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Title: The placental glycocalyx in pregnancies complicated with gestational diabetes *mellitus*: the effect of metformin on this *in utero* and *in vitro*.

Introduction: In the human placenta, a glycocalyx layer covers the apical surface of the syncytiotrophoblast (ST) facing the maternal blood, the basement membrane of ST and the paracellular and luminal surface of fetal vessels (FV) contained within placental villi. This allows hypothesis that the glycocalyx may be a critical regulator of materno-fetal transport of solutes across the placental barrier. In *diabetes mellitus* changes in the constituents and extent of endothelial glycocalyx have been shown in renal, pulmonary, retinal and brain microvessels and has been linked with increased vascular permeability. Metformin, an antidiabetic drug, has been shown to tighten the placental fetal vascular barrier by upregulation of tight junctional occludin (Villota SD, Toledo-Rodriguez M and Leach L, 2021), but its effect on the glycocalyx layer has not been shown.

Aim: The aim of the study was to investigate the effect of metformin treatment on placental glycocalyx both *in-utero* and *in vitro*, using term placenta from normal pregnancies and those complicated by GDM which was treated by diet or metformin.

Methods: Placental biopsies were taken from freshly delivered placenta (normal: n=7, GDM-Diet: n=7 and GDM-Metformin: n=3). They were 1) directly fixed in 1% PFA (T_0) and 2) chorionic villous trees were excised from the normal and GDM-Diet study groups and cultured in suspension, using net support in 12-well plates to mimic the villous environment. They were incubated with or without metformin (1.2µg/ml) for 24 hours (T_{24}) at 37°C (95% O_2, 5% CO_2) and then fixed. Cryosections (7µm thick) were immuno-stained with anti-PECAM-1(1µg/ml) and TRITC-conjugated secondary antibody followed by lectin staining (WGA-FITC; 10 µg/ml). Fluorescence Images were acquired (blinded). Quantitative analysis using systematic sampling was performed, with percentages of villous (ST) and vascular (FE) profiles positive for glycocalyx calculated for all study groups. One-way and two-way ANOVA were used for statistical analyses.

Results: At T_0 there was no significant difference between the percentages (±S.D) of villous profiles showing glycocalyx coverage on the ST ($p>0.05$) of normal (57.30%±5.55), GDM-Diet (52.68%±4.53) and GDM-Metformin (59.38%±2.79). In fetal vessels, GDM-Diet (46.94%±6.2) group showed significantly lower ($p<0.05$) vascular profiles with luminal endothelial glycocalyx compared to that of normal (64.20%±7.33) and GDM-Met (71.50%±4.46). 24 h incubation with or without metformin did not alter glycocalyx localisation on the apical border of ST for both normal and GDM-Diet study groups. Vascular profiles within chorionic villi, grown in the absence of metformin for 24h (T_{24}), maintained the significant difference measured at T_0. 24 h metformin treatment resulted in an increase in the number of vessels with luminal glycocalyx for both normal (75.33%±10.24) and GDM-Diet (66.28%±4.17) study groups. Furthermore, GDM-Diet explants showed a highly significant increase ($p\leq0.003$) in the percentage of lectin positive vascular profiles after metformin treatment. This was not seen for the normal study group.

Conclusions: Our data suggest that metformin treatment during GDM pregnancy may restore the loss of glycocalyx in placental fetal vessels. In vitro, addition of metformin to the GDM-Diet mimicked this restoration. Thus metformin, which readily crosses the placenta may be able to influence fetal vascular integrity in pregnancies complicated by gestational diabetes.