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Effect of Increasing Maternal Body Mass Index on Oxidative and Nitrative Stress in the Human Placenta

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Abstract

Maternal obesity is an increasing problem in obstetrics associated with adverse pregnancy outcomes and delivery complications. As an inflammatory state, where elevated levels of pro-inflammatory cytokines are found, obesity can lead to the increased incidence of oxidative and nitrative stress. These stresses may result in protein oxidation and protein nitration respectively, which are post translational covalent modifications that can modify the structure and subsequently alter the function of a protein.

The objective of this study was to examine whether placental oxidative and nitrative stress increase with increasing maternal body mass index.

Placental tissue was collected from three groups of patients categorized as lean, overweight and obese. The presence of nitrotyrosine residues, a marker of nitrative stress, and anti-oxidant enzymes, as markers of oxidative stress, were assessed by immunohistochemistry, western blot and ELISA. Protein carbonyl formation, a specific measure of protein oxidation, was measured by Oxyblot™ kit.

Nitrotyrosine residues were increased in obese compared to lean and overweight groups although localization was unaltered across the three groups. Superoxide dismutase enzyme expression, localization and activity was unaltered between the groups. Protein carbonyl formation was greater in the lean compared to the overweight individuals.

This study demonstrates that with increasing maternal body mass index there is an increase in placental nitrative stress. There does not appear to be a corresponding increasing in oxidative stress and indeed we demonstrate some evidence of a decrease in oxidative effects in these placenta samples. Potentially the formation of peroxynitrite may be consuming reactive oxygen species and reducing oxidative stress. There may be a shift in the balance between nitrative and oxidative stress, which may be a protective mechanism for the placenta.

Keywords

Nitrative stress; Oxidative stress; Obesity

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Introduction

Obesity, which is defined as a body mass index (BMI) of ≥ 30 kg/m^2, has become a major health problem in the US. Approximately 50% of reproductive age women are overweight (BMI range 25 – 29.9 kg/m^2) with a significant proportion of those being obese [1]. Obesity is an inflammatory condition which in pregnancy is linked to poor perinatal outcome and increased incidence of obesity, insulin resistance, hypertension and cardiovascular disease of the offspring in adult life [2]. Nutrient and metabolic surplus initiate the release of a similar set of molecules and signaling pathways to those seen in classical inflammation [3]. For example, obesity is associated with increased levels of pro-inflammatory cytokines such as Interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) and decreased levels of adiponectin, in the maternal bloodstream [4]. Increased macrophage accumulation and heightened levels of pro-inflammatory cytokines have also been demonstrated in the placenta of pre-gravid obese women compared to lean controls [5].

Along with cytokine production, inflammation is also linked to oxidative stress in the placenta [6]. Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS), such as superoxide, and the ability of anti-oxidant enzymes to scavenge ROS [7]. Pregnancy per se is a state of oxidative stress due to the high metabolic activity of placental mitochondria that generate ROS, and also to superoxide generation from NADPH oxidase [8]. Heightened ROS production occurs in a physiological role at certain stages of placental development as well as under certain pathological circumstances such as in preeclampsia, diabetes and intrauterine growth restriction [9-10].

There are several antioxidant defense mechanisms found within cells, these include the superoxide dismutase enzymes (SOD), of which there are three forms; manganese (MnSOD), copper zinc (CuZnSOD) and extracellular (ECSOD) (For a comprehensive review see [11-12]). MnSOD and ECSOD are localized in the mitochondrial matrix and on the outer surface of cell membranes respectively whilst CuZnSOD is found in the cytosol [13]. The SOD enzymes dismutate the superoxide radical to hydrogen peroxide, which is then reduced to H_2O by another antioxidant enzyme, glutathione peroxidase [14]. In the absence of SOD, superoxide is a fairly stable molecule with a half life of 5 seconds [13] indicating the potential of this free radical to cause extensive damage within a tissue if inappropriately scavenged.

Metal catalyzed oxidation of proteins results in the introduction of carbonyl groups (i.e. aldehydes and ketones) at arginine, lysine, proline and threonine residues in a site specific manner [15-16]. Measurement of carbonyl group formation is the most common indicator of protein oxidation [17]. As a result of oxidative covalent modifications the biochemical characteristics of proteins, for examples enzymatic activity and susceptibility to proteolytic degradation, may be altered.

