Research Square

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Fetal circulating human resistin increases in diabetes during pregnancy and impairs placental mitochondrial biogenesis

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Abstract

Background Diabetes during pregnancy affects placental mitochondrial content and function, which can impact fetal development and the long-term health of offspring. Resistin is a peptide hormone originally discovered in mice as an adipocyte-derived factor that induced insulin resistance. Unlike rodents, human resistin is primarily secreted by monocytes or macrophages. The regulation and roles of human resistin in diabetes during pregnancy remain unclear. Methods Human resistin levels were measured in cord blood of women with diabetes during pregnancy (n=42) and healthy controls (n=81). Secretion of resistin from cord blood mononuclear cells (CBMCs) were measured. The actions of human resistin in mitochondrial biogenesis were determined on placental trophoblastic cells (BeWo cells) or human placental explant. Results Concentrations of human resistin in cord sera were higher in diabetic pregnancies (67 ng/ml) compared to healthy controls (50 ng/ml, P< 0.05), and correlated (r=0.4, P=0.002) with a measure of maternal glycemia (glucose concentration 2 h post challenge). Resistin mRNA was most abundant in cord blood mononuclear cells (CBMCs). Secretion of resistin from cultured CBMCs was increased in response to high glucose (25 mM). Exposing BeWo cells or human placental explant to resistin decreased expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), mitochondrial abundance, and ATP production. Conclusions In summary, we have shown that resistin is increased in fetal circulation of infants exposed to the diabetic milieu potentially reflecting the response of monocytes/macrophages to hyperglycemia and metabolic stresses associated with diabetes during pregnancy. Increased exposure to resistin may contribute to mitochondrial dysfunction and aberrant energy metabolism characteristic of offspring exposed to diabetes in utero .

Background:

Diabetes during pregnancy, including pre-gestational diabetes and gestational diabetes (GDM), affects fetal growth, which is linked to the development of obesity, diabetes, and cardiovascular diseases in later life (1–4). Approximately 15% of pregnant women globally have diabetes during pregnancy (5), and the percentage continues to increase, contributing significantly to the increased prevalence of diabetes and obesity in subsequent generations. The placenta plays a key role in fetal growth and development by supplying nutrients and oxygen. Diabetes during pregnancy alters placental structure and function with aberrant vascularization, increased inflammation, and impaired energy metabolism (6–8). As the interface between maternal and fetal circulation systems, the placenta can be affected by changes in both maternal and fetal circulating factors in response to the diabetic milieu (9).

Resistin is a secreted protein implicated in the pathogenesis of obesity and type 2 diabetes. It was discovered in rodents as an adipocyte-derived factor which induces insulin resistance (10). Human and murine resistin only share 59% homology at the amino acid level (11). Unlike rodent resistin, human resistin is predominantly produced by peripheral blood mononuclear cells (PBMCs), macrophages, and bone marrow cells (12). Human resistin has been shown to induce expression of proinflammatory cytokines and adhesion molecules in the settings of inflammation and endothelial dysfunction. Given the strong relationship between inflammation and metabolism, there is mounting evidence suggesting a role

of human resistin in the pathological processes of metabolic diseases, including obesity, diabetes, and cardiovascular diseases (13, 14). However, the precise mechanism by which resistin impacts these processes has not been clearly defined as as several studies have failed to identify an association of resistin levels with obesity or type 2 diabetes (15, 16). Resistin has been implicated in the insulin resistance observed in normal pregnancy as the level of resistin increases with gestational age and decreases after delivery (17). The most recent meta-analysis suggests maternal resistin levels are associated with risk of GDM (18). However, current studies examining the fetal levels of resistin in diabetes during pregnancy are inconsistent (19–21). In addition, the regulation and function of human resistin in fetal circulation and how it affects placenta in diabetes during pregnancy remain poorly understood.

Our previous studies demonstrate that maternal diabetes is associated with decreased PGC-1α/TFAM/mitochondrial biogenesis signaling in human placenta (22). The present studies demonstrate increased resistin production from CBMCs, which has a role in inhibiting mitochondrial biogenesis and metabolism in placenta, providing evidence for resistin as a link between inflammatory response and energy metabolism in the context diabetes during pregnancy.

Methods:

Subjects for Cord serum Samples

Pregnant Native American or Hispanic women with Diabetes (N=42, including 31 gestational diabetes and 11 pre-gestational type 2 diabetes), or non-diabetic controls (N=81) were enrolled into a prospective longitudinal study on the impact of *in utero* exposure to DM, as previously described (23). Gestational or type 2 diabetes was diagnosed according to ADA guidelines (24). Women were excluded if they delivered prior to 37 weeks gestation, had type 1 diabetes, pre-eclampsia, chronic hypertension, renal disorders or a smoking history during pregnancy. They were also excluded if the infants were small for gestational age, had a major malformation, or chromosome abnormality. Demographics for participants providing cord blood samples are shown in Table 1. The participants with diabetes during pregnancy were older, had higher HBA1C, BMI, and slightly lower gestational age. There was no significant difference in the ethnicity and fetal sex between pregnant women with or without diabetes. Cord blood, maternal blood, and placental tissues were obtained after delivery. The protocol was approved by the Institutional Review Boards of the University of Oklahoma Health Science Center, the Chickasaw Nation, and the Choctaw Nation of Oklahoma.

