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Effects of maternal diabetes and fetal sex on human placenta mitochondrial biogenesis

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Abstract

Abnormal placental function in maternal diabetes affects fetal health and can predispose offspring to metabolic diseases in later life. There are fetal sex-specific differences in placenta structure and gene expression, which may affect placental responses to maternal diabetes. The present study examined the effects of maternal diabetes on indices of mitochondrial biogenesis in placentae from male and female offspring. Mitochondrial DNA (mtDNA) copy number and expression of key regulators of mitochondrial biogenesis were assessed in placentae from 19 diabetic and 23 non-diabetic women. The abundance of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and mitochondria transcription factor A (TFAM) were lower in female placentae compared to males, but not mtDNA content. In male offspring, maternal diabetes was associated with decreased placental PGC-1α and TFAM, and mitochondrial DNA (mtDNA) content. Male placental TFAM levels were highly correlated with PGC-1α and mtDNA content. However, despite decreased PGC-1α, concomitant changes in TFAM and mtDNA content by diabetes were not observed in females. In addition, TFAM abundance in female placentae was not correlated with PGC-1α or mtDNA content. In summary, placental PGC-1α/TFAM/mitochondrial biogenesis pathway is affected by maternal diabetes and offspring sex. Decreased PGC-1α in response to maternal diabetes plausibly contributes to impaired mitochondrial biogenesis in placentae of male offspring, which may affect long-term health and explain some of enhanced risk of future metabolic diseases in males.

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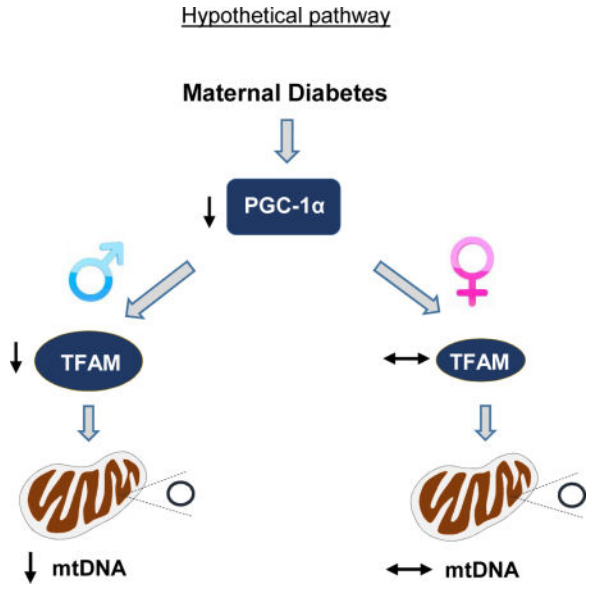
Author contributions:

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. CEA did the statistical analysis. All authors revised the manuscript and approved this version to be published.

Duality of interest:

There is no conflict of interest associated with this manuscript.

Graphical abstract



Keywords

Placenta; maternal diabetes; fetal sex; PGC-1α; TFAM; mitochondrial DNA

Introduction

Maternal Diabetes during pregnancy not only has short-term impacts on fetal development, but also increases the risk for offspring to develop type 2 diabetes, obesity, and other metabolic disorders later in life [1–6]. A sibling study in Pima Indians demonstrated the important role of the prenatal environment *per se* in metabolic programming, showing that offspring exposed to diabetes *in utero* had a 3.7-fold higher risk of diabetes compared to siblings born before their mother developed diabetes [7]. While the exact mechanisms underlying this phenomenon remain unclear, hyperglycemia during pregnancy is considered a major factor [1, 2, 8]. Among offspring, maternal hyperglycemia is associated with increased fetal/neonatal adiposity [9], lower insulin sensitivity, altered β-cell function [8], and higher long-term risks of type 2 diabetes [2, 10, 11] and overweight/obesity [12].

Fetal sex-associated differences have been observed. Pregnant women carrying a male fetus have poorer pancreatic beta cell function and are at increased risk of gestational diabetes [13]. In male infants born to women with gestational diabetes, maternal fasting blood glucose concentration is a major predictor of adiposity, but has little relationship to adiposity in female infants. Conversely, maternal BMI is the primary predictor of adiposity for female infants but has little effect in males [14].

