Melatonin Improves Mitochondrial Respiration in Syncytiotrophoblasts From Placentas of Obese Women

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Abstract

Maternal obesity is associated with increased oxidative stress but decreased placental mitochondrial respiration and expression of mitochondrial electron transport chain (ETC) complexes I to V. Melatonin acts as an antioxidant and prevents oxidative stress-induced changes in cytotrophoblasts. Placentas were collected at term by cesarean delivery from obese (first trimester body mass index [BMI] ≥30, n = 10) or lean (BMI < 25, n = 6) women. Cytotrophoblasts were isolated and allowed to syncytialize for 72 hours with or without melatonin (0.1-100 μM) for the last 24 hours. Mitochondrial respiratory parameters were measured in a Seahorse XF24. Expression of ETC complexes I to V and antioxidant enzymes was measured by Western blot. Maternal clinical characteristics of patients were similar except for BMI. No significant improvement in mitochondrial respiration occurred with addition of melatonin to trophoblasts of lean women. However, in trophoblasts from obese women, melatonin (10 and 100 μmol/L) significantly increased maximal respiration ($P = .01$ and $P = .009$, respectively) and spare capacity ($P = .02$ and $P = .003$, respectively) compared to the untreated control. No differences were detected in the expression of ETC complexes and superoxide dismutase 1 or 2 in trophoblasts treated with melatonin. The expression of glutathione peroxidase, which was significantly greater in trophoblast of obese compared to lean women ($P < .05$), was decreased back to the level seen in trophoblast of lean women with addition of melatonin ($P = .02$). Improved spare respiratory capacity, the cellular reserve, could impart a protective effect to the placenta and fetus in an adverse intrauterine environment or in response to additional stressors.

Introduction

The World Health Organization recognizes obesity as a global pandemic.[1] The number of reproductive-age women who are overweight or obese is increasing with 31.9% of reproductive-aged women (20-39 years) in the United States being obese (body mass index [BMI] ≥30 kg/m^2.[2,3] Obesity in pregnant women can lead to immediate complications for both mother and infant during pregnancy and may also have adverse long-term health consequences for the child. Overweight and obese women are at increased risk of gestational diabetes, hypertension, preeclampsia, and cesarean delivery.[4,5] Fetal complications of maternal obesity include prematurity, stillbirth, congenital anomalies, macrosomia, and subsequent childhood and adolescent obesity.[6-8]

Pregnancy is a state of oxidative stress,[9] which becomes exaggerated with pathologic conditions including obesity.[10] Oxidative stress is described as an imbalance in the production of reactive oxygen species (ROS) and the ability of the antioxidant defenses to scavenge them. The capacity of the placental antioxidant defenses to counteract the effects of highly reactive and potentially damaging free radicals is critical for healthy placental function and optimal growth and development of the fetus.[11] Placental mitochondria are a major site for ROS production and themselves become a target for ROS-induced damage and can be severely compromised by prolonged oxidative stress.[12-14] Mitochondrial respiration can be measured in intact syncytiotrophoblast from human term placentas and provides a physiologic readout of function.[15] Endogenous homeostatic ROS levels are tightly regulated by major

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antioxidant enzymes,[16] which are present in the human placenta.[10,17] These include the primary antioxidant enzyme superoxide dismutase (SOD) which converts superoxide to hydrogen peroxide and is found in both cytosol (SOD1) and mitochondria (SOD2) together with glutathione peroxidases (GPxs) found in both the mitochondrial matrix and the cytoplasm as part of a secondary defense mechanism catalyzing the conversion of hydrogen peroxide to water.[18] When the balance between prooxidant and antioxidant activities is lost, deleterious outcomes such as syncytiotrophoblast apoptosis occurs.[19]

Placental mitochondrial function is compromised in pregnancies complicated by obesity. Syncytiotrophoblasts from placentas of obese women have significantly reduced mitochondrial respiration, decreased adenosine triphosphate (ATP) production, decreased expression of mitochondrial electron transport chain (ETC) complexes I to V, decreased activity of mitochondrial ETC complex I, and abnormal metabolic flexibility.[20,21] In obesity, there are increased placental oxidative stress markers including increased levels of reduced glutathione and increased expression of catalase and SOD[22] as well as a 14-fold increase in ROS production.[20]

Melatonin, an indoleamine that is rhythmically secreted in regulation of circadian rhythm, is a potent antioxidant that participates in the physiologic regulation of mitochondrial homeostasis. Melatonin improves mitochondrial function, reduces oxidative stress, and increases activity of the respiratory chain[23,24] and acts directly by scavenging ROS and also indirectly by inducing gene expression of antioxidant enzymes including GPx, glutathione reductase, SOD, and catalase.[16,25-27] Extrapineal sources of melatonin include local synthesis of melatonin in human trophoblasts where its receptors are also expressed.[28] In vitro treatment of human cytotrophoblast with melatonin has been reported to reverse hypoxia/reoxygenation (H/R)-induced damage.[29] Fetal tissues, especially the brain, are vulnerable to the effects of ROS.[30] In an ovine model, maternal antenatal melatonin administration has been shown to improve fetal outcomes as evidenced by decreased newborn neurodevelopmental deficits and brain injury caused by intrauterine growth restriction (IUGR).[31] There are currently phase I trials underway using antenatal maternal melatonin administration to establish whether melatonin will clinically benefit women and pregnancies with early-onset preeclampsia and IUGR with pregnancy prolongation and to determine the oxidative stress response in maternal, placental, and fetal circulation.[32,33]

We hypothesized that addition of melatonin to syncytiotrophoblasts isolated from placentas of obese women would lead to (1) improved mitochondrial respiration, (2) increased expression of mitochondrial ETC complexes, and (3) alterations in the expression of the antioxidant enzymes SOD1, SOD2, and GPx4.