In addition to superoxide, the placenta can also produce nitric oxide ($NO^{\cdot}$) and this molecule in combination with excess superoxide can result in the production of peroxynitrite ($ONOO^-$), leading to nitrative stress. Peroxynitrite is a powerful pro-oxidant that can modify tyrosine residues within a protein sequence to give nitrotyrosine. This post-translational modification can subsequently alter the function of a protein and is referred to as protein nitration. Nitrotyrosine residues have been previously demonstrated in the placenta of pregnancies complicated by preeclampsia [10], pre-gestational diabetes [9] and chronic hypoxia at high altitude [18]. Additionally, we have previously shown nitration of several proteins in the human placenta and have demonstrated that the extent of nitration is increased in pregnancies complicated by preeclampsia [19-21]. Indeed nitration of p38MAP Kinase in the placenta results in its reduced catalytic activity i.e. downregulation of function in preeclampsia [19;22].

Thus it appears that in pathological conditions there is evidence for increased oxidative and nitrative stress in the human placenta. However it is not currently known whether oxidative or nitrative stresses are heightened in the placenta of obese individuals, where there is an inflammatory response. The objective of this study was to examine the effect of increasing maternal BMI on nitrative stress, anti-oxidant markers of oxidative stress and protein oxidation in the human placenta.

Materials and Methods

Materials

All reagents were analytical grade purchased from standard suppliers unless otherwise stated.

Sample collection

Human placentae were collected at term following informed consent and all studies were conducted in compliance with the Institutional Review Board of the University of Cincinnati. Samples were collected from three patient groups of differing BMI: lean (BMI 18.5-24.9 kg/m^2), overweight (BMI 25-29.9 kg/m^2) and obese (BMI 30-40 kg/m^2). Patient characteristics are summarized in Table 1. Patients in these groups had no other complications of pregnancy and no reported co-morbidities such as smoking or drug use. Villous tissue was dissected from beneath the basal plate at 3 distinct sites within 30 minutes of delivery, rinsed in PBS, flash frozen in liquid N_2 and stored at -80°C.

Immunohistochemistry

Placental villous tissue was sectioned at 7μm and immunostained using VectaStain Elite ABC kits. In brief, frozen sections were allowed to air-dry, re-hydrated in PBS and blocked with blocking serum for 30 minutes in a humid box at room temp. A minimum of five slides per patient group were incubated with anti-nitrotyrosine (1:100, Millipore), anti-MnSOD (1:100, Chemicon International), anti-CuZnSOD (1:50, Millipore), or anti-ECSOD (1:50, Sigma) primary antibody overnight in a humid box at 4°C. Slides were washed three times for 5 minutes each with PBS before 1 hour incubation with secondary antibody at 37°C. Slides were washed three times for 5 minutes with PBS prior to incubation with the ABC complex for 30 minutes at room temp followed by a further three 5 minute washes in PBS. Aminoethylcarbazole was used as the peroxidase substrate and allowed to develop for 3 minutes before a brief rinse in PBS and counterstaining with hematoxylin. Samples were mounted with glass coverslips using PBS/glycerol (1:9). Negative control sections were included in the absence of primary antibody.

Tissue preparation

Protein was extracted from villous tissue taken from three distinct areas of each placenta and pooled, following homogenization in lysis buffer containing 20mM Tris (pH 7.5), 1mM EDTA, 1mM EGTA, 20mM sodium fluoride, 0.15M sodium chloride, 0.5% Nonidet P-40, 0.5% Triton X-100, 200μM sodium orthovanadate, 2μM leupeptin, 5.8μM pepstatin, 200μM 4-(2-aminoethyl) benezenesulfonyl fluoride hydrochloride (AEBSF) and 5μM N-tosyl-L-lysine chloromethyl ketone (TLCK). Homogenization was carried out on ice using a polytron tissue tearer at high speed for 10 second intervals until smooth. Homogenate was centrifuged for 5 minutes at 20,000×g to remove cell debris from the placental samples. The pellet was discarded and supernatant stored at -80°C until use. Quantification of protein was determined using the BCA assay (Pierce).

Western Blotting

Protein samples (20μg) were heat-denatured at 95°C for 5 minutes in Laemmli buffer (125M Tris, 4.1% SDS, 40mM urea, 20% glycerol, 0.002% bromophenol blue) and separated on either 8-16% or 16% Tris-glycine pre-cast gels (Invitrogen) using a mini-gel electrophoresis system (Bio-Rad Laboratories) at 35mA per gel for 80 minutes. Protein was transferred to nitrocellulose membranes using the iblot dry transfer system (Invitrogen). Equal protein transfer was assessed using Ponceau S staining (Sigma-Aldrich). Following transfer, membranes were blocked for 2 hours with Tris buffered saline/0.1% Tween 20 (polyoxyethylene sorbitan monolaurate, TBST) containing 5% bovine serum albumin (BSA).

