Amino Acid Restriction Differentially Affects Insulin-like Growth Factor-1 (IGF-1) and IGF Binding Protein-1 (IGFBP-1) in the Maternal Decidua Compared to Fetal Liver

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Introduction: Maternal protein deficiency (MPD) is a major cause of fetal growth restriction (FGR). Mechanisms linking MPD to FGR are unknown, but may be related to amino acid response (AAR) activation and its effects on the insulin-like growth factor system. IGFBP-1 is expressed mainly in the fetal liver and maternal decidua in pregnancy. We *hypothesized* that AAR causes dysregulation of the IGF-1 and IGFBP-1 axis in the decidua and affects its development and functionality.

Methods: Human endometrial stromal cells (HESC) treated with decidualizing agents and human hepatocellular carcinoma cells (HepG2) were used as models of decidual stromal cells and fetal liver cells, respectively. Both were treated with halofuginone (HF) to stimulate AAR. Proline or leucine were used to rescue the effect of HF. IGFBP-1 expression was determined at mRNA level by qRT-PCR and at protein level and phosphorylation by immunoblotting. Cell integrity was determined by MTT assay.

Results: During decidualization, HESCs *increased* levels of IGFBP-1 (2.6-fold) and phospho-IGFBP-1 (0.72-fold). In decidualizing HESCs, HF *decreased* (i) IGFBP-1 mRNA (3.5-fold); (ii) IGFBP-1 secretion (2.9-fold); (iii) IGFBP-1 phosphorylation (0.56-fold). In HepG2 cells, HF *increased* (i) IGFBP-1 mRNA (0.3-fold); (ii) IGFBP-1 secretion (2.4-fold); (iii) IGFBP-1 phosphorylation (0.87-fold). Addition of proline but not leucine reversed these effects on IGFBP-1 secretion in HESCs (3.6-fold) and HepG2 (2.78-fold). Fold-change was represented relative to experimental controls. Cellular integrity was unaffected.

Conclusion: These findings indicate that AAR differentially affects IGFBP-1 biosynthesis in decidua and fetal liver, suggesting MPD may cause tissue/organ-specific effects *via* IGF-1/IGFBP-1 axis to result in FGR.

250 words.