Materials and Methods

Study Participants

We obtained placentas immediately following cesarean delivery at term without labor from otherwise uncomplicated

Table 1. Maternal and Pregnancy Clinical Characteristics.[a]

Descriptions	Lean (BMI < 25)	Obese (BMI ≥ 30)	P Value
n	6	10	
Maternal age, years	26.5 ± 2.0	27 ± 1.2	.15
Gravidity	3.0 ± 0.2	3.2 ± 0.3	.64
Parity	1.5 ± 0.2	1.7 ± 0.2	.57
Ethnicity (Hispanic/non-Hispanic)	6/0	10/0	
Early pregnancy BMI, kg/m^2	21.6 ± 1.0	36.9 ± 2.0	<.001
Gestational age at initial prenatal visit, weeks	14.5 ± 2.7	12.3 ± 1.8	.47
Gestational age at delivery, weeks	39.2 ± 0.1	39.3 ± 0.1	.48
Gestational weight gain, lb	24.9 ± 2.8	19.3 ± 3.1	.15
Placental weight, g	565 ± 32	693 ± 67	.09
Infant weight, g	3234 ± 62	3484 ± 91	.06
Infant sex, M/F	4/2	5/5	

Abbreviations: BMI, body mass index; M, male; F, female.
[a]Values are mean ± SEM.

pregnancies in women who were either lean (BMI < 25, n = 6) or obese (BMI ≥ 30, n = 10) during early pregnancy (<17 weeks' gestational age). Deidentified relevant medical information was also collected under a protocol approved by the University of Texas Health Science Center at San Antonio institutional review board, to which pregnant women were recruited following written informed consent.

Isolation and Culture of Primary Cytotrophoblasts

Cytotrophoblasts were isolated from placental villous tissue using trypsin/DNAse digestion and percoll purification as described previously.[15,20] Cytotrophoblasts were plated at a density of ~3.5 × 10^6 cells on 6-well plates or ~8 × 10^5 cells in Seahorse XF24 plates (Agilent, Santa Clara, California) and incubated for 72 hours to allow fusion and differentiation into syncytiotrophoblast. Cells were cultured at 37°C in 5% CO$_2$ in air and media (Dulbecco's modified Eagle medium/Hams F-12, supplemented with l-glutamine, penicillin, streptomycin, gentamicin, and 10% fetal bovine serum) was changed daily.[15,20,34] For the last 24 hours (48-72 hours in culture), cells were cultured with or without melatonin 0.1, 1.0, 10, and 100 μmol/L. Stock melatonin was dissolved in dimethyl sulfoxide (M5250; Sigma-Aldrich, Billerica, MA). Melatonin has been previously shown to have no effect on human chorionic gonadotropin (hCG) production (a biochemical measure of villous cytotrophoblast differentiation rate) of in vitro cultured primary cytotrophoblasts.[29] The melatonin concentrations were based on data from prior studies[29,35] as well the calculated human physiologic dose to a level that would be well below the maternal no-adverse-effect-level (NOAEL) at 100 mg/kg/d and fetal NOAEL established at ≥200 mg/kg/d.[36,37]

Assessment of Mitochondrial Respiration

Mitochondrial respiration of the cultured syncytiotrophoblasts was measured using a Seahorse XF24 analyzer
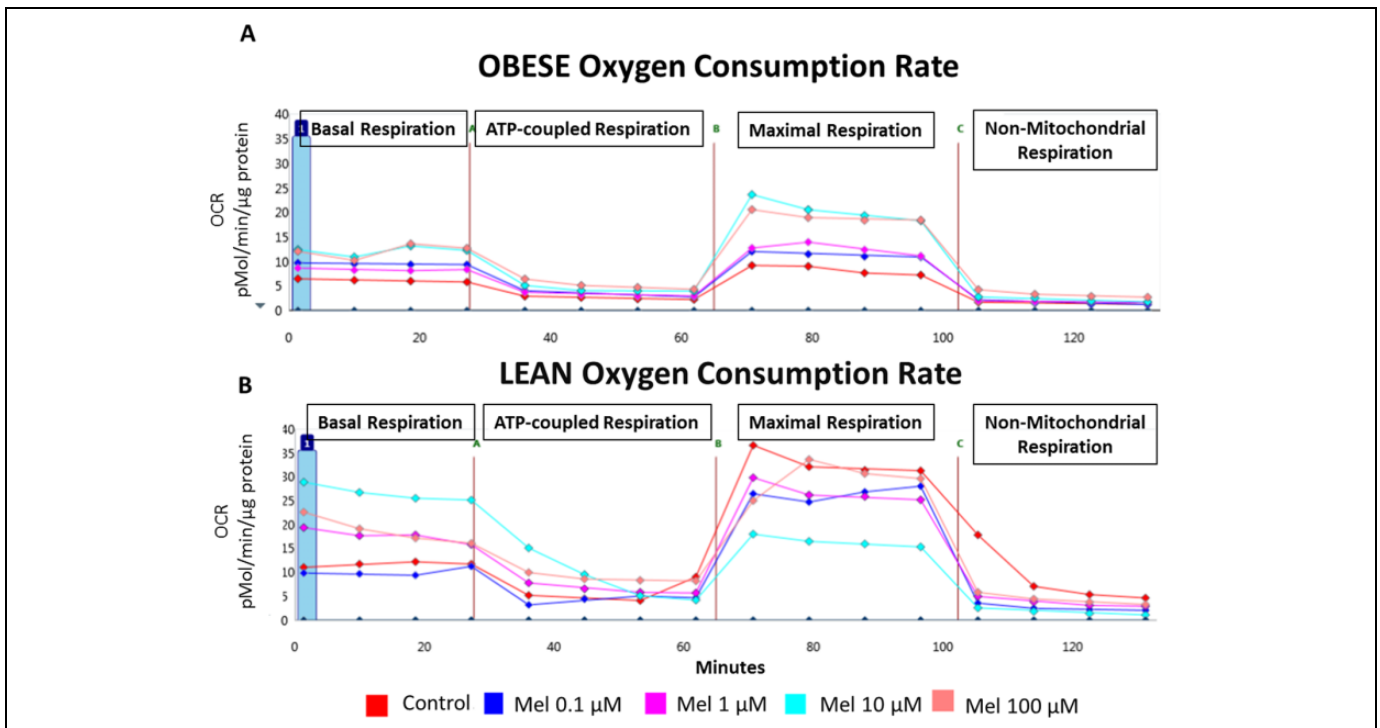
Figure 1. Effect of melatonin on mitochondrial oxygen consumption rate: representative traces of mitochondrial respiration of the cultured synctiotrophoblasts from (A) an obese woman and (B) a lean woman with or without increasing melatonin concentrations (0.1-100 μmol/L). Each data point is a specific oxygen consumption rates (OCR) measurement in time representing the parameters of basal respiration, ATP-coupled respiration, maximal respiration, and nonmitochondrial respiration. ATP indicates adenosine triphosphate.

