Insight Into the Pathogenesis of Fetal Growth Restriction in Placental Malaria: Decreased Placental Glucose Transporter Isoform 1 Expression

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Placental malaria, especially when complicated with intervillitis, can cause fetal growth restriction. Transplacental glucose transport by glucose transporter isoform 1 (GLUT-1) on the syncytiotrophoblast microvillous and basal plasma membranes regulates fetal growth. We found that GLUT-1 expression in the microvillous plasma membrane of Plasmodium falciparum–negative placenta biopsy specimens was comparable to that in P. falciparum–positive placenta biopsy specimens with or without intervillitis, whereas GLUT-1 expression in the basal plasma membrane was lowest in P. falciparum–positive placenta biopsy specimens with intervillitis, compared with the other 2 specimen types (P ≤ .0016). GLUT-1 expression in the basal plasma membrane also correlated negatively with monocyte infiltrate density (r = −0.43; P = .003) and positively with birth weight (r = 0.28; P = .06). These findings suggest that intervillitis, more than placental malaria per se, might cause fetal growth restriction, through impaired transplacental glucose transport.

Keywords. Plasmodium falciparum; intervillitis; fetal growth restriction; GLUT-1; syncytiotrophoblast; glucose transport; monocytes; pregnancy; malaria.

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Placental malaria can lead to fetal growth restriction and low birth weight, particularly when associated with intervillitis [1]. Little is known about the pathogenesis of placental malaria–associated fetal growth restriction, but impaired transport of oxygen or nutrients has been suggested [2]. While there is no evidence of placental hypoxia in placental malaria [3], we recently showed decreased amino acid transport in placental malaria, especially with intervillitis [4], and transport of other nutrients might also be altered.

An essential determinant of fetal growth is transplacental transport of maternal glucose across the syncytiotrophoblast, the transporting epithelium, and main barrier to maternal-fetal exchange to the fetal capillaries [5]. Transplacental glucose transport is primarily mediated by glucose transporter isoform 1 (GLUT-1) expressed in the microvillous (maternal facing) and basal (fetal facing) plasma membranes of the syncytiotrophoblast. The microvillous plasma membrane has a glucose transport capacity estimated to be 20 times that of the basal plasma membrane, owing to a larger surface area and higher GLUT-1 density than the basal plasma membrane. Therefore, the passage of maternal glucose across the syncytiotrophoblast is limited by GLUT-1 expression levels in the basal plasma membrane [5].

GLUT-1 expression in the basal plasma membrane was reported to be upregulated in diabetic pregnancies, predisposing newborns to macrosomia [6], whereas fetal growth restriction linked to placental hypoxia was associated with reduced GLUT-1 expression in the basal plasma membrane [7]. By contrast, GLUT-1 expression and glucose transport were unaltered in idiopathic fetal growth restriction [5], suggesting that fetal growth restriction of different etiologies might occur through distinct pathogenetic mechanisms.

We hypothesized that placental malaria–associated fetal growth restriction was supported by decreased GLUT-1 expression in the basal plasma membrane and compared GLUT-1 expression in syncytiotrophoblast microvillous and basal plasma membranes from uninfected or Plasmodium falciparum–infected placentas, with or without intervillitis.

MATERIALS AND METHODS

Recruitment of Participants
Pregnant women delivering a live singleton at the Queen Elizabeth Central Hospital, Blantyre, Malawi, were recruited into a case-control study. Inclusion and exclusion criteria have been described elsewhere [8]. Case patients were defined by the
presence of *P. falciparum* asexual parasites on a placental blood smear. Uninfected control patients (defined as women with both peripheral and placental blood smears negative for parasites) were age and gravidity matched to case patients. The College of Medicine Research Ethics Committee, University of Malawi, approved the study. Written informed consent was obtained from all participants.

Placental villous tissue biopsies (mean weight [± standard error], 7.2 ± 1.6 g; n = 46) were taken around the pericentral region within 1 hour after delivery, snap frozen in liquid nitrogen, and shipped to Melbourne, Australia, in liquid nitrogen dry vapor shippers. Another set was fixed in 10% neutral-buffered formalin for histological assessment for malaria as previously described [1].

**Sample Selection**

Samples were selected on the basis of tissue availability, after histological assessment of placenta specimens [1]. Intervillositis was defined as a monocyte count of ≥5% among all intervillous cells counted. Placental malaria cases were subgrouped into placental malaria with or without intervillositis. Uninfected placenta specimens showed no signs of intervillositis.