The placenta is the critical organ mediating all communications between mother and fetus, and thus must be involved in the effects of maternal diabetes on offspring [15]. Functional changes occurring in response to maternal diabetes include reduced fatty acid oxidation,

impaired mitochondrial function, and increased production of reactive oxygen species [16, 17]. Abnormal placental function could in turn affect nutrient transfer and alter the constituency of bio-active molecules released into the fetal circulation, ultimately affecting fetal growth and predisposing offspring to metabolic disease in later life [18–20]. Furthermore, sexual dimorphism in structure, function [21] and gene expression [22] in human placenta has been shown, suggesting that sex-specific placental responses and adaptation may mediate certain fetal sex-associated differences.

Our previous studies demonstrated a decrease in the catalytic subunits of AMP activated protein kinase (AMPKα1) in placentae of diabetic women [23]. AMPK plays an essential role in maintaining cell energy homeostasis and it regulates the expression and activation of PGC-1α, the master regulator of mitochondrial biogenesis and energy metabolism [24–26]. In the present work, we examined the effects of maternal diabetes and fetal sex on PGC-1α and the mitochondrial biogenesis pathway in human placenta. PGC-1α stimulates mitochondrial biogenesis by activating transcription factors that promote expression of TFAM, which directly regulates mitochondrial DNA transcription and replication. We compared the abundance of PGC-1α and TFAM proteins, and mitochondrial DNA copy number in the placentae of infants born to women with diabetes during pregnancy to controls and demonstrate that maternal diabetes impacts the placental PGC-1α/TFAM/mitochondrial biogenesis pathway in a sex-dependent manner.

Research Design and Methods

Subjects for Placenta Samples

Placental samples were obtained from self-identified Native American and Hispanic women with gestational diabetes (GDM), pregestational type 2 diabetes, or with normoglycemic pregnancies. Mothers and offspring were enrolled into a prospective longitudinal study on the impact of *in utero* exposure to diabetes, as previously described [27]. Gestational and type 2 diabetes in the mothers was diagnosed according to ADA guidelines [28]. Women were excluded if the infants were small for gestational age, had a major malformation, or chromosome abnormality. They also were excluded if they delivered prior to 37 weeks gestation, had type 1 diabetes, pre-eclampsia, chronic hypertension, renal disorders or a smoking history of more than 5 cigarettes per day during pregnancy. 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. Maternal fasting blood glucose concentrations (before the fasting oral glucose tolerance tests) were obtained retrospectively from medical records.

Placentae Dissection

Term placentae were dissected as soon as possible after delivery, generally within one hour, and processed as previously described [23]. An approximately three cm diameter core was obtained by cutting from the fetal surface down through the maternal surface. The core was cut into thirds such that one-third was fetal-side tissue, one-third was maternal-side tissue and the other the middle third. Only the fetal-side placenta samples were used in the present

study. The fetal membrane was removed and the remaining tissue washed with icecold saline, blotted dry and stored at −80°C.

Western Blot Analysis

Placental samples were lysed and homogenized in protein lysis buffer containing a protease and phosphatase inhibitor cocktail (Pierce Biotechnology, Rockford, IL; part number 78443). Protein concentrations were measured by BCA assay (Pierce, Rockford, IL). Thirty μg of protein lysate was treated with reducing agent (beta-mercaptoethanol), 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 (Abcam, Cambridge, MA), TFAM, AMPKα1, 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 real-time PCR, forward primer: 5′-CACCCAAGAACAGGGTTTGT; reverse: 5′-TGGCCATGGGTATGTTGTTA) and with that of the nuclear β2-macroglobulin gene (forward: 5′-TGCTGTCTCCATGTTTGATGTATCT; reverse: 5′-TCTCTGCTCCCCACCTCTAAGT).

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; chi-squared test for counts. The Generalized Linear Model (GLM) framework was used to determine the significance of the effect of diabetes on PGC-1α and TFAM levels after adjusting for the covariates listed in Table 1. Differences in the effect of diabetes due to sex of infant were assessed by fitting a diabetes × sex interaction. Correlations were calculated as standardized regression coefficients. Data analyses used Excel and IBM SPSS Statistics (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). P-values <0.05 were treated as statistically significant for the purposes of discussion.