(Seahorse Bioscience, Agilent, Santa Clara, CA) as we have described previously.[15] Data are expressed as oxygen consumption rate (OCR) in pmol/min. Total cellular protein was measured following each experiment by the Bradford method to normalize the mitochondrial OCR. The OCR was measured under basal conditions followed by the sequential addition of oligomycin (1 μmol/L), carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (1 μmol/L), and a mixture of rotenone (3 μmol/L) and antimycin A (1.5 μmol/L) to determine the ATP-coupled respiration, maximal respiration, spare capacity, and nonmitochondrial respiration (Mito stress kit).[15,20,34]

Western Blots

In addition, separate syncytiotrophoblast cultures treated with and without melatonin for the last 24 hours were collected with buffer-containing protease and phosphatase inhibitors (Sigma-Aldrich, Billerica, MA). Total protein concentration of each sample was determined using Bradford reagent (Bio-Rad, Hercules, CA). Proteins were separated on 4% to 20% gradient precast gels (Bio-Rad), transferred onto nitrocellulose membranes, and blocked with 5% milk in 0.1% Tween, 20 mmol/L Tris (pH7.5)-buffered saline (TTBS; w/v) for 1 hour. Blots were probed with primary antibody in 1% nonfat milk powder/TTBS overnight at 4°C and were detected using appropriate peroxidase-conjugated secondary antibody in 5% milk/TTBS for 1 hour. Mitochondrial ETC complexes antibody cocktail (Abcam ab110411, Cambridge, MA) was used at 1:5000

dilution, anti-SOD1 antibody was used at 1:2000 dilution (Abcam ab16831, Cambridge, MA), anti-SOD2 was used at 1:10 000 dilution (EMD Millipore 06-984); and anti-GPx4 was used at 1:5000 (Abcam ab125066, Cambridge, MA). Protein bands were visualized by enhanced chemiluminescence (Millipore, Billerica, Massachusetts, USA) and band intensity was measured by densitometry using Gene Tools software (Syngene, Frederick, Mary land) and ImageJ software (National Institutes of Health). All gels were run at the same time, in the same buffers, and incubated with the same primary and secondary antibodies. The density of each band was normalized to β-actin.

Statistical Analysis

There were a total of 16 study participants across the range of maternal BMIs. Summary data are presented as means ± standard error of the mean (SEM). Because our data did not significantly deviate from normality for most variables (as tested using *F*-test or Brown-Forsythe test), comparisons between 2 groups was performed using a Student *t*-test and comparison of more than 2 groups was performed using 1- or 2-way analysis of variance (ANOVA) and Dunnett post hoc test. Mann-Whitney *U* test or Kruskal-Wallis test was utilized for nonparametric data. Given that there may be sexual dimorphism in the adaptation of the placenta,[38] we also separated the samples by placental sex and evaluated for male and female differences. A *P* value of <.05 was considered significant.
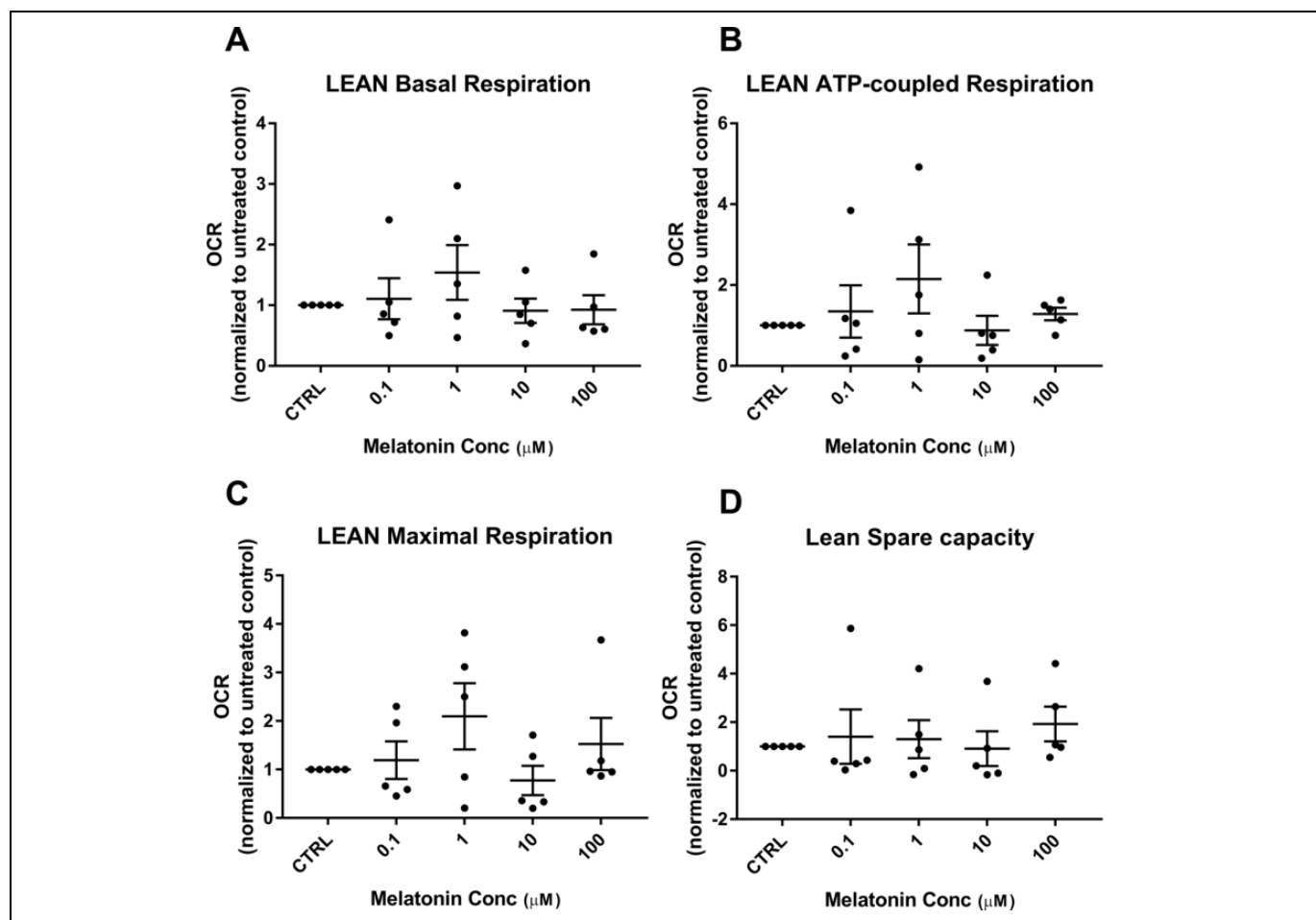
Figure 2. Effect of melatonin on respiratory parameters in trophoblasts of lean women: mitochondrial respiratory parameters of the syncytio-trophoblasts from lean women (n = 5), with and without melatonin (0.1-100 μmol/L) were measured in SeahorseXF24. Measurements in the presence of melatonin were expressed as a ratio normalized to the values in the untreated control, scatter plot with mean ± SEM. Basal respiration (A), ATP-coupled respiration (B), maximal respiration (C), and spare capacity (D). ATP indicates adenosine triphosphate; SEM, standard error of the mean.

