 9 of each sample were normalized against total protein concentrations.

Study of the intracellular insulin signaling pathway

The proteins of the amnion explants (obtained from nondiabetic fetal membranes) cultured for 1 hour in the presence and absence of insulin and LY294002 were extracted to study the phosphorylation

### Table 1. Specific Primers Used for PCR (Quantitative and Qualitative)

<table>
<thead>
<tr>
<th>Human Gene</th>
<th>Sequence (5’-3’)</th>
<th>Product Size (pb)</th>
<th>GenBank No.</th>
</tr>
</thead>
<tbody>
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<td>AQP0-S</td>
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<td>196</td>
<td>NM_012064.3</td>
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<tr>
<td>AQP0-AS</td>
<td>TAAATCATCCTCAGAGATGTG</td>
<td>224</td>
<td>NM_198098.2</td>
</tr>
<tr>
<td>AQP1-S</td>
<td>ATCACCTCCTCCTGACT</td>
<td>224</td>
<td>NM_001486.5</td>
</tr>
<tr>
<td>AQP1-AS</td>
<td>CAGCCAGTGTTAGCTAATGC</td>
<td>189</td>
<td>NM_001492.5</td>
</tr>
<tr>
<td>AQP2-S</td>
<td>CTCCTCCTCATAGCTTCTCT</td>
<td>151</td>
<td>NM_001650.4</td>
</tr>
<tr>
<td>AQP2-AS</td>
<td>ACAAGTGTTTTGGCCTTATTT</td>
<td>159</td>
<td>NM_001651.3</td>
</tr>
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<td>AQP3-S</td>
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<td>175</td>
<td>NM_001170.1</td>
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<tr>
<td>AQP5-S</td>
<td>CTAGCTCCCATCCTCCTCCTC</td>
<td>223</td>
<td>NM_001730.2</td>
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<tr>
<td>AQP5-AS</td>
<td>CCAGGTCAGAGACACACACCAG</td>
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<td>NM_173039.2</td>
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<td>AQP8-S</td>
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<td>NM_005544.2</td>
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<td>AQP9-S</td>
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<td>AQP11-S</td>
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<td>AQP12-S</td>
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</table>

Abbreviations: IR, insulin receptor; IRS, insulin receptor substrate.
profile of six proteins of the insulin signaling pathway: the kinase B protein (AKT), the S6 kinase protein (p70S6K), the glycogen synthase kinases 3α and β (GSK3α and GSK3β), the insulin-like growth factor 1 (IGF1) receptor, and the tuberous sclerosis complex protein 2 (TSC2). MILLIPLEX MAP kits (Merck KGaA), based on the Luminex XMAP technology were designed to target the well-known and published phosphorylation residues of each protein cited previously and were used according to the manufacturer’s recommendations.

Statistics

Statistical analysis was performed using Stata software, version 13 (StataCorp). The tests were two-sided, with a type I error set at α = 0.05. Quantitative data were presented as the mean ± standard deviation or the median [interquartile range] according to the statistic. Comparisons among independent groups were made using the test of Kruskal-Wallis followed by Dunn’s test because criteria for performing ANOVA were not met (normality by the Shapiro-Wilk test and homoscedasticity using the Bartlett test). A multivariate linear regression model was proposed to consider adjustment of covariables fixed according to univariate results and clinical relevance: BMI and age of the mother. Comparisons between independent groups concerning categorical data (% obese, macrosomic babies) were performed using the χ² test or Fisher’s exact test, followed when appropriate by the Marascuilo procedure. The nonparametric Mann-Whitney test was used to compare the expression of RNA or proteins (AQPs 3 and 9) in different groups (without insulin versus with insulin in the GD group, BMI < 30 kg/m² versus BMI > 30). When P < .05, subgroup analyses were explored.

Results

Clinical data linked to collection of fetal membranes

The main clinical data are noted in Table 2. All the T2D patients were treated by insulin. Concerning the GD group, 22 women were treated by insulin and nutritional recommendations and 19 by nutritional recommendations only. The mothers in the T2D and GD groups were significantly older than those in the nondiabetic and T1D groups. A significantly higher BMI was found in the T2D group than in the control and T1D groups. Likewise, the proportion of obese mothers (BMI > 30 kg/m²) was significantly greater in group T2D than in the other three groups, and greater in the GD group than in the control and T1D groups.