Table 1: Characteristics of Research Subjects Providing Cord blood Samples

		DM N=42 (Male 21; Female 21)	Control N=81 (Male 37; Female 44)	P-value (DM vs Control)
Maternal Age, Y		31	24.47	P<0.001
Maternal HbA1C, %		5.68	5.15	P<0.001
Maternal BMI		32.74	28.22	P<0.01
Gestational age, weeks		38.89	39.55	P<0.001
Race	Native American	24 (57%)	56 (69%)	P>0.05
	Hispanic	18 (43%)	25 (31%)	

ELISA

The concentrations of resistin in serum and cell culture media were measured using ELISA kit (R&D Systems, Minneapolis, MN) according to manufacturer's protocol and as previously described(25).

Cord blood mononuclear cell isolation

In a separate cohort, human cord blood mononuclear cells (CBMCs) were isolated from cord blood by Ficoll density gradient centrifugation. The cord blood was diluted 1: 3 in PBS (without Ca^{2+} and Mg^{2+}), layered over Ficoll buffer, and centrifuged at 400g for 35 minutes. The interphase cell layer was collected and washed in PBS for 3 times. The CBMCs were plated and cultured in Dulbecco's Modified Eagle Medium with 10% Fetal Bovine Serum followed by treatment with TNFα (100 ng/ml), high glucose (25 mM), palmitate acid (0.6 mM), or 4-Hydroxynonenal (4-HNE, 0.6 mM) for 16 hours.

RNA extraction

Total RNA was extracted from BeWo cells (a human placental trophoblast cell line that originates from choriocarcinoma) using commercially available kits (miRNeasy, Qiagen, Valencia, CA) according to the manufacturer's instructions. Isolated total RNA was quantified by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

qPCR analysis

Reverse transcription (RT) was done with SuperScript VILO cDNA Synthesis Kit according to the manufacturer's instructions (Invitrogen). Quantitative real-time PCR was performed using TaqMan Real-Time PCR Probes for PGC-1α or GAPDH (Life Technologies). Results were calculated using the $2^{-\Delta\Delta Ct}$ method normalized to endogenous control GAPDH.

Placental explant culture

In a separate cohort, two pieces of placental tissue were collected from healthy subjects right after delivery, stripped of connective tissues, and dissected to small pieces (about 2 mm). The placental villous explants were cultured in 6-well plate at 37°C in 5% CO_2 in Ham's F-12 medium (Gibco/Life Technologies, Grand Island, NY) supplemented with 10% FBS (Mediatech, Manassas, VA), 100 μM MEM Non-Essential Amino Acids (Gibco/Life Technologies, Grand Island, NY), and 0.5% penicillin/streptomycin/amphotericin B (Gibco/Life Technologies, Grand Island, NY) and were treated with indicated doses of resistin or vehicle for 24 hours in culture.

Western Blot Analysis

Western blot analysis was performed as method described previously (22). Placental explant samples or BeWo cells were lysed and homogenized in protein lysis buffer containing a protease and phosphatase inhibitor cocktail (Pierce Biotechnology, Rockford, IL). Protein concentrations were measured by BCA assay (Pierce, Rockford, IL). Thirty μg of protein lysate was reduced in laemmli sample buffer with dithiothreitol, and subjected to sodium dodecyl sulfated polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane followed by incubation with antibodies specific for PGC-1α, PDH, DNMT1, or β-actin (Cell Signaling Technology, Danvers, MA). The proteins of interest were detected by enhanced chemiluminescence (Pierce, Rockford, IL) and analyzed by imaging densitometry with Image Lab Software (Bio-Rad, Hercules, CA).

Mitochondrial DNA copy number

DNA was isolated from placental tissue using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, St. Louis, MO) with proteinase K and RNase treatment, according to the manufacturer's instructions. Mitochondrial DNA copy number was estimated by comparing the abundance of the mitochondrial $tRNA^{Leu(UUR)}$ gene (Determined by quantitative RT-PCR, forward primer: 5'-CACCCAAGAACAGGGTTTGT; reverse: 5'-TGGCCATGGGTATGTTGTTA) and with that of the nuclear β2-macroglobulin gene (forward: 5'-TGCTGTCTCCATGTTTGATGTATCT; reverse: 5'-TCTCTGCTCCCCACCTCTAAGT).

ATP measurement:

Cellular ATP levels were measured with Luminescent ATP detection assay kit (Abcam) according to manufacturer's protocol. The cells were cultured in the medium containing galactose instead of glucose and the readings were normalized to DNA abundance measured by Sybrsafe staining.

Statistical methods

Group descriptive statistics are presented as mean ± SD and group count (percentage). Differences in characteristics between two groups were assessed using Student's t-test for continuous measures. Data normality was tested using GraphPad Prism. Mann-Whitney test was used for non-normal distribution.

Correlations were calculated as standardized regression coefficients. P-values <0.05 were treated as statistically significant.

Results:

Cord blood resistin concentration is increased in offspring born to mothers with diabetes during pregnancy and correlates with maternal blood glucose levels

The level of resistin in cord blood of infants born to mothers with diabetes (67 ng/ml, n=42) were significantly higher than those born to control women (50 ng/ml, n=81) (Figure 1A) Cord blood resistin levels were significantly higher compared to the corresponding maternal blood resistin levels (Fig. 1B).

Concentrations of human resistin in cord sera correlated (r=0.4, P=0.002) with maternal glucose concentrations measured 2 hours after oral glucose challenge (OGTT, Fig. 2) during the second trimester of pregnancy. The correlation of cord blood resistin concentrations with maternal

Table 2: Correlation of maternal factors with Cord blood Samples

	Correlation with Cord resistin
Maternal Age, Y	P>0.1
Maternal HbA1C, %	P=0.08
Maternal BMI	P>0.1
Gestational age, weeks	P>0.1

HbA1C was approaching significance (P=0.08), whereas there were no significant correlations between cord blood resistin with maternal age, BMI, or gestational age at birth (Table 2).

