Journal of
Obesity & Weight Loss Therapy
ISSN: 2165-7904

Alanis et al., J Obes Wt Loss Ther 2012, 2:7
DOI: 10.4172/2165-7904.1000143

Research Article
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Maternal Obesity and Placental Oxidative Stress in the First Trimester

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Abstract

Objective: Maternal obesity is associated with adverse pregnancy outcomes affected by placental dysfunction. We sought to compare levels of placental oxidative stress between obese and lean women in the first trimester.

Study Design: Obese and lean women matched 1:1 for baseline variables and gestational age were enrolled between 8 and 13 weeks of gestation at the time of voluntary surgical abortion. The global cellular redox status was determined by measuring the total protein thiol content in placental homogenates and serum (ThioGlo-1).

Results: There were no differences in baseline variables between obese (n=22, median BMI 35.0) and lean controls (n=22, median BMI 22.0). The median level of placental oxidative stress was 31% greater in the obese group compared to the lean group (141.1 [117-156] vs. 203.7 [189-234] counts/sec/µg protein, respectively; p <0.001). A similar but statistically insignificant difference was noted in the serum (12.2 [9-15] vs. 13.6 [12-23] counts/sec/µcg protein; p=0.09).

Conclusion: Maternal obesity is associated with placental oxidative stress in the first trimester. Oxidative stress in the first trimester may reflect or contribute to impaired placentation and placental dysfunction in obese women.

Keywords: Obesity; Oxidative stress; Placenta; Redox; Protein sulfhydryls; Thiols; ThioGlo-1

Introduction

Pregnancy has been described as a state of oxidative stress due to increased metabolic activity of the placenta and decreased antioxidant capacity during normal pregnancy [1]. Rapidly dividing placental cells produce large amounts of Reactive Oxygen Species (ROS), including superoxide anion, as a byproduct of aerobic respiration by complexes I and III of the mitochondrial electron transport chain [2]. NAD(P)H oxidase (Nox) is the other main source of superoxide anion generation in the placenta and is expressed in the cell membrane of the syncytial layer, the vascular endothelium, and maternal granulocytes found at the maternal-fetal interface [3,4]. Collectively, these observations suggest that normal pregnancy is a state close to the limit at which oxidative stress may become pathological. Decreased antioxidant capacity and increased ROS are associated with placental dysfunction resulting in preeclampsia, intrauterine fetal hypoxia and growth restriction, and stillbirth [1-3,5-7].

Oxidative stress markers such as levels of plasma lipid peroxidation and urinary F2 isoprostanes are known to be increased in obese, non-pregnant women [8,9]. However, the relationship between maternal obesity and placental oxidative stress is unclear. Maternal obesity is associated with placental dysfunction, characterized clinically by preeclampsia, second trimester spontaneous abortion, and stillbirth, in a dose-dependent fashion with body mass index (BMI) [10-15]. More than 1 in 3 women in the United States are obese at the time of conception, defined as a BMI equal to or greater than 30 kg/m^2[16]. Thus, there is a broad interest among public health experts, physicians, and researchers in elucidating mechanistically based interventions to reduce the impact of maternal obesity on pregnancy outcome. The aim of this study was to determine the effect of maternal obesity on placental oxidative stress in the first trimester.

Methods

Subjects

Obese (BMI ≥ 30) and lean (18.5 ≤ BMI <35) patients undergoing voluntary surgical abortion were recruited from a single reproductive services clinic in this IRB approved study. Inclusion criteria included a gestational age between 8(0/7) and 13(6/7) weeks and signed informed consent. Exclusion criteria included co-existing diabetes mellitus, renal disease, and use of antibiotics in the previous 6 weeks, use of any non-steroidal anti-inflammatory drug in the previous 24 hours, use of oral or systemic steroids, or recent (<6 weeks) pelvic inflammatory disease. BMI was determined by height and weight measured immediately prior to the procedure. Obese subjects were enrolled consecutively, and a matched lean controls was subsequently enrolled for each obese subject. Matching was 1:1 by race/ethnicity, smoking status (yes/no in pregnancy), and gestational age (± 3 d). All gestational ages were confirmed by measurement of the crown-rump-length with ultrasound prior to the procedure.