Differential expression of AQPs in fetal membranes of diabetic women (T1D, T2D, and GD)

The expression profile of the messenger RNA (mRNA) for the 13 AQPs in the 129 fetal membranes was established by classical RT-PCR to identify any qualitative modification (“on-off effect”). No qualitative modifications in expression were found. Indeed, the AQPs expressed in the membranes were AQPs 1, 3, 8, 9, and 11 irrespective of the group (control, T1D, T2D, and GD). No significant differences were observed between the RNA expression of AQPs 1 and 8 in the diabetic groups and

Table 2. Clinical Data for the Collected Fetal Membranes

<table>
<thead>
<tr>
<th>Clinical data related to pregnancy</th>
<th>Control Group</th>
<th>T1D Group</th>
<th>T2D Group</th>
<th>GD Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average term of pregnancy in weeks of amenorrhea (sd)</td>
<td>39.5 (1.1)</td>
<td>37.4 (1.2)</td>
<td>37.6 (0.9)</td>
<td>39.1 (1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Average weight of the baby at birth in grams (sd)</td>
<td>3379 (384)</td>
<td>3580 (534)</td>
<td>3718 (744)</td>
<td>3371 (530)</td>
<td>NS</td>
</tr>
<tr>
<td>Percentage of macrosomic babies (number)</td>
<td>8% (3)</td>
<td>23% (8)</td>
<td>29% (5)</td>
<td>12% (5)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical data related to mothers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in years (sd)</td>
<td>28.4 (4.8)</td>
<td>29.3 (5)</td>
<td>33.5 (5.3)</td>
<td>32.6 (4.7)</td>
<td>T2D versus control&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean BMI of mother in kg/m² (sd)</td>
<td>21.9 (3.2)</td>
<td>24.3 (4)</td>
<td>33.7 (6.3)</td>
<td>28.1 (6.5)</td>
<td>T2D versus control&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percentage of obese mothers (number)</td>
<td>3% (1)</td>
<td>9% (3)</td>
<td>82% (14)</td>
<td>37% (15)</td>
<td>T2D versus control&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Macroesmia is defined by a weight more than 4000 g at term gestation or more than the 95th percentile for gestational age.

Abbreviations: NS, nonsignificant; NA, not applicable.

<sup>a</sup> P < .05.
that of controls (Figure 1, A and B). For AQP 3, we observed a 34% decrease in RNA expression in the T1D group relative to controls and statistically significant decreases of 62% for the T2D group and 56% for the GD group (Figure 1C). For AQP3, we found a 33% decreased protein expression relative to controls in the T1D group and statistically significant decreases of 58% for the T2D group and 60% for the GD group (Figure 1D). For AQP9, we observed a 25% decrease in RNA expression for the T1D group relative to controls, a decrease of 67% for the T2D group, and a statistically significant decrease of 98% for the GD group (Figure 1E). No significant difference relative to controls was found for the protein expression of AQP9 (Figure 1F).

In the GD group (n = 41), we compared the expression of AQP3s 3 and 9 in fetal membranes in two subgroups: one in which the mothers were treated solely by diet (n = 22) and one in which they were treated by diet plus insulin (n = 19). A statistically significant decrease in the expression of AQP3s 3 and 9 was observed in the subgroup “with insulin” for both RNA (Figure 2, A and C) and protein (Figure 2,
B and D). The level of expression of AQPs 3 and 9 was also tested in two other subgroups: one in which the mothers were not obese (BMI < 30 kg/m², n = 96) and one in which they were (BMI > 30 kg/m², n = 33). A statistically significant reduction in the expression of AQPs 3 and 9 was present in the subgroup “BMI more than 30” for both RNA (Figure 2, E and G) and protein (Figure 2, F and H).

Study of the effect of insulin on the expression and function of the genes for AQPs 3 and 9 in nondiabetic fetal membranes

After culturing in the presence and absence of insulin (at 10 or 100 nM) at different times (24 and 48 hours), we observed no effect of insulin in the chorion on the RNA or protein expression of the AQP3 and AQP9 genes, whether concentration-dependent or time-dependent (results not shown). For the amnion, comparing the RNA expression of the AQP3 gene for each incubation time between the untreated explants and those treated with insulin, we observed a 25% lower expression at 24 hours and a statistically significant decrease of 54% at 48 hours on treatment with 10 nM insulin. For the explants treated with 100 nM insulin, the differences were significant after 24 hours (51% lower) and 48 hours (48% lower) (Figure 3A). For the protein expression of AQP3, the difference was significant after 48 hours of treatment with 100 nM insulin (a decrease of 36%) (Figure 3B). For the amnion, comparing the expression of the transcripts of AQP9 for each incubation time between the untreated explants and those treated with insulin, we observed a 48% lower expression at 48 hours on treatment with 10 nM insulin (Figure 3C). For explants treated with 100 nM insulin, the differences were significant after 24 hours (a decrease of 32%) and 48 hours (a decrease of 78%) (Figure 3C). For the protein expression of AQP9, the difference was significant after 48 hours of treatment with 100 nM insulin (a decrease of 78%) (Figure 3D). After 48 and 72 hours of amnion explant culture in the presence and absence of insulin at 100 nM, the transfer of tritiated glycerol (mediated by AQPs) was statistically lowered (Figure 3E). This effect of insulin was no
longer observed in the presence of 0.3 mM HgCl₂, which inhibits the transmembrane transfer of glycerol in the amnion in the presence or absence of insulin (Figure 3E).

