Phenotyping the fetoplacental vasculature of a prepregnancy smoking mouse model using micro-computed tomography and automated vascular segmentation

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Aims
Maternal cigarette smoking is associated with numerous reproductive abnormalities including placental disorders and low birth weight infants. The main toxicants found in cigarettes are a group of carcinogens known as polycyclic aromatic hydrocarbons (PAHs). Importantly, these toxicants can accumulate in adipose and mammary tissue and therefore can slowly be released into the blood stream during pregnancy, even when a woman ceases smoking upon learning she is pregnant. In a mouse model, prepregnancy injections of PAHs lead to growth restricted fetuses and reduced surface area and volume of the fetal arterial vasculature of the placentas. The purpose of this study was to apply advances in micro-computed tomography (micro-CT) imaging and together with vascular segmentation analysis to phenotype the branching pattern of the fetoplacental arterial tree and to quantify the effect of prepregnancy PAH exposure on this vasculature. We subsequently used these data to predict the influence of such changes on fetoplacental vasculature resistance.

Method
Two groups of C57Bl6/J virgin female mice were randomly separated into vehicle control and PAH-treated groups. They were subcutaneously injected with vehicle (corn oil) or PAHs for a 9 week period at a total cumulative dose of 12 mg/kg, a dosage equivalent to ~7 cigarettes/day for 9 week in humans. Females were mated with no further injections given during pregnancy. At day 15.5 of gestation pregnant mice were sacrificed and the fetuses and placentas were removed from the uterus. A radio-opaque silicone rubber contrast agent (Microfil, Flow Tech, Carver, MA) was injected into umbilical artery using established methods [1]. After being fixed in 10% buffered formalin phosphate for 24-48 hours, the perfused specimens (n=10/group) were scanned using an eXplore Locus SP micro-CT scanner (GE Healthcare, London, ON, Canada). Three-dimensional (3D) datasets were acquired for each specimen with a voxel size of 13 microns. Individual isosurface rendering images of the arterial fetoplacental tree were generated using the Amira visualization package (Visage Imaging, San Diego, CA). Umbilical artery diameters and the span and depth of the fetoplacental arterial tree were measured directly from these surface renderings. We applied an automated vascular segmentation process that transformed the data into a tubular model for which diameters and lengths are known to identify vessel-like structures in the image [2]. The process is illustrated in Fig. 1. Vascular resistance was calculated based on vessel architecture through use of standard formulas for pipe flow and for resistances in parallel and in series [3].
Results
In comparison with the control group, PAH embryo weight was reduced by 23% (p<0.0001). There was a 27% decrease in the number of arteriole-sized (50-100 μm) vessels (p<0.01). No change was observed in the number of large, chorionic plate vessels. However, PAH exposure increased curvature of the chorionic plate vessels as shown by a significantly increased tortuosity ratio of the tree (p=0.001) (Fig.2). No changes were observed in the depth or span of the tree, the diameter scaling coefficient, or the segment length-to-diameter ratio. Arterial vascular resistance was increased by 30% (p=0.015). Assuming equal pressure in both groups, blood flow would be 19% lower in PAH-exposed placentas (p=0.01) (Fig. 3).

Fig. 2 Vascular tortuosity. A: Isosurface rendering of control specimen. B: PAH-exposed specimen, vessel tortuosity was quantified as the ratio of vascular path length (red line) to the beeline distance (green arrow) from the umbilical artery to each terminal vessel. C: A histogram of the vessel tortuosity ratio for controls (black bars) and PAH-exposed specimens (red bars) demonstrates a shift towards larger ratios in the PAH-treated group.
Fig. 3 Vascular hemodynamics. A: Total vascular resistance of the fetoplacental arterial tree of PAH-exposed (black bar) and control (gray bar) placentas. \( p = 0.015 \). B and C: Isosurfaces were color rendered to illustrate blood flow magnitude in control (B) and PAH-treated (C) placentas. The trees of PAH-exposed placentas had lower flows throughout. This was noticeable at the terminal vessels, which display more blue coloring compared with controls. Scale bar = 1 mm.

**Conclusion**

We used Micro-CT technology together with automated vascular segmentation to detect quantitative differences between control and PAH-exposed fetoplacental vessels, including vascular tortuosity, branching pattern, and distribution of vessel diameters. Computational flow calculations were used to estimate vascular resistance. Our findings show that PAH-exposed mice had a significant reduction in the number of small diameter intraplacental vessels, but not in the large chorionic plate vessels. An increase in vascular tortuosity was also found in PAH-exposed vascular tree. These changes in vascular geometry were predicted to increase arterial vascular resistance by 30%, and decrease blood flow by 19%. Low flow may contribute to the 23% reduction observed in fetal weight.

**References**