Blood and placenta collection

Blood samples were obtained at the time of intravenous access and prior to initiation of the procedure. Blood samples were placed on ice and allowed to clot for at least 30 minutes. Immediately following dilation and curettage, products of conception were floated in ice-cold phosphate buffered saline (PBS, pH 7.4). Placenta was identified by the consistent frond-like appearance of villi (Figure 1). Placental specimens were washed with ice-cold PBS then flash-frozen in liquid nitrogen. The entire tissue collection process was completed in less than 20 minutes after dilation and curettage in all cases. Placental specimens

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Received June 20, 2012; **Accepted** August 06, 2012; **Published** August 12, 2012

Citation: Alanis MC, Steadman EM, Manevich Y, Townsend DM, Goetzl LM (2012) Maternal Obesity and Placental Oxidative Stress in the First Trimester. J Obes Wt Loss Ther 2:143. doi:10.4172/2165-7904.1000143

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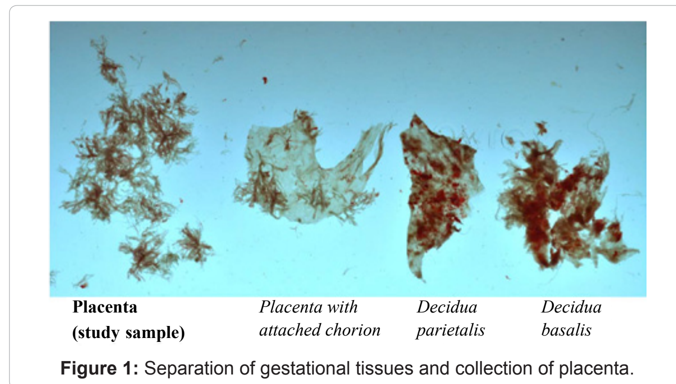


Figure 1: Separation of gestational tissues and collection of placenta.

were stored at -80° C until batched testing. Clotted blood samples were centrifuged at 4,000 rpm for 20 minutes at 4° C and serum fractions were kept for analyses.

Sample Preparation

Frozen placental samples were manually homogenized in lysis buffer containing 50 mMTris-HCl (pH 7.5), 2mM Ethylene Diaminetetraacetic Acid (EDTA), 2mM Ethylene Glycol Tetraacetic Acid (EGTA), 1% Triton X-100, 150 mMNaCl, 10 glycerol, and diluted 1:100 with Phenyl Methyl Sulfonyl Fluoride (PMSF). The lysis buffer was supplemented with a proteinase inhibitor cocktail containing 4-(2-amnioethyl) benzenesulfonyl fluoride (AEBSF), pepstatinA, E-64, bestatin, leupeptin, and aprotinin (Sigma-Aldrich, St. Louis, MO). Samples were incubated in the lysis buffer with periodic vortexing at 4^0C for 15 minutes. The incubated samples were then centrifuged at 15,000 rpm for 15 minutes to precipitate non-soluble material. The supernatant was collected and passed through a Biospin-6 (Bio-Rad Laboratories, Inc., Hercules, CA) size-exclusion chromatography mini-column and the protein concentration was determined using the Bradford Assay (Bio-Rad Laboratories, In., Hercules, CA). The resultant filtrate was diluted 1:100 with 20 mM PBS.

Fluorescent detection of oxidative stress

Oxidative stress was assessed through the measurement of reduced protein thiol content using the sulfhydryl-specific maleimide fluorescent dye, ThioGlo-1 (Calbiochem Inc., San Diego, CA) as previously described [17-18]. The total reduced protein thiol content is the reciprocal of the total oxidized protein content. The fluorescent emission of ThioGlo-1-protein sulfhydryl adducts, therefore, is an accurate measure of the global protein redox status [19]. Freshly prepared placental homogenates and thawed serum were analyzed using real-time kinetics mode of a QM-4 fluorometer (Photon Technology International, Inc., Piscatway, NJ; Figure 2). Saturation ThioGlo-1 fluorescence was normalized for the total protein content of each sample.

Statistical Analysis

Continuous data were described as medians (IQR), and categorical data were described by frequencies (column percents). The primary outcome was ThioGlo-1 emission, normalized for protein content (counts/sec/µg protein). Group comparisons between obese and lean women were performed using Wilcoxon rank sum tests or Chi-square tests as appropriate. Linear relationships between placental and serum ThioGlo-1 emission and between gestational age and ThioGlo-1 emission were assessed by Spearman rank correlation coefficients. P-values <0.05 were considered statistically significant for all tests (SAS 9.1.3 (Cary, NC)).

