Effect of Obesity and Diabetes on Nitrative Stress in the Placenta

Jessica A. Smith, Victoria H.J. Roberts, Brad A. Pitzer, Angela B. Heizer & Leslie Myatt
Department of Obstetrics and Gynecology, University of Cincinnati College of Medicine.

Both obesity and type 2 diabetes produce elevated pro-inflammatory cytokines, which leads to oxidative stress. The interaction of superoxide and nitric oxide, two reactive oxygen species (ROS), produces peroxynitrite, a potent long-lived oxidant. Peroxynitrite nitrates tyrosine residues in proteins, a post-translational modification that alters protein function, i.e. nitrative stress.
Objective: To examine whether placental peroxynitrite production increases with increasing BMI and the presence of diabetes.
Methods: Placental tissue was collected at term from lean (BMI 18.5-24.9 kg/m^2), overweight (BMI 25-29.9), obese (BMI 30-40), and obese diabetic (BMI 30-45) patients (n=6/group). Tissue was immunostained with anti-nitrotyrosine primary antibody or homogenized prior to SDS-PAGE or detection of nitrated proteins by dot blotting. Samples were derivatized using 2,4-dinitrophenylhydrazine (DNPH), separated on SDS-PAGE and oxidized proteins detected with anti-DNP Ab. Three oxidized proteins were analyzed by MALDI-mass spectrometry.
Results: Nitrotyrosine residues were immunolocalized in vascular endothelium, villous stroma, and weakly in syncytiotrophoblast. No significant difference was seen in staining intensity with increasing BMI. Dot Blotting revealed an overall difference in nitrotyrosine expression across the four groups ($p<0.002$, one-way ANOVA) being significantly increased in obese compared to lean ($p<0.002$), overweight ($p<0.003$) and diabetic ($p<0.01$, Tukey test). The concentration of oxidized proteins was however significantly greater in lean versus overweight patients ($p<0.02$, Mann-Whitney U test). One oxidized protein was tentatively identified as a member of the hydroxysteroid dehydrogenase family by mass spectrometry.
Discussion: With increasing BMI an increase in nitrative stress appears to occur in parallel with a decrease in oxidation of proteins. Potentially the formation of peroxynitrite (nitrative stress) may be consuming ROS and lowering oxidative stress.