In the presence of LY294002—an inhibitor of PI3K (involved in the intracellular transmission of the insulin pathway)—the decrease in the RNA expression of AQP3 in the amnion after 48 hours’ culture in the presence of 100 nM insulin was no longer found (Figure 3F). In addition, LY294002 led to an insulin-independent, statistically significant 3.4-fold increase in the expression of AQP3 transcripts (Figure 3F). Likewise, in the presence of LY294002, the decrease in the expression of AQP9 RNA after 48 hours culture in the presence of 100 nM insulin was absent (Figure 3G). Similarly, LY294002 brought about an insulin-independent, statistically significant 2.1-fold increase in the expression of AQP9 RNA (Figure 3G).

Study of the insulin signaling pathway in the nondiabetic amnion

The expression of mRNA of the insulin receptor and its 2 main substrates (IRS1 and IRS2) was found in both amnion and chorion by RT-PCR (results not presented).

For AKT, we observed a statistically significant 3.8-fold increase in the phosphorylation of serine 473 in the presence of insulin that was not observed in the presence of LY294002. In addition, LY294002 brought about an insulin-independent, statistically significant decrease in the phosphorylation of serine 9 in 94% (Figure 4A). For GSK3β, we found a statistically significant 2-fold increase in the phosphorylation of serine 473 of 94% (Figure 4A). For GSK3β, we found a statistically significant 2-fold increase in the phosphorylation of serine 9 in the presence of insulin that was not observed in the presence of LY294002. Also, LY294002 caused an insulin-independent statistically significant decrease in the phosphorylation...
of serine 9 of 77% (Figure 4B). For GSK3β, we found a statistically significant 2.3-fold increase in the phosphorylation of serine 21 in the presence of insulin that was not observed in the presence of LY294002, which led to a statistically significant decrease in the phosphorylation of serine 21 of 66% (Figure 4C). For p70S6K, the phosphorylation of threonine 412 was significantly increased 2.1-fold in the presence of insulin, this effect being inhibited in the presence of LY294002, which also brought about an insulin-independent, statistically significant 56% decrease in the phosphorylation of threonine 412 (Figure 4D). For TSC2, we observed a statistically significant 1.9-fold increase in the phosphorylation of serine 939 in the presence of insulin that was not found in the presence of LY294002, which led to a statistically significant decrease in the phosphorylation of serine 939 of 67% (Figure 4E). For the IGF1 receptor, insulin caused a statistically significant 28-fold increase in the phosphorylation of tyrosines 1135 and 1136, independent of LY294002 (Figure 4F).

**Discussion**

The amniotic fluid plays an essential role during pregnancy, and the normalcy of its volume is a good indicator of fetal well-being. Despite its fine regulation, volume anomalies may occur (oligoamnios or polyhydramnios) that are closely associated with increased fetal morbidity and mortality (5). The AQP s are known to play a major role in the regulation of water homeostasis in the body and to participate in the regulation of the amniotic volume (19). An expression of AQP s 1, 3, 8, and 9 in chorio-amniotic membranes has already been reported in healthy pregnancy (10); an increase in their expression has been observed in idiopathic polyhydramnios (12–14). In addition, polyhydramnios is known to frequently be associated with diabetes (5, 6). On the other hand, the role of the fetal membranes via the AQP s in regulating the amniotic volume in diabetic pregnant women has never been closely investigated, except for one recent study on a small sample, which did not allow for the different types of diabetes encountered during pregnancy, links with certain clinical or therapeutic data, or molecular and cellular studies of insulin involvement in the amnion (16). In our work, we have for the first time established in parallel an expression profile of the AQP genes in the fetal membranes from pregnant diabetic women (T1D, T2D, and GD), and demonstrated the role of insulin in the expression and function of aquaglyceroporins in these membranes. For this purpose, 129 fetal membranes were col-
lected from mothers in four groups: controls, T1D, T2D, and GD. That the mothers were significantly older and more obese in the T2D and GD groups (Table 2) is echoed in the literature (4), which tends to validate our cohort.