Results

A total of 44 subjects (22 matched pairs) were enrolled. There were no differences in background or demographic variables (Table 1). Maternal obesity was associated with a 31% median increase in placental oxidative stress compared to lean controls (Figure 3a). Thirty-two subjects (16 in each group) also had serum available for peripheral

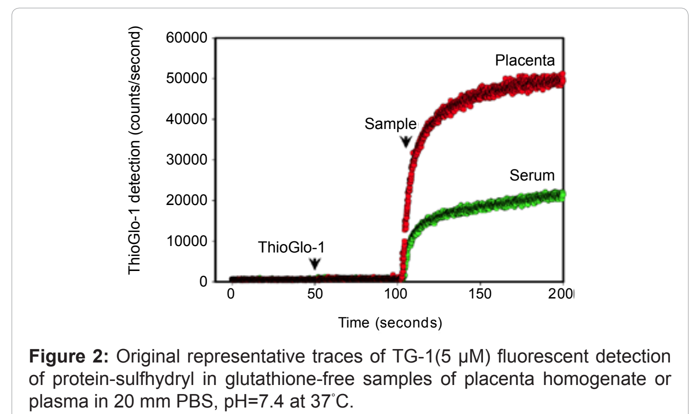
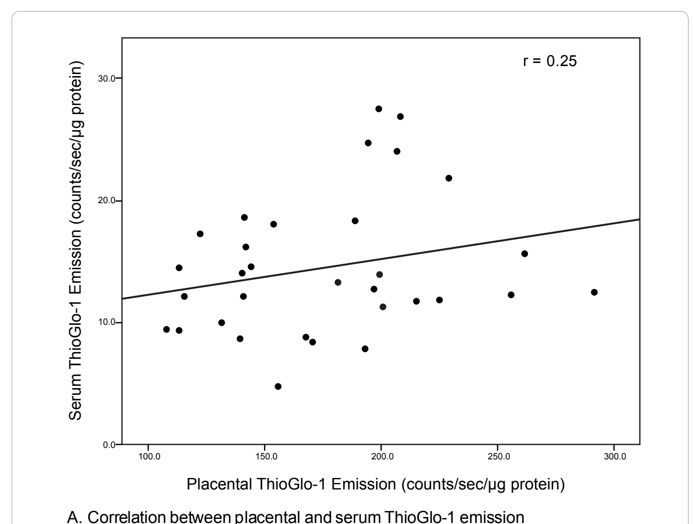


Figure 2: Original representative traces of TG-1(5 µM) fluorescent detection of protein-sulfhydryl in glutathione-free samples of placenta homogenate or plasma in 20 mm PBS, pH=7.4 at 37°C.

Background Characteristics and Placental Oxidative Stress			
Variable	Obese (n=22)	Lean (n=22)	p-value
Age (y)	22.5 (21-26)	21.5 (20-25)	.45
Gestational age (wk)	9.4 (9-10)	9.4 (9-10)	.82
BMI	35.0 (34-38)	22.0 (21-23)	<.001
Gravidity	2.0 (1-3)	1.5 (1-3)	.36
Parity	1.0 (0-2)	0.0 (0-1)	.06
Race/Ethnicity			
White	8 (31.8%)	8 (31.8%)	1.0
Black	14 (63.2%)	14 (63.2%)	
Smoking	3 (13.6%)	3 (13.6%)	1.0
Chronic hypertension	1 (5%)	0 (0%)	1.0

Table 1: Continuous variables presented as medians and interquartile range (IQR) and analyzed by Wilcoxon rank sum tests. Categorical variable presented as frequencies and column percents and analyzed by Chi-square tests.



