Placenta 51 (2017) 64–69

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Sexual dimorphism in the effect of maternal obesity on antioxidant defense mechanisms in the human placenta

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ARTICLE INFO

Article history:
Received 3 January 2017
Received in revised form
30 January 2017
Accepted 4 February 2017

Keywords:
Oxidative stress
Antioxidants
Obesity
Mitochondria
Placenta

ABSTRACT

Introduction: Maternal obesity creates an adverse intrauterine environment, negatively impacts placental respiration, is associated with a higher incidence of pregnancy complications and programs the offspring for disease in adult life in a sexually dimorphic manner. We defined the effect of maternal obesity and fetal sex on pro- and anti-oxidant status in placenta and placental mitochondria. Methods: Placental villous tissue was collected at term *via* c-section prior to labor from four groups of patients based on fetal sex and prepregnancy/1st trimester body mass index: lean - BMI 22.1 ± 0.3 (6 male, 6 female) and obese - BMI 36.3 ± 0.4 (6 male, 6 female). Antioxidant enzyme activity, mitochondrial protein carbonyls, nitrotyrosine residues, total and nitrated superoxide dismutase (SOD) and nitric oxide synthesis were measured. Results: Maternal obesity was associated with decreased SOD and catalase activity, and total antioxidant capacity (TAC), but increased oxidative (protein carbonyls) and nitrative (nitrotyrosine) stress in a sexually dimorphic manner. Placentas of lean women with a male fetus had higher SOD activity and TAC ($p < 0.05$) than other groups whereas obese women with a male fetus had highest carbonyls and nitrotyrosine ($p < 0.05$). Glutathione peroxidase and thioredoxin reductase activity increased with obesity, significantly with a male fetus, perhaps as a compensatory response. Conclusion: Maternal obesity affects oxidative stress and antioxidant activity in the placenta in a sexually dimorphic manner. The male fetus of a lean women has the highest antioxidant activity, a protection which is lost with obesity perhaps contributing to the increased incidence of adverse outcomes with a male fetus.

1. Introduction

The prevalence of maternal obesity (body mass index (BMI) ≥ 30) has been steadily increasing both in the US [1] and worldwide [2]. Maternal obesity deleteriously affects maternal health and increases delivery complications, as well as negatively impacting short term and long term health outcomes in the offspring [3]. As BMI increases, pregnant women have an increased incidence of complications such as preeclampsia, gestational diabetes, cesarean sections, stillbirths, and preterm deliveries [4], as well as increased healthcare costs due to increased admission of newborns to the neonatal intensive care unit [5–7] and longer hospital stays [8]. Newborns born to obese mothers have an increased prevalence of cleft palates, spina bifida, and congenital heart defects [4,9,10]. The challenge of intrauterine stress associated with maternal obesity can disrupt normal placental function thus impacting fetal growth and development by mechanisms that remain largely unknown [3].

The physiologic balance between production of ROS and scavenging by intracellular antioxidants is critical to cell survival. In mammalian cells, including trophoblast, a variety of antioxidant mechanisms operate to control ROS production and action [11]. The primary antioxidant is superoxide dismutase (SOD), which dismutates superoxide to hydrogen peroxide, and is found in both cytosol (SOD1) and mitochondria (SOD2). Glutathione peroxidases (GPx's) found in both the mitochondrial matrix and cytoplasm, and catalase (CAT) found in peroxisomes are part of a secondary defense mechanism catalyzing the conversion of hydrogen peroxide to water. These antioxidant enzymes operate in a coordinated manner to defend against ROS propagation and action. Pregnancy has been characterized as a state of oxidative stress, an imbalance of ROS molecules and antioxidant scavenging capabilities, due to increased

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http://dx.doi.org/10.1016/j.placenta.2017.02.004
0143-4004/

Abbreviations

SOD	superoxide dismutase
CAT	catalase
GPx	glutathione peroxidase
TrxR	thioredoxin reductase
TAC	total antioxidant capacity

placental mitochondrial activity and subsequent generation of reactive oxygen species (ROS) [12]. Oxidative stress was further increased in pregnancies complicated by preeclampsia and gestational diabetes, where obesity is a predisposing factor [12]. Placentas from preeclamptic women showed reduced enzymatic activity of SOD, GPx, and thioredoxin reductase (TrxR), as well as increased lipid peroxidation, an oxidative stress marker [13,14]. However there were no differences in another marker of oxidative stress, protein carbonyls [14]. Whereas one study of placentas from pregnancies complicated by gestational diabetes showed increased SOD activity and protein carbonyls but no differences in GPx activity [15] another study of gestational diabetes reported no differences in placental SOD and GPx activities but decreased CAT and antioxidant potential together with increased lipid peroxidation and xanthine oxidase activity [16]. Since it has been well established that obesity alone increases oxidative stress markers in the non-pregnant state [17], the aim of the present study was to examine the effect of maternal obesity on oxidative stress in the placenta in uncomplicated pregnancies.