Results

Characteristics of Study Population

Demographics for participants providing placental samples are shown in Table 1. Compared with non-diabetic controls, those with diabetes had higher HbA1c, were older, and delivered slightly earlier (about 4 days) in gestation. Among the diabetic mothers, five were treated with glyburide, seven with insulin, one with metformin, and six with diet alone. The diabetic

and control groups were no different with respect to ethnicity, body mass index, infant sex, birth weight, or mode of delivery.

Placental PGC-1α and TFAM expression is lower in female offspring

We initially compared placental PGC-1α/TFAM pathway and mitochondrial DNA content between male and female offspring of non-diabetic controls. Both PGC-1α and its downstream target TFAM [29] in female placentae were approximately 60% that in male placentae (Figure 1A and 1B). The level of AMPK phosphorylation (P-T172-AMPKα) was also lower in female placentae compared to males (Figure 1A and 1B). However, protein abundance of AMPKα1 (T-AMPKα1, Figure 1A and 1B) and mtDNA copy number (Figure 1C) were no different between placentae from females and males.

Maternal DM alters placental PGC-1α/TFAM and mitochondrial DNA content in a sex-dependent manner

We next examined the effects of maternal diabetes on indices of mitochondrial biogenesis. Overall, including values obtained from both male and female offspring, maternal diabetes was associated with a reduction in PGC-1α (by 38%, $p<0.01$) and TFAM (by 37%, $p<0.01$) abundance in placentae. After adjusting for the effects of each covariate listed in Table 1, the effect of diabetes on PGC-1α (p for diabetes effect < 0.02) and TFAM (p for diabetes effect < 0.007) remained significant regardless of covariate.

The effects of maternal diabetes were examined separately for each sex. In male offspring, PGC-1α (Figure 2A) and TFAM (Figure 2B) proteins, as well as mtDNA copy number (Figure 2C), were significantly lower in placentae of diabetic women than controls. Placental TFAM in males was highly correlated with PGC-1α (Figure 2D), and there was significant correlation between mitochondrial DNA copy number and TFAM protein abundance (Figure 2E). However, in female offspring, despite reduced placental PGC-1α abundance (Figure 3A), diabetes was not associated with a decrease in TFAM (Figure 3B), nor mtDNA copy number (Figure 3C). In addition, there was no significant association between placental PGC-1α and TFAM in females (Figure 3D), nor between mtDNA copy number and TFAM levels (Figure 3E). Additional analysis on the overall data of both males and females revealed that for TFAM, the diabetes × sex interaction term was significant ($p = 0.0021$), validating the differential effect of sex on the offsprings' response to diabetes exposure.

Negative correlation of maternal blood glucose concentrations with placental PGC-1α/TFAM and mitochondrial DNA content

The results of correlation analysis show that in pregnant women with male offspring, maternal fasting blood glucose concentration was inversely associated with placental PGC-1α ($P<0.05$) and TFAM ($P<0.05$) with statistical significance (Figure 4). There was a trend for negative relationship of maternal fasting blood glucose with placental mtDNA copy number ($P=0.067$).

Discussion

The placenta requires sufficient mitochondrial metabolic activity to meet the high energy demands of gestation required for fetal growth and development [15, 20, 30]. As a key regulator of mitochondrial biogenesis and energy metabolism, PGC-1α is positioned to play a central role in optimizing placental function. In this study, we report that the abundance of PGC-1α in the placentae is reduced in offspring of diabetic women, but the effects on the downstream targets (TFAM and mitochondrial DNA content) are varied depending on offspring sex.

The critical roles of PGC-1α as a determinant of mitochondrial function and energy metabolism have been extensively demonstrated in adipose tissue, skeletal muscle, heart, neurons, and pancreatic β-cells [31, 32]. Much less is known about the regulation and function of PGC-1α in the placenta, especially in the context of maternal diabetes. The placenta is not only the conduit of oxygen to the fetal circulation but is also a significant consumer of oxygen [33]. With the decreased placental blood flow in maternal diabetes [34, 35] the placenta undergoes metabolic remodeling to reduce mitochondrial oxygen consumption in order to preserve oxygen delivery to the fetus [33]. In this regard, the reduction in placental PGC-1α abundance in response to diabetes we report is a plausible mechanism for such placental remodeling. Interestingly. Kelstrup et al [36] recently reported PGC-1α gene expression was 40% lower in skeletal muscle of adults born to mothers with GDM, supporting the ideas that the changes we find in placentae also occur in other offspring organs, likely as an important component of the long-term effects of prenatal exposure to diabetes.