A. Correlation between placental and serum ThioGlo-1 emission

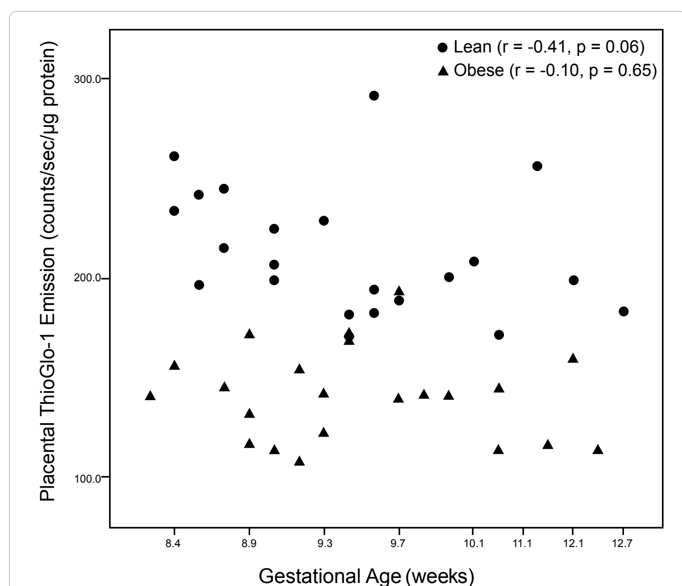
Figure 3a: First trimester ThioGlo-1 emission (513 nm) in obese compared to lean women. The top and bottom bars represent the full range of observations. placental homogenates (n=44, medians [IQR]=203.7 [189-234] and 141.1 [117-156] for normal weight and obese subjects, respectively, p<0.001).

oxidative stress assessment. A similar, but non-significant increase in serum oxidative stress was found (Figure 3b). There was no significant correlation between placental and serum oxidative stress either within groups (obese r=-0.11, p=0.67; lean r=-0.06, p=0.83) or overall (r=0.25, p=0.17, Figure 4a). There was also no correlation between gestational age and placental (r=-0.16, p=0.28) or serum (r=0.26, p=0.14) oxidative stress (Figure 4b). Overall, the level of oxidative stress measured in the placenta was far more pronounced than in the serum (Figure 3a, Figure 3b).

Discussion

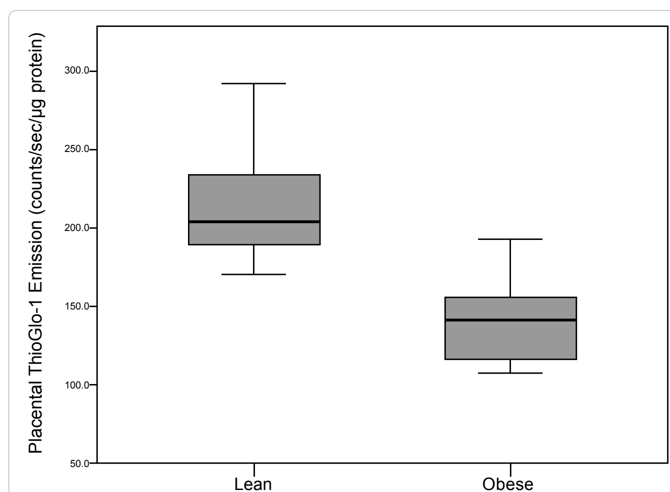
Maternal obesity is an emerging public health concern of enormous clinical impact and research interest. Our data shows for the first time that maternal obesity is associated with placental oxidative stress in the first trimester. This finding supports a first trimester origin for the observed increased rate of placental dysfunction noted in obese women later in pregnancy, although a direct relationship cannot be established from this investigation [10-15]. Oxidative stress is increasingly viewed as an upstream process resulting in inflammation and cellular injury. Indeed, maternal obesity is associated with robust placental inflammation at term Challier et al. [20] demonstrated that mRNA expression of TNF-α and other pro-inflammatory cytokines are elevated in placentas of obese women compared to lean women at term [20]. These investigators found that an accumulation of activated $CD14^+$ macrophages in the placenta was considered the primary source for these pro-inflammatory cytokines [20]. Other investigators have found that placental mitochondrial ROS production is stimulated by TNF-α [21]. Therefore, increased obesity-related placental inflammatory cytokines may promote further production of ROS and induce a feed forward cycle of placental cellular damage [22].