Secretion of resistin from cord blood mononuclear cells in response to metabolic stresses

The expression of resistin mRNA in fetal tissues and cells was examined by quantitative real-time PCR. As shown in Figure 3A, resistin was highly expressed in cord blood mononuclear cells (CBMCs). Placenta also expressed resistin but at much lower abundance, whereas expression of resistin was not detectable in mesenchymal stem cells isolated from umbilical cord Wharton's Jelly, nor in BeWo cells, a placental trophoblast cell line (Figure 3A). Treating CBMCs with high glucose, palmitate, or the inflammatory factor TNFα, but not oxidative stress inducer 4-HNE, resulted in increased levels of resistin in the culture media (Figure 3B).

Human resistin inhibits placental mitochondrial biogenesis

We previously reported a decrease in the PGC-1α/TFAM mitochondrial biogenesis pathway in placenta of mothers with diabetes(22). Treating human placental explants with resistin resulted in a decrease in PGC-1α protein abundance (Figure 4A) accompanied by a significant decrease in mitochondrial DNA copy number (Figure 4B), demonstrating a role of resistin in decreasing placental mitochondrial biogenesis.

Human resistin decreases PGC-1α and mitochondrial energy metabolism in placental trophoblasts

Trophoblasts are the placental cells which provide the major source of nutrients for the growing embryos. In a transformed trophoblast cell line, BeWo cells, human resistin treatment also decreased the PGC-1α protein abundance (Figure 5A) and its mRNA expression (Figure 5B). In addition, the protein level of pyruvate dehydrogenase (PDH) was decreased by resistin treatment (Figure 5A). Resistin also inhibited cellular ATP production (Figure 5D), further demonstrating the influence of resistin on mitochondrial energy metabolism.

Discussion:

An adverse maternal environment, such as diabetes during pregnancy, impacts fetal and placental development, which is associated with increased risk of metabolic diseases in offspring later in life (1–4). The present study demonstrates that an increase in cord blood resistin found in the presence of maternal diabetes may play a role in placental mitochondrial biogenesis and function.

A recent meta-analysis including 18 published studies (18) notes that resistin levels are elevated in maternal circulation in gestational diabetes. Much less is known about the determinants of resistin abundance in the fetal circulation and current reports regarding the association between cord resistin with diabetes during pregnancy remain discordant and unclear (19–21). The present study found an increase in cord blood resistin in maternal diabetes, which agrees with the reports by Shang et al and Oncul et al (19, 20). Furthermore, cord blood resistin levels were significantly associated with maternal blood glucose levels. We also demonstrated that resistin expression was highly enriched in cord blood mononuclear cells, in which high glucose treatment stimulated resistin secretion, suggesting that fetal mononuclear cells may likely be the main source of fetal circulating resistin. It has been reported that resistin is expressed by the placenta (17, 26). We also detected expression of resistin in placenta, but at much lower levels than the expression in mononuclear cells. As no expression was detected in a placental trophoblast cell line BeWo cells, we suspect that expression by placental macrophages, rather than trophoblasts, is responsible for placental resistin expression.

Hyperglycemia and hyperinsulinemia in GDM activate inflammatory cells and induce a pro-inflammatory status (27). In accord, we found that cord blood mononuclear cells secreted resistin in response to high glucose and other diabetes-related factors. Our findings further demonstrate resistin as an inflammatory cell-derived factor that responds to hyperglycemia and metabolic stresses associated with diabetic pregnancy.

Our study begins to examine the potential functions of resistin in the fetus, suggesting involvement in regulation of placental mitochondrial abundance and function. Mitochondria play a key role in placental function, and defects in placental mitochondrial function and content are associated with impaired placental energetics and increased oxidative stress, leading to adverse pregnancy outcomes (28–30). We previously demonstrated a decrease in PGC-1α/TFAM/mitochondrial biogenesis signaling in placenta of women with diabetes during pregnancy (22). Here we observed decreases in PGC-1α expression and mitochondrial DNA copy number when human placental explants were exposed to resistin. In addition,

resistin treatment reduced the abundance of pyruvate dehydrogenase (PDH) and ATP production. Pyruvate dehydrogenase is a mitochondrial enzyme that catalyzes pyruvate oxidation, linking glycolysis to the Krebs cycle for ATP generation to meet energy demands (31). As mitochondria are the primary source of ATP needed for placental growth, nutrient transport, and hormone synthesis, increased expression of resistin may contribute to impaired placental mitochondrial biogenesis and function, as well as offspring adverse outcomes in pregnancies complicated by diabetes.

The strengths of the present study are identification of resistin as a dysregulated fetal circulating factor that was derived from inflammatory cells in response to maternal diabetes, and demonstration of a role of human resistin in inhibiting placental mitochondrial metabolism. Limitations of the present study are that the specific downstream signaling and detailed mechanisms underlying resistin effects on mitochondrial biogenesis remain to be explored.

Conclusions:

Together, the present study provide evidence that resistin acts as a fetal circulating factor linking inflammation and energy metabolism when pregnancy is complicated by diabetes. Resistin can be an underlying factor contributing to the impaired placental mitochondrial metabolism in maternal diabetes. Thus, human resistin could be a potential therapeutic target and diagnostic marker for the short-term and long-term adverse pregnancy outcomes of diabetes during pregnancy.

Abbreviations

cord blood mononuclear cells (CBMCs); peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α); gestational diabetes (GDM); 4-Hydroxynonenal (4-HNE); pyruvate dehydrogenase (PDH).