PGC-1α stimulates mitochondrial biogenesis by increasing transcriptional activity of nuclear respiratory factor 1 which, in turn, increases expression of TFAM [29], which directly regulates mitochondrial DNA transcription and replication [37]. We found that maternal diabetes was associated with a significant decrease in TFAM protein levels in placentae of male offspring and was tightly correlated with PGC-1α expression and mitochondrial DNA copy number, consistent with the regulatory role of PGC-1α in TFAM expression and mitochondrial biogenesis. In contrast, although female offspring from diabetic women showed reduced placental PGC-1α, abundance of TFAM and mitochondrial DNA copy number were unaffected, revealing sexual dimorphism in the PGC-1α/TFAM/mitochondrial biogenesis system. This influence of offspring sex is additionally supported by our observation that PGC-1α and TFAM protein abundance, as well as AMPK activation status, were generally lower in female compared to male placentae.

The precise significance of the sex difference we report is unclear, however it is consistent with emerging evidence that the response of the fetus to environmental influence differs by sex [38]. Males born to mothers with GDM have higher cord blood levels of leptin and c-peptide [39] and increased adiposity both at birth [14] and at school age [40] compared to similarly exposed females. Sexual dimorphism of mitochondrial biogenesis has been demonstrated in the brain, cardiac muscle, liver, and brown adipose tissue of rodents [41, 42], but not previously reported in placenta in the context of maternal diabetes to our knowledge. Although speculative, the sex dependent effects on factors regulating

mitochondrial abundance or function, if persistent, offer a potential mechanism that could explain subsequent changes in body composition or metabolic state.

Our findings are consistent with a recent study by Muralimanoharan S. *et al* [43], which also reported a reduction in placental PGC-1α content in the context of GDM. However, the number of subjects they examined was likely too small to detect any sex differences. These authors also reported reduced oxygen consumption by cultured trophoblast from mothers with GDM, which would be anticipated given our observations of reduced PGC-1α and TFAM. Our study also demonstrated a negative association of maternal blood glucose levels with PGC-1α and TFAM content in placentae of males, supporting the important role of intrauterine hyperglycemia in affecting placenta mitochondrial biogenesis pathway.

The strengths of our study are the examination of relevant human tissue susceptible to the influence of maternal diabetes in a population at increased risk for type 2 diabetes, the determination of correlation between protein levels of key factors of mitochondrial biogenesis and mitochondrial DNA content, and the detection of fetal sex differences. The limitations are the intrinsic variability in expression of placental proteins and the somewhat limited sample size that could obscure other important relationships, such as the effects of medication during pregnancy. Further studies about the potential effects of various aspects of maternal status (e.g. degree of dysglycemia, form of treatment) are warranted and would require studies of larger scope and different design.

In summary, we found that the placental PGC-1α/TFAM/mitochondrial biogenesis pathway is affected by maternal diabetes and offspring sex. The effect on PGC-1α plausibly contributes to impaired placental mitochondrial biogenesis that may ultimately affect long-term health and explain some of enhanced risk of future metabolic disease in males.

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Abbreviations

mtDNA	Mitochondrial DNA
PGC-1α	Peroxisome proliferator-activated receptor-γ coactivator-1α
TFAM	Mitochondria transcription factor A
AMPK	AMP activated protein kinase
GDM	Gestational diabetes

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Highlights

1. Placental PGC-1α and TFAM proteins are lower in female offspring than males.
2. Placental PGC-1α is reduced in both male and female offspring of diabetic women.
3. The effects of maternal diabetes on TFAM/mtDNA content are fetal-sex dependent.