Our finding that global placental redox status in obese women is independent of gestational age was surprising. A common belief is that placental oxidative stress occurs only after 10 weeks of gestation, based on the observation that only after this time point does the fetal circulation come into direct contact with the uterine spiral arteries and the intervillous oxygen tension rises sharply (pO_2=50 mmHg) [23,24]. Prior to this period, the fetal environment is very hypoxic (pO_2<20 mmHg) [24].The reason for independence of oxidative stress for gestational age in obese women is unclear. There are two potential explanations for this phenomenon. The first is hypoxia-induced production of ROS from the placental mitochondrial electron transport chain [2]. The second involves *a priori* increased NADPH oxidase activity in the placenta of obese women. In fact, NADPH oxidase appears to be the major contributor of ROS production in non-pregnant obese women [8,25,26]. Our findings differ from those earlier reported by Roberts et al. [27] who found no direct relationship between lean, overweight, and obese BMI classes and oxidative stress [27]. The disagreement between our two studies most likely reflect that their study: 1) was limited to term gestations without pregnancy complications; 2) contained a very limited number of subjects (7 lean, 5 overweight, and 8 obese), which may have limited the power to detect increases in oxidative stress; 3) used quasi-quantifiable



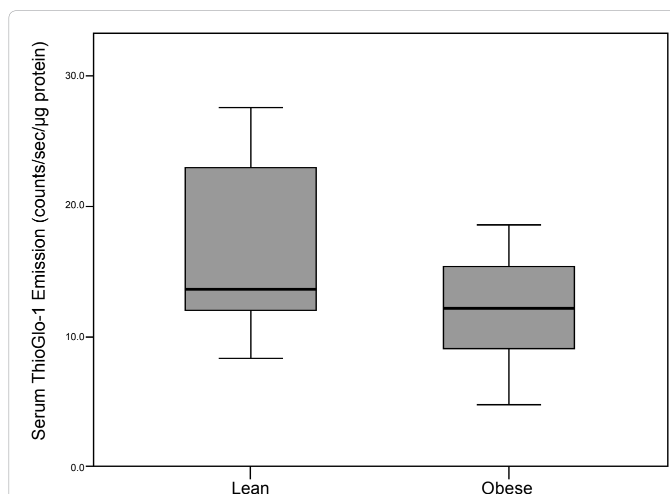
B. Correlation between placental ThioGlo-1 emission and gestational age

Figure 3b: Serum (n=32, medians=13.6 [12-23] and 12.2 [9-15] for normal weight and obese subjects, respectively, p=0.09).



A. Placental ThioGlo-1 emission

Figure 4a: Relationships between placental oxidative stress and peripheral oxidative stress and gestational age. Spearman-rank correlation between placental ThioGlo-1 emission (513 nm) and serum ThioGlo-1 emission (513 nm) (n=32, p=0.17).



B. Serum ThioGlo-1 emission

Figure 4b: Spearman-rank correlation between placental ThioGlo-1 emission (513 nm) and gestational age (n=44, overall p=0.26).

methods, such as western blot, to measure placental oxidative stress; and 4) recruited both laboring and non-laboring women [27]. In contrast, our study examines the induction of placental oxidative stress at the critical time period following placentation. Further, our study was not confounded by the increase in oxidative stress known to be associated with parturition [17,28]. Finally, Roberts et al. [27] selected for uncomplicated pregnancies [27], which may have reduced the likelihood of detecting placental oxidative stress, while our sample is unselected. Another advantage of our study is that our subjects were matched for important baseline variables that could potentially affect oxidative stress. Consistent with non-pregnant studies [8], the level of oxidative stress measured in the serum was slightly increased in our obese subjects. However, only a subset of subjects had serum available for analysis, limiting our power to establish statistical significance.

There are numerous advantages to the assessment of placental oxidative stress as described and performed in our study. First, Hansen et al. demonstrated the appropriateness of determination of the global cellular sulfhydryl status as an indicator of oxidative stress [19]. Protein cysteine residues (e.g. sulfhydryls) are considered to be redox switches and mediate oxidative and nitro sative stress-induced signaling events that are critical to cell fate. Rather than assaying a specific component of the oxidative stress (e.g. antioxidant levels), which may fail to detect oxidative stress, tissue assessment of protein redox status is highly sensitive. Second, this direct measurement of the total protein redox status is straightforward and reproducible. Third, samples can be stored and assayed in batch, further reducing variability within the assay.

We anticipate that our approach affords the opportunity to identify obesity-related mechanisms of placental oxidative stress in the first trimester. Further, our study implicates pre-pregnancy maternal obesity as a potential first-trimester marker for selection of those whom may be candidates for antioxidant trials for the prevention of adverse pregnancy outcome.

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