Membranes were incubated with either an anti-nitrotyrosine (1:2000, Millipore), anti-MnSOD (1:500, Chemicon International), anti-CuZnSOD (1:2000, Millipore) or anti-ECSOD (1:500, Sigma) primary antibody overnight at 4°C in 1% BSA prepared in TBST. (To confirm equal protein loading membranes were later stripped using Re-blot solution (Chemicon International) and incubated with anti-β-actin antibody (1:1000) in TBST containing 5% non-fat milk protein). Following primary antibody incubation the membranes were washed four times for 5 minutes each in TBST prior to incubation with a HRP-conjugated goat anti-mouse (nitrotyrosine and MnSOD) or donkey anti-rabbit (CuZnSOD and ECSOD) secondary antibody (Invitrogen) diluted 1:10,000 in TBST containing 5% non-fat milk protein (Mid-America farms) for 1 hour at room temperature. Signals were detected using an enhanced chemiluminescence detection system (ECL; Pierce) and detected on photosensitive film (Hyperfilm-ECL; Kodak). Western blotting was performed in all samples (Lean n=7, overweight, n=5 and obese, n=8) for each primary antibody and protein band intensity was measured using scanning densitometry (Alpha Imager).

Total nitrotyrosine measurement

Total nitrotyrosine residues in the placental homogenates was quantified by ELISA carried out according to manufacturer's instructions (Upstate). Samples (Lean n=6, overweight, n=5 and obese, n=6) were measured in duplicate against known standards and expression of nitrotyrosine was normalized to cellular protein content.

Total superoxide dismutase activity measurement

Total SOD enzyme activity in the placental homogenates was quantified by ELISA carried out according to manufacturer's instructions (Cayman Chemical). All samples (Lean n=7, overweight, n=5 and obese, n=8) were measured in duplicate against known standards and activity of SOD was normalized to cellular protein content.

Glutathione peroxidase activity measurement

Glutathione peroxidase enzyme activity was measured at 1 minute intervals over a period of 5 minutes in a kit-supplied (Cayman Chemical) standard control, allowing a calculation of the change in rate, which was used to determine the enzyme activity in each sample (Lean n=7, overweight, n=5 and obese, n=8), which was normalized to cellular protein.

Measurement of protein carbonyls

Samples were derivatized according to the OxyBlot™ kit instructions (Chemicon International). In brief, protein was prepared at the desired concentration and incubated 1:1 v/v in 12% SDS for 10 minutes. Five samples from each BMI group were incubated 1:1 v/v either in 2,4-dinitrophenylhydrazine (DNPH) or derivatization control solution for 15 minutes at room temperature. Neutralization solution was added to each sample to stop the reaction prior to addition of 2-mercaptoethanol (5% v/v) for protein denaturing. Samples were then loaded

for Western blotting as previously described with the exception of no heat denaturing. Primary and secondary antibodies were OxyBlot™ kit specific.

Statistical analysis

All data are reported as mean ± standard deviation. Statistical differences between the three BMI groups were analyzed using a one way ANOVA with Tukey post test or Kruskal Wallis with a Mann Whitney U test.

Results

Immunolocalization of nitrotyrosine, MnSOD, CuZnSOD, and ECSOD in the placenta

Nitrotyrosine residues were immunolocalized primarily in the endothelial cells of the fetal placental vasculature with some staining observed in both the villous stroma and to a lesser extent in the syncytiotrophoblast (Figure 1 A-C). Discreet MnSOD staining was observed both along the syncytiotrophoblast and in the endothelial cells of the fetal vasculature with more diffuse staining in the villous stroma in close proximity to the fetal vessels but not widespread throughout the stroma (Figure 1 E-G). CuZnSOD is the cytosolic form of SOD and was found diffusely on the syncytiotrophoblast and in some areas of the villous stroma (Figure 1 I-K). ECSOD was immunolocalized extracellularly throughout the stoma with minimal staining observed on the syncytiotrophoblast (primarily on the basal surface) and modest expression in the endothelial cells (Figure 1 M-O). No staining was observed in the absence of primary antibodies as negative controls (Figure 1 D, H, I and P). Overall the localization of nitrotyrosine, MnSOD, CuZnSOD and ECSOD immunostaining was unaltered across the three BMI groups.