In our previous study of the effect of maternal obesity on placental miR-210 expression we observed evidence of sexual dimorphism as miR-210 expression was increased in placentas of obese women with female but not male fetuses compared with placentas from lean women with a female fetus [18]. Other studies have demonstrated that male animals have higher oxidant levels under hypertensive [19] and obese [20] conditions. Therefore in the present study we investigated the role of sex as a biological variable on oxidative stress and antioxidant status in placenta and predicted sex-dependent outcomes with maternal obesity.

We have previously observed that as maternal adiposity increases there was a parallel increase in placental ROS generation [21] and nitrotyrosine residues [22], thus indicating both oxidative and nitrative stress occur in the placenta in obese women. Despite mitochondria being a major source of ROS and shown to cause oxidative stress during normal [12] and complicated pregnancies [23], little is known about the effect of maternal obesity on mitochondria in the placenta. Based on previous evidence, we also hypothesized that with maternal obesity superoxide and nitric oxide would be increased in placental mitochondria resulting in peroxynitrite generation leading to nitration of mitochondrial proteins thus causing further decreased antioxidant defense mechanisms.

2. Methods

2.1. Ethical approval and study participants

Placentas were collected from the Labor and Delivery Unit at University Hospital San Antonio under a protocol approved by the Institutional Review Board of the University of Texas Health Science Center San Antonio, with informed consent from the patients. Placentas were collected from patients with no history of smoking or drug use at c-section at term from uncomplicated pregnancies in the absence of labor. Prepregnancy/1st trimester body mass index (BMI) was used to determine adiposity, defined as lean (BMI 18.5–24.9) or obese (BMI 30–40). Following delivery, villous tissue was randomly sampled, flash frozen, and stored at −80°C.

2.2. Assays

Tissue was homogenized in isolation buffer (0.25Msucrose, 10mMTris-HCl, 1mMEDTA, pH7.4) with protease inhibitors (Roche, Indianapolis, IN). Placental homogenates were assayed according to the manufacturer's recommendation for SOD, GPx, TrxR and CAT activity, total antioxidant capacity (TAC), and nitric oxide (NO) (Cayman Chemical, Ann Arbor, MI).

2.3. Mitochondrial preparation

Placental mitochondria were extracted using differential centrifugation. Homogenates were centrifuged at 1,500 × *g* for 15 min at 4 °C, the supernatant was removed and transferred into a new tube then centrifuged at 16,000 × *g* for 10 min at 4 °C to pellet the mitochondria. The pellet was resuspended in resolving buffer (0.25Msucrose, 1mMTris-HCl, 1mMEDTA, pH 7.8) and centrifuged at 12,000 × *g* for 10 min at 4 °C. The pellet was washed with isolation buffer by centrifuging at 12,000 × *g* for 10 min at 4 °C. The pellet was then suspended in 2% CHAPS in TBS and agitated for 2 h at 4 °C to lyse mitochondria. Mitochondrial extracts were stored at −80°C.

2.3.1. Protein carbonyl assay

Centrifugation steps were the same as described above. However, mitochondria were extracted in the buffer recommended by the manufacturer for protein carbonyl (Cayman Chemical, Ann Arbor, MI). Resuspended mitochondria pellets were sonicated to lyse the mitochondria and stored at −80°C.

2.4. Protein assays

Protein was quantified from placental and mitochondrial extracts using Bradford protein analysis (Bio-Rad, Hercules, CA).

2.5. Western blotting

Placental mitochondria extracts (15 μg) were loaded onto 4–20%Tris-HCl gel, electrophoresed, and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). Membranes were blocked with 5%BSA in PBS for 1hr before being probed with nitrotyrosine (1:1000) or MnSOD (1:500) 1° antibody (Millipore, Temecula, CA), and normalized to voltage-dependent anion channel protein (VDAC, 1:1000) (Abcam, Cambridge, MA) overnight at 4 °C. Membranes were washed with 10%Tris-buffered saline with Tween20 (TBST), incubated with appropriate horseradish peroxidase 2° antibody (Cell Signaling, Danvers, MA) in 1%milk + TBST for 2 h at RT, then visualized using enhanced chemiluminescence (Roche, Indianapolis, IN).

2.5.1. Immunoprecipitation of nitrated proteins

Placental mitochondria extracts (200 μg) were incubated with anti-nitrotyrosine antibody(1:50) and EZview Red Protein A Affinity Gel (Sigma, St. Louis,MI). The immunoprecipitated nitrated proteins were then electrophoresed as previously described prior to western blotting with antibody to MnSOD and band density normalized to IgG used as the loading control.

2.6. Statistics

Results are expressed as mean ± SEM where n is the number of