Declarations

Ethics approval and consent to participate:

The studies on human cord serum samples (IRB protocol#1267), and the CBMCs and placental explants (IRB #2540) were approved by the Institutional Review Boards of the University of Oklahoma Health Science Center, the Chickasaw Nation, and the Choctaw Nation of Oklahoma.

Author contributions:

All authors contribute to the conception, design and interpretation of the data. SJ, AMT, and JBT performed the experiments. SJ and SDC wrote the manuscript. All authors revised the manuscript and approved this version to be published.

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Declarations:

There is no conflict of interest associated with this manuscript. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Fetita LS, Sobngwi E, Serradas P, Calvo F, Gautier JF. (2006) Consequences of fetal exposure to maternal diabetes in offspring. *J Clin Endocrinol Metab* **91:** 3718-3724.
2. Dabelea D, Crume T. (2011) Maternal environment and the transgenerational cycle of obesity and diabetes. *Diabetes* **60:** 1849-1855.
3. Damm P*, et al.* (2016) Gestational diabetes mellitus and long-term consequences for mother and offspring: a view from Denmark. *Diabetologia* **59:** 1396-1399.
4. Friedman JE. (2018) Developmental Programming of Obesity and Diabetes in Mouse, Monkey, and Man in 2018: Where Are We Headed? *Diabetes* **67:** 2137-2151.
5. Guariguata L, Linnenkamp U, Beagley J, Whiting DR, Cho NH. (2014) Global estimates of the prevalence of hyperglycaemia in pregnancy. *Diabetes Res Clin Pract* **103:** 176-185.
6. Jarmuzek P, Wielgos M, Bomba-Opon D. (2015) Placental pathologic changes in gestational diabetes mellitus. *Neuro Endocrinol Lett* **36:** 101-105.
7. Osmond DT, Nolan CJ, King RG, Brennecke SP, Gude NM. (2000) Effects of gestational diabetes on human placental glucose uptake, transfer, and utilisation. *Diabetologia* **43:** 576-582.
8. Muralimanoharan S, Maloyan A, Myatt L. (2016) Mitochondrial function and glucose metabolism in the placenta with gestational diabetes mellitus: role of miR-143. *Clin Sci (Lond)* **130:** 931-941.
9. Desoye G, Hauguel-de Mouzon S. (2007) The human placenta in gestational diabetes mellitus. The insulin and cytokine network. *Diabetes Care* **30 Suppl 2:** S120-126.
10. Steppan CM*, et al.* (2001) The hormone resistin links obesity to diabetes. *Nature* **409:** 307-312.
11. Ghosh S, Singh AK, Aruna B, Mukhopadhyay S, Ehtesham NZ. (2003) The genomic organization of mouse resistin reveals major differences from the human resistin: functional implications. *Gene* **305:** 27-34.
12. Schwartz DR, Lazar MA. (2011) Human resistin: found in translation from mouse to man. *Trends Endocrinol Metab* **22:** 259-265.

13. Lazar MA. (2007) Resistin- and Obesity-associated metabolic diseases. *Horm Metab Res* **39:** 710-716.
14. McTernan CL*, et al.* (2002) Resistin, central obesity, and type 2 diabetes. *Lancet* **359:** 46-47.
15. Gerber M*, et al.* (2005) Serum resistin levels of obese and lean children and adolescents: biochemical analysis and clinical relevance. *J Clin Endocrinol Metab* **90:** 4503-4509.
16. Pfutzner A, Langenfeld M, Kunt T, Lobig M, Forst T. (2003) Evaluation of human resistin assays with serum from patients with type 2 diabetes and different degrees of insulin resistance. *Clin Lab* **49:** 571-576.
17. Chen D*, et al.* (2005) Alterations of serum resistin in normal pregnancy and pre-eclampsia. *Clin Sci (Lond)* **108:** 81-84.
18. Hu SM, Chen MS, Tan HZ. (2019) Maternal serum level of resistin is associated with risk for gestational diabetes mellitus: A meta-analysis. *World J Clin Cases* **7:** 585-599.
19. Shang M, Dong X, Hou L. (2018) Correlation of adipokines and markers of oxidative stress in women with gestational diabetes mellitus and their newborns. *J Obstet Gynaecol Res* **44:** 637-646.
20. Oncul M*, et al.* (2013) Maternal and cord blood apelin, resistin and visfatin levels in gestational diabetes mellitus. *Minerva Med* **104:** 527-535.
21. Mohamed MH*, et al.* (2010) Cord blood resistin and adiponectin in term newborns of diabetic mothers. *Arch Med Sci* **6:** 558-566.
22. Jiang S, *et al.* (2017) Effects of maternal diabetes and fetal sex on human placenta mitochondrial biogenesis. *Placenta* **57:** 26-32.
23. Teague AM*, et al.* (2015) Cord blood adipokines, neonatal anthropometrics and postnatal growth in offspring of Hispanic and Native American women with diabetes mellitus. *Reprod Biol Endocrinol* **13:** 68.
24. American Diabetes A. (2003) Gestational diabetes mellitus. *Diabetes Care* **26 Suppl 1:** S103-105.
25. Jiang S*, et al.* (2014) Human resistin promotes neutrophil proinflammatory activation and neutrophil extracellular trap formation and increases severity of acute lung injury. *J Immunol* **192:** 4795-4803.
26. Erol O*, et al.* (2016) Serum level and placental expression of resistin in pregnancies complicated by preeclampsia: relationship with disease severity. *Clin Exp Obstet Gynecol* **43:** 516-521.
27. Pantham P, Aye IL, Powell TL. (2015) Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta* **36:** 709-715.
28. Mando C*, et al.* (2018) Impact of Obesity and Hyperglycemia on Placental Mitochondria. *Oxid Med Cell Longev* **2018:** 2378189.
29. Clemente DBP*, et al.* (2017) Prenatal ambient air pollution exposure, infant growth and placental mitochondrial DNA content in the INMA birth cohort. *Environ Res* **157:** 96-102.
30. Bijnens EM*, et al.* (2019) Placental mitochondrial DNA content is associated with childhood intelligence. *J Transl Med* **17:** 361.