Nitrotyrosine, MnSOD, CuZnSOD and ECSOD protein expression in the placenta

Western blotting was used to assess the expression of nitrated proteins in the lean, overweight and obese samples (A representative blot showing three samples from each group is shown in Figure 2Ai). Several nitrated protein products were observed across a range of molecular weights. All blots were stripped and re-probed for β-actin as a loading control (Figure 2Aii). There were no obvious differences between patterns of nitrated protein expression across the groups – i.e. the presence or absence of a band in one BMI category, however the number of bands detected made it difficult to quantify the expression of nitrated proteins.

MnSOD was detected as the 25kDa monomer form of this protein in the placenta samples. Expression of MnSOD varied across the three BMI groups (A representative example showing four samples from each group is shown in Figure 2Bi). Despite this apparent variation in expression, measurement of band intensity by scanning densitometry corrected for β-actin (Figure 2Bii) showed no overall difference across the three BMI groups (data not shown). CuZnSOD was detected at the expected protein product size of 16kDa in each placenta sample (A representative example showing four samples from each group is shown in Figure 2Ci), yet expression was unaltered across the three groups when densitometry data (not shown) were normalized to β-actin expression (Figure 2Cii). Similarly, the double protein band at the predicted molecular weights of 30-36kDa for ECSOD was detected in all patient samples (A representative example showing four samples from each group is shown in Figure 2Di). Measurement of band intensity by scanning densitometry corrected for β-actin (Figure 2Dii) showed no overall difference across the three BMI groups (data not shown).

Total nitrotyrosine expression

Total nitrotyrosine concentrations (mean ± SD in μg/mg of protein) quantified by ELISA were lean; 0.17 ± 0.03 (n=6), overweight; 0.15 ± 0.03 (n=5) and obese; 0.34 ± 0.09 (n=6, Figure 3A). There was an overall difference in total nitrotyrosine residues across the three groups

($p<0.001$ One Way ANOVA) and nitrotyrosine expression in the obese group was significantly increased compared to both the lean and overweight groups ($p<0.001$, Tukey post hoc test).

Total SOD activity

Total SOD enzyme activity (mean ± SD in U/mg protein) quantified by ELISA were lean; 0.34 ± 0.14 (n=7), overweight; 0.19 ± 0.06 (n=5) and obese; 0.37 ± 0.27 (n=8, Figure 3B). There was no statistical significant difference in SOD activity across the three BMI groups.

Glutathione peroxidase enzyme activity

Glutathione peroxidase enzyme activity (mean ± SD in nmol/min/mg protein) quantified by ELISA were lean; 4.6 ± 1.4 (n=7), overweight; 4.2 ± 2.2 (n=5) and obese; 7.3 ± 4.3 (n=8, Figure 3C). Despite the trend towards increased glutathione peroxidase activity in the obese group there was no statistically significant difference across the groups ($p=0.143$ one way ANOVA).

Oxidized protein expression

Several oxidized protein products were detected in the placenta samples over a range of molecular weights (A representative blot showing oxidized protein expression in three lean and three obese samples is given in Figure 4A). Oxidized protein expression was examined in five patients from each of the 3 BMI groups and three protein bands (Labeled 1-3 on Figure 4A) were selected for further analysis by densitometry. Densitometry data are presented as relative expression as a percentage of the most intense individual sample for each band (Figure 4B 1-3). Overall nonparametric analysis demonstrated the following significances, band 1 ($p<0.008$), band 2 ($p<0.03$), and band 3 ($p<0.02$, Kruskal-Wallis). Statistical differences between the groups were assessed by Mann-Whitney U test and are indicated on the individual graphs (Figure 4B) where we demonstrate a trend towards increased protein carbonyl formation in the lean compared to obese individuals (data did not reach significance) and significantly greater carbonyl expression in lean individuals compared to overweight. Additionally the combined band intensity from 1, 2, and 3 was analyzed for differences both overall ($p<0.008$, Kruskal-Wallis) and between groups (as indicated on Figure 4C, Mann-Whitney U test).

Discussion

Maternal obesity is becoming a major health consideration for successful pregnancy outcome. Many researchers are currently investigating the effect of maternal obesity on different aspects of placental function and fetal development. Here we have examined the effect of increasing maternal BMI on oxidative and nitrative stress in the human placenta. This study appears to demonstrate that with increasing maternal BMI there is an increase in nitrative stress yet the evidence for altered oxidative stress is conflicting. On the one hand it appears that antioxidant enzymes are not significantly altered by maternal BMI although there may be evidence to suggest a trend towards increasing expression of glutathione peroxidase with obesity. However, carbonyl formation, a protein modification resulting from oxidative stress, demonstrates the converse of this with higher amounts seen in lean individuals when compared to obese.

