

Differences in maternal and neonatal cardiometabolic markers and placenta status by foetal sex. The GESTAFIT project

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Virginia A Aparicio^{1,2}, Laura Baena-García^{2,3,4} ,
Marta Flor-Alemany^{1,2}, Luis J Martínez-González⁵ ,
Alfonso Varela-López¹, Cristina Sánchez^{1,2} and José L Quiles¹

Abstract

Aims: To explore the differences in some maternal-neonatal metabolic markers and placenta status by foetal sex.

Methods: One hundred thirty-nine Caucasian pregnant women from the GESTAFIT project and their new-borns were included in the present cross-sectional study. Serum cardiometabolic markers (i.e. lipid and glycaemic profile and uric acid) were analysed at late pregnancy and at birth. In placenta, telomeres length, proportion of deleted mitochondrial-DNA and mitochondrial-DNA density, some minerals and interleukin 8, epidermal growth factor, fibroblast growth factor-2 and vascular endothelial growth factor were measured. The study was run between November 2015 and April 2018.

Results: Mothers carrying a male showed higher serum triglycerides than mothers carrying a female at late pregnancy ($p < .05$). Serum total and low-density lipoprotein cholesterol were greater in males' umbilical cord blood artery compared to females' new-borns (both, $p < .05$). Mothers of males and male new-borns presented higher uric acid than mothers of females and female new-borns at birth ($p < .05$). Female's placentas presented greater placental-newborn weight ratio, manganese content and fibroblast growth factor-2 (all, $p \leq .05$), and evidence of statistical significance in telomeres length, which were 17% longer ($p = .076$).

Conclusion: Our findings show weak differences in some cardiometabolic and placental status markers by foetal sex. Notwithstanding, we observed a slightly more proatherogenic profile in both, mothers carrying males' foetuses and male new-borns. We also found lower serum uric acid and better placenta status in mothers carrying a female. These findings indicate that foetal sex might need to be considered for a more personalized follow-up of pregnancies.

Keywords

fibroblast Growth Factor, lipids, newborn, pregnancy, telomere, triglycerides

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¹Department of Physiology, Institute of Nutrition and Food Technology and Biomedical Research Centre, University of Granada, Granada, Spain

²Sport and Health University Research Centre, University of Granada, Granada, Spain

³Department of Nursing, Faculty of Health Sciences, University of Granada, Granada, Spain

⁴Institute of Biosanitary Research (IBS), Granada, Spain

⁵GENYO: Centre for Genomics and Oncological Research: Pfizer/University of Granada/Andalusian Regional Government, Granada, Spain

Corresponding author:

Laura Baena-García, Department of Nursing, Faculty of Health Sciences, University of Granada, Calle Cortadura del Valle s/n, 51001 Ceuta, Spain.
Email: lbaenagarcia@ugr.es



Introduction

Pregnancy implies a strict physiological regulation¹ in which any alteration in the maternofoetal environment may have consequences in the mother, foetus and newborn.² Therefore, environmental and behavioural factors (e.g. maternal exposure to toxics, diet and physical activity) may influence this maternofoetal status, increasing or preventing the risk of pregnancy-related complications.² In this context, foetal sex may also influence the different metabolic responses that occur throughout pregnancy. Previous evidence has suggested that relevant pregnancy outcomes are sex-dependent.^{3,4} Notwithstanding, literature is equivocal and elusive. Regarding birth-related outcomes, females present lower stillbirths and neonatal mortality and better outcomes in the perinatal period, particularly after preterm births.^{4,5} However, the differences in maternal and neonatal cardiometabolic profiles and placenta status by foetal sex requires further study.

Although hyperlipidaemia is an important risk factor for metabolic diseases during pregnancy,⁶ there is a lack of studies exploring the influence of foetal sex on maternal lipids, especially among pregnant women without complications.⁷ Moreover, to the best of our knowledge, only the study by Pac-Kożuchowska et al.⁸ has compared umbilical cord lipids by sex, showing that male newborns had lower levels of lipids. Regarding glycaemic profile, women carrying males seem to present higher rates of gestational diabetes mellitus (GDM)⁹ and worse glucose metabolism.⁹

Foetal sex (sexual dimorphism) might also influence placenta status.⁸ Some authors have hypothesized that male placenta is more efficient,^{10–12} while others failed to find such conclusion.¹³ Dysfunctional placenta ageing is also associated with obstetric complications. It seems that maternal dyslipidaemia might accelerate epigenetic ageing of the placenta specially in women carrying a female.¹⁴ Similarly, the adverse effect of placental inflammation might be greater in females.¹⁰ In contrast, others have found an interaction effect between maternal body mass index and foetal sex, with positive differences only in females.¹⁰ Placenta of male and female fetuses has different protein and gene expressions, especially in adverse conditions,^{12,13} which in turn suggests a sex-dependent physiological variation.¹³

Further studies are required to explore if maternal and neonatal glycaemic and lipid profile and uric acid, a powerful marker of cardiometabolic and perinatal risks^{15,16} and placenta status differ by foetal sex, especially among pregnant women without complications. This is of clinical interest to better understand metabolic pathways, and to design sex-specific guidelines and interventions. Consequently, the aim of the present study was to explore sex-related differences in maternal and neonatal

cardiometabolic markers during late pregnancy (34th gestational week, g.w.) and at birth, and on some markers of placenta status.

