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# Magnesium deficiency during pregnancy in mice impairs placental size and function☆

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ABSTRACT

*Objective:* Maternal magnesium (Mg) deficiency has been associated with fetal growth restriction. Using a mouse model of maternal Mg deficiency-induced fetal growth restriction, we sought to investigate the effect of Mg deficiency on placental physiology and function.
*Methods: In vivo*: Pregnant Swiss Webster mice were fed either 100% of the recommended amount of Mg (control) or 10%Mg (Mg-deficient) (8 per group). Dams were euthanized on gestational day 17 and placentas were collected, weighed and assessed for Mg concentrations, as well as nutrient transporter mRNA expression. For nutrient transfer studies, control and Mg-deficient dams (6 per group) were injected with $^{14}$C-amino acids and $^{3}$H-glucose and trans-placental passage was determined. In vitro: BeWo placental cells were grown in media containing 10%Mg to 100%Mg and the effects of Mg status on cell proliferation, oxidative stress and nutrient uptake were measured. Data were analyzed by Student's t-tests comparing controls vs. Mg-deficient animals or cells. For multiple comparisons, data were analyzed by ANOVA followed by Dunnett's post hoc testing.
*Results: In vivo*: Maternal Mg deficiency decreased placental Mg content, placental and fetal weights, ratio of fetal:placental weight ($P < 0.05$), placental *Slc7a5* transporter mRNA expression and trans-placental nutrient transport ($P < 0.05$). In vitro: Mg deficiency reduced BeWo nutrient uptake ($P < 0.01$) and cell proliferation ($P < 0.01$), and increased oxidative stress ($P < 0.01$).
*Conclusion:* These findings highlight the adverse effects of maternal Mg deficiency on fetal weight and placental function, including transport and proliferation and may explain the fetal growth restriction observed with moderate Mg deficiency in mice.



## 1. Introduction

The placenta is critical for promoting normal fetal development through numerous biological functions, including substrate and nutrient transport. Placental dysfunction, believed to affect approximately one third of all pregnancies [1], is associated with numerous conditions including, fetal growth restriction (FGR) [2]. In fact, compromised placental structure and function is proposed to be a major contributor to perinatal and maternal morbidity and mortality [3]. However, all of the factors contributing to healthy placental development are not understood.

Maternal nutrition can significantly affect placental development and hence, fetal development and neonatal outcomes [4].

*Abbreviations:* (DCFH-DA), dichloro-dihydro-fluorescin diacetate; (FGR), fetal growth restriction; (Mg), magnesium; (MgD), magnesium deficiency; (MeAIB), 2-methylaminoisobutyric acid.

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Numerous dietary nutrients (e.g. protein), vitamins (e.g. vitamin D and folic acid), fatty acids (e.g. docosahexaenoic acid, DHA), and micronutrients (e.g. zinc and magnesium) have been shown to be critical for placental development and health [5,6].

Magnesium (Mg) is an essential nutrient; it is a cofactor for >300 biological reactions, as well as the synthesis of nucleic acids and protein [7]. Most importantly, Mg is required for balancing oxidative stress [8]. While there are many benefits of consuming sufficient dietary Mg, recent studies report widespread inadequate Mg intakes, particularly among females of reproductive age [9]. In light of this, we explored the effects of moderate maternal Mg deficiency in mice and found that it was accompanied by FGR [10]. The objective of this study was to investigate the effects of maternal Mg deficiency on placental size and function, specifically transplacental transport, using in vitro and *in vivo* models.

## 2. Materials and methods

### 2.1. Mouse model of maternal Mg deficiency and placental nutrient transport studies

Animal experiments were approved by the Institutional Animal Care and Use Committee of the Feinstein Institute (#2010-018) and complied with the Guide for the Care and Use of Laboratory Animals. After a one week acclimatization period, outbred Swiss Webster female mice (9–14 weeks old, Taconic Farms, Germantown, NY) were mated with normal males. On gestational day 6 (GD6), pregnant dams were randomly assigned to either normal (control, containing 100% of the recommended amount, 500 mg/kg Mg) or the Mg-deficient (MgD, containing 10%Mg, 50 mg/kg Mg) diets, as previously described [10]. This model of moderate Mg deficiency was chosen because it leads to FGR and reduces maternal serum Mg by 40–45% [10]. Dams continued their respective diets *ad libitum* throughout pregnancy. On GD17 (9am-12pm), control and Mg-deficient dams (8 per group) were euthanized by $CO_2$ asphyxiation followed by exsanguination (via cardiac puncture) and placentas were collected and weighed. *Nutrient transport assays:* In separate experiments, control and Mg-deficient GD18 dams (6 per group) were weighed and anesthetized using ketamine (90–100 mg/kg)/xylazine (10–12.5 mg/kg) and injected retro-orbitally with $^{3}$H-methyl-glucose ($^{3}$H-glucose) and $^{14}$C-2-methylaminoisobutyric acid ($^{14}$C-MeAIB) (Perkin Elmer, Waltham, MA), as previously described [11]. Radioactivity was counted by β scintillation counting (Tri-Carb *1900*, Perkin Elmer/Packard, Waltham, MA) in Bioscint (National Diagnostics). The maternal-to-fetal unidirectional clearance ($K_{mf}$; $\mu l \bullet min^{-1} \bullet g^{-1}$) for each tracer was determined using the following formula: $K_{mf} = N_x/AUC_{0\text{-}x}$ X W, where $N_x$ is the counts to the fetus taken at time x when the dam was euthanized (dpm), $AUC_{0\text{-}x}$ is the area under the maternal isotope concentration curve from time 0 to the time of dam euthanasia ($dpm \bullet min \bullet \mu l^{-1}$) and W is the wet weight of the placenta (grams, g), as previously described [11].