The lowered expression of AQP3 and AQP9 genes in the fetal membranes from women with T2D or GD raises the question of insulin involvement. Insulin resistance and hyperinsulinism are physiopathological characteristics common to T2D and GD (20, 21). The repressive role of insulin on the expression of certain aquaglyceroporins has already been shown in essential tissues with glucidic metabolism (17, 22, 23). This regulation culminates in an inactivation of the Forkhead box a2 transcription factor, which is bound normally on an insulin response element on the promoter of the genes for AQP3 (24) and AQP9 (25). AQP9 has also been described as being repressed by insulin in the placenta, although its functional capacities do not seem to be impacted (26). In addition to physiopathological and bibliographical arguments in favor of a role of insulin in the decreased expression of AQP3 and 9 transcripts in the fetal membranes from women in the T2D and GD groups, we have obtained other results from our cohort of 129 membranes that support our hypothesis. In the GD group, we found a significantly decreased RNA and protein expression of the genes for AQP3 and 9 in the subgroup treated with diet plus insulin compared with the one treated with diet alone. In addition, we observed a significantly decreased RNA and protein expression of the genes for AQP3 and 9 in the subgroup of fetal membranes from obese women compared with that of fetal membranes from nonobese women; this clearly establishes that perturbations of the insulin pathway occur in obesity (20). Interestingly, a decrease of AQP9 transcripts for T2D and GD was not found for the AQP9 protein expression in any of the groups. The difference may be due to different posttranslational mechanisms between AQP3 and AQP9 and/or that these two AQPs apparently present an inverted ratio of “cytoplasm/cell membrane” protein expression, with most showing membrane-localized AQP3 and a strongly cytosolic AQP9 (unpublished results from Cheung’s group). According to these data and explanations, the decreased activity of AQP-mediated transfer of tritiated glycerol in amnion explants with insulin might account for the repression of AQP3 protein expression only.

Nevertheless, the transcriptional regulation of the expression of AQP3 and 9 by insulin is established by our work on cultures of isolated amnion explants. This a tissue-selective response because the AQPs were not transcriptionally regulated in the chorion (the second component of fetal membranes) despite it presenting the same molecular actors in terms of the insulin pathway. Although Zhang et al (19) hypothesized a repression of AQP3 and 9 by insulin in fetal membranes by the extrapolation of data previously reported for other tissues, we demonstrate it here for the first time for both expression and function. Although the expression of the insulin receptor was shown to be weak in earlier work (25), its ability to activate the mechanisms of intracellular phosphorylation is present. In the presence of insulin, we found an increase in the phosphorylation of AKT proteins on serine 473 and GSK3β on serine 9, GSK3α on serine 21, p70S6K on threonine 412, and TSC2 on serine 939. For these five proteins, this insulin effect disappeared in the presence of a PI3K inhibitor. Current knowledge concerning the signaling pathway of the insulin and IGF1 receptors can explain these results (27). This PI3K/AKT regulation pathway seems important for aquaglyceroporins AQP3, 7, and 9 because it has already been found in adipocytes, cutaneous fibroblasts, and an ovarian cell line, even though it is the culmination of other cascades initiated by other membrane receptors such as epidermal growth factor for AQP3 (16). An impairment of this intracellular phosphorylation pathway is described in a neighboring tissue, the placenta, to explain a deregulated expression of AQP9 in preeclampsia (28).

The results of our in vitro experiments support our hypothesis of a repressed transcriptional expression of AQP3 and 9 by insulin in fetal membranes. This effect is especially visible in the context of T2D or GD associated with hyperinsulinemia. We believe these membranes from T2D and GD pregnancies are insulin-sensitive, given their hybrid nature, being of both maternal and paternal origin. In addition, in our study, the RNA expression profile of AQP3 and 9 shows their inhibition in this context of hyperinsulinemia, whereas the literature describes an elevation of levels in insulin-resistant tissues (17). These fetal membranes thus appear as collateral victims that are sensitive to ambient hyperinsulinism and modulate the expression of target genes for their AQPs. A similar observation was made in the placenta of obese women where overexpression of the AKT/mammalian target of rapamycin pathway was found (29). We thus advance the hypothesis that through a strong insulin impregnation, aquaglyceroporins are repressed in the amnion, so contributing to the retention of amniotic fluid. This retention, added to fetal polyuria, may contribute to maintaining polyhydramnios. The AQP-insulin links in the fetal membranes therefore provide a new physiopathological explanation (further to others already described in the fetus, mother, and placenta) that may account for the anomalies in the homeostasis of the amniotic fluid during pregnancies in diabetic women.
Acknowledgments

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This work was supported by a research grant from Fondation Coeur et Artère, F-59 000, Lille, France (www.fondacoeur.com).

Disclosure Summary: The authors have nothing to disclose.

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