Methods

Design

These are secondary analyses from the GESTAFIT project¹⁷ where a concurrent exercise intervention (aerobic + resistance) was conducted. The entire methodology of the project and the inclusion-exclusion criteria has been previously published.¹⁷

Participants

From the 384 pregnant women contacted at the University Hospital during early pregnancy (12th g.w.) we included a final study sample of 139 for the present study (see complete flow-chart in Supplementary Figure S1).

Data collection

The study was run between November 2015 and April 2018. The evaluation and sample recollection procedures were performed on non-consecutive days. On the first appointment (15th–17th g.w.), the recruited participants came to the research centre, and sociodemographic and clinical data, blood pressure, and body composition were assessed. Before leaving, each participant was given an accelerometer to wear during 9 consecutive days. On the second appointment (one week later), participants attended our research centre to return the questionnaires and accelerometers. The third appointment took place in the second evaluation at 33th–35th g.w. where a 5 ml blood sample was collected under fasting conditions (further detailed in Supplementary Appendix S1). Finally, 3–5 minutes after birth, blood samples from the mother and the umbilical cord vein and artery were also drawn. Placental samples were collected immediately after birth. Placentas were visually examined ensuring completeness, consistency, absence of accessory lobes, placental infarction, tumours, and nodules and samples of 2 cm x 2 cm were obtained. The sample was taken from the central placental region, excluding areas with necrosis, signs of ischaemia or calcification. The samples were washed with saline solution (0.9% and 0.1% butylhydroxytoluene). All the samples, once prepared, were aliquoted and kept in a -80°C freezer until posterior analysis.

Sociodemographic and clinical data. A self-reported survey was employed to gather information on sociodemographic and clinical data of study participants. The research team provided instructions to participants to complete that survey properly.

Maternal and neonatal anthropometry. Pre-pregnancy body weight was self-reported. At the 16th g.w. maternal body weight and height were assessed using a scale (InBody R20; Biospace, Seoul, Korea) and a stadiometer (Seca 22, Hamburg, Germany), respectively. Body mass index was calculated as weight (kg) divided by squared height (m²). The neonate's body weight was measured with an adapted scale (Kern MBC20K10M, Germany).

Blood pressure and resting heart rate. Systolic and diastolic blood pressure and resting heart rate were assessed through a blood pressure monitor (Omron, The Netherlands), while women were seated. Measurements were taken twice, 5 minutes apart, and the mean heart value and the lowest value of the two blood pressure measurements was used if the difference between measurements was lower to 5 mm/Hg.

Maternal diet. The Mediterranean Diet Score¹⁸ was used to evaluate overall maternal diet quality. In pregnant women, this self-reported questionnaire ranges from 0 to 50, with higher scores indicating a healthier diet.

Sedentary time and physical activity. Objectively measured sedentary time and physical activity levels were assessed with a triaxial accelerometer (ActiGraph GT3X+, Florida, US) in the waist during 9 consecutive days, 24 hours/day following the methodology detailed elsewhere.¹⁹

Glucose, lipids and uric acid. Maternal serum glucose, total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), phospholipids and uric acid concentrations were measured using standard spectrophotometric enzyme assays (AU5822 Clinical Chemistry Analyzer, Beckman Coulter, Brea, CA, USA). At birth, these maternal and arterial and venous cord serum markers were also determined.

Insulin. Maternal insulin was assessed via paramagnetic-particle-based chemiluminescence immunoassays (UniCel DxI 800 Access Immunoassay analyser, Beckman Coulter, Brea, CA, USA). Standard formula²⁰ was employed to calculate the homeostasis model assessment (HOMA)-IR (insulin resistance).

Placenta weight. The placenta was weighed three times on a calibrated digital device to the nearest gramme (Kern MBC20K10M, Germany) within one hour after delivery. Placental-newborn weight ratio was calculated.

Placental angiogenesis and grow markers. We used Luminex xMAP technology (Millipore, Darmstadt, Germany) to measure some placenta markers (Interleukin 8, epidermal growth factor, fibroblast growth factor 2 and vascular

endothelial growth factor). For these determinations we used Human Sepsis Magnetic Bead Panel 3 Multiplex Assay (cat. No. HTH17MAG-14K). We prepared samples, reagents, and standards following the manufacturer's instructions. The amount of placenta extract used in the determinations was 25 µL. The plate was read on LAB-Scan 100 analyser (Luminex Corporation, Texas, USA) with xPONENT software for data acquisition. The average values for each set of duplicate samples or standards were within 15% of the mean.