31. Park S, *et al.* (2018) Role of the Pyruvate Dehydrogenase Complex in Metabolic Remodeling: Differential Pyruvate Dehydrogenase Complex Functions in Metabolism. *Diabetes Metab J* **42:** 270-281.

Figures

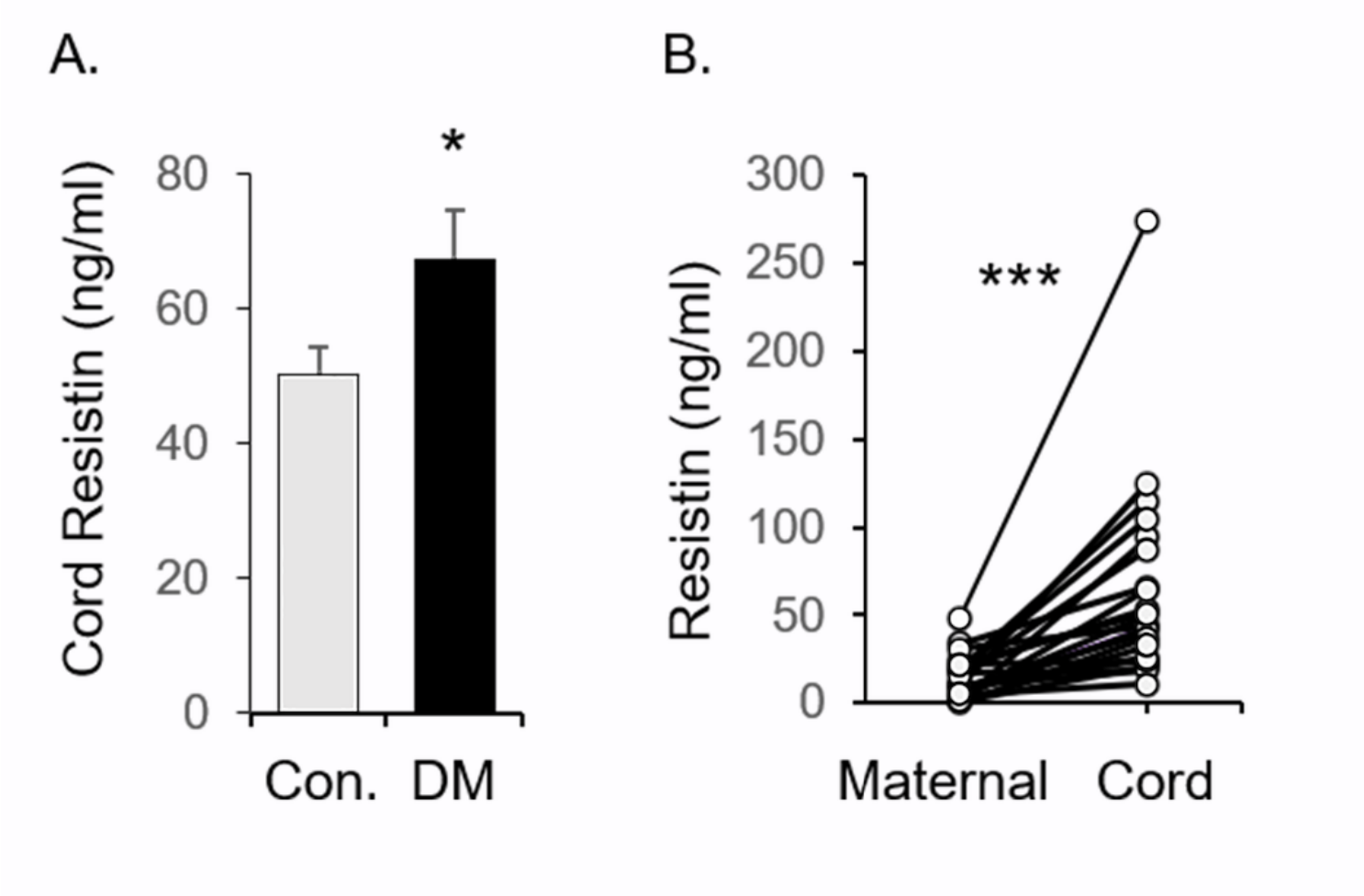

Figure 1

Serum resistin levels in cord and maternal blood. (A) Resistin concentrations were higher in the cord blood of diabetic women (DM) compared to healthy controls (Mean ± SEM, * P<0.05, N=81 in control, N=42 in Diabetes); (B) Pairwise comparison of resistin levels in maternal and cord blood (N= 24 pairs including 9 control and 15 Diabetes, *** P<0.001).