**Microvillous Plasma Membrane Purification**

Microvillous plasma membrane purification was performed as described previously [4], using MgCl₂ precipitation and differential centrifugation based on the method of Glazier et al [9] with modifications.

**Basal Plasma Membrane Purification**

Basal plasma membrane purification is described in detail in the Supplementary Materials. The first MgCl₂ precipitation pellet was lysed in phosphate-buffered saline and 5 mM ethylenediaminetetraacetic acid and centrifuged. The basal plasma membrane was then immunoprecipitated from the pellet, using an anti-calcium ATPase antibody (Sigma clone 5F10) and protein G–coated beads (PureProteome, Millipore). The basal plasma membrane was eluted twice in 100 mM glycine (adjusted to pH 2.5, using 10 M HCl). Both eluates were neutralized and stored at −20°C.

**Quality Control of Microvillous and Basal Plasma Membranes**

**Measurement of Protein Concentration**

The protein concentration of placental homogenate and microvillous plasma membrane fractions was determined by the Lowry method. The protein concentration of basal plasma membrane fractions was determined by SYPRO Ruby staining (see below).

**Enrichment in Microvillous and Basal Plasma Membrane Markers**

Purity of microvillous and basal plasma membrane preparations was assessed by the enrichment or depletion in the expression of microvillous plasma membrane– or basal plasma membrane–specific markers in each fraction relative to that in the starting placental homogenate. Placental alkaline phosphatase (PLAP) activity and β2-adrenergic receptor (β2-AR) expression were used as markers of the microvillous and basal plasma membranes, respectively (Supplementary Materials). PLAP activity was measured in placental homogenates and in microvillous and basal plasma membrane fractions as previously described [9]. β2-AR expression was quantified in placental homogenates and basal plasma membrane fractions by Western blot and normalized to protein input.

**Analysis of GLUT-1 Expression**

GLUT-1 expression in microvillous and basal plasma membranes was quantified by dot blot (Supplementary Materials). Membrane fractions were extracted in radioimmunoprecipitation analysis buffer before being diluted in Tris-buffered saline and centrifuged. Supernatants were loaded on a nitrocellulose membrane together with a serial dilution of pooled homogenates as reference to normalize signal intensity between blots. Proteins were fixed onto the membrane and then stained with SYPRO Ruby protein blot stain (Invitrogen) to quantify the amount of protein loaded per dot. After extensive washes, the membrane was blocked and probed with a rabbit anti–GLUT-1 antibody (0.2 µg/mL; Chemicon International), washed, and incubated with an anti-rabbit immunoglobulin G horseradish peroxidase–linked antibody (Sigma). Chemiluminescence substrate (Supersignal West Pico; Pierce) was added before visualizing the GLUT-1 signal on a film (ECL Hyperfilm; Amersham). The GLUT-1 signal density was quantified and normalized to protein input.

**Statistical Analysis**

Data were not normally distributed and are reported as medians and interquartile ranges (IQRs). Statistical analyses were performed using Stata software (version 10.1). Quantitative variables were compared across the 3 groups, using the Kruskal-Wallis test, and the Spearman rank correlation test was used to examine correlations between variables.

**RESULTS**

**Participant Characteristics**

Characteristics of the participants (Table 1) were comparable across all groups, except for maternal hemoglobin levels at delivery.

**Purity of Microvillous and Basal Plasma Membrane Fractions**

Fold enrichments in the activity of the microvillous plasma membrane marker PLAP relative to data for the starting placental homogenate were high for microvillous plasma membrane fractions (median, 14.76 [IQR, 12.08–19.83]) and not different.
Lositis was lower than in P. falciparum–P. falciparum (Supplementary Figure 1). Between microvillous and basal plasma membrane fractions, microvillous plasma membrane, with clear marker distinctions (Supplementary Figure 1) indicated negligible cross-contamination by basal plasma membrane. Immunoprecipitated using an anti-calcium ATPase antibody as a marker, confirming basal plasma membrane identity of this fraction. Furthermore, the lack of PLAP enrichment activity in basal plasma membrane isolates (Supplementary Figure 1) indicated negligible cross-contamination by microvillous plasma membrane, with clear marker distinctions between microvillous and basal plasma membrane fractions (Supplementary Figure 1).