Relative telomere length analysis. Relative telomere length was assessed by a quantitative reverse-transcription polymerase chain reaction (QRT-PCR) method developed by Cawthon et al.²¹ with slight modifications²² (further detailed in Supplementary Appendix S2). Amplification of telomeric DNA (T), together with that of the single-copy genomic Rplp0 gene (S) was performed on a Micro Amp Optical 384-well Reaction Plate (Applied Biosystems, Foster City, CA, USA) using Applied Biosystem's 7900HT Fast Real-Time PCR system. Mean quantification cycle (Cq) values and standard deviation were calculated for each sample and the absolute concentration of the telomere hexameric repeat (T) was divided by the absolute concentration of the Rplp0 gene (S) according to efficiency values calculated from their standard curves. The resulting value (T: S ratio) was divided by the T:S ratio determined from the calibrator DNA (one of our samples). The resulting ratio expresses the amount of telomere hexameric repeats, called Relative Telomere Length.

Proportion of deleted mitochondrial DNA (mtDNA) and mtDNA copy number

The proportion of deleted mtDNA and relative levels of mtDNA copy number were determined by using a QRT-PCR to amplify two mitochondrial genes, MT-ND4 and MT-ND1 and a single-copy nuclear gene, Rplp0, as previously described²³ using the instruments, material, conditions and reagents described in previous section, except for mitochondrial genes oligonucleotide primers (further detailed in Supplementary Appendix S3). Mean Cq for each sample was calculated using Sequence Detector Systems version 2.4 software. The proportion of deleted mtDNA was calculated for each well as the ratio between MT-ND4 and MT-ND1 amplification data considering reaction efficiency values. The resulting values were divided by the average obtained from control samples analysis. This allowed to obtain a relative MT-ND4: MT-ND1 ratio. In turn, the MT-ND1: Rplp0 ratio was calculated and multiplied per two to obtain mtDNA copy number for each sample.

Minerals. Samples for quantitative determination of metals were first lyophilized in a vacuum pump (Telstar, Madrid,

Spain) and then 0.2 g of lyophilized sample were mineralized by attack with HNO₃ and H₂O₂ of supra-pure quality (Merck, Darmstadt, Germany), in a microwave digester (Milestone, Sorisole, Italy). The determination of total content of these essential elements (Mn, Fe, Zn and Se) in placenta samples was performed by an ICP-MS instrument (Agilent 7500, Agilent Technologies, Tokyo, Japan) fitted with a Meinhard type nebulizer (Glass Expansion, Romainmotier, Switzerland) and equipped with a He collision cell (further detailed in Supplementary Appendix S4).

Data analysis

Descriptive statistics (number of women (%) for categorical variables and mean (standard deviation) for quantitative variable) were employed to describe baseline characteristics of the participants. Baseline comparisons between pregnant women carrying a male versus a female were performed by using Student's *t*-test in quantitative variables, and by chi-square test in categorical variables. Analyses of covariance (ANCOVA) were employed to assess differences in maternal serum and placental markers between pregnant women carrying a female and male foetus. Since a concurrent physical exercise programme was performed from 17th g.w. until birth in the GESTAFIT project,¹⁷ we adjusted all the comparisons for the exercise intervention (control or intervention). Neonatal and placenta markers were additionally adjusted for gestational age. The analyses were performed by using SPSS 22.0 (IBM, NY, USA) and the level of significance was set at $p \leq 0.05$.

Validity, reliability and rigour

The quantitative instruments used for the measurement of the study variables (e.g. physical activity, cardiometabolic and placental markers or telomere length) are validated and fully described in the supplementary appendices.

Results

From all interested participants ($n=384$), the final study sample was composed of 139 Caucasian pregnant women (mean age 33.0 years old, standard deviation=4.6) and their neonates. However, some placenta and blood samples could not be extracted or analysed, which means a loss of data in those outcomes (see Supplementary Figure S1).

The sociodemographic, anthropometric and clinical characteristics of the study sample (mothers and neonates) by foetal sex are shown in Table 1.

Table 2 shows differences in maternal serum cardiometabolic markers grouped by foetal sex at the 34th g.w. and at birth. At the 34th g.w., maternal serum triglycerides concentration was 16% higher in mothers carrying a male

compared with mothers of females ($p < .05$). At birth, serum uric acid levels were 25% greater in mothers carrying a male ($p < .001$).

Serum biomarkers in umbilical cord vein and artery by the new-born sex are shown in Table 3. Total cholesterol concentrations were higher in umbilical cord artery of male compared with female neonates ($p < .05$). Females also showed 25% lower LDL-cholesterol concentrations in the cord artery compared with males ($p < .05$). Serum uric acid concentration was 17% higher in the umbilical cord artery of males compared with females' neonates ($p < .05$). No differences between groups were found in the vein umbilical cord serum (all, $p > .05$).

Table 4 shows placenta status accordingly to the new-born sex. The results showed evidence of statistical significance in placenta telomeres length, which were 17% longer in placentas from mothers carrying a female ($p = .076$). Female's placentas had greater placental-new-born weight ratio ($p = .047$) and manganese ($p = .051$) and fibroblast growth factor 2 concentrations ($p = .048$).