It is important to note the mode of delivery in our patient groups (Table 1) as each BMI group contains samples from both vaginal deliveries and Caesarian section deliveries. There is some evidence in the literature demonstrating that labor contributes to increased oxidative stress in the placenta [23]. In this particular study Cindrova-Davies and colleagues [23] examined the effect of duration of labor in short (<5hrs) and long term labor (>15hrs) groups in comparison to a non laboring Caesarean section group of women, on a wide range of markers of oxidative stress and antioxidant enzymes. Interestingly their data showed that indeed several placental markers of oxidative stress where increased as a result of labor yet in some cases e.g. MnSOD long labor returned protein expression levels to those of the non-labor patients [23]. Clearly

oxidative effects are susceptible to many uncontrollable variables and in collection of data from human subjects it is difficult to avoid individual patient variables. Valid statistical comparisons are difficult to perform on small data sets however all of our data were analyzed with the values stratified by mode of delivery and no statistical significances were demonstrated (data not shown in this format). Regardless, the possibility of some contribution of labor to our results can not be ruled out.

Also noted is the fact that the patient BMI's are reported at delivery rather than pre-pregnancy. This was unavoidable as pre-pregnancy weights were not available in 4 out of the 20 cases. With these 4 individuals their delivery BMI's were at the high end of the BMI classification range and if appropriate weight gain is assumed it is considered likely that they began pregnancy in the same BMI grouping. In all other cases we ensured that each patient began and finished pregnancy within the same BMI group. Indeed this is one reason why the overweight group has a slightly lower ‘n’ than the lean and obese groups.

Immunohistochemical localization of nitrotyrosine residues has previously been examined in the human placenta as a means of demonstrating the formation and action of peroxynitrite in this tissue [24]. In the present study nitrotyrosine was immunolocalized primarily to the endothelial cells of the fetal placental vasculature with faint staining in the villous stroma, and minimal staining in the syncytiotrophoblast. This appears to concur with previous findings where, for example, in pre-gestational diabetes nitrotyrosine residues were localized in the endothelial cells and the villous stroma surrounding the fetal vessels and again minimal nitrotyrosine residues were found on the syncytiotrophoblast [9]. Peroxynitrite has a relatively short diffusion distance of approximately 5μm. Therefore the discrete localization of nitrotyrosine residues indicates local generation of peroxynitrite and its predominant site of action to be in the vascular endothelium. MnSOD was immunolocalized primarily to the syncytiotrophoblast and the vascular endothelium. CuZnSOD was diffuse on the syncytiotrophoblast and ECSOD immunostaining was somewhat more diffuse in the extracellular space of the villous stroma and similar to MnSOD in the fetal vasculature. The localization of the SOD enzymes is indicative of principal sites of superoxide generation.

Several nitrated protein products were detected in the placenta samples used in this study thus demonstrating that nitrative stress and the subsequent nitration of proteins is fairly abundant in the human placenta. Quantification of the intensity of nitrated protein expression demonstrated by Western blot was made difficult by the number of individual bands therefore using a quantitative ELISA method we demonstrated an increase in nitrotyrosine residues with increasing BMI. This was in agreement with our original hypothesis which suggests that maternal obesity is linked to increased nitrative stress and altered protein function. Conversely we did not find a corresponding increase in total SOD or glutathione peroxidase enzyme activity with increasing BMI. We demonstrated the presence of several oxidized proteins in the placenta samples. The intensity of protein carbonyl formation was significantly greater in the lean versus overweight patients and although the data were not statistically significant we consistently saw a trend of increased protein carbonyls in the placenta of lean compared to obese individuals.

Our expectations were that both oxidative and nitrative stress would increase with obesity. Formation of nitrotyrosine has been extensively examined in a wide variety of organ systems and is well documented as an indicator of nitrative stress. However, the choice of possible candidates, as markers of oxidative stress, is far greater making it harder to choose a clear endpoint to examine. Decreased total SOD activity has been demonstrated in placenta from pregnancies complicated by preeclampsia [25] and from pregnancies at high altitude (chronic hypoxia) [18]. Data such as these suggest that decreased activity reflects an impairment of antioxidant enzyme action, possibly due to consumption of scavenging oxidants, and would result in increased oxidative stress. However, Zamudio and colleagues were unable to

demonstrate an association between the decreased antioxidant activity seen with chronic hypoxia and increased lipid peroxide content and protein carbonyl formation i.e. protein oxidation effects [18]. Furthermore glutathione peroxidase activity in preeclamptic placentae has been shown to be both downregulated [25] and upregulated [26]. Conflicting data could raise the question as to whether the presence of these antioxidant enzymes is indicative of increased ROS and thus the need for more scavengers, or whether more enzyme reflects an excess in a circumstance where ROS production is low and scavenging is minimally required.