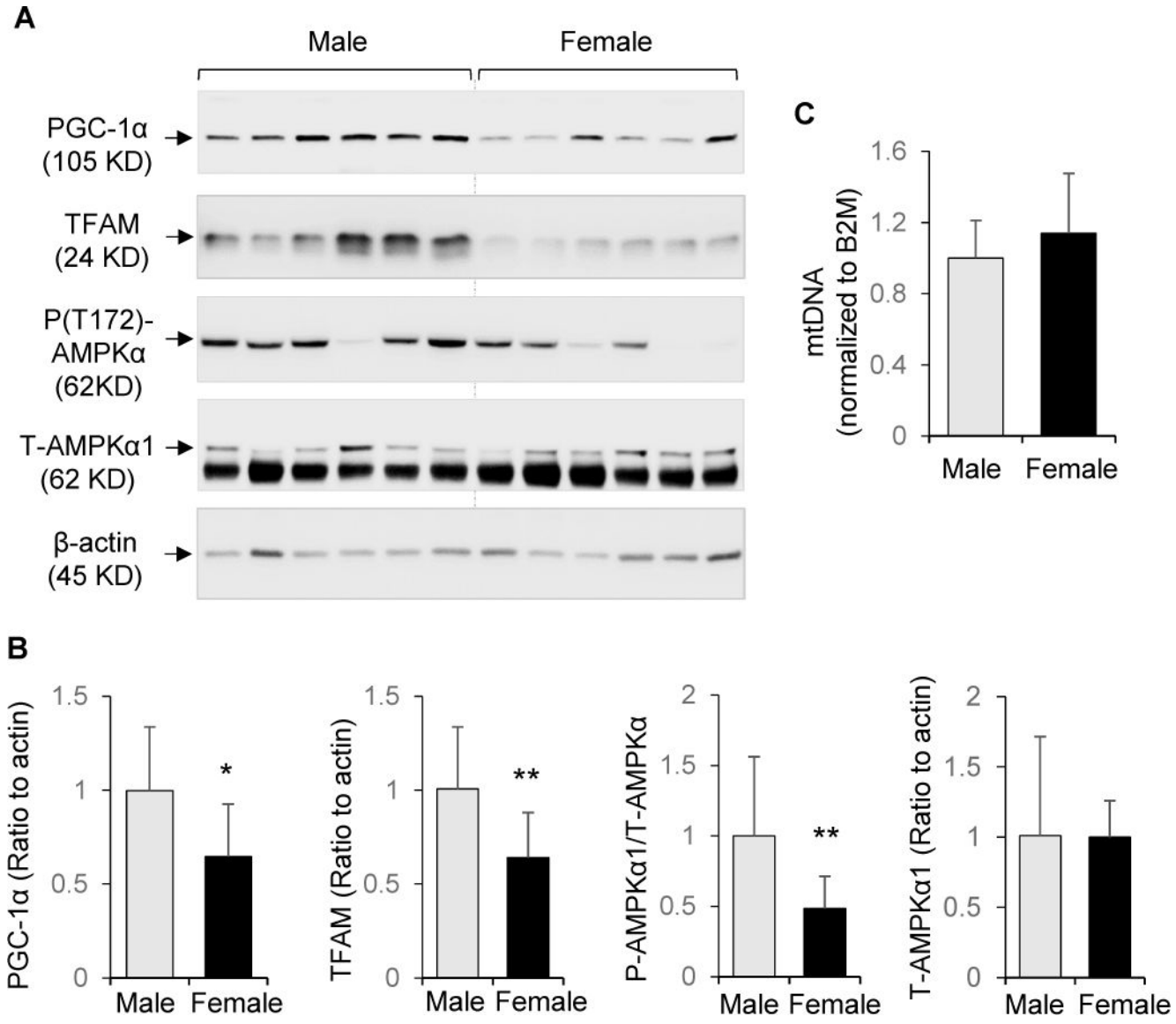

Figure 1.

PGC-1α and TFAM protein abundance in placentae from controls. Protein extracts were subjected to Western blot analysis and DNA extracts were analyzed by quantitative RT-PCR. A: Representative blots from male and female offspring. B: Quantitation of PGC-1α, TFAM, P-(T172)-AMPKα, and total AMPKα1 (T-AMPKα1, mean ± SD) from Western blots, using β-actin as reference. C: Fold change of mitochondrial $tRNA^{Leu(UUR)}$ gene DNA copy number normalized to nuclear β2 microglobin (B2M). For males, n=10. For females, n = 13. * P<0.05; ** P<0.01.