Results

Demographic Data and Birth Outcome

Placentas were obtained from 6 lean (BMI < 25) and 10 obese (BMI ≥ 30) women at term at the time of scheduled repeat cesarean delivery. The demographics of the participants involved in this study are summarized in Table 1. As per study design, obese women had a significant higher maternal BMI (36.9 ± 2.0, mean ± SEM) at their initial prenatal visit when compared to lean pregnant women (21.6 ± 1.0, P < .001). Lean and obese women were otherwise matched for maternal age, gravidity, and gestational age at delivery. All of the women had an early entry to prenatal care including similar gestational age at initial visit. The mean weight gain during pregnancy of each group, although not statistically different, followed the Institute of Medicine recommendations.[6] There were no differences in placental or infant weight. All of the pregnancies were otherwise uncomplicated, and there were no cases of gestational diabetes, preeclampsia, macrosomia, IUGR, or poor immediate fetal or neonatal outcomes.

The Effect of Melatonin on Placental Mitochondrial Respiration in Trophoblasts of Obese Women

We have previously shown that mitochondrial respiration is reduced in trophoblasts of obese versus lean women.[20] Representative tracings of mitochondrial respiration of the cultured syncytiotrophoblasts from an obese and a lean woman, as measured by the Seahorse XF24 analyzer and showing the effect of addition of increasing concentrations of melatonin, are shown in Figure 1. Addition of melatonin to trophoblasts of lean women did not have a significant effect with either improvement or detrimental decrease in basal respiration, ATP-coupled respiration, nonmitochondrial respiration, maximal respiration, and spare capacity (Figure 2A-D). Melatonin also had no effect on basal respiration or ATP-coupled respiration in trophoblasts
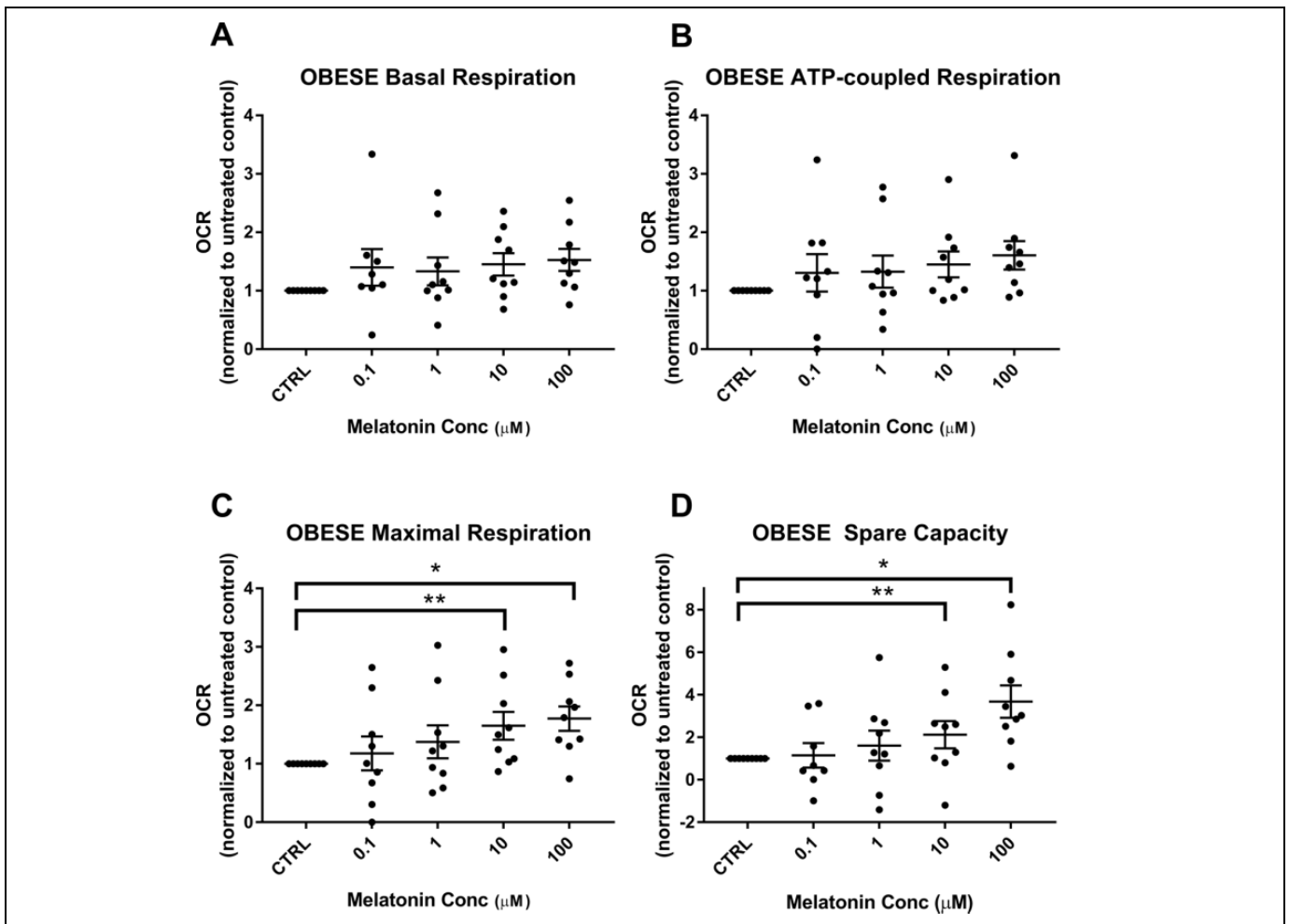
Figure 3. Effect of melatonin on respiratory parameters in trophoblasts of obese women: mitochondrial respiratory parameters of the synctiotrophoblasts from obese women (n = 9), with and without melatonin (0.1-100 μmol/L) were measured in SeahorseXF24. Measurements in the presence of melatonin were expressed as a ratio normalized to the values in the untreated control, scatter plot with mean ± SEM. Basal respiration (A), ATP-coupled respiration (B), maximal respiration (C), and spare capacity (D). C, Obese maximal respiration melatonin versus control: 10 μmol/L (**P = .01) and 100 μmol/L (*P = .009). D, Obese spare capacity melatonin versus control: 10 μmol/L (**P = .02) and 100 μmol/L (*P = .003). ATP indicates adenosine triphosphate; SEM, standard error of the mean.