Although in strict chemical terms peroxynitrite is a powerful oxidizing agent that gives nitration, one possible explanation of our findings is that as peroxynitrite forms the reactive oxygen species that contribute to oxidative stress are consumed by their reaction with reactive nitrogen species and thus nitrative stress effects increase whilst ‘oxidative’ effects, in this case the formation of protein carbonyls, diminish. However, although peroxynitrite predominantly causes nitration of tyrosine residues it has also been shown to be one of several molecules that can contribute to oxidation of proteins [27]. Thus the explanation for our findings may not be this simplistic as nitrative and oxidative stress do not arise independently of one another. However it seems possible that there may be a shift in the balance between oxidative and nitrative stress and this may in fact be indicative of a protective mechanism adapted by cells to reduce the harmful effects of free radicals. Further investigation into the balance between superoxide and nitric oxide consumption and interaction, in relation to maternal BMI, is needed to more clearly demonstrate the relationship between oxidative and nitrative stress in the human placenta and the possible subsequent effects on protein modification and altered function.

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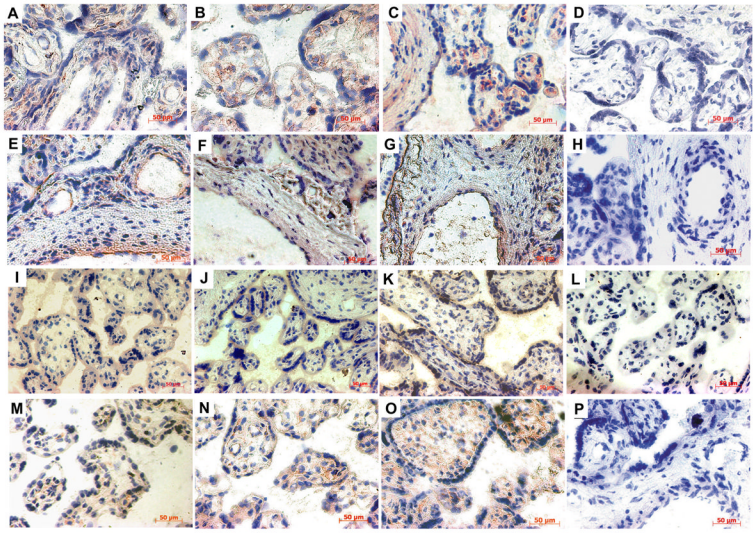

Figure 1.

Immunolocalization of nitrotyrosine residues (A-C), MnSOD (E-G), CuZnSOD (I-K) and ECSOD (M-O) in placental sections from lean (LN; A, E, I, M), overweight (OV; B, F, J, N) and obese (OB; C, G, K, O) patients. Negative control sections where primary antibody was omitted (D, H, L, P). Original magnification 200×; scale bar = 50μm. Positive staining is indicated by the arrow heads (▶).

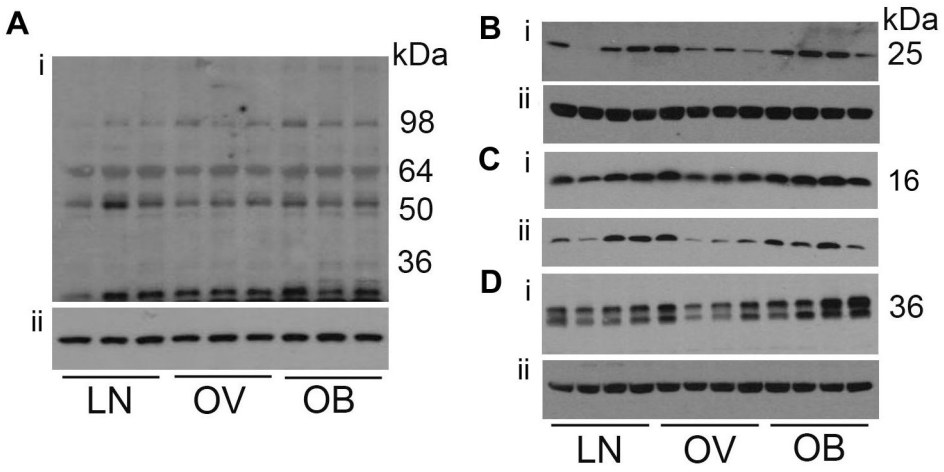

Figure 2.