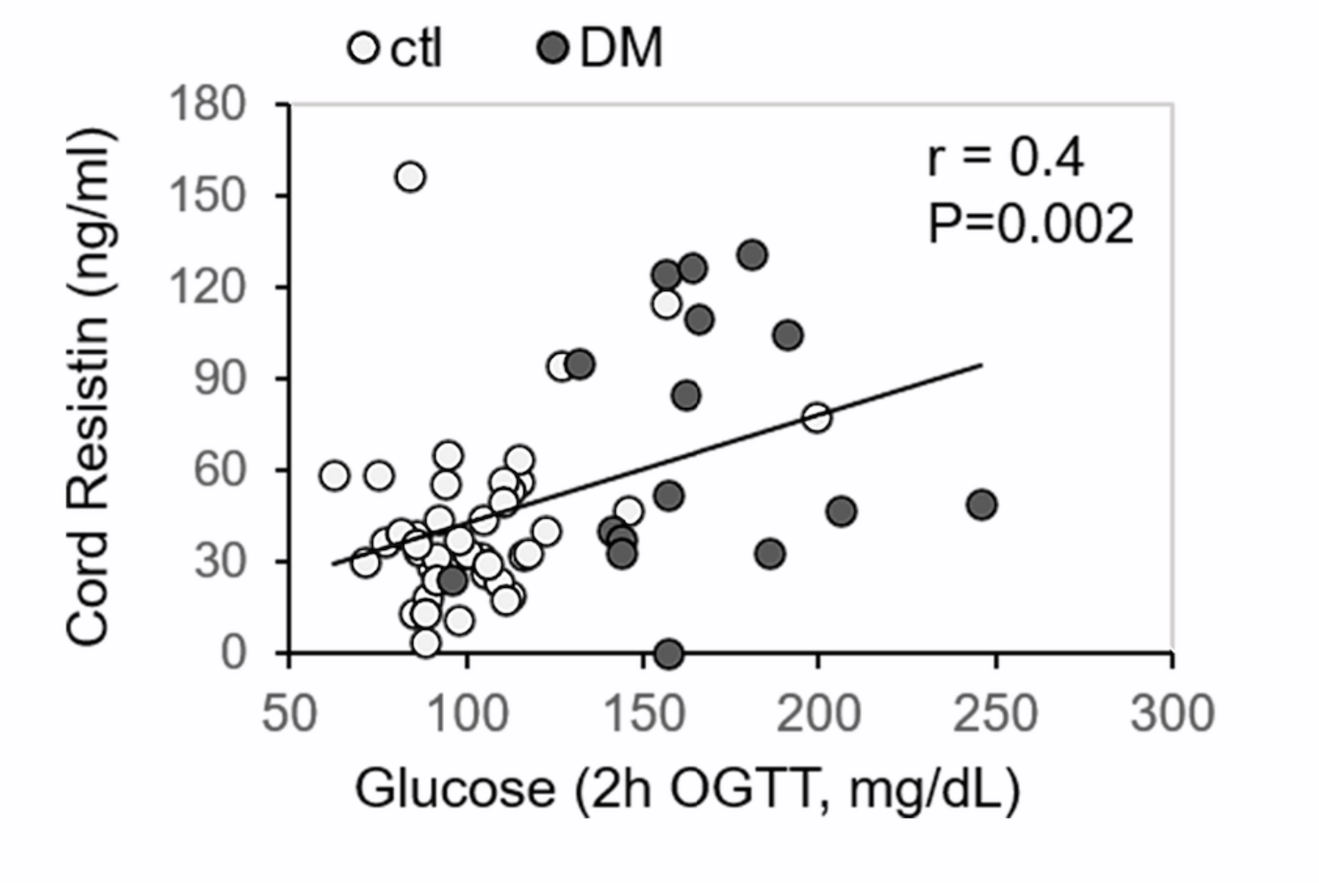

Figure 2

Positive correlation between cord blood human resistin and maternal blood glucose levels during pregnancy. Correlation of cord blood resistin levels with maternal glucose concentration measured at 2 h post OGTT. N= 59 including 43 control and 16 Diabetes.