of obese women (Figure 3A and B). However, in trophoblasts from obese women, which we have previously shown to have significantly lower maximal respiration compared to trophoblasts of lean women,[20] there is a significant concentration-dependent increase in maximal respiration upon addition of melatonin as shown by 1-way ANOVA (P = .008) which reached significance at melatonin concentrations of 10 and 100 μmol/L (P = .01 and P = .009; Figure 3C). Correspondingly, evaluation of spare capacity in trophoblasts from obese women demonstrates a significant concentration-dependent increase in spare capacity upon addition of melatonin (one-way ANOVA, P = .003), which reached significance at 10 and 100 μmol/L melatonin (P = .02 and P = .003; Figure 3D). Indeed, maximal respiration and spare capacity of trophoblasts from obese women is increased by melatonin to a level similar to that found in trophoblast of lean patients (Figure 4A and B).

When stratifying the data by placental sex, there were also no sex-specific differences detected in OCR in either lean or obese placentas with the addition of melatonin (data not shown).

Expression of Mitochondrial ETC Complexes and Antioxidant Enzymes

To determine the expression of ETC complexes and antioxidant enzymes, trophoblasts were cultured from placentas of lean (n = 4) and obese (n = 7) women and treated with or without melatonin 100 μmol/L for 24 hours. Unfortunately, cellular homogenates were not available from all samples to study. Western blotting using an antibody cocktail recognizing epitopes of purified selected subunits of complexes I to V on syncytiotrophoblasts from lean and obese placentas was performed (Figure 5A). We were unable to visualize complex II on
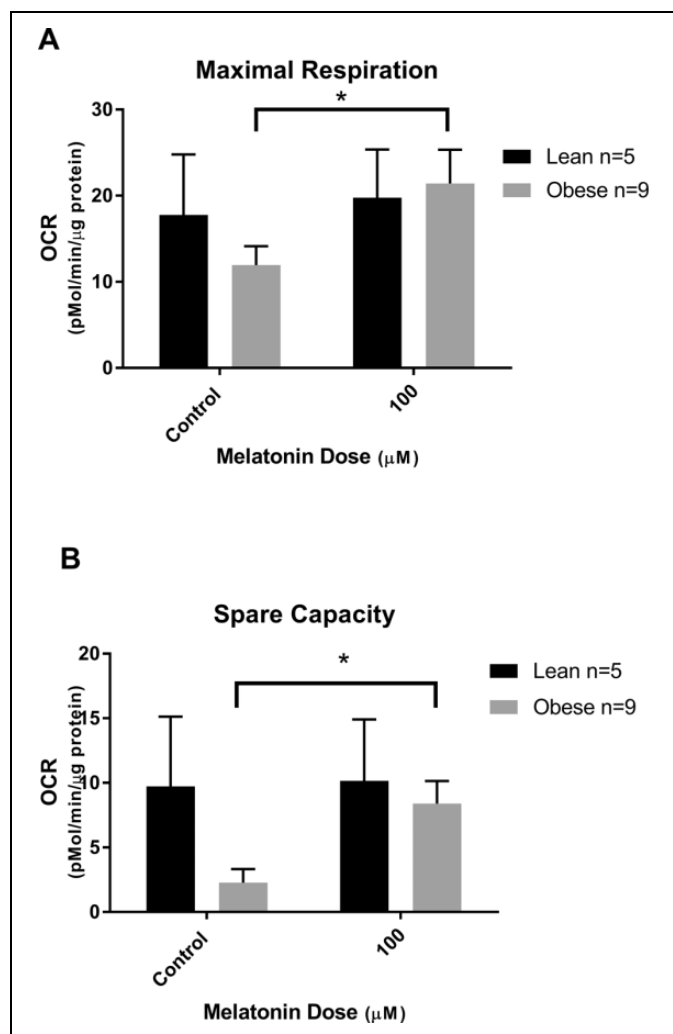
Figure 4. Improvement in maximal respiration and spare capacity by melatonin: oxygen consumption rate (OCR) in trophoblasts from lean and obese women with or without addition of 100 μmol/L melatonin. (A) Maximal respiration 100 μmol/L melatonin versus control (*P = .009) and (B) spare capacity 100 μmol/L melatonin versus control (*P = .003). Mean ± SEM. SEM indicates standard error of the mean.