Representative Western blots demonstrating A (i); nitrated protein expression, B (i); MnSOD monomer expression, C (i) CuZnSOD and D (i); ECSOD expression in lean (LN), overweight (OV) and obese (OB) patient placental samples. A-D (ii); Corresponding blots from A-D (i) stripped and re-probed with β-actin as a protein loading control. Molecular weight markers are indicated (kDa).

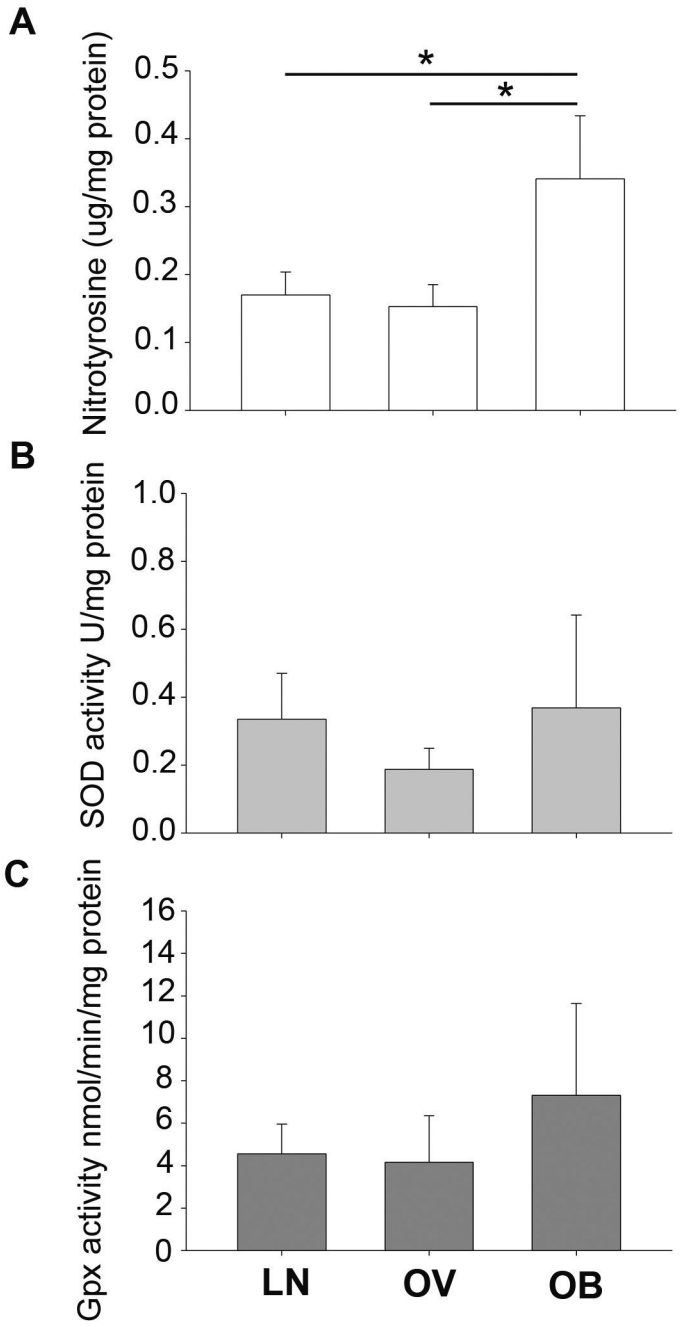

Figure 3.

ELISA data showing A; total nitrotyrosine residue levels, B; total superoxide dismutase (SOD) enzyme activity and C; glutathione peroxidase (Gpx) enzyme activity in lean (LN), overweight (OV) and obese (OB) patient placental homogenate samples. *p<0.001 one way ANOVA with Tukey post hoc test.