**Table 1. Participant Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Uninfected (n=18)</th>
<th>PM Without IVS (n=8)</th>
<th>PM With IVS (n=20)</th>
<th>P^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk</td>
<td>40 (38–40)</td>
<td>40 (38–40)</td>
<td>38 (38–40)</td>
<td>.57</td>
</tr>
<tr>
<td>Age, y</td>
<td>18.5 (18–19)</td>
<td>20.5 (18–21)</td>
<td>18.0 (17–20)</td>
<td>.51</td>
</tr>
<tr>
<td>Gravity</td>
<td>1 (1–1)</td>
<td>1 (1–1)</td>
<td>1 (1–1)</td>
<td>.46</td>
</tr>
<tr>
<td>Maternal Hb level, g/dL</td>
<td>13.1 (12.1–13.6)</td>
<td>13.2 (11.9–14.7)</td>
<td>10.8 (9.0–11.9)</td>
<td>.0006^b</td>
</tr>
<tr>
<td>Maternal weight at enrollment, kg</td>
<td>56 (50–59)</td>
<td>56 (53–60)</td>
<td>52 (49–56)</td>
<td>.135</td>
</tr>
<tr>
<td>Fetal weight, g</td>
<td>3025 (2700–3500)</td>
<td>2950 (2775–3150)</td>
<td>2850 (2550–3000)</td>
<td>.38</td>
</tr>
<tr>
<td>Placental weight, g</td>
<td>545 (450–580)</td>
<td>475 (435–520)</td>
<td>505 (430–580)</td>
<td>.336</td>
</tr>
<tr>
<td>Fetal to placental weight ratio</td>
<td>5.82 (5.2–6.48)</td>
<td>6.04 (5.78–6.76)</td>
<td>5.79 (5.19–6.06)</td>
<td>.463</td>
</tr>
</tbody>
</table>

Data are median (interquartile range).

Abbreviations: Hb, hemoglobin; IVS, intervillositis; PM, placental malaria.

^a By the Kruskal-Wallis test.

^b Women with PM with IVS had lower Hb levels than infected women without IVS (P = .0032) and uninfected women (P = .0008).

(P = .19) between groups, suggesting high and comparable microvillous plasma membrane purity in all groups. The absence of significant contamination of the basal plasma membrane fractions by microvillous plasma membrane was ascertained by the low PLAP enrichment factors for basal plasma membrane (median, 0.03 [IQR, 0–0.59]). These were comparable across groups (P = .97). It was not possible to assess the purity of basal plasma membrane fractions by the classically used 3H-dihydroalprenolol binding assay because of the limited amount of basal plasma membrane protein obtained and the assay requirement for a relatively high protein input. Instead, we investigated the expression of the basal plasma membrane marker β2-AR by Western blot (Supplementary Figure 1). All basal plasma membrane samples included in this study demonstrated enrichment in β2-AR expression, compared with the starting homogenate. β2-AR enrichment factors (median, 21.7 [IQR, 12–318]) were comparable across groups (P = .35; data not shown). Therefore, the basal plasma membrane fractions, immunoprecipitated using an anti-calcium ATPase antibody as a basal plasma membrane–specific marker, demonstrated enriched expression of β2-AR, another well-characterized basal plasma membrane–specific marker, confirming basal plasma membrane identity of this fraction. Furthermore, the lack of PLAP enrichment in basal plasma membrane isolates (Supplementary Figure 1) indicated negligible cross-contamination by microvillous plasma membrane, with clear marker distinctions between microvillous and basal plasma membrane fractions (Supplementary Figure 1).

**GLUT-1 Expression**

GLUT-1 expression in the microvillous plasma membrane was comparable between groups (Figure 1A; P = .82). However, levels of GLUT-1 in the basal plasma membrane of P. falciparum–positive placenta biopsy specimens with intervillositis was lower than in P. falciparum–positive specimens without intervillositis (Figure 1B; P = .016) and uninfected placenta specimens (P = .0005). GLUT-1 expression in the basal plasma membrane from the 2 latter specimen groups was comparable (P = .82). GLUT-1 expression in the basal plasma membrane correlated negatively with the density of monocyte infiltrate in intervillous blood (Figure 1C; r = −.43; P = .003) and positively with birth weight (Figure 1D; r = 0.28; P = .06), especially in P. falciparum–positive placenta specimens with intervillositis (r = 0.53; P = .017).