### 2.2. Determination of placental Mg concentrations

Total placental Mg analyses were performed at the Biomarker Mass Spectrometry Facility of University of North Carolina at Chapel Hill using inductively-coupled plasma mass spectrometry (ICP-MS). Briefly, placentas were digested in 70% nitric acid, as previously described [12] and diluted for measurements by ICP-MS, which were performed using blanks and internal standards [13].

### 2.3. Evaluation of placental oxidative stress and gene expression for markers of anti-oxidant activity and nutrient transport

Placental oxidative stress was determined by western blotting. Briefly, placentas (100 mg per sample) were homogenized in lysis buffer (Tris buffered saline pH 7.4 containing 150 mM NaCl, 0.25% Triton X-100 and protease and phosphatase inhibitor cocktail) using 0.9–2.0 mm stainless steel beads and the Bullet Blender. Lysates (40μg/lane) were separated by SDS-PAGE electrophoresis and western blotted using anti-4-HNE (4-hydroxy-2-nonenal) (Abcam, Cambridge, MA) and anti-GAPDH antibodies (Cell Signaling Technology, Danvers, MA). Blots were revealed using the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE), as previously described [12]. Collectively, the ratio of the band densities of the 4HNE-modified proteins-to-the corresponding GAPDH band density was assessed for each placenta using ImageJ software (NIH); the mean ratio±SD for controls and Mg-deficient placentas are shown.

For gene expression studies, placental RNA was isolated using the RNeasy Plus Universal Mini kit with DNase treatment. qRT-PCR was performed in triplicate using RNA (with 260:280 and 260:230 ratios ≥1.8.) using specific primers (Table 1), as previously described [10,12]. Relative changes in gene expression were calculated as relative mRNA expression using *Hprt1* and *Gapdh* housekeeping genes and the comparative Ct (ΔΔCt) method [14].

### 2.4. BeWo cell proliferation in vitro

BeWo choriocarcinoma (placental) cells (ATCC, Manassas, VA) were plated in 96-well plates ($1 \times 10^4$/well) in F12K media (containing 1.6 mM $MgSO_4$ [100%]) with 10%FBS, penicillin-streptomycin and glutamine (PSQ)). Four hours later, the media was replaced with minimum essential media (MEM) containing 10%FBS, Ca (2.7 g/dL), non-essential amino acids, PSQ and 10%–100% of the recommended amount of Mg (100%Mg = 1.6 mM, as $MgSO_4$) (n = 6 per condition) for 3 days. Cell number was

**Table 1**
qPCR primers used to assess gene expression.

| Gene | Forward Sequence<br>Reverse Sequence | GenBank Accession #[a] | Probe# |
|---|---|---|---|
| *Gapdh* | 5-gccaaaagggtcatcatctc-3<br>5-cacacccatcacaaacatgg-3 | NM_001289726.1 | 29 |
| *Glut1* | 5-gaccctgcacctcattgg-3<br>5-gatgctcagataggacatccaag-3 | NM_011400.3 | 99 |
| *Glut3* | 5- tttggtgctggagagaggtta-3<br>5-tgactcacaggccaccagta-3 | NM_011401.4 | 88 |
| *Gpx3* | 5-gtgaacggggagaaagagc-3<br>5-tgagcccaggagttctgc-3 | NM_008161.3 | 51 |
| *Hprt1* | 5-tcctcctcagaccgctttt-3<br>5-cctggttcatcatcgctaatc-3 | NM_013556.2 | 95 |
| *Slc7a5* | 5-cgaacctggcctatttcact-3<br>5-ccaagtggtagttcccgaag-3 | NM_011404.3 | 5 |
| *Slc27a4* | 5-ggcagtgagatggcctca-3<br>5-cagagcagaagaggctgagtg-3 | NM_011989.4 | 3 |
| *Slc38a1* | 5-cttcagccataaaatccctcat-3<br>5-catcgacgtaccaggctga-3 | NM_134086.4 | 88 |
| *Slc38a2* | 5-caatgagatccgtgcaaaag-3<br>5-tgcttccaatcatcaccact-3 | NM_175121.3 | 2 |
| *Slc38a4* | 5-tgcatggtgtttttcgtcag-3<br>5-ttccgttgttgtgatccaga-3 | NM_027052.3 | 13 |
| *Sod3* | 5-ctcttgggagagcctgaca-3<br>5-gccagtagcaagccgtagaa-3 | NM_011435.3 | 102 |

Forward and reverse primers for mouse genes with GenBank Accession numbers and Roche Universal Probe numbers used for assessing mRNA expression in mouse placentas by qPCR.

[a] National Center for Biotechnology Information (NCBI) EntrezGene (http://www.ncbi.nlm.nih.gov/gene).