the Western blot, and there were no differences detected in the expression of the mitochondrial, ETC complexes I, III, IV, and V in trophoblasts from lean or obese women treated with melatonin (Figure 5B to E, lean not shown). To determine whether melatonin's effect on OCR is mediated by alteration in the expression of antioxidant enzymes, Western blotting of SOD1, SOD2, and GPx4 was performed (Figure 6A and B). In trophoblasts of both lean and obese women, addition of melatonin resulted in no significant alteration in the expression of SOD1 or SOD2 (Figure 6C and D, lean not shown). While expression of GPx4, which has a high preference for lipid hydroperoxides, was significantly greater in untreated trophoblast of obese compared to lean women ($P < .05$), the addition of melatonin significantly reduced ($P = .02$) GPx4 expression to a level not significantly different from that in lean women (Figure 6E). There was no effect of melatonin on GPx4 expression in trophoblast of lean women.

Discussion

We investigated the effect of a potent antioxidant, melatonin, on the mitochondrial respiratory function of trophoblasts from obese women in vitro. We have shown that certain respiratory parameters in syncytiotrophoblasts from obese women were significantly improved with the addition of melatonin. Maximal respiration is, as the name suggests, the maximal mitochondrial oxygen consumption a cell can achieve, and was significantly improved in trophoblasts of obese placentas upon addition of melatonin. Spare capacity, or reserve respiratory capacity, is the difference between ATP produced by oxidative phosphorylation at the basal resting state and at maximal activity[39] and represents the ability of cells to increased work and to respond to stress.[15] Many cells operate at a basal level that only requires a part of their total bioenergetics capability. It is perhaps not surprising then that there is no change in basal OCR of trophoblast from obese women or from lean women upon addition of melatonin. Similarly, Lanoix et al found that melatonin had no effect on cytotrophoblasts under normoxic conditions but did lead to improved oxidative stress and apoptosis-induced changes in trophoblasts that had been stressed via H/R.[29] Exhaustion of spare capacity has been correlated with variety of pathologies including heart disease,[40] neurodegenerative disorders,[41,42] and cell death in smooth muscle.[43] If the energy requirement supersedes what the spare capacity can provide, the cell and affected tissue risk cell death.[39] Improved spare respiratory capacity could impart a protective effect to placenta and fetus in response to additional stressors, such as the medical comorbidities and complications that occur in conjunction with obesity including preeclampsia, chronic hypertension or other vasculopathies, IUGR, or macrosomia.

There also appears to be different overall mitochondrial OCR response to the addition of melatonin in syncytiotrophoblasts from lean versus obese women. In syncytiotrophoblasts of obese women, a significant concentration-dependent increase in OCR from 0.1 to 100 μmol/L melatonin is seen. However, in trophoblasts of lean women, there is no significant effect with an increasing concentration of melatonin. There is considerable variability in response in trophoblasts of lean women, and we acknowledge that an increase number of samples may have reduced this variability. We have previously shown decreased respiratory parameters in placental mitochondria from obese compared to lean women,[20] and the overall aim of this study was to determine whether the depressed respiratory function in obesity could be improved with addition of melatonin. The ROS induces pathology as part of oxidative stress, but they also serve as signaling molecules to regulate biological and physiological processes.[44] In rat vascular smooth muscle, ligand binding of growth factors stimulates a burst of ROS, and inhibition of the ROS rise was shown to block normal signaling.[45,46] Therefore, alterations in this homeostasis, in a cell without dysfunctional oxidative stress, could lead to detrimental cellular effects. The lack of effect of melatonin in trophoblasts from lean women perhaps reflects their low level of oxidative stress but also that there are no
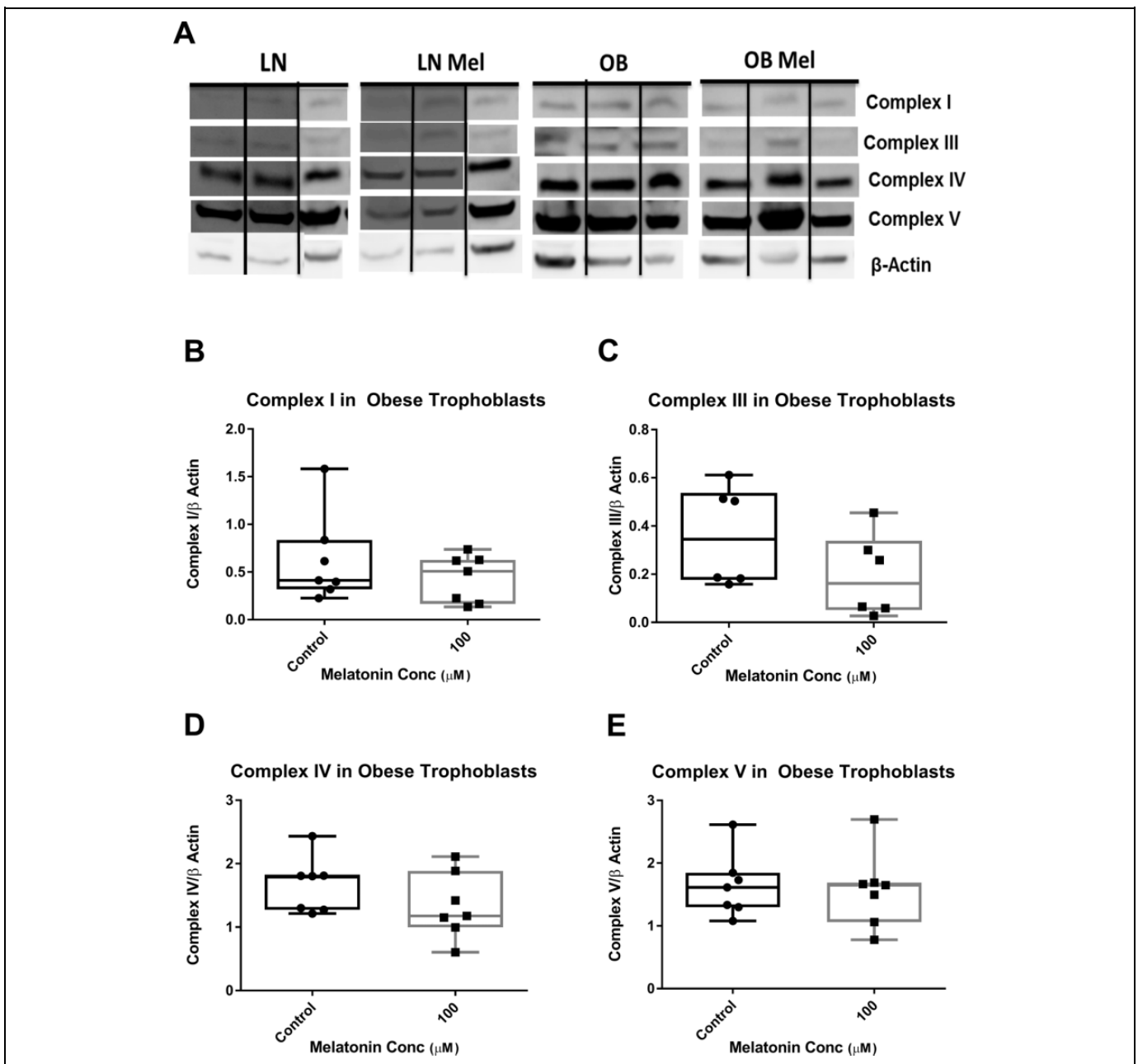
Figure 5. Expression of mitochondrial electron transport chain complexes: (A) representative Western blots of complexes I, III, IV, and V in trophoblast of lean (n = 4) and obese (n = 7) women treated with and without melatonin 100 μmol/L for 24 hours. Relative protein expression of complex I (B), complex III (C), complex IV (D) or complex V (E) in trophoblasts of obese women. Represented in box and whiskers plot with median, minimum, and maximum values shown due to nonparametric distribution of data.