**DISCUSSION**

We hypothesized that placental malaria–associated fetal growth restriction was supported by decreased GLUT-1 expression in the basal plasma membrane. Because transplacental glucose transport is orchestrated by placental syncytiotrophoblast GLUT-1 expression, we examined the effect of placental malaria and associated intervillositis on GLUT-1 levels in microvillous and basal plasma membranes.

The placental samples used here were small snap-frozen biopsy specimens, rather than large volumes of fresh placental tissue, which have been used in other studies [5–7]. This made the purification of microvillous and basal plasma membrane fractions particularly challenging. Microvillous plasma membrane purification was relatively easily achieved using the MgCl2 precipitation and differential centrifugation technique, demonstrating this can be usefully applied to small placental biopsy specimens that have been frozen. Immunoprecipitation with calcium ATPase as a highly polarized marker of basal plasma membrane [10] yielded small amounts of basal plasma membrane protein deemed to be of high purity, based on the enrichment of β2-AR and lack of PLAP enrichment activity. Overall, our marker analyses indicated that microvillous and basal plasma membrane fractions were of good and similar purity across all groups.
Because GLUT-1 expression parallels transporter activity [6], comparable levels of GLUT-1 expression in the microvillous plasma membrane across groups suggests that neither placental malaria nor intervillositis altered entry of glucose into the syncytiotrophoblast. The unaltered GLUT-1 expression in the microvillous plasma membrane but reduced GLUT-1 expression in the basal plasma membrane in placental malaria with intervillositis could lead to an accumulation of glucose in the syncytiotrophoblast.

Given that GLUT-1 expression in the basal plasma membrane limits the rate of transplacental glucose transport [5, 11] and positively correlates with placental glucose transport capacity [6], we measured levels of GLUT-1 expression in the basal plasma membrane as an index of transplacental glucose transfer. The reduced GLUT-1 levels in the basal plasma membrane we observed in P. falciparum–positive placenta biopsy specimens with intervillositis could lead to an accumulation of glucose in the syncytiotrophoblast.

GLUT-1 expression in the basal plasma membrane negatively correlated with monocyte count and was specifically decreased in P. falciparum–positive placenta specimens with but not those without intervillositis. This suggests that monocytes and/or their products negatively regulate GLUT-1 expression in the basal plasma membrane. For example, tumor necrosis factor α, a cytokine that is produced by monocytes and expressed at increased levels in placental malaria–associated fetal growth restriction [12], inhibits glucose uptake and signaling downstream of the insulin receptor [13]. Insulin-like growth factor I, a ligand of the insulin receptor, appears to enhance carrier-mediated glucose uptake by a cell line of first trimester cytotrophoblast-like cells, and this change may be mediated by GLUT-1 [14]. Decreased maternal insulin-like growth factor I levels in cases of placental malaria with intervillositis, compared with cases of placental malaria without intervillositis and cases without placental malaria [15], could, in part, contribute to the lower basal plasma membrane

Figure 1. Quantification of glucose transporter isoform 1 (GLUT-1) expression. GLUT-1 expression levels were quantified in microvillous plasma membrane (MVM) and basal plasma membrane (BM) from control patients without Plasmodium falciparum–infected placenta biopsy specimens and case patients with P. falciparum–infected placenta biopsy specimens, without or with intervillositis (IVS). A, GLUT-1 levels in the MVM were similar across groups (P = .82, by the Kruskal-Wallis test). B, GLUT-1 levels in the BM were lower in P. falciparum–infected placenta biopsy specimens with IVS, compared with the other 2 specimen types. The horizontal line represents the median. C, The density of the monocyte infiltrate in the intervillous spaces negatively correlated with GLUT-1 expression in the BM (Spearman r = −0.43; P = .003). D, GLUT-1 expression in the BM was positively correlated with birth weight (Spearman r = 0.28; P = .06), especially in case patients with placental malaria with IVS (Spearman r = 0.53; P = .017). Uninfected specimens are represented by circles, P. falciparum–positive specimens without IVS are represented by squares, and , P. falciparum–positive specimens with IVS are represented by triangles.
GLUT-1 expression during placental malaria with intervillusitis. It is currently unknown whether the downregulation of GLUT-1 expression in the basal plasma membrane is due to decreased GLUT-1 transcription, translation, or targeted expression to the basal plasma membrane.

Our study highlights a role for placental malaria–associated inflammation in reducing GLUT-1 expression in the syncytiotrophoblast basal plasma membrane. This could be one mechanism by which placental malaria impairs placental function, leading to the serious clinical consequences of fetal growth restriction and low birth weight.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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