Discussion

Main findings

The major finding of the current exploratory study indicates that at late pregnancy, mothers carrying a male presented higher serum triglycerides than those carrying a female. Total cholesterol and LDL-cholesterol concentrations were also greater in the umbilical cord artery of males. Moreover, male new-borns and mothers of males showed higher uric acid concentrations compared with female new-borns and mothers of females, respectively. Regarding placenta status, female's placentas showed greater placental new-born weight ratio, 17% longer telomeres, and a higher concentration of fibroblast growth factor 2 and manganese.

Maternal and neonatal uric acid

In the last years, serum uric acid concentrations have been proposed as a marker of cardiovascular disease and all-cause mortality risk in different populations.²⁴ During pregnancy, this biomarker has also been associated with pregnancy-induced hypertension and preeclampsia,^{15,16,25} preterm labour and low-birth weight,²⁶ intrauterine growth restriction,¹⁶ and increased risk of foetal morbidity and mortality.¹⁶ Mothers of males and male new-borns had greater concentrations of uric acid, which reinforces the idea that female foetuses induce a lower perinatal cardiometabolic risk for the mother, the new-born, and perhaps to the child.²⁷ Indeed, in a study performed with adolescents, those who were born prematurely (23th–24th g.w.) with very-low birth weight showed higher

Table 1. Sociodemographic, anthropometric and clinical characteristics of the study sample by foetal sex (n = 139).

	Mothers of males (n = 67)	Mothers of females (n = 72)	p
Age (years)	32.7 ± 4.6	33.2 ± 4.6	.514
Body mass index at early pregnancy (kg/m ²)	24.9 ± 3.8	25.2 ± 4.5	.612
Systolic blood pressure (mm/Hg)	108.5 ± 9.3	107.1 ± 8.6	.374
Diastolic blood pressure (mm/Hg)	63.7 ± 7.7	64.3 ± 7.7	.651
Gestational weight gains (kg)*	8.5 ± 3.6	9.0 ± 3.1	.498
Gestational age (weeks)	39.4 ± 1.4	39.7 ± 1.2	.110
New-born birth weight (g)	3346 ± 483	3267 ± 477	.336
<i>Small for gestational age, (yes, n (%))</i>	5 (7.4)	6 (8.5)	.811
<i>Large for gestational age, (yes, n (%))</i>	9 (13.2)	10 (14.1)	.884
Epidural administration, (yes, n (%))	46 (70.8)	47 (69.1)	.836
Marital status, n (%)			
<i>Single</i>	34 (50.7)	46 (63.9)	.117
<i>Married (or with partner)</i>	33 (49.3)	26 (36.1)	
Number of children, n (%)			
0	43 (64.2)	41 (56.9)	.357
1	22 (32.8)	25 (34.7)	
2	2 (3.0)	6 (8.3)	
Professional status, n (%)			
<i>Worked full/part time</i>	50 (74.6)	47 (66.2)	.700
<i>Unemployed/Retired/Student/Housekeeper</i>	17 (25.4)	24 (33.8)	
Education level, n (%)			
<i>Primary school</i>	8 (11.9)	9 (12.5)	.590
<i>Professional training</i>	10 (14.9)	15 (20.8)	
<i>Secondary school</i>	11 (16.4)	7 (9.7)	
<i>University medium degree</i>	17 (25.4)	14 (19.4)	
<i>University higher degree</i>	21 (31.3)	27 (37.5)	
Sedentary lifestyle and physical activity levels			
<i>Sedentary time (min/day)</i>	3560 ± 776	3575 ± 558	.907
<i>Moderate-vigorous physical activity (min/wk)</i>	285 ± 354	265 ± 143	.671
<i>Total physical activity (min/wk)</i>	3002 ± 712	2968 ± 531	.771
Smoking habit (yes, n (%))	7 (10.4)	6 (8.5)	.688
Mediterranean Diet Score (0–50)	29.6 ± 3.9	29.1 ± 3.5	.446

Continuous variables are presented as Mean ± Standard Deviation and categorical variables as Number (Percentage); *, weight gains from 16th until 34th gestational weeks.

serum uric acid levels than those born at term with normal birth weight.²⁶

Maternal and neonatal lipids

At late pregnancy, serum triglycerides were greater in mothers carrying a male. However, at birth mothers did not present sex-related differences in the lipid profile despite the same trend was observed. This lack of statistical significance could be partially explained by the high physiological stress induced by the labour,^{19,28} the maternal diet previous to labour (they were not in fasting conditions such as in 34th gestational week), or the lower sample size of maternal serum samples at birth, regardless of new-born sex.

Differences in artery umbilical cord serum lipids are the result of the foetal metabolism and the contribution

of fatty acids and cholesterol synthesis by the foetus itself.²⁹ In our study, male new-borns showed higher arterial cord total cholesterol and LDL-cholesterol. Considering that arterial cord cholesterol and LDL-cholesterol are likely to be from foetal origin, our findings suggest that male foetuses have a more lipogenic metabolism. Interestingly, as mentioned above, we also observed that serum triglycerides were more elevated in mothers of males at late pregnancy, which has not been confirmed by Lima et al.⁷ under the DALI study (performed in obese pregnant women). This might be relevant if we consider that hyperlipidaemia but especially hypertriglyceridemia is an important risk factor for metabolic diseases along pregnancy.^{6,30} In fact, triglycerides concentrations are significantly elevated in women with GDM and hypertensive gestational disorders.^{6,30} High concentrations of triglycerides during late pregnancy

Table 2. Serum cardiometabolic markers in pregnant women by foetal sex at late pregnancy and at birth.