detrimental effects of melatonin. Similarly, in Lanoix et al's study, the addition of melatonin to cytotrophoblasts in vitro showed no effect on hCG production and synctialization.[29]

The enzymes synthesizing melatonin and melatonin receptors are expressed in the human placenta throughout pregnancy, and melatonin promotes syncytiotrophoblast formation.[47] Hence, melatonin likely plays an essential role in the normal function of the placenta and therefore may be a viable treatment option to protect against oxidative stress generated in the setting of maternal obesity. Maternal melatonin administration

has been shown to improve outcomes in animal models in conditions of increased oxidative stress and ameliorates placental insufficiency and the detrimental newborn neurodevelopmental effects of IUGR.[31,48] Administration of melatonin in rats with undernourished pregnancies, a model known to promote oxidative stress, leads to improved placental efficiency and restored birth weight.[31] Antenatal maternal melatonin treatment normalized myelination and rescued axonopathy within IUGR lamb brains and gave functional improvements with reduced time to suckle at birth.[48]
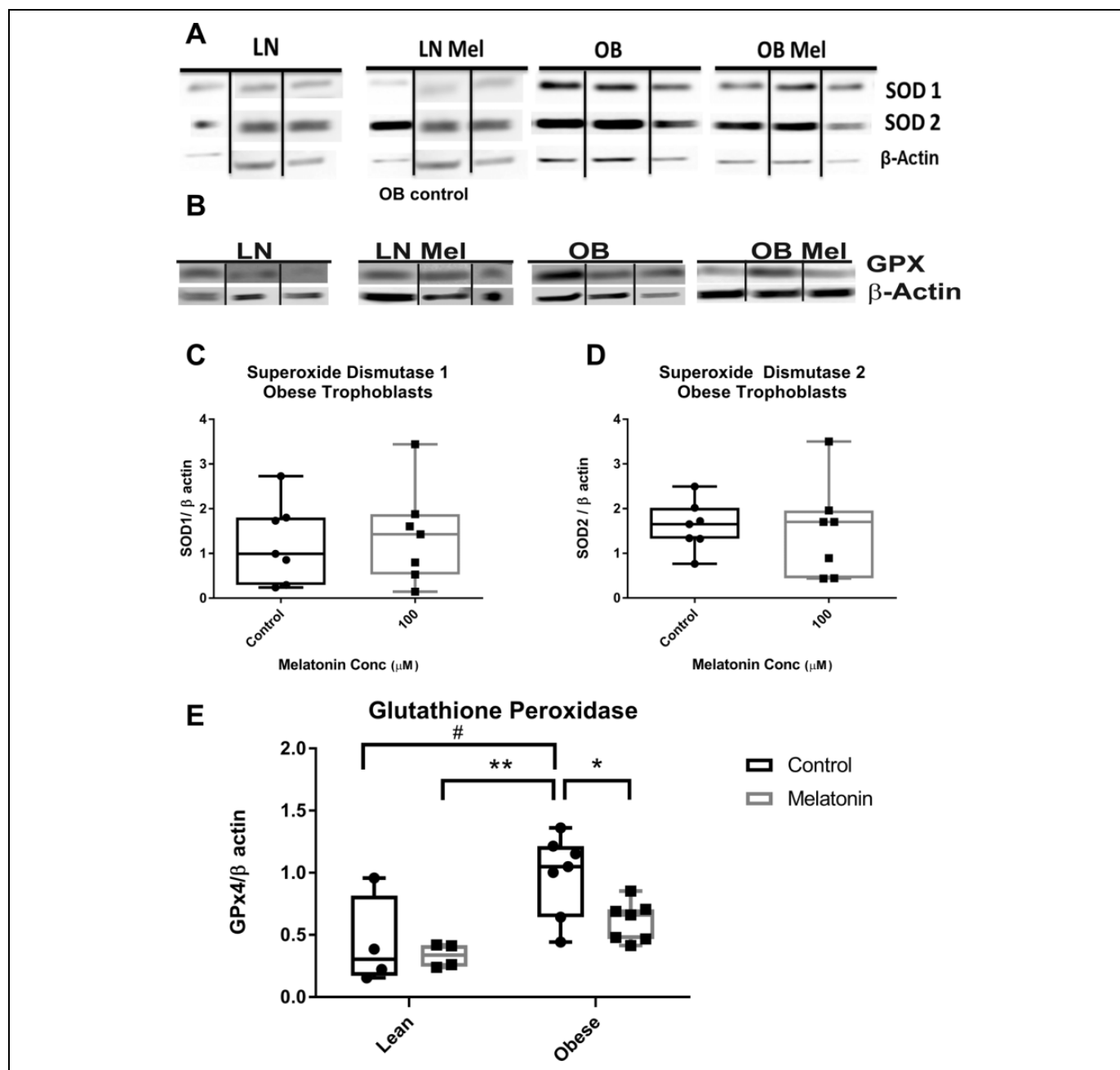
Figure 6. Expression of antioxidant enzymes: (A) representative Western blots of SOD1 and SOD2 and (B) GPx4 in trophoblast of lean (n = 4) and obese (n = 7) women with and without the addition of melatonin (100 μmol/L) for 24 hours. Relative protein expression of SOD1 (C) and SOD2 (D) in trophoblasts of obese women. E, GPx4 protein expression was greater in trophoblast of obese compared to lean women (#P < .05) and the addition of melatonin significantly reduced (*P = .02) GPx4 expression to a level not significantly different from that in lean women. Represented in box and whiskers plot with median, minimum, and maximum values shown due to nonparametric distribution of data. SOD indicates superoxide dismutase; GPx, glutathione peroxidase.