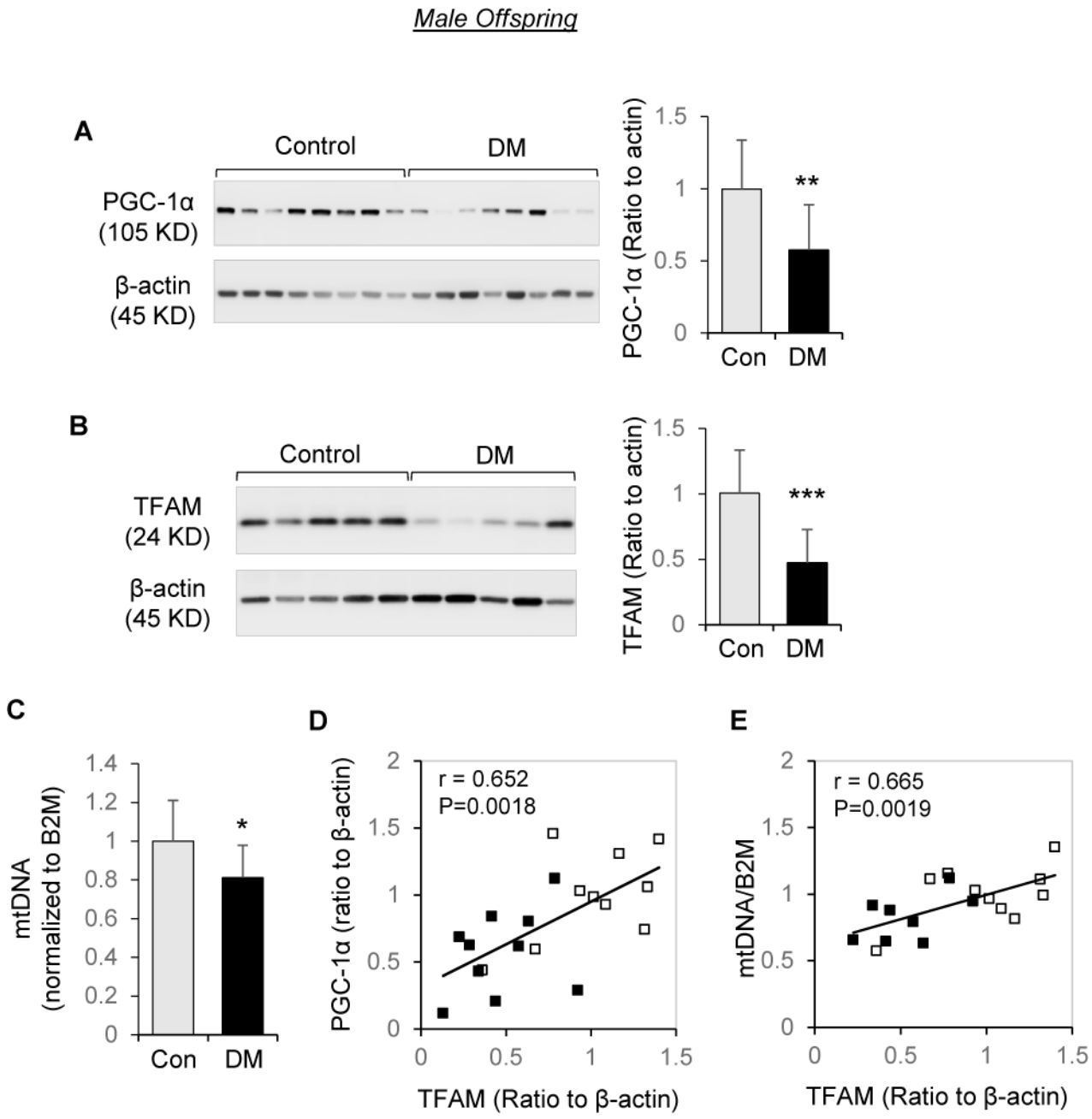

Figure 2.