	Mothers of males	Mothers of females	<i>p</i>
34th gestational week	(n = 53)	(n = 55)	
Total cholesterol (mg/dL)	276.7 ± 5.3	272.1 ± 5.2	.531
Triglycerides (mg/dL)	221.5 ± 10.3	192.2 ± 10.1	.046
HDL-cholesterol (mg/dL)	66.8 ± 1.5	66.4 ± 1.5	.855
LDL-cholesterol (mg/dL)	165.1 ± 5.0	167.0 ± 4.9	.794
Phospholipids (mg/dL)	215.7 ± 8.5	209.2 ± 8.0	.587
Glucose (mg/dL)	75.4 ± 1.6	73.6 ± 1.6	.421
Insulin (microUI/dL)	11.5 ± 2.5	8.05 ± 2.5	.328
Uric acid (mg/dL)	0.83 ± 0.07	0.75 ± 0.07	.421
HOMA-IR	2.73 ± 0.76	1.55 ± 0.75	.278
Birth	(n = 21)	(n = 25)	
Total cholesterol (mg/dL)	214.1 ± 12.4	191.3 ± 11.3	.185
Triglycerides (mg/dL)	187.5 ± 17.1	170.5 ± 15.7	.471
HDL-C (mg/dL)	83.8 ± 5.9	71.4 ± 5.4	.132
LDL-C (mg/dL)	48.3 ± 3.4	45.5 ± 3.2	.556
Phospholipids (mg/dL)	212.7 ± 10.1	195.1 ± 9.3	.208
Glucose (mg/dL)	89.0 ± 7.0	93.0 ± 6.4	.675
Insulin (microUI/dL)	-	-	-
Uric acid (mg/dL)	5.8 ± 0.27	4.3 ± 0.25	< .001

HDL: high density lipoprotein; LDL: low density lipoprotein; C: cholesterol; HOMA-IR: homeostasis model assessment for insulin resistance. Values expressed as mean ± standard error. Model adjusted for the exercise intervention programme.

Table 3. Serum biomarkers in umbilical cord vein and artery for male and female new-borns.

	Males	Females	<i>p</i>
Umbilical cord vein	(n = 17)	(n = 24)	
Total cholesterol (mg/dL)	66.5 ± 11.2	61.7 ± 9.4	.744
Triglycerides (mg/dL)	43.7 ± 5.9	48.7 ± 5.0	.518
HDL-cholesterol (mg/dL)	24.9 ± 2.9	26.1 ± 2.5	.754
LDL-cholesterol (mg/dL)	7.97 ± 1.9	8.76 ± 1.6	.751
Phospholipids (mg/dL)	100.7 ± 9.4	97.8 ± 8.0	.817
Glucose (mg/dL)	65.2 ± 5.9	57.8 ± 5.0	.339
Uric acid (mg/dL)	4.29 ± 0.4	3.99 ± 0.3	.521
Umbilical cord artery	(n = 9)	(n = 19)	
Total cholesterol (mg/dL)	81.2 ± 8.3	55.2 ± 5.7	.017
Triglycerides (mg/dL)	52.0 ± 8.9	51.2 ± 6.7	.811
HDL-cholesterol (mg/dL)	30.9 ± 2.9	25.9 ± 2.0	.176
LDL-cholesterol (mg/dL)	11.0 ± 1.1	7.73 ± 0.7	.021
Phospholipids (mg/dL)	94.7 ± 7.8	97.6 ± 5.8	.764
Glucose (mg/dL)	52.5 ± 7.3	62.8 ± 5.0	.259
Uric acid (mg/dL)	5.70 ± 0.3	4.67 ± 0.2	.019

HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

Values expressed as mean ± standard error. Model adjusted for the exercise intervention programme and gestational age.

have been also associated with large for gestational age new-borns.^{30,31} We observed (with evidence of statistical significance) lower gestational age in male compared with female new-borns (they were born one week earlier), which may concur with the potentially increased risk of preterm birth attributed to males.^{5,32} Nonetheless, our findings contrast with the study by Pac-Kożuchowska et al.⁸ where cord blood concentration of triglycerides, HDL-cholesterol and LDL-cholesterol was higher in

females. They did not control their analyses for gestational age, and they did neither specify where umbilical cord blood samples were taken (presumably venous cord blood). To note, associations between maternal metabolic parameters and neonatal adiposity vary between different periods during pregnancy, and time-dependency is different between sexes.⁷ Further studies are required to confirm or contrast these findings and the mechanisms involved.

Table 4. Placenta status in pregnant women by foetal sex.