We found that acute melatonin treatment for 24 hours had no significant effect on the expression of mitochondrial respiratory chain complexes. The effect of melatonin may therefore be via reduction in oxidative stress or increased respiratory chain activity rather than increased expression of the complexes in this time period. In vitro administration of melatonin to mouse liver cells has been shown to increase the activity of respiratory complexes I, III, and IV.[35] Melatonin administration has been reported to prevent the alteration of antioxidant enzymes induced by H/R including prevention of the H/R-induced reduction in SOD1, SOD2, GPx mRNA and protein expression, and enzymatic activity in cytotrophoblasts.[29] We find no effect of melatonin on either the cytosolic or the mitochondrial isoforms (SOD1 and 2) of SOD in trophoblasts from lean or obese women. However, GPx4 was significantly increased in trophoblasts of obese compared to lean women, probably as a

response to the increased oxidative stress but was reduced, back to the levels seen in the lean placentas, with the addition of melatonin. This would imply that melatonin decreased oxidative stress and the need for increased antioxidant enzymes. We have recently (Evans and Myatt) shown decreased SOD1 activity and protein expression in placental homogenates from obese women compared to lean women, which is significant when the fetus is male. In contrast, placental GPx activity was increased with obesity and significantly so if the fetus is male, which we suggest is a compensatory response to attempt to decrease oxidative stress.[49] In the present study, GPx4 expression is increased in the placenta of obese compared to lean women which agrees with this concept and is further reinforced by the overall antioxidant effect of melatonin, reducing the compensatory increase of GPx4. The GPxs are selenoenzymes thought to play an important role in protecting trophoblast mitochondria from oxidative stress. Indeed prior incubation of trophoblasts with Na selenite and selenomethionine mitigated a significant dose-dependent decrease in GPx activity after induced oxidative stress with rotenone and antimycin.[50] The primary effect of melatonin may not be via induction of antioxidant enzyme expression per se, but by another mechanism of reducing oxidative stress to which GPx4, which specifically targets lipid hydroperoxides, appears to be secondarily responsive.

Strengths of the study include the use of tissue collected at cesarean delivery to avoid labor-induced oxidative stress, the ethnically homogeneous population, the absence of confounding medical conditions other than obesity, and the absence of adverse pregnancy outcomes. Limitations of this study include an ethnically homogenous population that may not represent effects seen in other ethnicities, and the small sample size, which also limits stratification by placental sex. The participants have an ethnic background which is representative of the patient population in our region. However, the effect of maternal obesity on the placenta may show ethnic variances. For example, in a cohort of predominantly African American women, maternal obesity was reported to be associated with placental macrophage accumulation and inflammation, whereas a similar study in caucasian women failed to find infiltration of immune cells in the placentas of obese women.[51,52] In the setting of poor clinical outcome, more progressive placental dysfunction and abnormalities may be seen and is an area that should be expanded in future research. Indeed, we have already described further decrements in trophoblast respiration in placentas of women with GDM compared to their obese BMI-matched controls.[34] In a recent meta-analysis, even modest increases in maternal BMI were associated with increased risk of fetal death, stillbirth, and neonatal, perinatal, and infant death, but the greatest risk was observed in the category of severely obese women—women with a BMI of 40 had an approximate 2- to 3-fold increase in the relative risk (RR) of these outcomes versus those with a BMI of 20, with absolute risks in the range of 0.69% to 2.7% for BMI of 40 versus 0.20% to 0.76% for BMI of 20.[8] Placental oxidative stress and mitochondrial dysfunction may play a role in this pathophysiology. Although the clinical outcomes were normal, the mitochondrial function of placentas from obese women was decreased and could be rescued with melatonin administration in vitro.

Authors' Note

Acknowledgments

Declaration of Conflicting Interests

Funding

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