Effects of maternal diabetes on PGC-1α/TFAM/Mitochondrial DNA abundance in placentae of male offspring. Protein extracts subjected to Western blot analysis and DNA extracts were analyzed by quantitative RT-PCR. A and B: Representative Western blots and quantitation of PGC-1α (A) and TFAM (B) in male placentae of diabetic women (DM, n=10) and non-diabetic controls (n=10). C: Fold change of mitochondrial $tRNA^{Leu(UUR)}$ gene DNA copy number normalized to β2-microglobin (B2M); n=9 for DM, n=10 for control. D: Correlation between PGC-1α and TFAM protein levels. E: Correlation between mtDNA copy number to TFAM protein levels. Open square: control group; Solid square: DM. Bar graphs are presented as mean ± SD, * P<0.05; ** P<0.01; *** P<0.001.

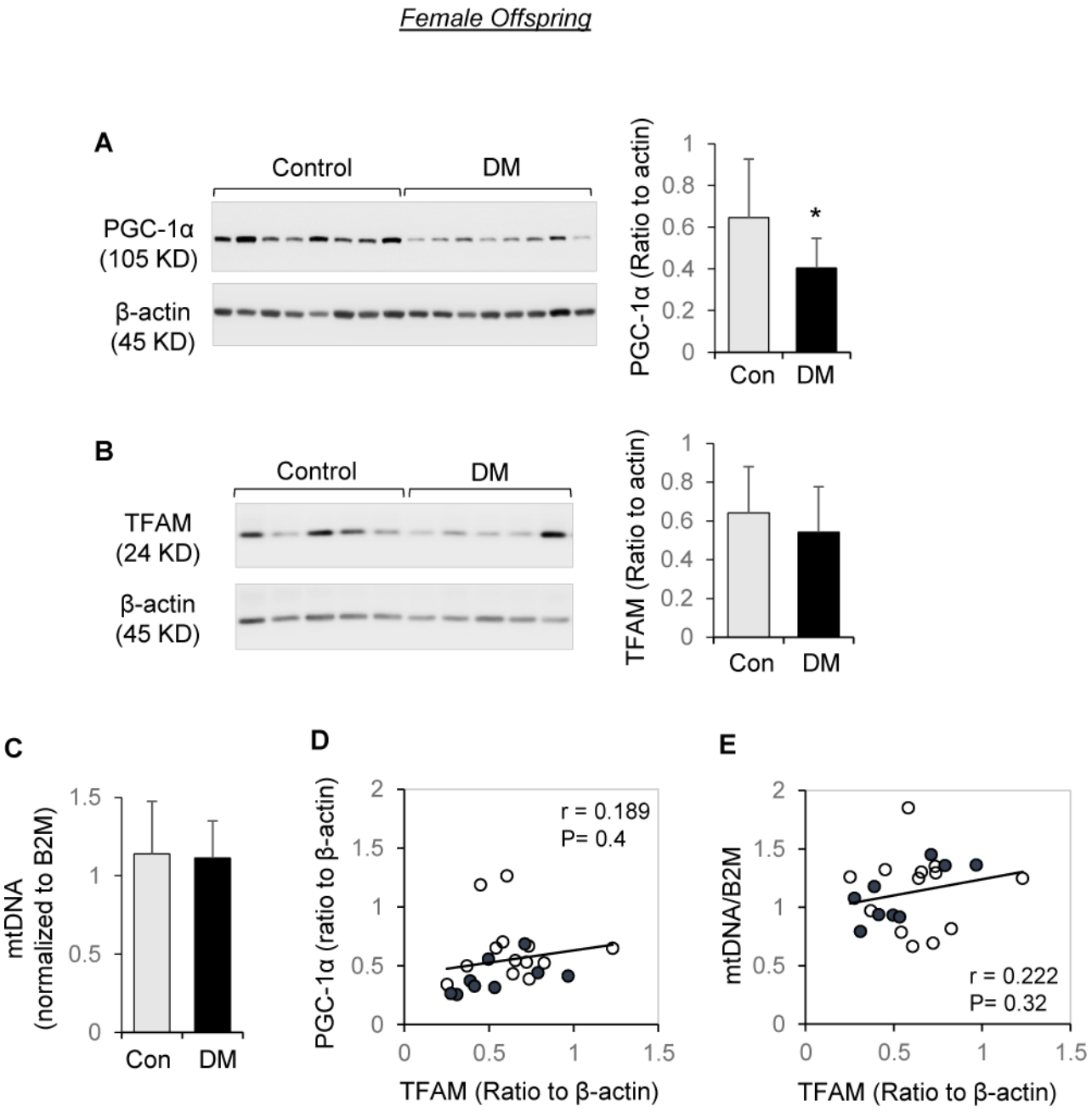

Figure 3.