	Males	Females	<i>p</i>
Placenta weight and efficiency	(<i>n</i> = 50)	(<i>n</i> = 52)	
Weight (g) (<i>n</i> = 50 vs 52)	559.2 ± 14.3	586.3 ± 14.0	.180
Placental-newborn weight ratio	0.167 ± 0.004	0.178 ± 0.004	.047
Genetics	(<i>n</i> = 42)	(<i>n</i> = 45)	
Telomere length (Rel T/S ratio)	0.553 ± 0.05	0.670 ± 0.05	.076
Mitochondrial DNA copy number	0.91 ± 0.07	0.98 ± 0.07	.400
Mitochondrial DNA deletions	1.10 ± 0.10	1.16 ± 0.10	.642
Angiogenesis and growth markers	(<i>n</i> = 39)	(<i>n</i> = 36)	
Interleukin 8 (pg/ml)	5.3 ± 1.7	5.7 ± 1.6	.319
Epidermal growth factor (pg/ml)	33.4 ± 2.4	32.1 ± 2.5	.701
Fibroblast growth factor 2 (pg/ml)	4750 ± 337	5728 ± 346	.048
Vascular endothelial growth factor (pg/ml)	44.7 ± 9.1	55.5 ± 9.1	.493
Minerals	(<i>n</i> = 22)	(<i>n</i> = 27)	
Manganese (mg/kg)	0.14 ± 0.03	0.23 ± 0.03	.051
Iron (mg/kg)	640 ± 9.0	763 ± 8.1	.306
Zinc (mg/kg)	74.0 ± 7.1	81.0 ± 6.4	.441
Selenium (mg/kg)	1.53 ± 0.2	1.72 ± 0.1	.352

Values expressed as mean ± standard error. Model adjusted for the exercise intervention programme and gestational age.

Maternal and neonatal glycaemic profiles

We did not find differences in glucose and insulin concentration and resistance between mothers carrying males compared to females, which may be explained by the high heterogeneity of this condition.²⁹ Several studies show a male predominance for GDM,⁴ whereas others failed to find such association.³³ Female new-borns are believed to be more insulin resistant,³⁴ which might explain why males are more affected by in utero exposure to GDM.³⁵

The small number of pregnant women with glycaemic complications in the present study hinder us to understand how sex could increase the risk for GDM. In the DALI study,⁷ at 35th–37th g.w., obese pregnant women carrying a female showed greater fasting glucose, without foetal sex-dependant differences regarding lipids. Contrary, at 30th g.w., Retnakaran et al.³⁶ observed that women carrying a male had poorer β-cell function and higher blood glucose. Therefore, more studies with greater sample sizes are necessary before reaching any solid conclusion.

Foetal sex and placenta status

Sexual dimorphism might have also effects on the placenta. It is believed that males present heavier placentas than females in normal pregnancies,¹¹ which neither concurs with our findings, where placenta weights were similar. Some authors have hypothesized that this heavier placenta could mean that male placenta is more efficient.¹¹ However, male foetuses seem to be at greater risk of alterations when nutrients are limited.¹² Nonetheless, in a study with singleton uncomplicated pregnancies, the authors found an interaction effect between maternal body mass index and foetal sex on placental weight and efficiency, as positive

differences in placental parameters were present only in females.¹⁰ They also reported a difference in placental adaptation depending on foetal sex, with advantages only in females. This is relevant because this fact could explain why female foetuses do better than males in terms of survival.¹⁰

In our study, we also observed a trend towards longer telomeres in placentas from women carrying a female. Moreover, we observed higher placental new-born weight ratio and fibroblast growth factor 2 in female's placentas, which could indicate lower placenta ageing and a greater placental functional volume, with greater villous, angiogenesis and overall development,^{37,38} as reported for placentas exposed to maternal exercise.^{39,40} In agreement with our findings, Pac-Kożuchowska et al.⁸ also described greater placental-foetal weight ratio in females.

Very related to placental function is the integrity of mitochondria and mtDNA. Alterations in mtDNA density have been associated to a higher risk of placental abruption. Moreover, mitochondrial mutations such as deletions, may be important as placenta maturation and aging happen. In this sense, we did not confirm any sex-related difference in both mtDNA density neither mtDNA deletions, which must be confirmed in future studies. Nonetheless, it should be highlighted that our study sample was mainly composed by pregnant women without metabolic alterations. Notwithstanding, the higher manganese content observed in female's placentas might indicate a better antioxidant activity in this tissue,⁴¹ which has been previously found in the cord blood of females.³ It should be taken into account that manganese is present in the mitochondrial antioxidant enzyme superoxide dismutase. In fact, in the present sample (data not shown), manganese content was associated with lower mtDNA deletions and greater mtDNA density.

This study has limitations that must be underlined. First, only interested participants were involved, and the sample size was heterogeneous and relatively small. Consequently, results must be interpreted cautiously. Second, due to the difficulty to obtain samples, we lack serum maternal and umbilical cord blood at birth, as well as some placentas samples. This study has also several strengths. We show several metabolic markers under the same study, and we do it in different tissues and times. Moreover, we assessed potential confounders such as maternal diet and objectively measured physical activity. Therefore, results provide a comprehensive examination of the differences that depend on foetal sex that could be used in the future to understand some pregnancy outcomes.