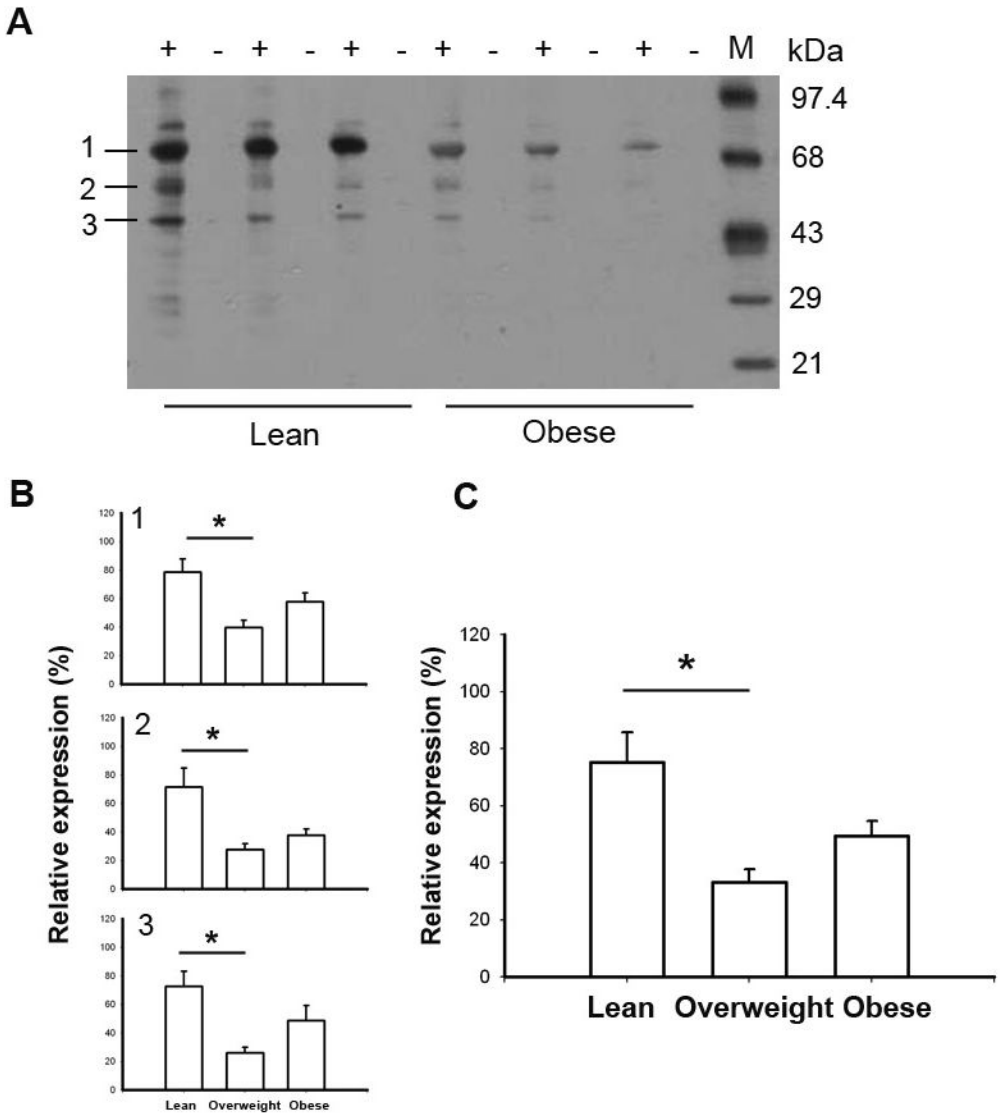

Figure 4.

A; Representative Oxyblot showing derivatized (+) and paired non-derivatized controls (-) for three lean and three obese patients. Molecular weight markers are indicated (M) and sizes reported (kDa). Three bands were selected for densitometric analysis (labeled 1-3). B; Graphs showing densitometry data for bands 1, 2 and 3, * $p<0.05$ Mann Whitney U test. C; Graph showing the combined total densitometry data for bands 1-3, * $p<0.05$ Mann Whitney U test.

Table 1

Clinical characteristics of the subjects and their offspring.

Characteristic	Lean (n=7)	Overweight (n=5)	Obese (n=8)
Maternal age (years)	24 ± 7	22 ± 2	24 ± 5 *NS*
Gestational age (weeks)	39 ± 1	39 ± 2	39 ± 1 *NS*
BMI at delivery (kg/m^2)	23.6 ± 1.3 §	28.5 ± 0.7 §	37.2 ± 2.2 §
Fetal weight (grams)	2906 ± 383 ¶	3167 ± 319	3607 ± 696 ¶
Mode of delivery (VD:CS)	2:5	3:2	2:6

All data are mean ± SD.

NS not significant,

§ p<0.001 all groups,

¶ p<0.05 lean v obese (one way ANOVA with Tukey post hoc test). VD; vaginal delivery and CS; Caesarian section.