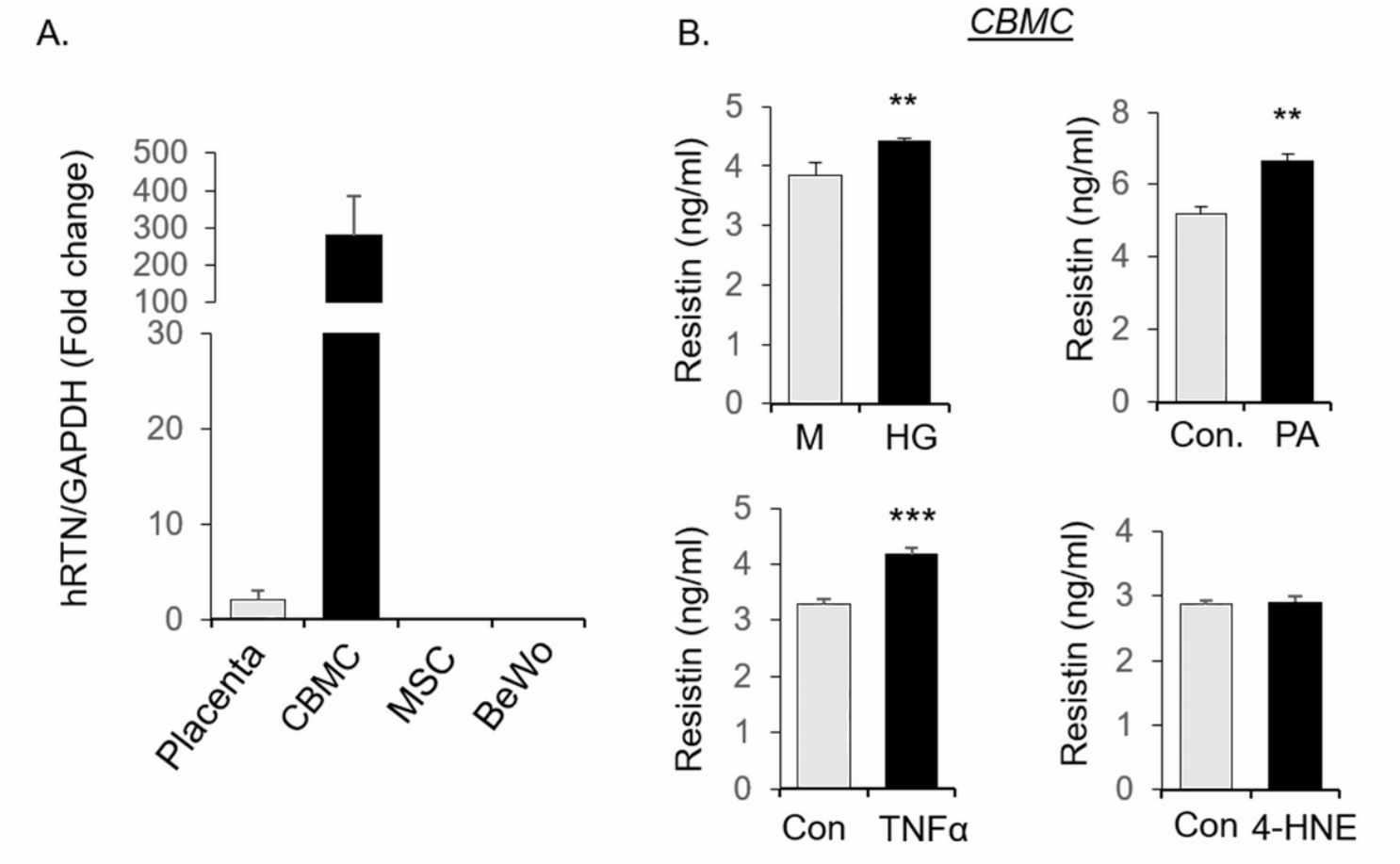

Figure 3

Secretion of resistin from cord blood mononuclear cells in response to metabolic stresses. (A) Expression of resistin mRNA in placenta and cord blood mononuclear cells (CBMCs), cord tissue mesenchymal stem cells (MSC) of healthy subjects (N=3 - 5), and BeWo cells (trophoblast cell line) were measured by quantitative RT-PCR; (B) Mononuclear cells were isolated from cord blood of healthy pregnant women and were treated with TNFα (100 ng/ml), high glucose (25 mM), palmitate acid (0.6 mM), or 4-Hydroxynonenal (4-HNE, 0.6 mM) for 16 hours, followed by measuring resistin levels in culture media with ELISA. Mean ± SD, ** P<0.01; *** P<0.001; N=4.