Conclusion

Overall, female new-borns and mothers of females seem to present better cardiometabolic and placenta metabolism. This study provides a novel insight supporting that foetal sex should be considered in the monitoring of pregnancies, which might favour the prevention of any potential metabolic disruption, especially among pregnant women carrying a male. If future studies confirm these results in similar and bigger samples of pregnant women and their new-borns, health professionals should consider the implementation of strategies aimed at improving healthcare in pregnant women carrying a male.

Declarations

Ethics approval and consent to participate

The GESTAFIT project was approved by the Clinical Research Ethics Committee (code: GESTAFIT-0448-N-15, approved on 19/05/2015). A written informed consent was obtained from all participants before enrolling in the study.

Consent for publication

Not applicable.

Author contribution(s)

Virginia A Aparicio: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing.

Laura Baena-García: Formal analysis; Investigation; Methodology; Writing – original draft; Writing – review & editing.

Marta Flor-Aleman: Formal analysis; Investigation; Methodology; Writing – review & editing.

Luis J Martínez-González: Formal analysis; Methodology; Writing – review & editing.

Alfonso Varela-López: Investigation; Methodology; Writing – review & editing.

Cristina Sánchez: Data curation; Investigation; Methodology; Resources; Writing – original draft; Writing – review & editing.

José L Quiles: Data curation; Investigation; Methodology; Resources; Writing – review & editing.

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Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

The dataset used and analyzed in this study is available from the corresponding authors without restriction.

ORCID iDs

Laura Baena-García  <https://orcid.org/0000-0002-4895-567X>

Luis J Martínez-González  <https://orcid.org/0000-0003-2202-0662>

Supplemental material

Supplemental material for this article is available online.

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References

1. Nelson SM, Matthews P and Poston L. Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. *Hum Reprod Update* 2010; 16(3): 255–275.
2. Hanson MA and Gluckman PD. Developmental origins of health and disease – global public health implications. *Best Pract Res Clin Obstet Gynaecol* 2015; 29: 24–31.
3. Diaz-Castro J, Pulido-Moran M, Moreno-Fernandez J, et al. Gender specific differences in oxidative stress and inflammatory signaling in healthy term neonates and their mothers. *Pediatr Res* 2016; 80(4): 595–601.
4. Al-Qaraghoul M and Fang YMV. Effect of fetal sex on maternal and obstetric outcomes. *Front Pediatr* 2017; 5: 144.
5. Ingemarsson I. Gender aspects of preterm birth. *BJOG* 2003; 110(Suppl. 20): 34–38.
6. Shen H, Liu X, Chen Y, et al. Associations of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: a prospective longitudinal cohort study. *BMJ Open* 2016; 6: e013509.