Effects of maternal diabetes on PGC-1α/TFAM/Mitochondrial DNA abundance in placentae of female offspring. Protein extracts subjected to Western blot analysis and DNA extracts were analyzed by quantitative RT-PCR. A, B, and C: Representative Western blots and quantitation of PGC-1α (A) and TFAM (B), and fold change of mitochondrial $tRNA^{Leu(UUR)}$ gene DNA copy number normalized to β2-microglobin (C) in female placentae of diabetic pregnancies (DM, n=9) and non-diabetic controls (n=13). Bar graphs are presented as mean ± SD, * P<0.05. D: Correlation between PGC-1α and TFAM protein levels. E: Correlation between mtDNA copy number to TFAM protein levels. Open circle: control group; Solid circle: DM.

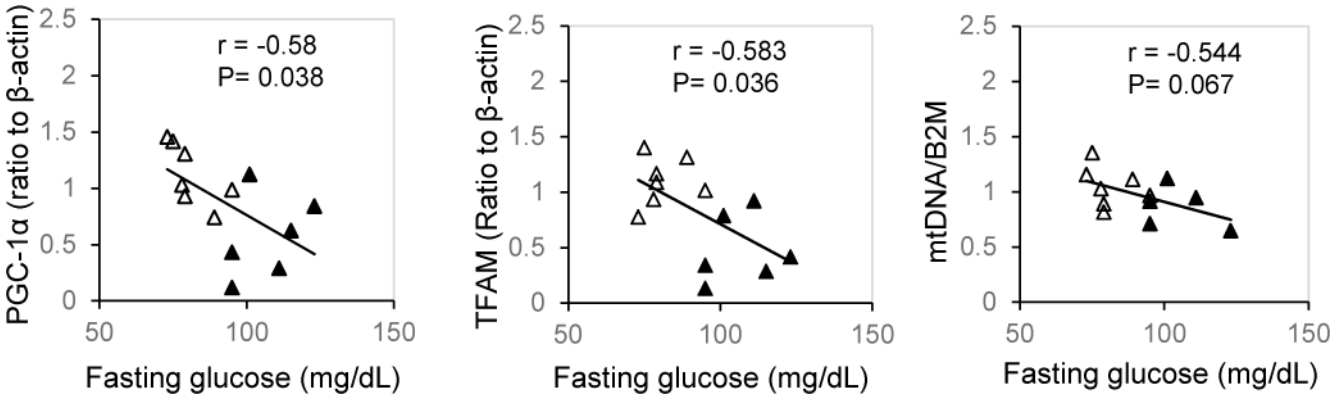

Figure 4.

Relation between maternal fasting blood glucose concentrations with PGC-1α/TFAM/ Mitochondrial DNA abundance in placentae of male offspring. Graphs show correlation of maternal fasting blood glucose concentrations with placental PGC-1α, TFAM protein levels, and mtDNA copy numbers. Open triangle: control group; Solid triangle: DM.

Table 1

Characteristics of Research Subjects Providing Placenta Samples

	DM	Control	P-value DM vs Control
n	19 (16 GDM, 3 Type 2 diabetes)	23	
Maternal Age, yrs	31 ± 5	27 ± 5	0.021
Maternal race			0.48
American Indian	13 (68%)	17 (74%)	
Hispanic	6 (32%)	6 (26%)	
Maternal HbA1C, %	5.6 ± 0.7	5.1 ± 0.2	0.013
Maternal BMI	34.7 ± 5.3	33.5 ± 4.3	0.40
Gestational age, weeks	38.9 ± 0.7	39.4 ± 0.8	0.045
Mode of delivery			0.77
vaginal	14 (74%)	16 (70%)	
cesarean	5 (26%)	7 (30%)	
Sex of infant			0.55
male	10 (53%)	10 (43%)	
female	9 (47%)	13 (57%)	
Birth weight, kg	3.47 ± 0.60	3.36 ± 0.44	0.50

Values are means ± SD. BMI: Body Mass Index (kg/m2). DM: diabetes