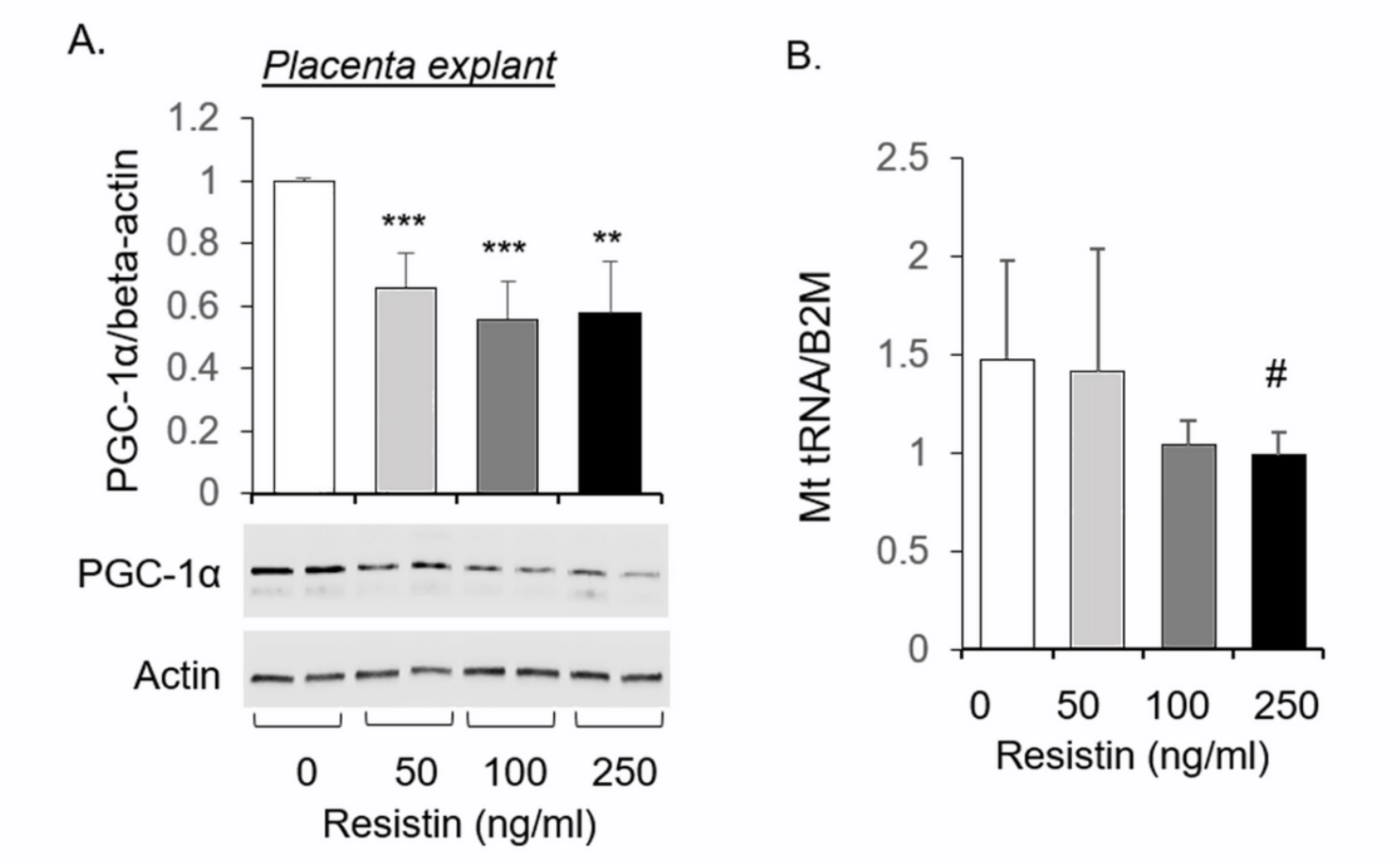

Figure 4

Human resistin inhibited mitochondrial biogenesis in placental explant. Human placental explants were treated with indicated doses of human resistin for 24 hours. (A) protein lysates were subjected for Western blot analysis; (B) total DNA were extracted and mitochondrial DNA copy number was determined by fold change of mitochondrial tRNALeu(UUR) gene DNA copy number normalized to nuclear β2 microglobin (B2M) with quantitative RT-PCR. Mean ± SD, ** P<0.01; *** P<0.001; N=4. # P<0.05 with one-tail T-test.

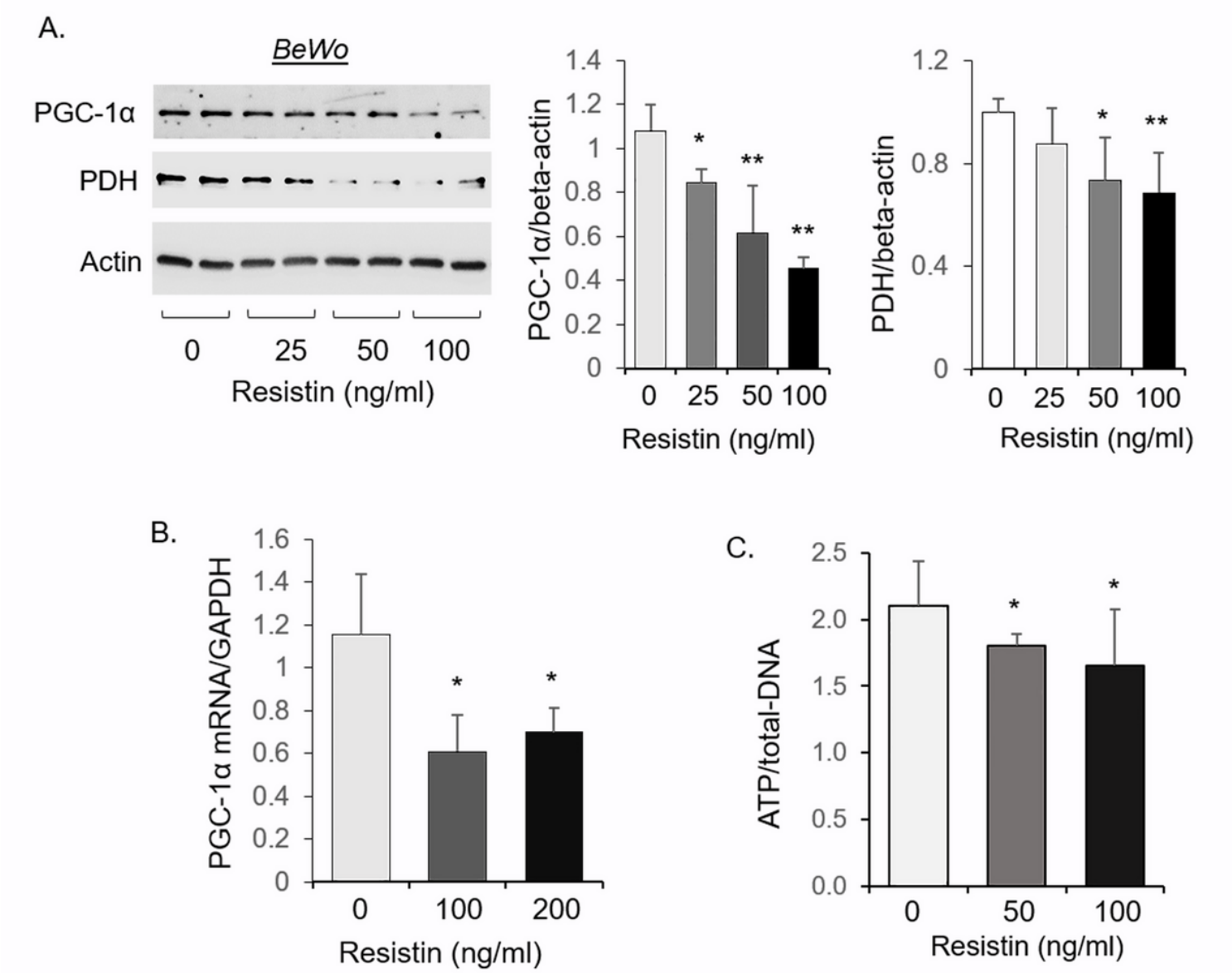

Figure 5

Human resistin decreased PGC-1α and mitochondrial metabolism in placental trophoblasts. BeWo cells were treated with indicated doses of human resistin for 24 hours. (A) Protein lysates were subjected for Western blot analysis (N=4 in each group); (B) total RNAs were reverse-transcribed and subjected for RT-PCR (N=4 in each group); (C) BeWo cells were treated with indicated doses of human resistin in the medium containing galactose for 24 hrs followed by assay for ATP levels (N=8 in each group). Mean ± SD * P<0.05; ** P<0.01.