7. Lima RA, Desoye G, Simmons D, et al. Temporal relationships between maternal metabolic parameters with neonatal adiposity in women with obesity differ by neonatal sex: secondary analysis of the DALI study. *Pediatr Obes* 2020; 15(7): e12628.
8. Pac-Kożuchowska E, Rakuś-Kwiatosz A and Krawiec P. Cord blood lipid profile in healthy newborns: a prospective single-center study. *Adv Clin Exp Med* 2018; 27(3): 343–349.
9. Weissmann-Brenner A, Simchen MJ, Zilberberg E, et al. Combined effect of fetal sex and advanced maternal age on pregnancy outcomes. *Med Sci Monit* 2015; 21: 1124–1130.
10. Leon-Garcia SM, Roeder HA, Nelson KK, et al. Maternal obesity and sex-specific differences in placental pathology. *Placenta* 2016; 38: 33–40.
11. Kalisch-Smith JI, Simmons DG, Dickinson H, et al. Review: sexual dimorphism in the formation, function and adaptation of the placenta. *Placenta* 2017; 54: 10–16.
12. Mandò C, Calabrese S, Mazzocco MI, et al. Sex specific adaptations in placental biometry of overweight and obese women. *Placenta* 2016; 38: 1–7.
13. Alur P. Sex differences in nutrition, growth, and metabolism in preterm infants. *Front Pediatr* 2019; 7: 22.
14. Shrestha D, Workalemahu T and Tekola-Ayele F. Maternal dyslipidemia during early pregnancy and epigenetic ageing of the placenta. *Epigenetics* 2019; 14(10): 1030–1039.
15. Kumar N and Singh AK. Maternal serum uric acid and calcium as predictors of hypertensive disorder of pregnancy: a case control study. *Taiwan J Obstet Gynecol* 2019; 58(2): 244–250.
16. Moreno Santillan AA, Briones Garduño JC, Diaz de Leon Ponce MA. Uric acid in pregnancy: new concepts. *Contrib Nephrol* 2018; 192: 110–115.
17. Aparicio VA, Ocón O, Padilla-Vinuesa C, et al. Effects of supervised aerobic and strength training in overweight and grade I obese pregnant women on maternal and foetal health markers: the GESTAFIT randomized controlled trial. *BMC Pregnancy Childbirth* 2016; 16: 290.
18. Panagiotakos DB, Pitsavos C and Stefanadis C. Dietary patterns: a mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. *Nutr Metab Cardiovasc Dis* 2006; 16(8): 559–568.
19. Acosta-Manzano P, Coll-Risco I, Van Poppel MNM, et al. Influence of a concurrent exercise training intervention during pregnancy on maternal and arterial and venous cord serum cytokines: the GESTAFIT Project. *J Clin Med* 2019; 8: 1862.
20. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28(7): 412–419.
21. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002; 30: e47–e47.
22. Varela-Lopez A, Pérez-López MP, Ramirez-Tortosa CL, et al. Gene pathways associated with mitochondrial function, oxidative stress and telomere length are differentially expressed in the liver of rats fed lifelong on virgin olive, sunflower or fish oils. *J Nutr Biochem* 2018; 52: 36–44.
23. Rivas-García L, Quiles JL, Varela-López A, et al. In vitro study of the protective effect of manganese against vanadium-mediated nuclear and mitochondrial DNA damage. *Food Chem Toxicol* 2020; 135: 110900.
24. Konta T, Ichikawa K, Kawasaki R, et al. Association between serum uric acid levels and mortality: a nationwide community-based cohort study. *Sci Rep* 2020; 10: 6066.
25. Shiozaki A, Matsuda Y, Satoh S, et al. Comparison of risk factors for gestational hypertension and preeclampsia in Japanese singleton pregnancies. *J Obstet Gynaecol Res* 2013; 39(2): 492–499.
26. Washburn LK, Nixon PA, Russell GB, et al. Preterm birth is associated with higher uric acid levels in adolescents. *J Pediatr* 2015; 167(1): 76–80.
27. Martínez AD, Ruelas L and Granger DA. Association between body mass index and salivary uric acid among Mexican-origin infants, youth and adults: gender and developmental differences. *Dev Psychobiol* 2017; 59(2): 225–234.
28. Miller N, Asali AA, Agassi-Zaitler M, et al. Physiological and psychological stress responses to labor and delivery as expressed by salivary cortisol: a prospective study. *Am J Obstet Gynecol* 2019; 221(4): 351e1–351.
29. Herrera E and Ortega-Senovilla H. Implications of lipids in neonatal body weight and fat mass in gestational diabetic mothers and non-diabetic controls. *Curr Diab Rep* 2018; 18: 7.
30. Lei Q, Niu J, Lv L, et al. Clustering of metabolic risk factors and adverse pregnancy outcomes: a prospective cohort study. *Diabetes Metab Res Rev* 2016; 32(8): 835–842.
31. Hou RL, Zhou HH, Chen XY, et al. Effect of maternal lipid profile, C-peptide, insulin, and HBA1c levels during late pregnancy on large-for-gestational age newborns. *World J Pediatr* 2014; 10(2): 175–181.
32. Sheiner E, Levy A, Katz M, et al. Gender does matter in perinatal medicine. *Fetal Diagn Ther* 2004; 19(4): 366–369.
33. Schaefer-Graf UM, Meitzner K, Ortega-Senovilla H, et al. Differences in the implications of maternal lipids on fetal metabolism and growth between gestational diabetes mellitus and control pregnancies. *Diabet Med* 2011; 28(9): 1053–1059.
34. Walsh JM and McAuliffe FM. Impact of maternal nutrition on pregnancy outcome – does it matter what pregnant women eat. *Best Pract Res Clin Obstet Gynaecol* 2015; 29(1): 63–78.
35. Macaulay S, Munthali RJ, Dunger DB, et al. The effects of gestational diabetes mellitus on fetal growth and neonatal birth measures in an African cohort. *Diabet Med* 2018; 35(10): 1425–1433.
36. Retnakaran R, Kramer CK, Ye C, et al. Fetal sex and maternal risk of gestational diabetes mellitus: the impact of having a boy. *Diabetes Care* 2015; 38(5): 844–851.
37. Ehsani V, Mortazavi M, Ghorban K, et al. Role of maternal interleukin-8 (IL-8) in normal-term birth in the human. *Reprod Fertil Dev* 2019; 31(6): 1049–1056.
38. Reynolds LP and Redmer DA. Angiogenesis in the placental. *Biol Reprod* 2001; 64: 1033–1040.
39. Clapp JF 3rd, Kim H, Burciu B, et al. Continuing regular exercise during pregnancy: effect of exercise volume on fetoplacental growth. *Am J Obstet Gynecol* 2002; 186(1): 142–147.
40. Clapp JF. The effects of maternal exercise on fetal oxygenation and fetoplacental growth. *Eur J Obstet Gynecol Reprod Biol* 2003; 110(Suppl): S80–S85.
41. Li L and Yang X. The essential element manganese, oxidative stress, and metabolic diseases: links and interactions. *Oxid Med Cell Longev* 2018; 2018: 7580707.